Molecular Mechanisms of Glucocorticoids Action: From Basic Research to Clinical Implications

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Abstract: Glucocorticoid hormones (GCs) have pleiotropic activities in the body playing important roles in metabolism and modulating/regulating the stress and immune responses. Upon stimuli that trigger immune or inflammatory responses there is a concomitant activation of the hypothalamus-pituitary-adrenal axis ultimately manifested by an increase in the synthesis and release of GCs to the systemic circulation. GCs play a pivotal role in the interface between the neuroendocrine and immune systems by modulating the final outcome of the immune response. The successful resolution of an immune response depends on the fine tune interplay between GCs and cytokines. The interaction between intracellular signals elicited by cytokines and the activated glucocorticoid receptor (GR) results in the induction or repression of gene transcription coordinating an effective immune response, and then its resolution avoiding excessive deleterious reactions. Herein, we described recent knowledge regarding basic research in the complex interaction between GCs and components of the immune system at cellular and molecular levels, as well as their clinical implications for health and disease. The benefits of therapeutic GCs controlling immune disorders as well as their misconduct are also discussed in terms of considering the benefits and adverse effects to control disease and inflammation.

Keywords: Glucocorticoids, transcription factors, molecular interaction, inflammation.

INTRODUCTION

An important feature of immune and inflammatory responses is the marked increase in cytokine synthesis. These cytokines activate the hypothalamic-pituitary-adrenal (HPA) axis, causing an increase of systemic glucocorticoids (GCs). GCs are steroid hormones produced mainly by the adrenal gland having a wide range of metabolic functions including the regulation of glucose, fat and protein metabolism. These actions are known to contribute to the side effects when GCs are applied at pharmacological doses. Because of their anti-inflammatory and immunosuppressive actions GCs are used as therapeutic drugs in a range of inflammatory, immune and allergic diseases. GCs participate actively in the interaction between the neuroendocrine and immune systems. The goal of this counter-regulatory interplay is to assure a fine tune regulation to maintain homeostasis of the whole body and avoid deleterious inflammatory responses [1]. Cytokines and hormones act as messengers that send systemic information at all cell effectors. The final outcome of an adaptive response will depend on the satisfactory integration of this information at the intracellular level, which occurs through the molecular interaction between immune/inflammatory components and steroid receptor signaling [2-7].

There are different levels of interaction between GCs and immune signals with the final outcome of regulation of gene expression [6]. One such interaction involves the cross-talk between the activated glucocorticoid receptor (GR) and transcription factors (TFs) involved in the regulation of inflammatory mediators. This interplay usually results in repression of inflammatory gene transcription. Also, this cross-talk can occur by interference with the activity of signal transduction components such as downstream kinases and phosphatases ultimately regulating GR and TF activies. A further level of mutual regulation between GCs and immune signals may be posttranslational modifications such as acetylation, methylation and phosphorylation together with specific protein components of the chromatin remodeling machinery, modifying corepressors or coactivators recruitment which finally leads to gene repression or activation. Some of these regulatory systems operate together, imparting variability and complexity to the regulation of the transcriptional activity of target promoters.

The anti-inflammatory activity of GCs is mediated through cytoplasmic GR that after activation influences gene expression. The frequent clinical use of GCs analogs to beat inflammation is due to their ability to diminish the expression of proinflammatory genes by a mechanism named transrepression. This mechanism refers to activation of the GR by binding of its cognate ligand and then, inhibition of the activity of TFs that goverrn proinflammatory gene expression, such as NF-κB and AP-1 [8]. The interaction between NF-κB or AP-1 and GR may impair their capacity to activate gene expression in a GR-dimerization independent manner.

Interestingly, several studies have shown that some of the effects of the GCs are observed after such a short time that they cannot be explained by the classical genomic

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mechanism of action [9]. For example there are rapid GC effects on actin structures, neuronal membranes, transmembrane currents [10-12] and rapid activation of protein kinases, such as phosphatidylinositol-3 kinase and Akt [13]. These non-genomic GC activities can be subclassified further into three modes of action: by specific interaction with the cytosolic GR (cGR) [11], by nonspecific interactions with cellular membranes [12, 14, 15] and by specific interactions with membrane-bound GR (mGR) [16, 17].

