Published in final edited form as:
Trends Endocrinol Metab. 2013 March ; 24(3): 109–119. doi:10.1016/j.tem.2012.11.005.

The five Rs of glucocorticoid action during inflammation: ready, reinforce, repress, resolve, and restore

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Abstract

Glucocorticoids are essential for maintaining homeostasis and regulate a wide variety of physiological processes. Therapeutically, synthetic glucocorticoids are widely prescribed for the treatment of inflammation, autoimmune disorders, and malignancies of lymphoid origin. In this review we examine emerging evidence highlighting both proinflammatory and anti-inflammatory actions of glucocorticoids on both the innate and adaptive immune systems. We incorporate these findings into the more traditional anti-inflammatory role attributed to glucocorticoids, and propose how the two seemingly disparate processes seamlessly work together to resolve cellular responses to inflammatory stimuli. These ideas provide a framework by which glucocorticoids ready and reinforce the innate immune system, and repress the adaptive immune system, to help to resolve inflammation and restore homeostasis.

Inflammation and the glucocorticoids

Inflammation is a physiological response to the detection of a foreign antigen or pathogen. Increases in the release or expression of cytokines, chemokines, adhesion molecules, receptors, and enzymes are critical steps for both vascular changes and leukocyte infiltration that occur in response to inflammatory stimuli [1]. Although initially beneficial, an unrestrained or chronic inflammatory condition can also be detrimental, often requiring pharmacological intervention [2]. It was recognized in the 1940s that glucocorticoids have potent anti-inflammatory properties and, as such, both natural and synthetic glucocorticoids have become one of the most prescribed classes of anti-inflammatory medications worldwide [3].

Under normal physiological conditions, glucocorticoids act on nearly every tissue in the body and are important regulators of carbohydrate, fat, and protein metabolism. In addition, glucocorticoids impact upon the cardiovascular, immune, reproductive, and central nervous systems [4], and are critical for lung development [5]. Glucocorticoids exert their anti-inflammatory actions via a complex interplay between glucocorticoid receptor (GR)-mediated transcriptional regulation and signal transduction within target tissues [6]. A major focus of recent studies on the anti-inflammatory actions of GR and glucocorticoids has centered on their ability to tether to and inhibit the activity of transcription factors, but without physically binding to DNA, a process known as ‘transrepression’ (see Glossary). Here we review what is known about the anti-inflammatory actions of glucocorticoids and highlight how recent discoveries have provided evidence for additional unrecognized proinflammatory actions of these steroids. Readers are referred to recent publications for a

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more comprehensive review of the anti- [6-8] and proinflammatory effects of glucocorticoids in the central nervous system [9,10].

Glucocorticoids ready the innate immune system and repress adaptive immunity

In mammals, the immune system is divided into two parts: the innate and the adaptive immune systems. The innate immune system is critical for the initial immune response upon infection or tissue damage. Through invariant pattern-recognition receptors (PRRs), the innate immune system is activated immediately upon detection of evolutionarily conserved structures known as pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) [11]. By contrast, the adaptive immune system serves as a second line of defense, relying on the expansion of antigen-specific T and B cells that effectively neutralize and remove specific pathogens and help to form immunological memory [12]. There is considerable crosstalk between the innate and adaptive immune system that helps to shape the nature and duration of the inflammatory response [13].

Owing to their lipophilic nature, glucocorticoids diffuse freely across the plasma membrane and exert their effects through activation of GR, a member of the nuclear receptor (NR) superfamily of ligand-dependent transcription factors [14]. Glucocorticoids, by virtue of almost ubiquitous GR expression, can affect nearly every cell of the immune system, depending on differentiation or state of activation [8,15]. Glucocorticoids target specific cell populations to combat hyperactivation of the immune system or systemic infections, at both the transcriptional and cellular level (Box 1). For example, by blocking the expression of cyclooxygenase-2 (Cox-2), glucocorticoids target T cells to control hyperactivation in response to excessive T cell receptor (TCR) binding or superantigen [16]. Glucocorticoid-induced apoptosis of T cells reduces inflammation associated with experimental autoimmune encephalomyelitis [16,17]. By contrast, glucocorticoids target macrophages to ensure survival in response to lipopolysaccharide (LPS)-induced sepsis, or to suppress inflammation associated with contact allergy [18,19]. Collectively, these actions are in line with the classical notion that glucocorticoids are able to repress and resolve inflammatory conditions, ultimately restoring homeostasis.

Although glucocorticoids are clearly anti-inflammatory in situations of ongoing inflammation, their role in the normal physiology of the immune system is less understood. The circadian and ultradian changes in the circulating levels of glucocorticoids lead to a dynamic pattern of chromatin occupancy by GR and transcriptional ‘bursts’ that are lost with chronic hormone treatment or upon administration of synthetic hormone [20]. How this changes the transcriptional output of GR is only now beginning to be understood. However, it stands to reason that, under basal conditions, glucocorticoids may well be protective by ensuring the immune system is ready to respond to pathogens. This appears to be the case, at least with respect to the innate immune system where glucocorticoids are not strictly immunosuppressive [21].

