Glucocorticoids in the regulation of transcription factors that control cytokine synthesis

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Abstract

The interaction at different levels between intracellular signals elicited by cytokines and activated glucocorticoid receptors (GR) is essential for the regulation of immune responses. We describe different levels of interaction between glucocorticoids and cytokines which result in the induction or repression of gene transcription. These include the regulation of cytokine receptor expression, the molecular cross-talk between the GR and transcription factors (TFs) activated by cytokine signaling, the interaction with several signaling pathways and also posttranslational modifications of both GR and TFs. Also, an overview of the implications of chromatin remodeling in this interplay is discussed. The complexity of the intricate network involved in the interaction between GR and TFs is pivotal for the final outcome of cytokines biological action.

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1. Glucocorticoids: sources and mechanism of action

1.1. Natural glucocorticoids

Steroid hormones influence several metabolic, reproductive, immune and neuroendocrine responses both in health and disease. Steroids are a group of small lipophilic compounds derived from a common precursor, cholesterol. The four major types of steroids are the progestins, androgens, estrogens and corticoids, which differ in the number of carbon atoms they contain, the receptors they bind and the biological activities they possess. Corticoids may be divided into two groups: mineralocorticoids, which regulate ion transport and glucocorticoids (GCs), which have many activities, including stress resistance, regulation of intermediary metabolism, and immunosuppressive and anti-inflammatory effects [1,2]. The first limiting step in steroid biosynthesis is the cleavage of the side chain of cholesterol by P450scc enzyme to generate the steroid pregnenolone. P450scc expression is limited to steroidogenic tissues such as the adrenals, placenta, gonads, brain and thymus. Pregnenolone is hydroxylated by P450c17, resulting in two possible pathways of corticoid synthesis. In adult rodents P450c17 expression is not detectable in the adrenal cortex, so the major circulating GC in mice is corticosterone which slightly differs from the preponderant circulating GC in most species, including human which is cortisol. Progesterone is hydroxylated to yield 11-deoxycorticosterone, which has little GC activity and is then converted in the mitochondria to the active GC corticosterone (or cortisol).

An important feature of immune or inflammatory responses is the marked increase in cytokine synthesis. These cytokines activate the hypothalamic–pituitary–adrenal system, causing an elevation of systemic GC levels. GCs participate actively in the interaction between the neuroendocrine system with the cellular components of the immune
system. The goal of this regulatory interplay is to assure a fine tune regulation to maintain homeostasis of the whole body avoiding excessive deleterious inflammatory/immunological effects. Within this network of communications, GCs and cytokines hold a prominent role coordinating an effective immune response against infections but avoiding excessive destruction and inflammation [3]. GCs inhibit both cytokine gene expression and pleiotropic actions on target cells, acting as immunosuppressive and anti-inflammatory agents that contain over-reactions of the immune system, as well as autoaggressive responses.

When environmental changes such as infectious states occur, organisms need to develop an adequate adaptive biological response. Through cytokines, cells receive information about the quality and magnitude of the aggression. Meanwhile, hormones give information about the changes suffered by different systems as result of the environmental alterations. Thus, cytokines and hormones act as messengers that send systemic information to all cellular effectors. The final outcome of an adaptive response will depend on the satisfactory integration of this information at the intracellular level, which occurs through a molecular interaction between cytokines and steroid receptor signaling, which is discussed in deep in this review.