Exposition to high long term GCs use may end in undesirable side effects as a result of lingand-GR complexes targeting genes *via* a transactivation mechanism [18]. The affected genes by GCs are responsible for side effects and correspond to those genes involved in the metabolism of sugars, proteins, fat, bone and muscle *via* transactivation. Of note, metabolic disorders because of chronic GCs administration are between the most common undesirable effects. In fact, glucocorticoid response elements (GREs) are present within the promoter regions of genes controlling metabolic pathways such as tyrosine aminotransferase (TAT) and phosphoenolpyruvate carboxykinase (PEPCK). Among the general use of GCs for treatment of several inflammatory diseases, there is a particular concern over patients under chronic GCs administration on insulin resistance and diabetes, mainly by induction gluconeogenesis.

This has led to the search of selective GR modulators, such as dissociated GR ligands, that selectively transrepress thus, reducing the appearance of wide range of side effects. There is a great interest in the pharmaceutical industry to find steroidal analogs without side effects but maintaining their therapeutic efficacy. Herein, we sumarized the understanding of the molecular basis that underlie the therapeutic and side effects involved in the action of GCs. This knowledge is being exploited in the development of new effective and safe drugs for the treatment of inflammatory, autoimmune and allergic disorders, avoiding systemic effects.

Induction of Gene Expression by GCs

GCs exert their anti-inflammatory and immunomodulatory actions through binding to the GR, a member of the nuclear receptor family. Similar to other steroid receptors, the GR has three distinct functional domains [19]. The N-terminal region has a hormone-independent transcriptional activation function (AF-1). The central part contains highly conserved zinc finger DNA binding domain (DBD), a nuclear localization signal and a dimerization motif. The N-terminal zinc finger is involved in DNA interaction, whereas the C-terminal region has a ligand-binding domain (LBD) with an additional transcriptional activation function (AF-2) [20]. The LBD of the GR is associated in the cytosol to heat shock proteins (e.g. Hsp90, Hsp70, Hsp40 and Hsp23) and immunophilins (e.g. FKBP51, FKBP52 and PP5) [21] (Fig. **1**). This complex serves to maintain the GR in an adequate conformation for high-affinity hormone binding. Interestingly, instead of being kept inactive in the cytoplasm of the cell, a rapid nucleocytoplasmic shuttling of the receptor underlies its localization [22]. Upon interaction with GCs, GR conformation is modified allowing its release from the complex and translocation to the nucleus [23, 24]. This conformational change includes alterations within the LBD region creating surfaces for best interactions with coregulators [25] thus, nucleating the assembly of multi-protein regulatory complexes. Recently, a nuclear retention signal (NRS) in the hinge region of GR that actively opposes the nuclear export of GR as well as the nuclear export mediated through an ectopic CRM1 (chromosome region maintenance 1) /exportin1-dependent nuclear export signal (NES) has been defined [26]. Also, this study suggests that active nuclear retention of GR plays an important role in the regulation of GR-mediated signaling.

In the nucleus GR binds as a homodimer to specific DNA motifs, the GREs. The GR-GRE complexes are continuously disassembled, by active processes driving GR apart from GREs. The LBD of the GR regulates GR exchange with GREs but this interaction is independent from ligand dissociation [27]. Several factors are implicated in the dissociation of GR from chromatin, including chaperones p23 and Hsp90, the proteasome, and Switch/Sucrose-Non-fermenting (SWI/SNF) chromatin remodeling complexes. *In vitro,* chaperones and SWI/SNF complexes dissociate receptors from DNA [28-30]. In addition, p23 and Hsp90 localize to genomic response elements in a hormone-dependent manner *in vivo* and disrupt receptor-mediated transcriptional activation *in vivo* and *in vitro* [30]. With multiple factors targeting on the LBD, some stabilizing and other destabilizing the interaction GR-chromatin, the balance of these factors influences the dynamics of the GR-chromatin interaction [27].

GR binding to DNA occurs at nuclease-accessible sites of hypersensitivity to DNase I (DHS) [31]. Several mechanisms render these sites available for GR binding. Interestingly, the accessibility of GR binding sites is either constitutive or inducible. Within each category, some DHS sites require the multimeric chromatin remodeling ATPase Brahma Related Gene 1 (BrgI), a homologue of yeast SWI/SNF, but others are BrgI-independent, implicating a different remodeling complex. The histone variant H2A.Z is highly enriched at both inducible and constitutive DHS sites and is subject to exchange during hormone activation. Moreover, the DHS profile is highly cell specific, implicating cell-selective organization of the chromatin structure as a critical determinant of tissue-selective receptor function, revealing another layer of regulation of the transactivation mechanism. These findings highlight the importance of nucleoprotein reorganization in the global GR response and that GR interactions throughout the genome involve a number of distinct remodeling systems [31].

