Glucocorticoid receptors: finding the middle ground

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Glucocorticoids (GCs; referred to clinically as corticosteroids) are steroid hormones with potent anti-inflammatory and immune modulatory profiles. Depending on the context, these hormones can also mediate pro-inflammatory activities, thereby serving as primers of the immune system. Their target receptor, the GC receptor (GR), is a multi-tasking transcription factor, changing its role and function depending on cellular and organismal needs. To get a clearer idea of how to improve the safety profile of GCs, recent studies have investigated the complex mechanisms underlying GR functions. One of the key findings includes both pro- and anti-inflammatory roles of GR, and a future challenge will be to understand how such paradoxical findings can be reconciled and how GR ultimately shifts the balance to a net anti-inflammatory profile. As such, there is consensus that GR deserves a second life as a drug target, with either refined classic GCs or a novel generation of nonsteroidal GR-targeting molecules, to meet the increasing clinical needs of today to treat inflammation and cancer.

Introduction

Glucocorticoids (GCs) are steroid hormones that are derived from cholesterol and secreted by the zona fasciculata of the adrenal glands. GC production occurs in a circadian- and stress-associated manner and is regulated by the hypothalamic-pituitary-adrenal (HPA) axis (1–3). Various physiologic processes, including glucose metabolism and metabolic functions in fat, muscle, and bone, are under GC control. Both natural and exogenous GCs function through activation of the GC receptor (GR), a transcription factor (TF) encoded by the NR3C1 gene. Alternative splicing and translation start sites (TSSs) give rise to several GR transcripts (4–6), with the full-length GRα as the predominant active isoform.

GCs diffuse freely through the cellular membrane and bind to the cytoplasmic GRα complex. Ligand binding induces a conformational change in the receptor, exposing nuclear localization signals and (ex)changing interaction partners, after which the receptor translocates to the nucleus. HSP70/90-based chaperone machinery functions with cochaperones to guarantee proper folding, maturation, nuclear accumulation, and DNA binding of the receptor (7, 8). Further, chaperone and cochaperone composition of this complex alters GR sensitivity. For example, FK506 binding protein-5 (FKBP5) association with the receptor complex decreases affinity for cortisol and results in less efficient nuclear translocation (9, 10). Because of their role in GR activation, chaperones are now being investigated as potential drug targets in the pathophysiology of stress-related psychiatric disorders (9), but they may also be relevant in other disease settings where GR activities are important. Once in the nucleus, GR target genes (e.g., FKBP5) are activated via receptor homodimer binding onto cognate DNA sequences, termed GR response elements (GREs). This mechanism is referred to as transactivation. Investigating GR occupancy on GRE elements in A549 human lung cells showed that 63% of GREs are more than 10 kb from the TSS. Furthermore, both the core GR binding sequences as well as the GRE architecture harbor gene-specific regulatory information (11). Besides acting as a genuine TF, activated GR can also influence target genes in the nucleus via other mechanisms (ref. 12 and discussed below).

GCs are primarily used in the clinic for their potent anti-inflammatory actions (1, 2, 13). Indications range from short-term treatments for conditions such as skin rashes (14), seasonal allergic rhinitis (15) and relapses of multiple sclerosis (16) to long-term treatments for diseases such as severe asthma (17) and rheumatoid arthritis (18). GCs still suffer from a bad reputation in the clinic: patients fear the long-term consequences of GC-based therapy, not in the least because of the appearance-changing psychological impact caused by water retention, resulting in a typical moon face and imbalanced fat build-up (Figure 1 and ref. 19). Recently, the multidisciplinary European League Against Rheumatism (www.EULAR.org) critically reviewed the evidence on the four most worrisome adverse effects of GC therapy from the clinician’s perspective, which are osteoporosis, hyperglycemia/diabetes mellitus, cardiovascular diseases, and infections. The risk of harm is especially elevated for patients taking long-term dosages equivalent to over 10 mg prednisone per day; at dosages of 5 to 10 mg/day, patient-specific characteristics determine the risk of harm (18).

In the current Review we focus on transcriptional mechanisms that explain how the intracellular mediator of GC actions, the GR, modulates gene expression to control inflammation. Furthermore, we emphasize the need for research into strategies to improve the safety profile of GRs.