Toll-like receptors (TLR1–10) are well-known PRRs that play a critical role in the detection and subsequent reaction to PAMPs. Activation of TLRs induces an intracellular signaling cascade that culminates in the activation of the AP-1, NF-κB, and IRF family of transcription factors. In general, glucocorticoids act to suppress TLR-mediated signaling through the induction of endogenous inhibitors (e.g., MKP-1 and GILZ) or through inhibition of AP-1, NF-κB, and IRF [22]. Interestingly, glucocorticoids induce the expression of TLR2, which is enhanced by the presence of proinflammatory cytokines (e.g., TNF-α or IL-1β) or *Haemophilus influenzae* [22]. Based on tissue-specific increases in TLR2, glucocorticoids can be viewed as being both pro- and anti-inflammatory and, as such,

are crucial for the initiation and resolution of the inflammatory response. The increase in TLR2 on epithelial cells enhances the secretion of IL-6 and IL-8 [22] (Figure 1a), cytokines critical for induction of the acute-phase response and chemotaxis of a variety of cells, respectively. By contrast, the induction of TLR2 (and TLR4) within the adrenal gland is directly involved in the release of cortisol and corticosterone [22] (Box 2), providing a positive feedback loop for the resolution of the inflammatory process. Therefore, the balance between pro- and anti-inflammatory effects of the glucocorticoids in response to bacterial infection would be predicted to depend on the phase of the response in which the glucocorticoids are introduced.

The NOD-like receptors (NLR), an intracellular family of PRRs, respond to both PAMPs and DAMPs. Of the 22 members, only three (NLRP1, NLRP3, NLRC4) are able to form the central component of the inflammasome, which is responsible for the maturation of IL-1β from its pro- to mature form [23]. It was recently demonstrated that glucocorticoids positively regulate the expression of NLRP3 in both cultured and primary macrophages [24]. The NLRP3 inflammasome is activated by a wide variety of molecules, including PAMPs, DAMPS, and particulate matter (e.g., aluminum). The glucocorticoid-dependent increase in NLRP3 expression sensitized macrophages to extracellular ATP, a DAMP commonly released following cellular damage or necrosis (Figure 1b). In addition to sensitizing cells to respond to lower concentrations of ATP, glucocorticoids significantly enhance the ATP-dependent secretion of the proinflammatory cytokines, IL-1β, TNF-α, and IL-6 [24]. Interestingly, glucocorticoids also induce the expression of the purinergic receptor P2Y2R, enhancing downstream signaling and subsequent IL-6 secretion from endothelial cells following stimulation with ATP [25] (Figure 1c). Thus, the induction of TLR2, NLRP3, and P2Y2R provides a mechanism by which glucocorticoids ready the innate immune system for rapid activation and enhancement of the acute-phase response (Figure 1).

Coactivation of GR and proinflammatory transcription factors reinforces the inflammatory response

Recent evidence suggests that the interaction between GR and proinflammatory transcription factors is much more complex. Chromatin immunoprecipitation assay coupled to deep-sequencing (ChIP-Seq) analysis has shown that, in the absence of inflammatory stimuli, AP-1 can direct the binding of GR following hormone activation by regulating chromatin accessibility [26]. In fact, 51% of the GR-bound sites were co-occupied by AP-1 and included both composite [glucocorticoid response elements (GRE) and AP-1 sites] and non-composite interactions (AP-1 sites only). It remains to be determined whether or not these interactions are transcriptionally functional. Surprisingly, loss of AP-1 activity significantly reduced GR occupancy at the co-bound sites, and attenuated ~50% of the glucocorticoid-regulated genes, suggesting a functional role for AP-1 in transcriptional pathways activated by GR [26]. Though not directly tested, this data has broader implications for GR-mediated signaling following activation of AP-1 by proinflammatory molecules because the GR-mediated inhibition of AP-1 is selective for some, but not all, of the AP-1 regulated genes, and vice versa [6]. How the timing of AP-1 or GR activation affects this cooperativity at composite or non-composite elements warrants further investigation.

Does the cooperation between GR and proinflammatory transcription factors allow the regulation of glucocorticoid-responsive genes not previously accessible? Some insight into this question comes from the recent ChIP-Seq analysis of GR and NF-κB binding sites in HeLa cells following treatment with the synthetic glucocorticoid triamcinolone acid (TA), TNF-α, or both [27]. Compared to either treatment alone, the majority of identified binding sites were conserved upon cotreatment with TA and TNF-α. Interestingly, a significant

proportion of GR and NF-κB binding sites were identified only when TA and TNF-α were administered concomitantly. Occupancy at these 'gained' GR and NF-κB sites was highly dependent on the presence of NF-κB and GR, respectively. In fact, NF-κB and AP-1 response elements were enriched at the sites that were co-occupied by GR and NF-κB. In line with the anti-inflammatory actions of GR, a subset of TNF-α-regulated genes were downregulated by cotreatment with TA. However, a subset of genes, including proinflammatory genes, were also synergistically regulated by the combined treatment. One caveat to this study is that GR was activated before NF-κB, and this could alter the transcriptional output compared to simultaneous stimulation or the initiation of TNF-α signaling before GR activation. For example, glucocorticoids could have a prophylactic effect when administered before NF-κB activation, skewing the response towards being anti-inflammatory. By contrast, activating NF-κB (and/or AP-1) before or at the same time as GR may shift the transcriptional profile of repressed/induced genes and expand the synergistically regulated subset. Further characterization of these genes and the functional significance of the observed synergism are warranted.

Interestingly, whole-genome microarray analysis identified ~900 genes that are regulated by concomitant administration of glucocorticoids and TNF-α [28]. Of these genes, more than two-thirds were coregulated by glucocorticoids and TNF-α. Of particular interest was serpinA3, a secreted acute-phase protein involved in several inflammatory diseases [29]. The expression of serpinA3 was induced by both glucocorticoids and TNF-α alone. However, coadministration of glucocorticoid and TNF-α led to a synergistic increase in the level of mRNA and protein *in vitro* and *in vivo* [28]. Despite having predicted GREs, the synergistic regulation of serpinA3 appears to occur through enhanced recruitment of GR and RNA polymerase II (Pol II) to the transcription start site. The question of whether or not NF-κB has a similar role to AP-1 in regulating GR-mediated signaling in the basal state remains open.