Once the GR is bound to GRE, the transactivation domains of the GR serve for the recruitment of transcriptional coactivators that either directly remodel chromatin or facilitate the initiation of transcription (Fig. **1**). The AF-1 domain recruits BrgI in a ligand independent manner [32, 33] which in turn, assembles coactivators CREB binding protein (CBP)/p300 and the histone acetylases p300/CBP-associated protein (P/CAF). Upon hormone binding the AF-2 domain recruits members of the p160 family of coactivators such as, steroid receptor coactivator (SRC)-1, SRC-2/ GR interacting protein (GRIP)1/ transcriptional intermediary factor (TIF)2 and RAC3/ACTR/

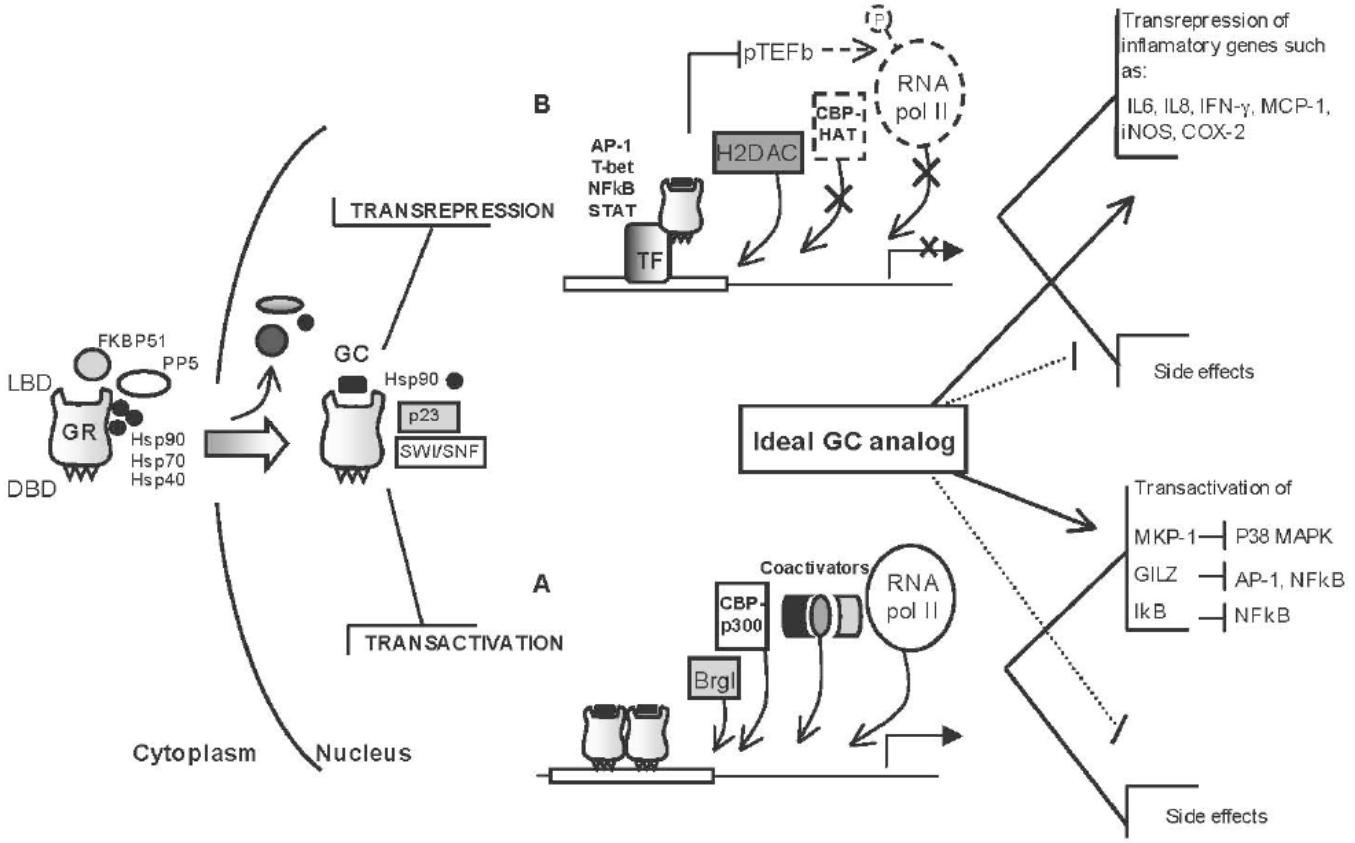

Fig. (1). The two major pathways of GR-dependent transcriptional regulation: transrepression *vs* transactivation. The LBD of the GR is associated in the cytosol to heat shock proteins (e.g. Hsp90, Hsp70, Hsp40) and immunophilins (e.g. FKBP51 and PP5). Receptor activation occurs upon lingand binding which initiates substitution of one immunophilin (FKBP51) for another (FKBP52) and concomitant recruitment of dynein, leaving Hsp90 unchanged. Translocation of the hormone-generated GR-Hsp90-FKBP52-dynein complex from cytoplasm to nucleus precedes dissociation of the complex within the nucleus. Once interacting with GREs, several factors are implicated in the dissociation of GR from chromatin, including chaperones p23 and Hsp90, the proteasome, and the SWI/SNF chromatin remodeling complexes. Transactivation and transrepression have different mechanisms leading to immunomodulation. *A*, Transactivation ocurrs when GR is bound to GRE serving for recruitment of transcriptional co-activators that remodel chromatin or facilitates transcription of anti-inflammatory genes such as MKP-1, GILZ or IκB. *B*, On the other hand, GR interaction with AP-1, T-bet, NF-κB or STAT directly interferes with transcription of inflamatory genes without affecting TF-DNA binding. Taking into consideration that both pathways have undesirable effects, related to the metabolic function of GCs, an ideal GC analog should enhance the anti-inflamatory actions without causing undesirable side effects. GC: glucocorticoids, GR: glucocorticoid receptor, GRE: glucocorticoid response elements, Hsp: heat shock protein, SWI/SNF: Switch/Sucrose-Non-Fermenting, PP5: protein phosphatase 5, FKBP: FK506 binding protein, TF: transcription factor, RNApol: RNA polymerase, HDAC2: Histone deacetylase 2, CBP-HAT: CREB binding protein with histone acetyl transferases activity. BrgI: brahma-related gene 1, LBD: ligand-binding domain, DBD: DNA-binding domain, P-TEFb: positive transcription elongation factor b, NF-κB: nuclear factor κB, AP-1: activator protein 1, STAT: signal transducers and activators of transcription, IFN-γ: Interferon-gamma, MKP-1: mitogen activated protein kinase phosphatase-1, GILZ: GC inducible leucine zipper, IκB: inhibitor kappa B. IL: interleukin, MCP-1: Monocyte chemotactic protein-1, COX-2: cyclooxygenase-2, iNOS: inducible nitric oxide sintase, p38 MAPK: p38 mitogen activated protein kinase, P-: phosphate. Dotted blunt ended arrows represent lack of effects and full arrows represent induction.