Anti-inflammatory effects via gene suppression

GCs exert their anti-inflammatory actions at multiple levels. At the cellular level, they suppress cytotoxic T lymphocytes (20) and impair DC maturation (21). Effects on immune and nonimmune cells involved in inflammation obviously differ. Historically, the bulk of GC anti-inflammatory effects were linked to GR-mediated gene suppression because the activated GRα typically interferes with the activities of various pro-inflammatory TFs, including NF-κB, activator protein 1 (AP-1), and interferon regulatory factors.

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Alternative mechanisms that explain transcriptional repression include indirect mechanisms wherein GR competes with other TFs such as NF-κB, IRF3, or AP-1 for essential (co-)activators such as CREB binding protein, nuclear receptor coactivator 1, GRIP1, or p53 (35–41). Additionally, pioneering work by Yamamoto and colleagues demonstrated that GR can also bind to DNA recognition sequence half sites, i.e., only part of the classic palindromic GR-binding sequence motif, as a monomer (42). A recent study by Steger and colleagues showed that GR monomers bind DNA, thereby facilitating transient contacts with nearby TFs in a process known as half-site–facilitated tethering (43). An alternative role for the partner proteins with which GR can tether was proposed by Hager and colleagues, who identified AP-1 as a facilitator of productive GR/chromatin interactions, following the observation that GR binding takes place at particular chromatin regions that are accessible even prior to hormone treatment (44). These studies demonstrate that GR is a versatile and mechanistically creative transcriptional repressor of the activity of other TFs.

Anti-inflammatory effects via gene activation

It has long been known that GR can activate genes encoding proteins that oppose different aspects of inflammatory signaling (45), e.g., lipocortin I (encoded by the ANXA1), the inhibitor of NF-κB (IκBα, encoded by NFKBIA), and IL-10. A common feature of the GR-supported gene activation mechanism is that the effects are all indirect and involve increased production of specific anti-inflammatory mediators. The contribution of this mechanism to the resolution of inflammation has historically been considered to be minimal; however, the primacy of GR-mediated transrepression of genes as a predominantly anti-inflammatory mechanism has been called into question by recent studies. GR-mediated transactivation of anti-inflammatory genes has been shown to be essential in curbing inflammatory diseases. Indeed, by inhibiting de novo protein synthesis and thereby curtailing anti-inflammatory protein production, the ability of GC to effectively resolve inflammation is attenuated (46–49). Furthermore, a number of candidate GR target proteins with anti-inflammatory function carry classic GRE elements in their promoters. For example, GCs induce the anti-inflammatory protein MAPK phosphatase 1 (Mkp1, also referred to as DUSP1) (Figure 2), which dephosphorylates the MAPK JNK2, thereby inhibiting intestinal epithelial cell apoptosis (58). These studies indicate that GCs act at different levels in the inflammation pathway to suppress ongoing inflammation, including via transrepression of pro-inflammatory TFs (as described above) and by targeting distal regulators such as MAPK kinases via upregulation of Mkp1 (see above) or upregulation of IκBα, the endogenous inhibitor of NF-κB (Figure 2 and refs. 59, 60). Moreover, at the posttranscriptional level, GCs destabilize pro-inflammatory cytokine mRNAs by inducing the zinc finger protein 36 (ZFP36, also known as tristetraprolin) (Figure 2 and ref. 60). These studies indicate that GCs act at different levels in the inflammation pathway to suppress ongoing inflammation, including via transrepression of pro-inflammatory TFs (as described above) and by targeting distal regulators such as MAPK kinases via upregulation of Mkp1 (see above) or upregulation of IκBα, the endogenous inhibitor of NF-κB (Figure 2 and refs. 59, 60). Moreover, at the posttranscriptional level, GCs destabilize pro-inflammatory cytokine mRNAs by inducing the zinc finger protein 36 (ZFP36, also known as tristetraprolin) (Figure 2 and ref. 60).
61). These may be cell-specific mechanisms, since an exclusively nuclear transcriptional mechanism that is independent of de novo protein synthesis was identified in other cell types (62).