As indicated in Box 2, the 11β-hydroxysteroid dehydrogenase (HSD) enzymes are critical in controlling the bioavailability of intracellular glucocorticoids. Interestingly, obesity and proinflammatory cytokines induce a significant increase in the expression and activity of 11β-HSD1 in macrophages and in pre- and mature adipocytes [30,31]. Surprisingly, the expression and activity of 11β-HSD1 is necessary for cytokine and LPS-induced secretion of several proinflammatory cytokines. In adipocytes specifically, glucocorticoid reamplification enhanced NF-κB and MAPK activity and may contribute to the persistent inflammation seen in obesity [31].

The observation that GR co-occupies a significant proportion of binding sites with proinflammatory transcription factors is not restricted to AP-1 and NF-κB. To determine the crosstalk between STAT3 and GR, whole-genome tiling array analysis was performed in cells stimulated with leukemia inhibitory factor (LIF), a member of the IL-6 family, and/or dexamethasone (Dex). This analysis identified hormone-activated GR at a significant number of LIF-activated STAT3 binding sites [32]. In fact, glucocorticoids significantly potentiated the number of genes induced by LIF, which alone regulated very few genes. In particular, one cluster of late-onset genes stood out. These genes, which comprise the cell defense response, were only regulated when both LIF and glucocorticoids were administered. Moreover, *in vivo*, the extent to which this cluster of genes was coregulated by LIF and Dex was similar to their induction by LPS. Furthermore, glucocorticoids also enhanced IL-6-activated STAT3 activity [32,33], potentially by both enhancing STAT3 binding [32] and repressing suppressor of cytokine signaling 3 (SOCS3) expression [33]. Classically, it is recognized that GR tends to repress the activity of these transcription factors (see below). However, the ability of these transcription factors to shape the glucocorticoid response (Figure 2) provides evidence that the initial burst of glucocorticoids

elicited by an inflammatory response could actually be to reinforce the proinflammatory environment, ensuring that proper clearance and removal of pathogen is achieved.

Glucocorticoid-mediated repression of transcription factors and signaling pathways involved in inflammation

Proinflammatory molecules released during inflammation initiate signaling cascades that ultimately activate the transcription factors AP-1 and NF-κB. In turn, these transcription factors regulate the synthesis of proinflammatory molecules, providing a positive feedback loop that propagates the inflammatory response [34,35]. The basic tenet and much of the research on the anti-inflammatory effects of the glucocorticoids has focused on GR-mediated repression of the transcriptional activity of AP-1 and NF-κB (Figure 3). However, GR is able to interact with and alter the transcriptional activity of several other factors potentially involved in the inflammatory response, such as the interferon (IFN) regulatory factor 3 (IRF3) [36,37], T-box expressed T cells (T-bet) [38], and GATA3 [39], among others [40]. Additional genome-wide studies are necessary to identify these alternative mechanisms [22,40].

AP-1, one of the key mediators of the inflammatory response, functions as a homo- or heterodimer composed of the basic leucine-zipper transcription factors Fos (cFos, Fos B, Fra-1, and Fra-2), Jun (c-Jun, v-Jun, Jun B, and Jun D), activating transcription factor (ATF2, ATF3, B-ATF, JDP-1, and JDP-2), or MAF (MAFA, MAFB, c-MAF, NRL, MAFF, MAFG, and MAFK) [35,41]. These proteins are differentially expressed, altering transcriptional output depending on the subunit composition of the AP-1 dimer [35]. However, the most common form of AP-1 formed downstream of inflammatory cytokine signaling is the c-Fos/c-Jun heterodimer. This heterodimer is formed following activation of the c-Jun N-terminal kinase (JNK), a member of the MAPK family of proteins [42]. Once activated, the AP-1 heterodimer regulates numerous proinflammatory genes by binding to AP-1 response elements. In the classic model of GR-mediated inhibition, the transcriptional activity of AP-1 is suppressed as a result of a direct interaction with the c-Jun subunit of AP-1, which results in the reciprocal antagonism of GR-mediated signaling [43] (Figure 3a). Although the DNA-binding domain (DBD) (Box 1) of GR is necessary for this interaction, it does not seem to require GR binding to GREs within the promoter of the target gene, does not compete for coactivators [44,45], nor does it inhibit AP-1 from binding to its response element within endogenous promoters [6].

Similarly to AP-1, NF-κB plays a crucial role in initiating and amplifying proinflammatory signals. The NF-κB family consists of five members: p65 (RelA), RelB, c-Rel, NF-κB 1 (p50/p105), and NF-κB 2 (P50/p100) [34]. In the canonical pathway (i.e., TNF-α-activated), the transcriptionally active NF-κB dimer (p65–p50) is held in the cytoplasm by a member of the inhibitor of NF-κB (IκB) family [34]. Signaling through proinflammatory stimuli results in the activation of IκB kinase (IKK), phosphorylation and degradation of IκB, and thus the release and nuclear shuttling of NF-κB. Once in the nucleus, NF-κB binds to its response elements to regulate numerous proinflammatory molecules. The exact mechanism by which glucocorticoids are able to inhibit the activity of NF-κB is unclear, although several hypotheses have been proposed (Figure 3b). Similarly to AP-1, GR is proposed to physically interact with RelA and inhibit its transcriptional activity [46]. GR is able to block the formation of the p65/IRF3 complex [36], perhaps by recruiting the GR-interacting protein (GRIP) [37,47]or inhibiting phosphorylation of IRF3 [48]. GR has been reported to block the phosphorylation of the C-terminal domain (CTD) of Pol II by competing with the CTD kinase pTEFb [49,50]. Similarly, GR can recruit histone deacetylases to NF-κB -dependent promoters [51,52], or compete with NF-κB for binding to CREB-binding protein and p300

[53]. Finally, p53 was also shown to be involved in GR-mediated repression of NF-κB activity through the regulation of the transcriptional activity of GR [54].