p/CIP that possess histone acetylase (HAT) activity, leading to disruption of nucleosomes. Recently, an additional factor called STAMP was shown to be associated with coactivators TIF2 and SRC-1 and it is a downstream component of GR action in both gene activation and expression [34]. Other chromatin-modifying enzymes such as CBP/p300 and the histone arginine methylase, coactivator associated arginine methyltransferase 1 (CARM1) [35, 36] interact with the GR. However, the desition of which co-activator/chromatin complexes are finally present on gene promoter regions depends on the availability of coactivators in a particular cellular context, the abundance of other TFs competing for the same coactivators, the stage of chromatin condensation and phosphorylation status of the AF-1 domain [37, 38].

Transactivation is key for the metabolic effects of GCs and this mechanism is also important for the anti-inflammatory role of GCs [39] (Fig. **1**). GCs-mediated gene expression is required to induce mRNA desestabilization of inflammatory genes such as interleukin (IL)-6, IL-8, monocyte chemoattractant protein-1 (MCP-1), cyclo-oxygenase-2 (COX-2) [40-44]. AU-rich elements (AREs) present in the 3′ untranslated regions of mRNAs may mediate desestabilization by GCs [44, 45]. In this respect, mitogen activated protein kinase phosphatase-1 (MKP-1) is induced by GCs and desphosphorylates p38 MAPK which in

turn plays an important role in the production of proinflammatory cytokines and is involved in ARE-mediated stabilization of inflammatory genes [46-48]. Also, p38 mitogen activated protein kinase (MAPK) induces the expression of inflammatory TFs such as activating transcription factor (ATF)-1, ATF-2, activator protein (AP)-1 and nuclear factor (NF)-κB. Therefore, MKP-1induction by GCs may lead to the repression of inflammatory genes regulated by these TFs [39, 49, 50]. GC inducible leucine zipper (GILZ) is another GC-inducible gene with anti-inflammatory activity and represses AP-1, NF-κB, Ras-1 and Raf [51-53]. GILZ may associate with Raf-1 inhibiting the activation of downstream extracellular signal-regulated kinase (ERK) MAPK and therefore, blocking inflammatory gene transcription [54]. Taking into account that the knowledge underlying precisely the molecular mechanism of GC-mediated transactivation is still poor, a more detailed comprehensive mechanistic analysis is required due to the increasing number of new recognized GC-induced genes.