In severe asthma and other inflammatory airway diseases that are commonly treated with GCs, TNF-α mRNA and protein expression is elevated (63, 64). To understand the impact of GCs on negative feedback control of TNF, Gerber and colleagues investigated how the expression of anti-inflammatory TNF targets such as TNFα-induced protein 3 (TNFAIP3, encoding A20) and NFKBIA (encoding IκBα) is selectively spared or augmented by dexamethasone (DEX) treatment in airway epithelial cells. Their data fit a model in which the expression of anti-inflammatory targets of TNF is maintained during treatment with GCs through a context-dependent cooperation between GR and IκBα (65). The inactive kinase IRAK-M is induced upon TLR stimulation and negatively regulates TLR signaling (66). Notably, activated GRα also induces the expression of IRAK-M (Figure 2) in both airway epithelial cells and macrophages (66), thereby counteracting inflammatory TLR signaling. The GR monomer-inducing compound A (67, 68) could not enhance IRAK-M expression, suggesting a GR dimerization-dependent mechanism (65). These findings collectively show that, in addition to cytokine repression, GR-mediated induction of immune regulators can assist in halting pro-inflammatory signaling pathways at various levels of the signal transduction cascade.

Besides directly targeting pro-inflammatory pathways, GCs can also induce anti-inflammatory, protective mechanisms at the cellular level. For example, GCs promote the restoration of endothelial barrier integrity in the lung by inducing the expression of sphingosine kinase 1 (SPHK1) in both macrophages and endothelial cells, thereby inhibiting leukocyte infiltration. SPHK1 expression has proven to be crucial for GR-mediated anti-inflammatory actions in the lung, as downregulation of pro-inflammatory cytokines alone was not enough to resolve inflammation (69). Other anti-inflammatory proteins induced by GCs include the GC-induced leucine zipper (GILZ) (70, 71), the ectonucleotide pyrophosphatase/phosphodiesterase NPP1 (72, 73), annexin 1 (74), and the secretory leukocyte proteinase inhibitor (75). In trying to identify targets through which GCs exert therapeutic effects in asthma, Kadiyala and colleagues found that GR recruits p65 to dimeric GR binding sites across the genome to augment gene expression. GR targets regulated by this mechanism include key anti-inflammatory and injury response genes such as SERPINA1 and FOXP4, the latter being an inhibitor of mucus production. Thus, cooperative anti-inflammatory gene regulation by GR and p65 contributes to GC efficacy, diminishing the role of GR/p65-tethering mediated gene repression as a means to resolve inflammation (76).

Feedback and feedforward loops in GC signaling

Pro-inflammatory pathways enhance many of the aforementioned GC-induced anti-inflammatory genes, thereby initiating feedback or feedforward loops. A well-known feedback loop triggered by inflammation is the augmented synthesis and secretion of GCs themselves via direct activation of the HPA axis by numerous pro-inflammatory cytokines (24). Additionally, DEX synergizes with nontypical Haemophilus influenzae to induce the binding of both GR and p65 at the promoter of II4R, with both factors working cooperatively to attenuate bacteria-induced inflammation (65). Gene regulatory interactions were also identified by Vettorazzi and colleagues for several other pro-inflammatory stimuli (69). In combination
with GCs, TNF-α and activators of TLR2, -3, and -4 drive SPHK1 expression (Figure 2) during lung inflammation. This cooperation is restricted to macrophages and is dependent on the p38 MAPK- and mitogen- and stress-activated protein kinase 1 (MSK1) signaling pathway as well as on optimal GR dimerization (69). Furthermore, the expression of the serine protease inhibitor α1-antichymotrypsin (encoded by serpin A3), a secreted acute-phase protein that is active in many inflammatory diseases, was enhanced by simultaneous challenge with DEX and TNF-α (77). Finally, the pro-inflammatory cytokine IL-1β cooperates with DEX to induce the feedback regulator MKP1 (50–52), which inactivates pro-inflammatory MAPK signaling, and the feedforward anti-inflammatory regulator ZFP36 (61, 78, 79). ZFP36 is itself upregulated by inflammatory stimuli and subsequently targets the AU-rich 3′-untranslated region of the TNF transcript to promote its degradation.