In addition to transrepression, glucocorticoids are able to induce the expression of proteins that can antagonize proinflammatory processes at the post-transcriptional level. The induction of MAPK phosphatase-1 prevents the phosphorylation and activation of JNK [55-58]. Although initial evidence suggested that glucocorticoids could induce the expression of IκBα to sequester p65/p50 in the cytoplasm, this response seems to be cellspecific and is not a universal mechanism to regulate NF-κB signaling [59]. Finally, glucocorticoids influence mRNA stability through the induction of tristetraprolin (TTP), which stimulates the degradation of transcripts with AU-rich elements such as TNF-α [60]. In the context of inflammation, glucocorticoids then repress the activity of these and other proinflammatory transcription factors directly, as well as by inducing proteins that antagonize inflammatory signaling pathways. In doing so, glucocorticoids ensure that there is not a prolonged or exaggerated production of inflammatory cytokines, allowing the ultimate resolution of inflammation and restoration of a homeostatic environment.

Glucocorticoids, resolution, and tissue restoration following an inflammatory response

The final phase of inflammation, resolution, is an active process involving multiple biochemical pathways [61]. Some of the key hallmarks of resolution are the active repression of neutrophil recruitment to the inflammatory site, the secretion of bioactive lipids known to have proresolving actions, and the nonphlogistic recruitment of monocytes which play a role in tissue repair [61]. Glucocorticoids affect each of these processes at the cellular and molecular level. For example, glucocorticoids suppress the expression of both endothelial- and neutrophil-expressed adhesion molecules, thus preventing extravasation [8]. Additionally, glucocorticoids induce the expression of Annexin-1, which induces neutrophil apoptosis [62]. Finally, prolonged glucocorticoid exposure promotes an antiinflammatory phenotype with enhanced phagocytic activity in resident macrophages, contributing to the resolution of inflammation by removing apoptotic cells and tissue repair [8] (Figure 4).

Concluding remarks

As a pharmacological intervention, glucocorticoids are the first line of defense to treat chronic inflammatory diseases. However, how to reconcile the physiological role of glucocorticoids as major factors released in response to stress (inflammation included) with their anti-inflammatory actions is a question that remains to be resolved. The ability of molecules to have both a pro- and anti-inflammatory role in both normal physiological and pathological conditions is not unprecedented. For example, both IL-6 and leptin are known to have pro- and anti-inflammatory properties [63-65]. It is becoming clear that the actions of glucocorticoids are much more pleiotropic than previously thought, and thus cannot be simply categorized as antiinflammatory (Figure 5). The regulation of components of the innate immune system, namely TLR2, NLRP3, and P2Y2R [22,24,25], provides evidence that glucocorticoids ready the immune system to respond quickly to both bacterial infection and tissue damage. In addition, the complete absence of glucocorticoids (i.e., adrenalectomy) renders animals much more susceptible to LPS-induced systemic inflammation [66], suggesting that glucocorticoids are necessary both to respond to infection as well as to prevent a massive release of cytokines and prevent the adaptive immune system from over-reacting.

We hypothesize that the shift in the ability of glucocorticoids to regulate pro- versus anti-inflammatory gene programs lies in the concomitant signals received by the cell, the

duration and magnitude of glucocorticoid signaling, and the duration and magnitude of the proinflammatory stimulus. The inflammatory process is critical for an organism to respond to and remove pathogens. It is also crucial that the process proceeds unfettered through to resolution. Therefore, rather than acting strictly as an anti-inflammatory mediator, GR should be considered as a cellular rheostat. Specifically, in response to inflammatory stimuli, the transcriptional output elicited by glucocorticoids is fine-tuned by the microenvironment [27,32,67], ligand bioavailability [31], receptor expression [4], concomitant hormonal input [66], chromatin state [26,68], receptor dynamics [69], and cellular binding partners [26,27,32,54,70] (Figure 5). Thus, glucocorticoids ready and reinforce the innate immune system to respond quickly (proinflammatory actions), but also act systemically to repress the adaptive immune system and help restore homeostasis (anti-inflammatory actions). Deciphering these mechanisms will provide a great deal of insight into the overall regulation of transcriptional profiles and physiological responses elicited by glucocorticoids.

Acknowledgments

We would like to extend our apologies to those colleagues whose work we were unable to cite owing to space limitations. We would like to thank and acknowledge these individuals for all of their work that significantly contributed to our current understanding of GR action.

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Glossary

Damage-associated molecular patterns (DAMPs)	a diverse set of endogenously derived products that alert the innate immune system to tissue damage
Extravasation	the movement of cells from the blood vessel into surrounding tissue
Glucocorticoid response element (GRE)	a short, palindromic DNA sequence that is bound by the liganded receptor, in this case GR. GREs can be found with the promoter sites, introns, or exons of target genes. The DNA sequence is 5′ AGAACAnnnTGTTCT 3′
Inflammasome	a macromolecular complex responsible for the activation of caspase-1 and -5 and subsequent processing and release of the cytokines interleukin (IL)-1β, IL-18, and IL-33
NOD-Like receptors	a family of cytoplasmic proteins that can function as pattern-recognition receptors to regulate inflammatory and apoptotic processes
Nonphlogisitic	the clearance of leukocytes in the absence of or induction of an inflammatory response
Pathogen-associated molecular patterns (PAMPs)	a diverse set of microbialderived products that share conserved features that alert the innate immune system to intruding pathogens