On the other hand, cytokine activated MAPK signaling can phosphorylate GR regulating its turnover and transcriptional activity [20, 55, 56]. Site specific (Serine 211) phosphorylation of the GR enhances its interaction with a protein of the DRIP/TRAP coactivator complex, clearly suggesting that site-specific phosphorylation is regulating GR transcriptional activity [56]. Also, the phosphorylation status of the GR has a profound effect on the GR stability affecting its half-life and ligand-dependent stabilization, indicating that phosphorylation is involved in receptor turnover [55]. Therefore, this posttranslational modification may have significant biological effects on GR expression and transactivation of its target genes.

Multiple cyclin-dependent kinase (CDK), c-Jun N-terminal kinase (JNK) and ERK have been found to phosphorylate different serine/threonine residues within GR, modifying its transcriptional activity [57-59]. Suppression of the GR function by activated p38 and JNK MAPK is a physiologically relevant mechanism of GCs-resistance observed in a subgroup of patients with chronic inflammatory disorders, such as asthma [60, 61]. Also, phosphatases might play a role in regulating dissociation between GR and Hsp90 and its nucleocytoplasmic shuttling, its DNA binding and transcriptional regulatory activities [46, 62-64]. The phosphorylation of the GR at multiple sites by different kinases affects a range of distinct receptor activities, such as dimerization, DNA binding, subcellular localization, or interaction with other proteins, including those involved in the transcriptional machinery. Each of these functions could be affected either positively or negatively, thereby adjusting the receptor response to environmental changes and becoming an important player of GR regulation [20].

Repression of Gene Transcription by GCs

Protein-protein interaction is one of the mechanisms through out GR modulates gene responses, which is commonly responsible for repression of transcription of inflammatory genes. This mechanism is called transrepression and involves the association between GR and other TFs such as AP-1, T-bet, NF-κB, cAMP response element-binding protein (CREB) and signal transducers and activators of transcription (STAT) family members (Fig. **1**). These TFs are involved in the activation of proinflammatory and immunoregulatory genes, such as cytokines, cytokine receptors, chemotactic proteins and adhesion molecules [6, 23, 65-68]

Several reports have shown that GCs inhibit NF-κB activity on IL-8, inducible nitric oxide synthase or rat cytokine-induced-neutrophil chemoattractant gene expression by repression of NF-κB DNA binding activity [69-71]. On the contrary, other studies showed that inhibition of intercellular adhesion molecule-1 and E-selectin also involved NF-κB but the mechanism is independent of NF-κB binding to DNA [72, 73]. Some of these effects may be due to inhibition of expression of the NF-κB subunit p50 [41, 74], p65 [74, 75], or induction of the expression of inhibitor-κB (IκB) [76, 77]. Although GCs directly interact with AP-1, it does not affect binding of AP-1 with DNA [78]. Analysis of AP-1 DNA occupancy revealed no effect of GCs, but direct interference of transcription (known as tethering negative GRE) might be involved. This mechanism best represents the current model of transrepressive inhibition of AP-1 [79] and also NF-κB activity [23].