It seems counterintuitive for a transcriptional boost of particular anti-inflammatory proteins such as ZFP36 (78), MKP1 (79, 80), and SPHK1 (69) to depend on a pro-inflammatory MAPK pathway. Even more puzzling, MKP1 exerts its anti-inflammatory action through inhibition of MAPK signaling and thus halts the above-mentioned cooperative effects (51, 81). However, anti-inflammatory actions of MAPK pathway components such as the p38 MAPK (82, 83) and the downstream MSK (80) have previously been described in some forms of inflammation. Newton and colleagues recently reported that a DUSP1-dependent negative feedback control reduces the feedforward control mediated by ZFP36 (79). Although these interactions constitute an incoherent feedforward loop, wherein an activator regulates both a gene and a repressor of a gene, this loop appears to serve as a sensor to prevent excessive inflammation (79, 84). Incoherent loops are actually believed to be necessary for negative and positive pulse generation, accelerated responses, and sensing of fold change (85, 86). The continuous competition between feedback and feedforward control should be interpreted not by following separate paths, but as an integrated network, wherein the temporal interplay between competing regulatory processes dictates the ultimate inflammatory status. Loss of one factor (e.g., MKP1) will lead to other systems taking over, (e.g., ZFP36), allowing for an efficient dampening of the inflammatory process and ensuring the return to homeostasis (79).

The mechanisms described above indicate that anti-inflammatory pathways are indirectly affected by GR target proteins, the expression of which is controlled by transactivation or other gene upregulation mechanisms. These anti-inflammatory pathways are required to fully control inflammation.

**Acute versus chronic inflammation**

Conditions characterized by sustained inflammation, such as autoimmune disorders, typically require a chronic GC treatment regimen. It is unknown whether these diseases can be managed without eliciting GRE-dependent pathways that are detrimental, such as those contributing to increased blood glucose levels. Additionally, some acute inflammatory diseases such as sepsis remain refractory to GC treatment for reasons still not fully understood. In mouse models in which GC dimerization is compromised (GRdim/dim), coping with inflammation becomes a life-threatening challenge (87). These findings support a framework wherein enhancement of GR dimerization may be suitable for the treatment of acute inflammatory disorders, while the induction of GR...
monomers (which prevents clinically problematic metabolic side effects) may be suitable for the management of chronic inflammatory diseases (Figure 1 and ref. 13). The reasoning for why GR monomers may be the way forward for chronic diseases includes not only the concept that the GR monomers can mediate classic transrepression (on NF-κB and AP-1) but also the concept that GR monomers can drive mechanisms whereby GR can work via half-site association to activate a subset of anti-inflammatory target genes. In contrast, palindromic GRE-instructed GR dimer binding remains a mechanism to avoid, especially when considering hyperglycemia as a GC-associated side effect that is known to rely on this particular GR dimer-dependent mechanism.

Nongenomic mechanisms of GC signaling
In addition to gene-targeted pathways, GCs exert rapid, nongenomic actions that do not require protein synthesis. For example, ligand binding to GRα not only induces activation of the receptor but also releases components of the multiprotein chaperone complex. These accessory proteins set in motion secondary signaling cascades such as induction of EGFR signaling through the actions of c-Src (1, 2, 88). Several other nongenomic GC signaling mechanisms have been described, including signaling through a membrane-bound form of GR in human peripheral blood mononuclear cells (PBMCs) (89), interactions with or regulation of the subcellular localization of particular kinases (90, 91), regulation of apoptosis by mitochondrial translocation of the activated GRα in thymocytes (92), or translocation of GRα to caveolin-1–dependent lipid rafts, which affects cell proliferation (93, 94). Follow-up studies are warranted to further unravel the implications of these nongenomic GC-mediated actions.

Potential pro-inflammatory actions of GCs
In spite of the well-known net anti-inflammatory outcome of GR actions, it is becoming increasingly clear that the receptor plays a dual role in immune gene regulation (Figure 3A). This notion is evidenced by GC-mediated enhancement of pro-inflammatory genes (23, 95), some examples of which are provided below.