1.2. Synthetic glucocorticoids

Due to their important anti-inflammatory and immunosuppressive actions, as for the endogenous natural GCs, the pharmaceutical industry has developed a number of synthetic analogs of GCs such as dexamethasone, betamethasone, triamcinolone, prednisone, prednisolone and methylprednisolone among others. These analogs are widely used in the therapy of inflammatory, autoimmune and allergic diseases and for transplanted patients [4–6]. They are also used to reduce organ allotransplant rejection and to treat brain edema, shock conditions, certain types of blood cancer (B- and T-cell lymphoma), as well as in diseases involving the adrenal cortex insufficiency. Their therapeutic use is based on their known effects as anti-inflammatory and immunosuppressive agents and for their role in induction of cell cycle arrestment and apoptosis. Dexamethasone relieves inflammation (swelling, heat, redness, and pain) and is used to treat certain forms of arthritis; skin, blood, kidney, eye, thyroid, and intestinal disorders (e.g., colitis), severe allergies and asthma. Dexamethasone is also used to treat certain types of cancer. Betamethasone and triamcinolone are commonly used for the relief of itching and inflammation associated with a wide variety of skin conditions. Prednisone is used alone or with other medications to treat the symptoms of certain types of cancer. Prednisolone is used to achieve prompt suppression of inflammation in many inflammatory and allergic conditions (e.g., rheumatoid arthritis, systemic lupus erythematosus, acute gouty arthritis, psoriatic arthritis, ulcerative colitis, and Crohn’s disease). It is also used for severe allergic disorders that fail conventional treatment, chronic skin diseases and chronic allergic and inflammatory conditions. Prednisolone is also used in the treatment of blood cell cancers (leukemias) and lymph gland cancers (lymphomas). Blood diseases such as idiopathic thrombocytopenia purpura and autoimmune hemolytic anemia can also be treated with prednisolone. Finally, prednisolone is used as a hormone replacement in patients whose adrenal glands are unable to produce sufficient amounts of corticosteroids. Methylprednisolone is used to achieve prompt suppression of inflammation (e.g., rheumatoid arthritis, systemic lupus erythematosus, acute gouty arthritis, psoriatic arthritis, ulcerative colitis, and Crohn’s disease). Severe allergic conditions that fail conventional treatment also may respond to methylprednisolone (e.g., bronchial asthma, allergic rhinitis, drug-induced dermatitis and contact and atopic dermatitis). Chronic skin conditions treated with methylprednisolone include dermatitis herpetiformis, pemphigus, severe psoriasis, and severe seborrheic dermatitis. Chronic allergic and inflammatory conditions of the uvea, iris, conjunctiva and optic nerves of the eyes also are treated with methylprednisolone. The knowledge of the molecular pathways involved in the action of GCs as we will discuss, is a major milestone for the development of new effective and safe drugs.

1.3. Molecular basis of glucocorticoid action

GCs are lipophilic compounds with the capability of diffusing freely through the cell membrane and binding their cytoplasmatic receptors. GCs exert their biological effects through binding to the GC receptor (GR), which is a ligand activated transcription factor (TF) regulating either positively or negatively the expression of target genes [1,7,8].

The GR belongs to a large superfamily that includes receptors for other steroid hormones, thyroid hormone, vitamin D3, retinoic acid and orphan receptors such as Nur77. The GR exists in the cytosol in an inactivated form as part of a complex with heat shock proteins and immunophilins. Upon interaction with GCs, the GR dissociates from the complex and translocates to the nucleus [1,8]. In the nucleus the GR binds as homodimer to specific palindromic DNA consensus sequence, the glucocorticoid response elements (GREs) (Fig. 1). Genes positively regulated by GR have GREs in the promoter which consists of two conserved 6-nucleotide halves separated by three nonconserved bases (5'-GGTACAnnnTGTTCT-3'). This mechanism is named transactivation (Fig. 1). Direct transcriptional repression can be achieved by the interaction of monomeric GR with negative GREs (nGRE) (Fig. 1) [9]. For some of these genes the mechanism also involves GR-dependent
displacement of another factor. Other DNA regulatory components, are tethering elements, where GR without binding to DNA acts through protein–protein interactions with other TFs that are specifically bound to DNA responsive sites. In the composite sites, GRE and other TF binding sites are involved and have some degree of overlapping, where GR can act either enhancing or repressing transcription of the involved gene. However, the GR can modulate gene responses also by protein–protein interaction, which is commonly responsible for repression of transcription of target genes. This mechanism is called transrepression and involves direct physical association between GR and other TFs, such as activating protein-1 (AP-1), nuclear factor-κB (NF-κB) and signal transducers and activators of transcription (STAT) family members (Fig. 1).

2. Cytokine regulation by glucocorticoids

There are different levels of interaction between GCs and cytokines with the final outcome of regulation of gene expression. The first upstream level of interaction starts with the selective regulation (induction or repression) of the expression of cytokine receptor genes. A further level of interaction involves the cross-talk between the activated GR and TFs implicated in the regulation of cytokine synthesis and function. This interaction results in the induction or repression of gene transcription. Also, when cytokine-induced transduction signals interact with the activated GR there may be an enhancement or an inhibition of GRE regulated genes. A further level of mutual regulation may be the ubiquitin–proteasome and sumoylation systems, which regulate GR transactivation modifying receptor trafficking and turnover. Other posttranslational modifications such as acetylation, methylation and phosphorylation together with specific protein components of the chromatin remodeling machinery have a role in maintaining the turnover of the GR and associated factors at active sites of transcription and consequently modifying cytokine gene expression. In this article we will review these different levels of regulatory interaction between GCs and cytokines, discussing the molecular mechanisms implicated and the functional consequences of this interaction.