Pattern-recognition receptors (PRRs)	germline-encoded receptors that recognize structures conserved among microbes or endogenous molecules released from damaged cells
Pre-receptor ligand metabolism	a series of enzymatic reactions controlling the intracellular concentration of active hormone, in this case cortisol. Cortisol is converted to its inactive metabolite, cortisone, through the actions of 11β-hydroxysteroid dehydrogenase (HSD) type 2. The reverse reaction, which converts cortisone back to cortisol, is regulated by 11β-HSD type 1
T cell receptor (TCR) ligation	recognition of an antigen/major histocompatibility complex (MHC) by the TCR. This leads to the activation of signaling pathways that result in clonal expansion, differentiation, and the secretion of cytokine and cytotoxic molecules
Th1 lymphocytes	a differentiated subset of T cells. T helper (Th)1 cells are primarily made in response to microbes and viruses that activate macrophages and natural killer (NK) cells. They produce cytokines such as interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) to reinforce an inflammatory response by activating effector T cells, natural killer cells, and macrophages
Th2 lymphocytes	a second differentiated subset of T cells. Th2 cells are primarily made in response to helminthes, allergens, and extracellular microbes and toxins. They produce cytokines such as IL-4, IL-5, and IL-13 and stimulate B cells, eosinophils, and mast cells during allergic responses
Toll-like receptors	pattern-recognition receptors expressed on cells of the immune system that recognize microbial structures that are evolutionarily conserved and activate the inflammatory response
Transrepression	repression of gene expression induced by indirect association (tethering) rather than by DNA-specific binding of the nuclear receptor with target genes

Box 1

Glucocorticoid biology, the basics

Glucocorticoid receptor (GR) structure: structurally, GR consists of three modular domains: an N-terminal transactivation domain (NTD), a central DNA-binding domain (DBD), and a C-terminal ligand-binding domain (LBD) [71]. The NTD interacts with cofactors and components of the basal transcriptional machinery via a transcriptional activation function (AF1) exposed upon ligand binding [71]. The DBD, which shares the highest degree of sequence identity with other members of the nuclear receptor (NR) superfamily, contains two zinc-finger motifs responsible for binding DNA at glucocorticoid response elements (GREs) [71]. In addition, the second zinc finger of the DBD contains residues that constitute the dimerization or D-loop and that aids in receptor dimerization following activation [71]. Following a short hinge region, the 12 α-helices and four β-sheets of the LBD form a hydrophobic pocket that allows high-affinity binding of glucocorticoids [72]. This region also contains residues that contribute to receptor dimerization [72] and a second activation function domain (AF2) that interacts with various coregulators in a ligand-dependent manner.

Glucocorticoid signaling: in the absence of ligand, GR is primarily located in the cytoplasm as part of a large multiprotein complex [73]. Upon activation, GR is actively transported into the nucleus in an importin-dependent manner [73,74]. It was generally thought that a conformational change triggered in GR upon glucocorticoid binding resulted in dissociation of GR from the complex, exposure of nuclear localization sequences, and import into the nucleus [75]. However, emerging evidence suggests that components of the chaperone complex are also needed for efficient nuclear translocation [74]. Once in the nucleus, GR homodimers bind to GREs on target genes and stimulate transcription. However, not all GR–DNA interactions induce gene expression. In fact, whole-genome microarray analysis *in vivo* and *in vitro* has shown that ~50% of the glucocorticoid-regulated genes are repressed [66,67], partly due to GR interacting with negative GREs to suppress gene activation [76]. Further fine-tuning of the GR response is accomplished through structural changes induced by the ligand and the GRE sequence itself [77,78], which help to coordinate the recruitment of coregulators and chromatin-remodeling complexes that influence Pol II-dependent transcription [4].

Interestingly, not all glucocorticoid-regulated genes contain GREs [79] because GR is also able to positively or negatively influence gene expression by physically interacting with other transcription factors, in other words through protein–protein interactions, a mechanism known as tethering [4]. For other transcription factors there is a composite regulation where GR needs to bind both to a GRE and to the transcription factor bound at an adjacent promoter site to effect gene transcription [4].

GR signaling can be directly attributed to the genomic effects described above. However, nongenomic (i.e., occurring independently of gene transcription) effects also exist. These effects are thought to occur via either nonspecific interaction of glucocorticoids with membrane components or perhaps through membrane-bound GR [80,81]. Additionally, GR is able to interact with and alter the activity of kinases such as JNK [82], Src [83], ERK [84,85], and PI3K [86,87], affecting signaling pathways independently of gene transcription [40]. Although the mechanisms and physiological outcomes of nongenomic glucocorticoid signaling are not well-defined, these responses could have important roles in the overall actions of glucocorticoids.

Box 2

The hypothalamic–pituitary–adrenal axis and glucocorticoids

To maintain homeostasis, organisms have developed an exquisite means to cope with various stressors. Key to this is the activation of the hypothalamic–pituitary–adrenal (HPA) axis, culminating in the synthesis and secretion of cortisol (in humans) or corticosterone (in rodents) [88]. Once secreted, the majority of circulating cortisol (~90%) is bound to corticosteroid-binding globulin (CBG). Free or loosely bound cortisol (~10%) diffuses across cellular membranes and exerts the biological effects of glucocorticoids. However, there is evidence that CBG-bound cortisol provides a means for delivery to particular microenvironments [89]. In healthy individuals, the synthesis and secretion of cortisol displays a circadian rhythm following the pulsatile changes in ACTH, totaling ~10 mg/day, and can increase ~10-fold in response to stress [90]. Importantly, several proinflammatory cytokines are known to activate the HPA axis, resulting in the secretion of glucocorticoids [91].