Danger-associated molecular patterns and pattern recognition receptors. Endogenous molecules released following cellular damage or stress, e.g., extracellular ATP or uric acid crystals, are danger-associated molecular patterns (DAMPs) that can initiate innate immune responses by binding pattern recognition receptors (PRRs). The NLR family pyrin domain containing 3 (NLRP3) gene encodes an intracellular PRR (called NALP3) that is part of the NALP3 inflammasome, a multiprotein oligomer that also contains caspase protease family members. NALP3 detects products of damaged cells, after which the activated receptor triggers immune responses. In differentiated macrophages, GCs rapidly and directly enhance expression of NALP3. Exposure of GC-sensitized macrophages to extracellular ATP in combination with LPS, which is an inflammasome-activating signal, enhances the release of IL-1β and other cytokines. This finding highlights a novel role for GCs as sensitizers or priming agents of an initial inflammatory response in the innate immune system (96). Priming or sensitization commonly refers to sequential signals whereby exposure to the first signal exacerbates the response to the second signal. These signals may be of the same nature — for example, initial cytokine/TLR signals triggering inflammatory responses may exacerbate inflammatory responses to cytokine/TLR signals in a second wave — or of different natures. In the field of neuro-inflammation, a GC-mediated priming of innate immune cells was identified in the hippocampal microglia and possibly other CNS macrophages. Acute (97, 98) or chronic (99–101) exposure to exogenous or endogenous GCs increases NLRP3 mRNA expression in microglia. NLRP3 expression shifts the microglia activation via upregulation of myeloid markers, including MHC-II. Subsequent exposure to LPS potentiates microglial pro-inflammatory responses such as the secretion of TNF-α, IL-1β, and IL-6. As the endogenous or exogenous GCs alone fail to induce IL-1β expression, this augmentation reflects a priming of neuro-inflammatory processes wherein GCs act as an endogenous danger signal to prepare the organism to cope with subsequent immunological threats.

Specific intracellular proteins also serve as DAMPs. A possible mechanism for neuro-inflammatory priming may be the GC-induced release of DAMP molecules such as high mobility group box-1 by damaged neurons, which is also actively secreted by innate immune cells. These findings on neuro-inflammation, together with the work on peripheral macrophages, indicate that macrophages throughout the body may be primed by GCs, regardless of their microenvironment (96, 101). Similarly, GCs mediate sensitization in human microvascular endothelial cells by transiently enhancing the expression of the purinergic GPCR P2YR, which is activated by extracellular ATP. Exposure to extracellular ATP induces GC-primed cells to produce enhanced levels of inflammatory mediators such as IL-6, IL-8, and intercellular adhesion molecule-1. Remarkably, this increase is highly stimulus specific, as LPS-induced release of IL-6 remains suppressed by GCs (73).

GCs also affect the activity of PRRs that react with pathogen-associated molecular patterns. For example, GCs cooperatively enhance TNF-α-induced expression of TLR2 in the lung epithelial cell line A549. The mechanism for the GC/TNF-α cooperation includes an interaction between GRα, NF-κB, and STAT5 at the TLR2 promoter. This pro-inflammatory augmentation is gene specific, as NF-κB-driven proinflammatory Il8 mRNA expression in the same cells under identical conditions is repressed by DEX. The TLR2 promoter region contains several GRE half-sites, which upon mutagenesis lead to the loss of cooperativity between GCs and TNF-α in transient TLR2 reporter assays (102). GCs in combination with a TLR2 agonist alter early signaling events of the TLR2 receptor. TLR2 phosphorylation is increased and Akt phosphorylation is diminished, via a mechanism involving a direct interaction between GRα and PI3K (103). GCs still induce anti-inflammatory pathways in parallel, such as the induction of MKP-1 in the A549 cells and TNAIFAP3 (A20) in microvascular endothelial cells (51, 55, 64, 73, 102). In DCs, GCs enhance the expression of both TLR2 and TLR4 but impair TLR-induced maturation and production of pro-inflammatory cytokines (21), indicating that TLR signaling is blocked further downstream of the receptor. Additionally, these findings demonstrate the cell specificity of GC-induced signaling: in endothelial cells GCs augment the TLR pathway, while in DCs GCs suppress effects downstream of the TLR itself. The interference of GRα with TLRs is a well-known anti-inflammatory action of GR, and GC-mediated regulation of endogenous inhibitors of TLR pathways could occur through several mechanisms, as...
described above and as summarized by Chinenov and Rogatsky (24). These mechanisms include the already described GC-mediated upregulation of MKP1, the upregulation of the suppressor of cytokine signaling 1 (SOCS1), which is able to inhibit JAK/STAT pathways triggered by both pro- and anti-inflammatory cytokines, and the inhibition of AP-1/NFκB by the GILZ protein (see “Anti-inflammatory effects via gene activation” above).