2.1. Cytokine receptors regulation by glucocorticoids

Cytokines are synthesized and secreted by many kind of cell types in the body. They constitute critical components of the immune system being efficient messengers in the communication between immune cells and peripheral
tissues, and helping innate and adaptive immune responses (as extensively reviewed in Refs. [10–12]). They do have immune cells as targets, but they also exert different biological activities in a wide variety of tissues affecting growth, metabolism, hematopoiesis, proliferation and cell differentiation in health, and fulfill a pivotal role in the pathophysiology of a wide spectrum of clinical disorders. A substantial knowledge of data regarding the biological role of several cytokines became by contrasting their role during pathological situations and health. Consistent with the notion that immunity is a tightly controlled and dynamic process, several aspects of cytokines activity should be observed as a whole including its regulated expression, synergism, sharing of subunits and subunits receptors, and importantly, their role at well defined stages of disease development (initiation-, maintenance-, and end-stages). Nowadays, cytokines are divided into several few classes which do not show any systematic relationship between each other: interleukins, tumor necrosis factors (TNFs), colony stimulating factors (CSFs), interferons (IFNs), and chemokines. Cytokines may be also classified in line with binding to their specific receptors. The families of class I and II type of cytokine receptors can be structurally differentiated between each other by distinctive extracellular region conserved motifs, including those residues for ligand binding. Thus, both types of cytokine receptors bind ligands often designated as type I (the IL-4 and IL-6/12 families, IL-2, IL-7, IL-9, IL-15, IL-21, and LIF) and type II (type I IFNs, IFN-γ, IFN-λ, IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, and IL-28/29) cytokines. Other important groups include the IL-1/toll-like receptors, TNF-α and TGF-β families of cytokines. A new family of cytokines comprises IL-17 and its six related members. This classification is particularly useful when a new unknown member comes up and, based on an already known cytokine-receptor/ligand pair this classification allows speculations faced to discover its putative biological function. However, we speculate that only a small number of unknown cytokines remain to be discovered since both the human and mouse genome sequencing are already completed. The multiple functionality observed for several cytokines are attributed to the usage of common subunits and receptor subunits. Moreover, members belonging to the same family of cytokines have been shown to use the same receptor complex to trigger different biological responses [13]. Notwithstanding that they are able to employ the same receptor they also show distinct biological functions, suggesting that receptor-complexes may be associated to unique signal transduction pathways which might result to be cell-type specific. Cytokine receptors are a first level of regulation as will be discussed.

The regulation of cytokine receptors expression by GCs is a complex matter and there are several aspects to consider. Some receptors expression is inhibited by GC treatment. This is the case for IL-2, IL-4, and IL-12 receptors. On the other hand, it has been described that GCs may also induce cytokine receptor expression, being the case of IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), colony stimulating factor 1 (CSF-1), and IL-7 on different cell types. The action of GCs on IL-1/IL-1 regulatory receptors system is more complex due to the existence of not only the IL-1 receptor type I (IL-1RI), but also the presence of IL-1 receptor type II (IL-1RII), which is unable to transduce any signal, and the IL-1 receptor antagonist (IL-1ra) which acts as an endogenous antagonist of the IL-1 cytokine [14].

GCs inhibit IL-2 receptor expression [15,16] likely at the posttranslational level [17]. GCs significantly downregulate mitogen-induced or IL-4-induced IL-4 receptor α (IL-4Rα) chain mRNA and protein levels in both mononuclear and T cells [18,19]. Particularly, the receptor for IL-12, a key cytokine involved in the differentiation and maintenance of T helper 1 (Th1)-type responses is inhibited by dexamethasone, a potent synthetic GC agonist. It has been shown that GCs inhibit responsiveness of activated human monocytes and T cells to IL-12 through the suppression of cell surface expression of IL-12 receptor β1 (IL-12Rβ1) and IL-12 receptor β2 (IL-12Rβ2) mRNA expression, consistent with the striking anti-inflammatory properties of GCs [20].