The bioavailability of cortisol is regulated by the 11β-hydroxysteroid dehydrogenase (11β-HSD) enzymes in a process known as ‘prereceptor ligand metabolism’. The conversion of cortisol to cortisone, the inactive metabolite, is catalyzed by 11β-HSD type 2 (11β-HSD2). By contrast, 11b-HSD type 1 (11β-HSD1) catalyzes the reverse reaction, converting cortisone to cortisol [92]. These enzymes are differentially expressed *in vivo*, with 11β-HSD2 expression being highest in the kidney to prevent cortisol from binding to the mineralocorticoid receptor, which has a higher affinity for cortisol than does the glucocorticoid receptor (GR) [90,93]. By contrast, 11β-HSD1 expression is highest in the glucocorticoid-responsive tissues (i.e., liver, adipose, and muscle) to ensure intracellular cortisol bioavailability [94].

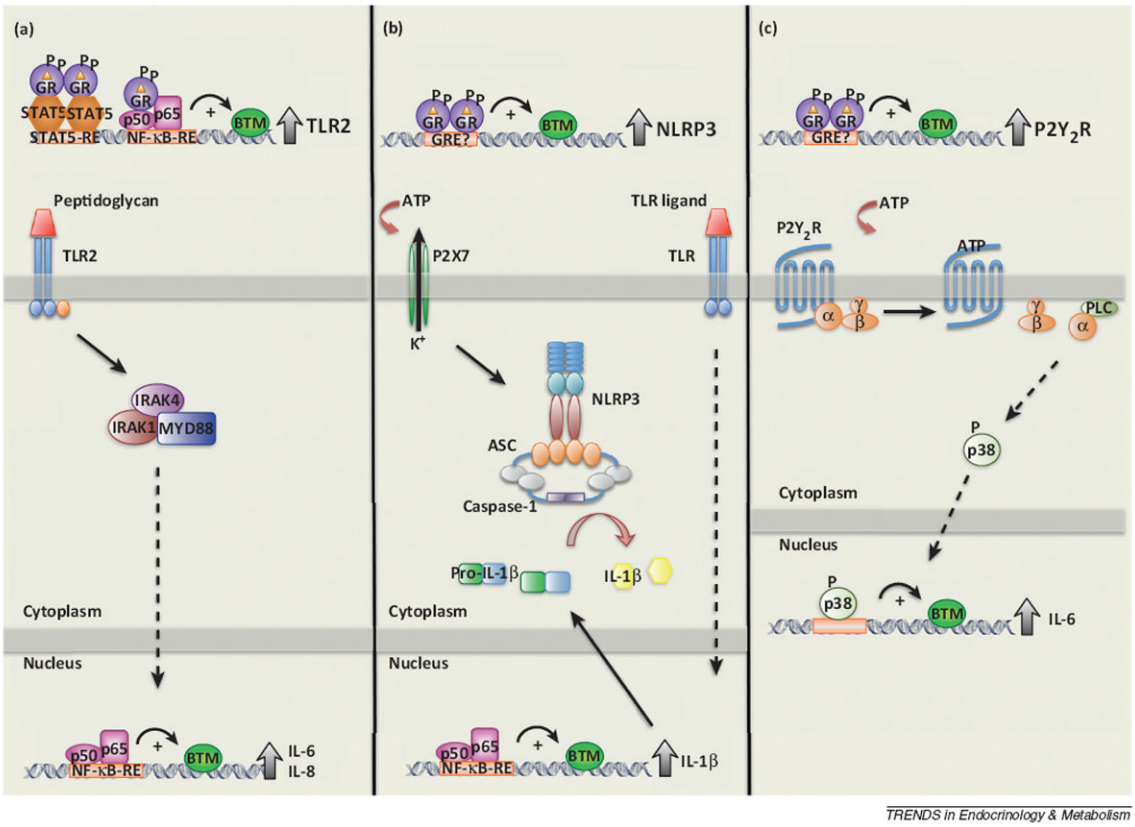

Figure 1.

Glucocorticoids ready the innate immune system. Glucocorticoids induce the expression of proteins involved in responding to the detection of microbial products and cellular damage. **(a)** Proposed mechanism of Toll-like receptor (TLR)-2 induction. Glucocorticoids are able to induce the expression of TLR2 alone, or by acting synergistically with STAT5 and NF-κB activated by interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α). This synergistic induction of TLR2 enhances interleukin-6 (IL-6) and IL-8 production induced by peptidoglycan, a TLR2 agonist. **(b,c)** Glucocorticoids enhance the ability of cells to respond to cellular damage through induction of NLRP3 and P2Y2R, respectively, via an unknown mechanism. (b) The induction of the intracellular pattern-recognition receptor, NLRP3, sensitizes macrophages to extracellular ATP, a known danger signal, enhancing the secretion of IL-1β in the presence of TLR activation. (c) Glucocorticoids induce the expression of the purinergic receptor, P2Y2R. P2Y2R, a G-protein-coupled receptor, is activated by extracellular ATP, and glucocorticoids enhance the ATP-dependent activation p38, enhancing the expression and secretion of IL-6.