**Cytokine-induced gene expression by GCs.** GCs coregulate genome-wide gene expression in concert with inflammatory mediators such as TNF-α (77), leukemia-initiating factor (LIF) (104), LPS (86), and IL-6 (105). The activity exerted by combined GCs and IL-6 can be pro-inflammatory, by mediating induction of acute-phase inflammatory proteins, as well as anti-inflammatory, by dampening IL-6-induced SOCS3 expression in primary hepatocytes (105). In the case of LIF, combined action with GCs enhances the induction of a class of genes involved in the hepatic acute-phase response and the innate cellular defense system. This particular augmentation of gene expression is mainly dependent on de novo protein synthesis and is thus categorized as a secondary response, delayed response, as relevant mediators need first to be synthesized before the innate cell defense response genes can be augmented (104). In macrophages, DEX and LPS coregulate several early genes, both pro- and anti-inflammatory, in a cooperative or antagonistic fashion. A number of these early genes code for transcriptional regulators such as members of the KLF family (86). Thus, GRα, in combination with other TFs, triggers numerous feedforward loops and other regulatory networks, further increasing the complexity of gene regulation and leading to an anti- or pro-inflammatory outcome that is dependent on the timing of these gene responses.

Generally, when GCs act in a pro-inflammatory manner, they do so by activating components of the innate immune pathways, thereby priming the immune system under basal conditions to better control subsequent dangers. GCs further reinforce the innate immune system and acute-phase response during the early stages of inflammation by synergistically enhancing cytokine-mediated gene expression. During ongoing inflammation, GCs exert systemic anti-inflammatory effects on the main pro-inflammatory pathways by repressing NFκB signaling and inducing anti-inflammatory signaling proteins (e.g., MKP-1). Furthermore, GC actions on the adaptive immune system generally tend to be anti-inflammatory, preventing excessive inflammation and tissue damage and ultimately restoring homeostasis (84). This distinction between innate and adaptive immune effectors was also seen by Galon and colleagues in human PBMCs, wherein GCs induced the expression of innate immune-related genes, including scavenger and toll-like receptors, but repressed the expression of adaptive immune-related genes (95).

Relevant to the translation of the above findings to a more clinical setting is the finding that the upregulation of apparently pro-inflammatory cytokines, chemokines, and receptors is now firmly documented in the human airways in vivo following the inhalation of the GC budesonide (106). Taken together, opposite processes controlled both by GC-activated GR are installed to prepare the immune system to respond to a stressor (i.e., GC pro-inflammatory effects) and subsequently restore homeostasis (i.e., GC anti-inflammatory effects).

**Flipping the coin: anti- or pro-inflammatory outcomes**

GCs set in motion opposing forces by simultaneously inducing both pro- and anti-inflammatory pathways, ultimately producing a pro- or anti-inflammatory response as part of a pro-resolving and homeostasis-reaching strategy. The effect of GRα activation is highly gene specific, cell specific, and stimulus specific. Predicting the physiologic outcome of GC treatment is further complicated by secondary effects, as the receptor acts as a hub that influences numerous highly branched regulatory networks, which are themselves cell specific. Even within the same cell type, the effect of GCs can vary depending on the activation state of the cell (95), cofactor recruitment patterns (39), or differentiation stage (96). Additionally, the effects of GC exposure change over time due to changes in the expression of interacting components and the existence of various feedback and feedforward loops, among other mechanisms. The ability of GCs to downregulate their own receptor, a process known as homologous downregulation, is a physiologic phenomenon involving a decrease in GR mRNA and protein levels (112). This phenomenon can have wider consequences. For example, long-term exposure to GCs leads to compensatory GRα downregulation in the frontal cortex and hippocampus. This downregulation accelerates neuro-inflammation via NLRP1 inflammasome activation and subsequent neuronal degeneration (108). Furthermore, the timing of treatment administration is of importance, as exemplified by a recent study revealing a regulatory mechanism linking the circadian clock and GC hormones together in the control of pulmonary inflammation and bacterial infection responses (113). The severity of the GC-inducing stress-
or also affects the final outcome (84, 86). Contributing factors are summarized in Figure 3.