GCs have been implicated in the regulation of various cancer and immune-derived pathologies. GCs induce not only the expression of membrane-bound IL-6 receptor but also a soluble form of IL-6 receptor, which can increase IL-6-induced signaling by forming a complex with IL-6 membrane bound gp-130 (the receptor β chain which transduces signals). This receptor was implicated in prostate carcinomas and in the pathology of some autoimmune diseases [21]. GM-CSF is a weak inducer of major histocompatibility complex (MHC) class II expression. Interestingly, GCs synergize with GM-CSF to up regulate MHC class II by induction of GM-CSF receptor expression which might result in a faster cellular immune response [22]. Expression of CSF-1 and its receptor, have been observed in a variety of neoplasms of epithelial origin. It has been shown that GCs, which are essential humoral regulators of normal mammary epithelial cell differentiation, induce the expression of CSF-1 receptor in several breast carcinomas cell lines, suggesting a mechanism by which GCs regulate the proliferation and differentiation of neoplastic mammary epithelial cells [23]. IL-7 receptor expression is strictly regulated during the development and maturation of lymphocytes. Thus, it has been shown that GCs induce IL-7 receptor expression on the T-cell surface, modulating survival and function of these cells [24].

It has been described that GCs inhibit IL-1 secretion and action, and that GCs and IL-1ra levels increase during bacterial infection being both instrumental for repressing the pathological sequel of exaggerated IL-1 action [25,26]. Finally, GCs shut down IL-1ra production and reset the monocyte responsiveness [25,26]. These apparently paradoxical actions of GCs underscore their physiological importance in the final control of the immune response. In
parallel to the inhibition of IL-1ra, GCs enhance IL-1RII expression ensuring the shut down of IL-1 signaling [27].

For IL-10 receptor, it has been reported that GCs can both suppress and even enhance its expression, the latter favoring a shift towards an anti-inflammatory response [28,29].

Therefore, at this point it is important to note that contradictory conclusions on the effect of GCs on cytokine receptors may be found in the literature. This is due in part by mistaken comparison using different GCs concentrations, different routes of administration, or prolonged or shorter periods of administrations. To add complexity to the system, GCs not only regulate expression but also signaling downstream of cytokine receptors, as will be discussed below.

2.2. Molecular interplay between the glucocorticoid receptor and inflammatory cytokines: involvement of TFs

During the past few years an overwhelming amount of scientific data has shed light into the modulating role of cytokines during inflammation. Accordingly, these immunoregulatory molecules are classified as pro-inflammatory cytokines during inflammation. Therefore, these immu-

The anti-inflammatory and immunosuppressive actions of GCs result from the inhibition of the activity of TF, such as AP-1, NF-κB and STAT family members, involved in activation of proinflammatory and immunoregulatory genes such as cytokines, cytokine receptors, chemotactic proteins and adhesion molecules. The first transrepressive mechanism described to explain the anti-inflammatory activity of GCs involves the physical interaction between GR and AP-1 [36–38]. NF-κB is present in a wide range of cell types within the immune system and regulates a wide repertoire of inflammatory cytokine genes. Therefore, the inhibition of NF-κB activity by GCs is relevant for the GR-mediated anti-inflammatory action. There are two main molecular mechanisms involved in GC-mediated inhibition of cytokines under NF-κB regulation. The first one involves physical interaction between GR and NF-κB [39–42]. The second mechanism involves the induction of IκB synthesis which retains NF-κB proteins in the cytoplasm and blocks NF-κB transcriptional activity [43–45]. The direct interaction between GR and AP-1 or NF-κB, results in the inhibition of inflammatory cytokine genes such as TNF-α and IL-1 [41].

Other inflammatory cytokines and chemokines targets of GR repression through inhibition of NF-κB transcriptional activity are IL-6, intercellular adhesion molecule (ICAM-1) and IL-8 promoters [41,46,47]. Modulation of the IL-2 promoter is a well established example of AP-1 and GCs mutual regulation, in which AP-1 together with the nuclear factor of activated T cells (NFAT) induces gene expression acting on NFAT sites and GCs inhibit the activation by direct protein–protein interaction affecting the cooperative binding between NFAT and AP-1 dimers [48]. A similar mechanism involving NFAT or GATA-3/NFAT/AP-1 sites was described for IL-4 and IL-5 [49–51]. IFN-γ expression is also inhibited by GCs and involves a tethering AP-1/CREB/ATF site [52]. Although the transrepressive mechanism involved in AP-1 inhibition is very similar to transrepression of NF-κB, a mutation in the first zinc finger of GR is able to affect NF-κB, but not AP-1 inhibition [53] showing that the requirements for transrepression are not identical.