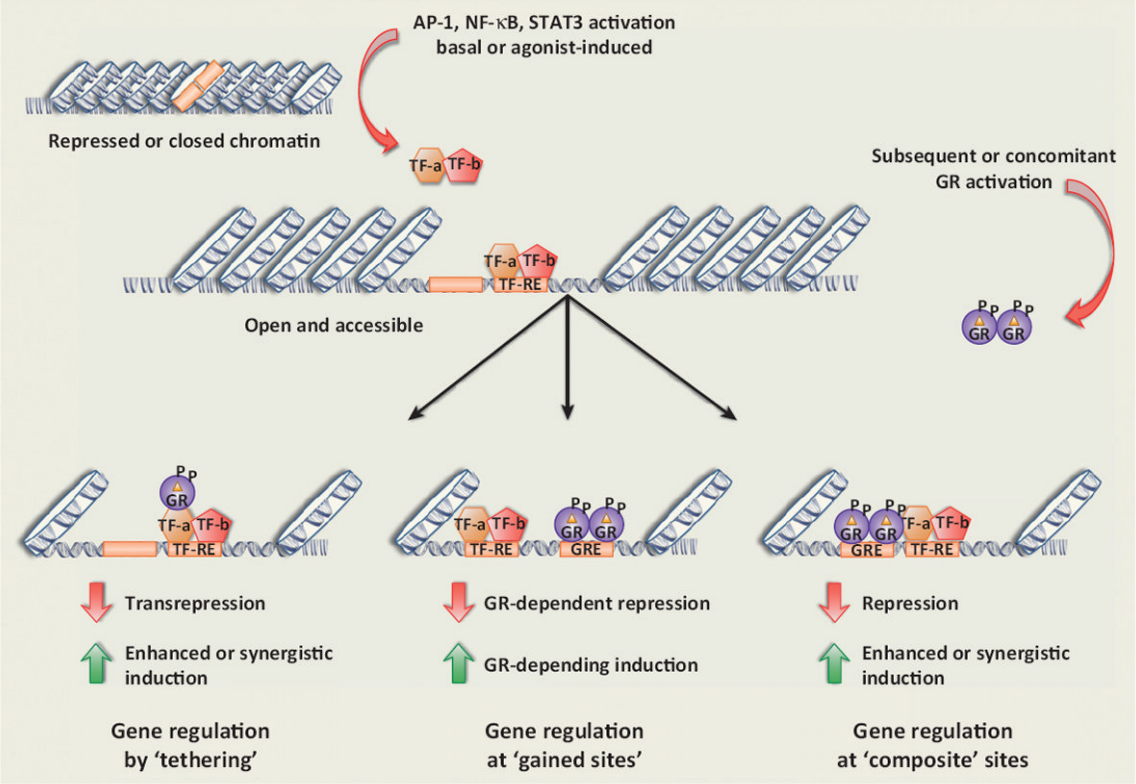

Figure 2.

Concomitant activation of proinflammatory transcription factors and the glucocorticoid receptor reinforces inflammation. The ability of the glucocorticoid receptor (GR) to activate gene transcription relies on its ability to interact with glucocorticoid response elements (GREs). (Top) Chromatin, in its inactive state, prevents GR from accessing a GRE and alters the transcriptional response. (Bottom) Proinflammatory transcription factors (TF) promote an open chromatin state in the absence (AP-1) or presence (NK-κB or STAT3) of an inflammatory stimulus, allowing GR to now access chromatin and elicit a response. Some of the glucocorticoid-responsive changes occur as a result of tethering (left) or as a result of a composite regulation (right) where GR is able both to bind to a GRE and tether to the transcription factor. The profile of these complex interactions will shape the immune response and can be either stimulatory or repressive.

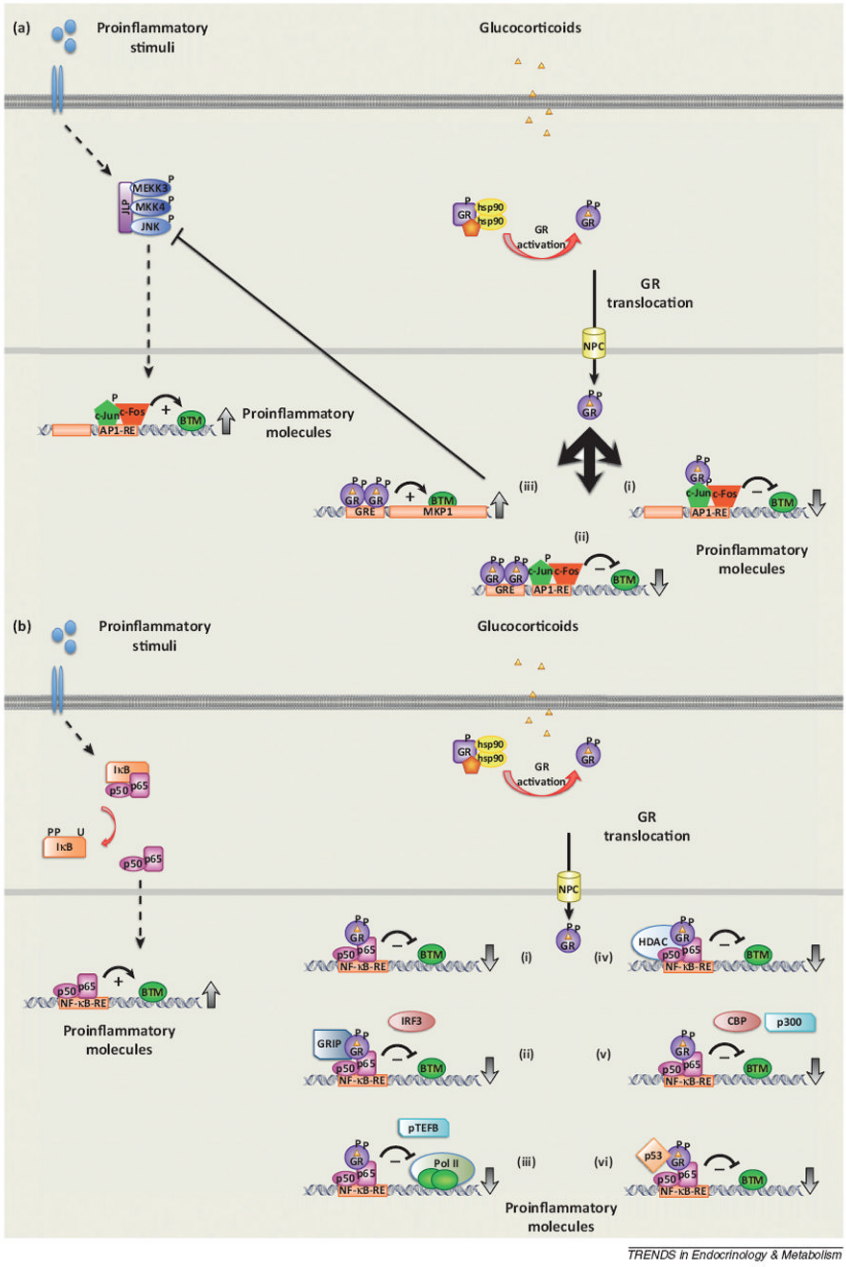

Figure 3.