Also, gene repression and activation are regulated by acetylation of histones. In the resting state, DNA is tightly packed around histones, forming a dense nucleosomal structure due to electrostatic attraction between negatively charged DNA and positively charged lysine residues. Acetylation of histones removes this charge, allowing the opening of the chromatin structure. Histone acetylation is mediated by transcriptional coactivators, which have intrinsic histone acetyltransferase (HAT) activity, whereas repression is induced by histone deacetylases (HDACs), which reverse this acetylation, allowing repackaging of the nucleosomes. Therefore, it is generally accepted that histone deacetylation is related with transcriptional repression, and that histone acetylation is required for activated transcription [80]. SRC-1 and GRIP1 act as adaptor proteins for GR with other cofactors, e.g. p300 and CBP (histone acetyl transferases). These cofactors modulate GR activity in a tissue-specific manner [81]. As mentioned before, steroid receptor coactivators, recruited by GR, can in turn bind other coactivators, such as p300/CBP and it subsequently, recruits SWI/SNF (a large multi-subunit protein complex) and mediator complexes. SWI/SNF enables chromatin remodeling to occur in an adenosine triphosphate (ATP)-dependent manner, but histone acetylation by p300/CBP facilitates the recruitment of SWI/SNF and mediator complexes. Thus, cofactor–cofactor interactions are essential for effective gene expression [82]. NF-κB binds to specific κB recognition sites in promoter regions of responsive genes and subsequently, recruits transcriptional coactivators such as CBP or p300/CBP-associated factor, that have intrinsic HAT activity. This results in acetylation of lysines in core histones, leading to recruitment of large protein complexes, including RNA polymerase II (RNA pol II), and in turn increments of inflammatory genes transcription. Nuclear events that mediate transrepression may involve the interference with the basal transcriptional machinery, *via* recruitment of the p160 family member GRIP [23, 59, 83, 84]. Also, GCs may decrease acetylation at inflammatory gene promoters by acting both as a direct inhibitor of CBP-associated HAT activity and by recruiting HDAC2 to the

p65-CBP HAT complex [85, 86]. GCs prevent phosphorylation of serine 2 residues within the C-terminal domain of the RNA pol II reducing its capacity to cause mRNA elongation and reinitiation. These may mediate promoter selective inhibition of NF-κB transcription *via* the GR dependent loss of a regulatory kinase complex, the Ser2 CTD kinase, positive transcription elongation factor b (P-TEFb) [87, 88].

Recently, a novel mechanism for GC transrepression has been found. GCs are able to modulate chromatin structure *via* interference with the recruitment of the nuclear kinase mitogen-and stress-activated protein kinase-1 (MSK-1) at inflammatory gene promoters, resulting in the inhibition of NF-κB transactivation and histone H3 S10 phosphorylation [89].

Interestingly, different regions of the GR DBD are apparently involved in AP-1 and NF-κB repression. Although the N-terminal zinc finger of the GR DBD is critical for AP-1 transrepression [90], it does not significantly contribute to the repression of NF-κB activity, as shown by point mutation experiments within the N-termianl zinc finger of GR [91]. In addition, previous data showed that GR mutants with deletion of the N-terminal domain could efficiently inhibit NF-κB but not AP-1 [92, 93]. Therefore, different mechanisms account for GC-mediated repression of AP-1 and NF-κB activities.

It has been described that GCs are able to inhibit the transcriptional activity of T box expressed in T cells (T-bet), a master T helper (Th)1 TF which has an important role in inflammation and autoimmune disorders. GCs inhibit T-bet activity by a transrepression mechanism involving protein-protein interaction between the activated GR and T-bet, and diminished DNA binding. GCs also inhibit T-bet mRNA and protein expression [68]. The master anti-inflammatory TF GATA-3 is also inhibited by GCs by means of a different molecular mechanism. GCs inhibit p38 MAPK-induced GATA-3 phosphorylation [94] by inducing MKP-1 mediated GATA-3 phosphorylation and nuclear translocation [95].

Transactivation *versus* Transrepression in Therapeutic Approaches

It is well accepted that the beneficial anti-inflammatory actions of GCs are generally sustained by transrepression mechanisms, whereas their side effects are mediated by GR binding to GREs located in promoter regions of featured genes. Assuming this principle, the ideal ligand-GR activators for therapeutic purposes must be those possessing only high transrepression and at the same time, with very low residual transactivation properties causing minimal side effects. This statement, although, might represent a simplistic mechanism of GCs action. In fact, full gene expression induced by GCs may require additional binding of protein complexes within promoter GREs regions as is the case for monoamine oxidase A [96].

The separation between transactivation and transrepression has been demonstrated using selective GR mutants. For example, mutation of Alanine 458 to Threonine within the dimerization D loop of the GR allows transrepression but not GRE-mediated transactivation [90]. Upon the replacement of the wild-type GR with a dimerization defective GR in mice (GR dim mice), a defective GC-induced GRE-dependent transcription and endogenous TAT gene expression was found but there was still the repression of AP-1 and NF-κB dependent inflammatory genes [97-99]. However, GR dim mice may allow induction of other GC-inducible genes such as, phenylethanolamine N-methyltransferase required for epinephrine synthesis [100] and MKP-1 because these are GC-inducible genes by the GR dim mutant [101, 102]. Therefore, on a subset of GR-responsive promoters, e.g. the phenylethanolamine N-methyltransferase (PNMT) gene, GR may form concerted multimers in a manner that is independent of receptor dimerization. Protein-DNA and protein-protein interactions supporting such complexes may be essential for activation of this kind of genes. Therefore, GR dims can bind specifically to a class of GREs and stimulate transcription, contradicting the conclusion that GR dims are globally deficient in DNA binding-dependent transactivation [101].