The interaction between GR and the STAT family members result in regulation of the expression of cytokine
receptors or cytokine-mediated signaling. As mentioned before, GCs up-regulate IL-6 receptor, in part due to the modulation of several intracellular signaling pathways in which TFs, such as STAT-3 and STAT-5 are involved [54]. GCs block IL-2 signaling via the Janus kinase (Jak)-signal transducer and STAT pathway [55]. It has also been reported that GCs inhibit the IL-4 signaling pathway by down-regulation of the IL-4-induced IL-4 receptor expression and STAT-6 activation at the level of tyrosine phosphorylation and target DNA binding. This is consistent with the previously described functional antagonism between steroid receptor and STAT-6 for their transcriptional activity [19,56].

Therefore, the cross-talk between GR and STATs results in synergistic enhancement or inhibition of the transcriptional activity of genes regulated by STAT TFs. STAT-5 and GR synergize at the β-casein promoter but antagonize GC-induced mouse mammary tumors virus-long terminal repeat (MMTV-LTR) promoter activity [57,58]. STAT-3 synergize with GCs on the γ-fibrinogen promoter and the MMTV-LTR promoter [59]. STAT-1, GR and the Ets family TF PU.1 cooperate to enhance IFN-γ-induced expression of FcγRII [60]. Also, GCs are able to inhibit IL-12-induced STAT-4 phosphorylation and IFN-γ production without affecting IL-4-induced STAT-6 phosphorylation [61,62]. On the other hand, GCs enhance IFN-β-induced STAT-4 activation and IL-10 production. These effects are associated with a down-regulation of IL-12β1 expression and up-regulation of IFN-β receptor. Therefore, the effect of GCs on the STAT-4 signaling pathway depends on the stimulus activating this pathway. These results may account for GCs suppressive action on the Th1 cellular immune response and may help to explain the shift toward the Th2 humoral immune response by GCs [63,64]. GATA-3 TF is the master driver of Th2 differentiation [65] and T-bet the master regulator of the Th1 response [66]. We have shown that GCs selectively regulate these TFs, the inhibition of T-bet being instrumental for the inhibition of IFN-γ and Th1 cells. Interestingly, GCs and IL-4 signaling pathways are known to interact, as shown by GCs inhibition of IL-4-induced proliferation and IL-4 suppression of GCs-induced apoptosis. The molecular basis for this reciprocal inhibition is due to GR and STAT-6 physical association in T-lymphocytes [56].

Cytokine-induced signaling and TFs not only regulate cytokine action and expression but also affect the response of GR-mediated transcription. TNF-α and IL-1 increase the transcriptional activity of the GR, affecting its biological action [45,67]. Mutual regulatory interplay of GR and TFs that control and mediate cytokine action, account for their biological outcome (Table 1).

2.3. Glucocorticoids, phosphorylation and kinases: mutual regulatory interaction

Steroid hormones and cytokines activate various signaling pathways through intracellular or membrane receptors, respectively. In immune cells, the interplay between these signaling pathways affects fundamental cellular processes such as proliferation, differentiation and apoptosis. However, mechanisms underlying the interactions between GCs and cytokines signal transduction pathways remain poorly understood in immune cells. Cytokines can trigger mitogen activated protein kinase (MAPK) cascades which lead to activation of several TFs, such as AP-1, NF-κB and STAT family members. As we already discussed for several cytokines, these TFs can activate multiple genes involved in inflammation and regulation of cytokines, cytokine receptors, chemotactic proteins and adhesion molecules. They can also establish protein–protein interactions with the GR independently of DNA binding, regulating the outcome of the immune response (Table 1). On the other hand, cytokine activated MAPK signaling can phosphorylate GR regulating its turn over and transcriptional activity. In turn, the GR can also regulate several MAPKs activities (Fig. 1).