**GC side effects and resistance**

As most of the cooperative and synergistic effects between GCs and pro-inflammatory stimuli discovered so far ultimately promote anti-inflammatory programs or induce life-saving mechanisms at early stages of inflammation, chronic GC usage still precipitates many adverse side effects, including GC resistance. The list of GC-associated adverse effects, including osteoporosis, diabetes, glaucoma, skin atrophy, and depression is long due to the pleiotropic functions of the GRs in various biological processes (Figure 1 and refs. 1, 19, 111). Disruption of the naturally occurring circadian and ultradian changes in circulating endogenous GC levels with exogenous GCs or chronic stress breaks the delicate control systems and provokes unwanted effects (113–115). This dysregulation is particularly clear during treatment of arthritis, due to the essential role of GR in bone homeostasis, as reviewed in depth by Hartmann and colleagues (115).

Chronic GC treatment or chronic stress elicits tissue-specific GC resistance, causing diminished therapeutic capacity while often retaining detrimental side effects. Many studies point to a GC-mediated GRs downregulation as a primary mechanism for acquired resistance (70, 108, 116–118). For example, in periodontal tissue that is inflamed due to chronic stress, the loss of GRs expression enhances Akt phosphorylation, which promotes TLR4 transcription and LPS-induced NF-κB activation, consequently accelerating the pathologic progression of periodontitis (117). Some inflammatory disorders such as chronic obstructive pulmonary disease and cystic fibrosis are intrinsically GC resistant. Various mechanisms for resistance, including cytokine-induced upregulation of the dominant-negative inhibitor GRβ isoform, are summarized by Barnes (119, 120).

Continuous efforts to circumvent these two main problems of GC treatment are based on diverse strategies, including the use of selective GC ligands (121–124), targeting of the GR interactome (125), tissue-specific targeting, and strategies to influence post-translational modifications and isoform expression (1). Several combination treatments have also been shown to be beneficial (49, 126–128), for example ginsenoside Rh1, which potentiates the long-term DEX-mediated anti-inflammatory effects in a collagen-induced arthritis model while diminishing hyperglycemia (127).

**Future prospects**

Given the clinical need, novel GCs are still being brought to the market. These drugs differ in half-life, potency, or (for topical applications) the amount of systemic action. To design more refined drugs that influence GR signaling in a desired manner, it remains crucial to combine all layers of information in order to understand the complex mechanisms that govern GR activity. Herein we reviewed the integration of various signals by GRs, including those from other receptors involved in immune regulation. Interestingly, as recently reviewed by Hapgood et al. (129), this integration can even occur in the absence of GCs, contributing to a shift in the sensitivity of target cells to subsequent GC exposure.

As suggested by the Yamamoto team, future approaches to developing GC-based treatment strategies will necessitate gathering and integrating data on priming, (de)sensitization, and (in)activation of GR, as well as synergies and cross-talk with other signaling pathways, for each individual, allowing for a personalized medicine approach in the nearby future (130). Such studies will be made possible by a combination of interdisciplinary approaches in biological, physical, engineering, and computer and health sciences (130). Bearing in mind the above-described complexities of GR-mediated responses, future clinicians will have to make pragmatic choices informed by the knowledge being generated today. Patient stratification will become essential to decide on the need for a GR dimer–favoring drug (for acute disease) and GR monomer–favoring drug (for chronic disease) (13). Besides the dose and frequency of treatment, additional parameters may influence the healing process and may depend on a more precise timing of treatment (morning versus evening), the nutritional state of the patient, and disease dynamics that may require a switch to another GR-modulating drug at some point during the disease course.

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