GR transactivation and transrepressive functions may also be dissociated by ligands, derivates of RU486 that have limited GRE transactivation but can still transrepress. Such is the case of RU24858 which represses NF-κB and AP-1 transcriptional activity. RU24858 does not modify IκB expression nor interfere with NF-κB DNA-binding ability. This GR ligand shows anti-inflammatory and immunosuppressive activities *in vivo* [103, 104]. However, RU24858 was equipotent compared with GCs in eliciting side effects (e.g., weight loss, thymus involution, osteopenia of the femur growth plate), suggesting that *in vitro,* separation of transrepression from transactivation activities does not necessary translate to an increased therapeutic benefit for GCs *in vivo* [103]. However, although the parameters chosen in this study represent some of the undesirable side effects of steroids, it has not been studied the safety profile on other known side effects of GCs such as diabetes mellitus, glaucoma, opportunistic infection and behavioral changes. If such parameters were diminished together with a significant retention of the anti-inflammatory activity, this would be an important development [103]. Given the possible therapeutic benefits of a dissociated steroid, the contradictory data generated to date should only serve as a stimulus for future research in this area.

Recently, Compound A (CpdA), a plant-derived phenyl aziridine precursor, was shown to exert an anti-inflammatory potential by down-modulating TNF-induced proinflammatory gene expression, such as IL-6 and E-selectin, but, did not enhance GRE-driven genes or induce GR binding to GRE-dependent genes [105]. The specific gene-repressive effect of CpdA depends on the presence of functional GR, displaying a differential phosphorylation status. The anti-inflammatory mechanism involves both a reduction of DNA-binding activity, as well as an interference with the transactivation potential of NF-κB. Finally, CpdA was shown to counteract acute inflammation *in vivo* and did not cause a hyperglycemic side effect. Also, CpdA was shown to inhibit joint inflammation without inducing hyperinsulinemia in rheumatoid arthritis in a collagen induced arthritis mouse model as compared with dexamethasone [106]. Also, CpdA suppressed experimental autoimmune neuritis by a mechanism involving regulatory T cells and M2 macrophage cells [107]. Taken together, this

compound may hold great potential for therapeutic use. Further studies involving anti-inflammatory genes and side effects must be performed preferentially *in vivo*.

Numerous steroidal and nonsteroidal ligands of GR where reported to have dissociated function based on standard reporter assays. These compounds showed anti-inflammatory activity but detailed description with respect of inducible genes or side effects are deficient. Despite the poor GRE-dependent transactivation these ligands may induce genes related to side effects or may have other unknown inflammatory properties [39].

GCs can induce gene transcription not only by binding to GRE elements but also in combination with other TFs and *via* promoter elements that do not necessary involve GR dimerization or DNA interaction. Therefore, ligands that seem to be silent at simple GREs may induce transcriptional responses from these other promoters [108]. Importantly, if a ligand is completely incapable of transactivation, this may reduce the expression of some anti-inflammatory genes such as MKP-1 or GILZ, reducing anti-inflammatory efficacy. Therefore, the design of future GR ligands should balance between undesirable and desirable transactivation and transrepressive properties.

Common Clinical Use of GCs

The situation of a strong immune response followed by an excessive tissue inflammation process plays a critical role in the pathogenesis and development of chronic inflammatory disorders. Compelling experimental as well as clinical evidence emphasize the key role of the hypothalamus-pituitary-adrenal (HPA) axis and the GCs-cytokine interplay during inflamation. In fact, activation of the HPA axis by proinflamatory cytokines increases GCs levels, which contributes to maintain homeostasis during immune response [109]. Under the situation of unresolved inflammation, despite the existence of a normal physiological response able to release high levels of endogenous steroids into circulation, administration of GCs analogs are often employed in the clinic and consist the first line drugs used to help control the organism homeostasis.