Phosphorylation is a posttranslational modification important for the regulation of protein function, by inducing conformational changes in protein structure. The GR is a phosphoprotein, and phosphorylation modulates its activity. GR phosphorylation occurs in a hormone-dependent manner, at serine/threonine residues located within the DNA binding domain [68]. The location of many of these sites suggests a role of phosphorylation in transactivation [69]. Single or multiple phosphorylation site mutations have little effect on receptor expression, subcellular distribution, ligand-dependent nuclear translocation, or on the ability to activate GC-mediated transcription of the complex MMTV promoter, whereas decreased phosphorylation in the GR decreases transactivation of a simple GRE-containing reporter, suggesting that the effect of GR phosphorylation on transcriptional activation is promoter-specific [70]. It is known that GR transcriptional activity is determined in part by interactions with coactivators and corepressors. Vitamin D receptor-interacting protein 150 (DRIP 150) activates GR transcriptional activity through the DNA binding domain. Interestingly, site-specific (Serine 211) phosphorylation of the GR enhances its interaction with a protein of the DRIP/
Cytokine signaling through their membrane receptors activate cyclin-dependent kinases (CDK) and MAPK that target GR potential phosphorylation sites. This phenomenon leads to the regulation of GR transcriptional activity probably resulting in GR anti-inflammatory and immunosuppressive actions. Multiple cyclin/CDK complexes, as well as c-Jun N-terminal kinase (JNK) and extracellular signal-regulated protein kinase (ERK) have been found to phosphorylate different serine/threonine residues within GR modifying its transcriptional activity [72,73]. Suppression of the GR function by activated p38 and JNK MAPK is a physiologically relevant mechanism of resistance to GCs observed in many patients with chronic inflammatory disorders [74,75]. Also, phosphatases might be controlling GR phosphorylation by regulating GR dissociation from Hsp90 and its translocation to the nucleus where it can target GRE-regulated genes involved in immune responses [76]. Cytokines and GCs signaling pathways interfere with each other in the regulation of apoptosis and gene expression in the immune system. Several MAPKs have been identified as potential targets for negative regulation by GCs. Some reports show that GCs inhibit the entire MAPK/phospholipase A2 pathways that may account for some of the anti-inflammatory actions [77]. It has been shown that GCs antagonize TFs involved in cytokine expression, such as AP-1 by inhibiting the activation/phosphorylation of JNK and ERK signaling pathways [78,79]. Dexamethasone, specifically inhibits LPS-induced JNK/stress-activated protein kinase (SAPK) activity but not that of p38, ERK1 and ERK2, or MAPK activators MAPK/ERK kinases 3, 4, and 6 (MEK3, MEK4, and MEK6) [80]. The finding that GCs inhibit the IL-1β-induced phosphorylation and activation of MAPKs p38 and JNK via up-regulation of MAP Kinase Phosphatase-1 (MKP-1) may also contribute to the understanding of the signaling mechanisms underlying the GC-mediated attenuation of MAPKs [81]. Also, it has recently been shown that IL-2, through the Jak/STAT and MAPK pathways, activates STAT-5 and AP-1, respectively, which are known to repress GR activity, at least in part, through protein–protein interactions. Thus, STAT-5 plays a critical role in IL-2 regulation of GC-dependent transactivation [82]. The latter could be of importance to elucidate how cytokines modulate expression of GC-regulated genes in pathological situations, i.e., asthma or lymphoma, or in physiological situations, i.e., apoptosis.

As already discussed above, GCs play an important role in suppressing innate and Th1-mediated cellular responses by inhibiting Th1 and enhancing Th2 cytokine secretion. This effect is partially due to GCs inhibition of IL-12-induced phosphorylation of STAT-4 without altering its expression, but not by affecting IL-4-induced STAT-6 phosphorylation. Inhibition of STAT-4 phosphorylation by GCs does not alter other early steps of IL-12 signaling, namely the expression of IL-12 receptor and IL-12-induced tyrosine kinase 2 (Tyk2) and Jak2 phosphorylation. Therefore, blocking IL-12-induced STAT-4 phosphorylation, appears to be a new suppressive action of GCs on the Th1 cellular response and may help to explain the GC-induced shift towards the Th2 humoral response by selectively inhibiting phosphorylation of TFs involved in immune regulation [62]. Interestingly, GCs induce synthesis of phosphatase enzymes capable of inactivating the MAPK cascade showing a novel mechanism for regulating MAPK activity [83]. Indeed, GCs inhibit STAT-4 phosphorylation through a number of potential mechanisms, including the modulation of expression and activity of different factors that play a role in the attenuation of cytokine signaling, such as suppressors of cytokine signaling proteins (SOCS), or protein inhibitors of activated STATs (PIAS). Hence, multiple signaling mechanisms are likely to underlie the regulation of MAPK by GCs (Fig. 1).

3. Implications of chromatin remodeling and posttranslational modifications in the cross-talk between GC and transcription factors

Upon immune stimulation, cells of the immune system orchestrate the integration of diverse signals resulting in a biological response which may involve DNA structural changes and consequently, gene repression or activation. Cytokines and GC actions converge at the level of DNA. The molecular interaction between cytokine signaling pathways and GCs implies the correct recruitment of activators, chromatin modifiers/remodelers, corespressor complexes, coactivators and TFs that culminate with several chromatin remodeling events triggering gene repression/activation. Thus, the mechanisms modulating chromatin structure might be essential to regulate the immune response [84].

Since, a condensed DNA form is related with gene repression, a relaxed form is associated with available DNA sequences for protein binding which may result in active gene expression. The chromatin structure depends on and responds to environmental changes. Histone tails protrude out of the nucleosomes and are susceptible to several posttranslational modifications, such as acetylation, methylation, ubiquitination, phosphorylation and sumoylation. Different classes of histone modifiers are involved in these processes: HATs (histone acetyl transferases), HDACs (histone deacetyl transferases), histone methyl transferases, histone kinases and others [85]. Such modifications alter the
equilibrium between both condensed and relaxed chromatin states. In general, acetylation is correlated with gene activation since it reduces DNA–histone interaction leading to an open structure facilitating transcription. Deacetylation has the opposite effect. The presence of methyl groups is associated with repressive chromatin structure in terms of gene transcription. Thus, histone tails modifications have been proposed as events that regulate access to target DNA sequences. Chromatin remodeling complexes are recruited to target promoters in response to specific stimuli leading to DNA decondensation and by this mean, facilitating binding of transcriptional coactivators that bridge TF and components of the basal transcription machinery [86], consequently regulating cytokine gene expression (Fig. 1).

GCs are the mainstay of asthma therapy and mediate the repression of a number of cytokine genes, such as IL-4, IL-5, IL-13, and GM-CSF, which are central to the pathogenesis of asthmatic airway inflammation. The regulation of IL-4, IL-5, and IL-13 is controlled by the key Th2 TF GATA-3, which has been shown to induce changes in the chromatin structure at the IL-4 locus and subsequently transactivate the IL-5 and IL-13 promoters [87]. IL-10 may be regulated by GATA-3 independently of the IL-4/IL-5/IL-13 cluster, by inducing chromatin remodeling and acetylation of histones H3 and H4 at the IL-10 locus [88]. Transcriptional repression of the IL-5 gene by GCs involves recruitment of GR within the IL-5 proximal promoter, which is bound by NFAT and AP-1. GR recruitment has a profound effect upon the activation capacity of GATA3, which has a binding site close to the NFAT/AP-1 domain in both IL-5 and IL-13 promoters. Repression of this transcriptional activity by GR involves HDAC1 recruitment [51]. It is generally accepted that histones deacetylation is related to transcriptional repression and HDACs are considered as corepressors. HDAC1 is associated with the GR complex at promoter regions upon hormone stimulation and an increase of acetylated HDAC1 becomes evident and correlates with down regulation of gene expression. Since, acetylation of HDAC1 prevents its deacetyl transferase activity, this posttranslational modification plays an important role in GR-mediated transcriptional regulation of target genes [89]. However, it has also been reported that deacetylated HDAC1 serves as GR coactivator [89]. As discussed above, GCs inhibit NF-κB and this is achieved by interfering with NF-κB transcriptional regulation by a DNA independent mechanism which involves protein–protein interaction. GR becomes acetylated after ligand binding and HDAC2-mediated GR deacetylation abolishes GR binding to the NF-κB complex. Therefore, HDAC2 regulates NF-κB-mediated inflammatory gene expression but does not affect GR transactivation activity [90]. After contacting the DNA, GR recruits an array of cofactors that modulate the chromatin remodeling characterized by intrinsic HAT activity that results in a more relaxed chromatin structure promoting gene activation [91]. Some of these cofactors are also implicated in the functionality of the basal transcription machinery, and in the assembly of the pre-initiation complex (PIC) formation allowing access of the RNA polymerase (RNApol) to the transcription start site. Proteins such as CBP/p300, steroid receptor coactivator-1 (SRC-1), GR-interacting protein-1, transcriptional intermediary factor-2 (GRIP1/TIF2), RAP-46 and STAT-5, all have GR coactivator activity. Limiting nuclear amounts of these cofactors used by GR and other TFs might restrict transactivation. Therefore, chromatin-remodeling events targeted by GR probably modify cytokine gene expression. TNF-α stimulated cells induce NF-κB activation following NF-κB binding to its response element on the IL-8 and ICAM promoters. Dexamethasone treatment induces GR binding to Rel-A-occupied NF-κB response elements. GR repress NF-κB IL-8- and ICAM-mediated activation not by interfering with NF-κB DNA binding nor with PIC formation but by inhibition of RNApol III carboxy-terminal domain (CTD) Ser-2 phosphorylation [8,47], a key event for cytokine gene transcription. The association of GR with several components of the chromatin remodeling machinery, such as the SWI/SNF complex, has also already been described [92,93]. All of these examples point that the immunomodulatory action of GCs is also mediated by interfering with the dynamics of DNA architecture.