GCs and their available synthetic wide range analogs of cortisol are among the most effective employed anti-inflammatory drugs for the treatment of allergic, inflammatory, allograft survival, lymphoproliferative diseases and autoimmune disorders, including inflammatory bowel disease, asthma, rheumatoid arthritis, and several other autoimmune disorders. However, not all patients suffering chronic allergic and autoimmune processes respond well to pharmacological doses of GCs. Compelling evidence indicates that even in disorders that usually respond successfully to GCs-treatment there is up to 30% of patients that are insensible/resistant to high dose treatments [110]. Moreover, several inflammatory conditions are completely refractory to the action of GCs (Table **1**). Although, the term GC resistant is coined to individuals that do not resolve inflammation despite high dose and long-term GC therapy, the phenomenon of tissue refractory response to GC actions may also be manifested in healthy individuals and in non-inflammatory disorders, including major depression, chronic stress [111, 112] and healthy aging [113]. Interestingly, it has been demonstrated that GC resistance is independent of inflammation severity and it shows a wide spectrum of intensity between individuals [114].

Table 1. Summary of Common Chronic Inflammatory Diseases Showing Dysregulated Immune Balance and their Response to GCs Treatment

GCs-Responsive Chronic Inflammatory Diseases	References
Asthma	[106]
Rheumatoid arthritis*	[122]
Systemic lupus erythematosus	[123]
Ulcerative colitis*	[124]
Uveitis	[125]
Crohn's disease*	[126]
Atopic dermatitis	[127]
Inflammatory bowel disease	[128]
Multiple sclerosis	[129]
GCs-Resistant Inflammatory Diseases	
Interstitial pulmonary fibrosis	[130]
Cystic fibrosis	[131]
Chronic obstructive pulmonary disease (COPD)	[132]
Atherosclerosis	[133]
Acute respiratory distress syndrome	[134]

*A subgroup (25-30%) of these patients has been shown to have GCs-resistance.

The importance of GCs resistance resides in its complication for effective clinical management of patients afflicted by chronic inflammatory diseases. Despite GC resistance within a local site of inflammation, the risk for peripheral side effects still persists in patients frequently exposed to high dose and long-term use of GC. Therefore, side effects are much more frequently observed in GC resistance patients. It is now well known that many different molecular mechanisms take place in glucocorticoid resistance. Diverse molecular mechanisms by which GC-resistance takes place have been identified. Some of them may have a genetic origin and indeed, polymorphisms of GR have been related to impaired GC responses [115]. Also, activation of molecular pathways by pro-inflammatory cytokines may increase GR-phosphorylated Ser-residues by action of increased activity of several kinases such as p38 MAPK and ERK/JNK [60]. Inhibiting kinases might warrant therapeutic intervention at this level. Also, the increase GRβ-isoform expression correlates with GC-resistance [116]. Post-translational modifications of GR such as, ubiquitination and nitrosylation have demonstrated influence on GR activity *in vitro* [117, 118]. However, the latter has not been described in GC-resistance diseases, yet. All these mechanisms may modify GR affinity for GC, GR stability, nuclear translocation and binding to GRE in response genes. Successful treatment of GC-resistant patients is very difficult to achieve and therefore, a best knowledge of the molecular mechanisms involved in GC resistance is warranted. Clinically, the affected individuals are difficult to manage and they deserve the discovery and development from the

scientific community of novel alternative therapeutic drugs to inhibit tissue inflammation. Blocking underlying mechanisms of GCs resistance might be a putative strategy to reverse GC resistance employing novel dissociated therapeutic GCs.

Finally, it is important to mention that GCs are frequently employed in the treatment of pregnancies at risk of preterm delivery. The aim in this situation is to enhance lung maturation and reduce preterm mortality. However, antenatal GCs administration has been linked with several permanent tissue malfunctions during adulthood. Noteworthy, the (HPA) axis is severely influenced by antenatal GCs administration and alterations in HPA axis activity are associated with higher blood pressure, hyperlipidaemia and glucose-metabolism disorders [119, 120]. Therefore, *in utero* exposure to high exogenous GC levels or even due to stress conditions might represent a mechanism linking foetal development and adult life pathophysiology. Epidemiogical evidence and experimental work in rodent animals favor the concept of early life programming by GCs [121].

CONCLUSIONS

The separation between transactivation and transrepression has lately been the goal to generate anti-inflammatory agents with safe profiles. There are many GC-induced genes that exert anti-inflammatory effects (Fig. **1**). Therefore, drugs that loose their transactivation properties might loose effectiveness and a balance considering less side effects together with reduced anti-inflammatory properties should carefully be analyzed. On the other side, although the transrepression mechanism is strongly associated to anti-inflammatory actions, protein-protein interactions has also been associated to side effects such as GC induced bone loss [38]. Also, as mentioned before, for each analog, not all of the possible side effects have been investigated. Future research may focus on GR-ligands that present the most favorable balance between better anti-inflammatory and lesser-associated side effects.

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