GCs exert their anti-inflammatory action by means of downregulation of proinflammatory gene expression. As pointed above, GCs achieve this effect, at least in part by induction of IκB synthesis [43–45]. Before activation by GCs, the IκB promoter possesses an open structure. DNase I footprinting studies on the IκBα promoter reveals that GC treatment is able to modify the accessibility at several DNA sites favoring and increasing the recruitment of TFs. Therefore, activation of IκBα promoter by GCs facilitates the accessibility and stability of TF binding at the proximal promoter leading to gene activation [94]. The ubiquitin–proteasome and sumoylation systems may be potential regulators of nuclear receptor transactivation, modifying both receptor trafficking and turnover. These and other posttranslational modifications, such as acetylation, methylation and phosphorylation, as well as proteins of chromatin remodeling machines, chaperones and proteasomal subunits may all have a role maintaining the turnover of receptors and associated factors at active sites of transcription. Nuclear and upstream proteins of many cytokine-signaling pathways are sumoylated as well as steroid receptors [95–97], suggesting that SUMO-mediated posttranslational modification could also play a role in GR–cytokine interaction. Accordingly, GR is modified by SUMO and this covalent modification regulates the stability of the GR and potentiates its transactivation activity [98]. On the other hand, sumoylation regulates processes including subcellular localization, transactivation activity and protein–protein interactions of many TFs. Interestingly, in some specific cases, target lysines for SUMO attachment are the same for ubiquitin attachment, thus sumoylation antagonizes ubiquitin-mediated degradation, being the case of IκB [99].
Therefore SUMO modification of IκB blocks ubiquitination and creates a pool of IκB, which is resistant to signal-induced degradation, inhibiting NF-κB dependent gene transcription. We anticipate that further regulatory pathways involving these posttranscriptional mechanisms will be discovered and will contribute to understand the complex GC/cytokine interaction.

4. Conclusions

The cross-talk between the TFs induced by cytokine signaling and the GR, results in the modulation of GRE regulated genes and in regulation of genes targeted by these specific TFs (Table 1). The latter may occur either by physical association with the GR or through the modulation of regulatory proteins. Also, this interaction may take place between the interaction of the GR and the signaling pathways elicited upstream of the involved TFs including a further upstream regulation involving cytokine receptor expression. Several posttranslational modifications of the GR or even the TFs also modulate the final outcome of the immune response. GR–TF-mediated chromatin remodeling and modification of the assembly of the pre-initiation complex may also regulate cytokine gene activation.

Despite the effectiveness of GC treatment for inflammatory disorders, explaining their widespread clinical usage, an important drawback of their long-term administration involves the occurrence of range debilitating side effects. Pharmacology is therefore interested in alternative therapies which would offer a better side effect profile. In order to reach this goal, a detailed understanding of the molecular mechanisms underlying the anti-inflammatory and immunosuppressive effects of GCs is essential. Thus, the detailed study of the molecular interactions implicated in the interplay among these pathways will further explain GCs immunosuppressive and anti-inflammatory actions and probably will localize new molecular targets for alternative therapies which would avoid undesirable effects.

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