Glucocorticoid receptor (GR)-mediated repression of the proinflammatory transcription factors AP-1 and NF-κB. **(a)** Proinflammatory stimuli trigger a signaling cascade that results in the activation of the transcription factor, AP-1, a heterodimer composed of c-Jun and c-Fos. This drives the transcription of several proinflammatory molecules. Activation of the GR results in its translocation to the nucleus where it can now repress AP-1 activity via one of three mechanisms: (i) at some promoters, GR physically interacts with c-Jun in a process known as tethering, which represses the activity of AP-1 and represses the transcription of proinflammatory genes; (ii) at some promoters, GR is able to simultaneously bind to a GRE and tether to c-Jun to repress the transcriptional activity of AP-1; and (iii) GR induces the expression of MKP-1, a phosphatase, that is able to dephosphorylate and inactivate the kinase JNK. **(b)** Proinflammatory stimuli trigger a signaling cascade that results in the

activation of the transcription factor, NF-κB, a heterodimer composed of the p50 and p65 subunits. This drives the transcription of several proinflammatory genes. Although the exact mechanism is not known, there are several theories as to how GR can inhibit NF-κB activity: (i) similarly to AP-1, GR can physically interact with and repress the activity of NF-κB; (ii) GR is able to block the formation of an NF-κB/IRF3 heterodimer, possibly through the recruitment of GRIP; (iii) GR is able to block the recruitment of the C-terminal tail kinase, pTEFb, thus preventing RNA polymerase II (Pol II) phosphorylation and activation; (iv) GR is able to repress NF-κB activity by recruiting a histone deacetylase (HDAC); (v) GR is able to block the ability of NF-κB to interact with p300 and CPB; and (vi) p53 is able to interact with GR, altering its transcriptional activity, and thus preventing NF-κB activity.

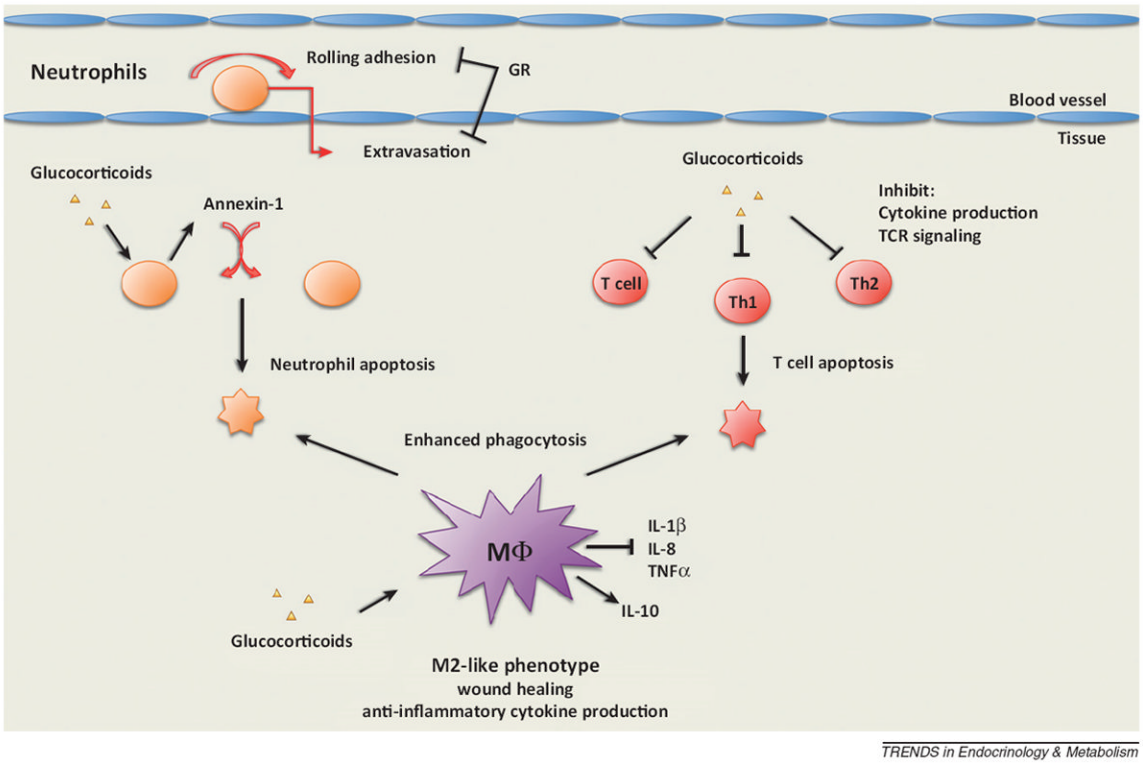

Figure 4.

Glucocorticoids promote the resolution of inflammation and restore homeostasis. Glucocorticoids affect nearly every cell type by virtue of nearly ubiquitous expression of the glucocorticoid receptor (GR). During the course of inflammation, glucocorticoids are able to promote resolution by repressing the expression of adhesion molecules, preventing rolling adhesion and extravasation of neutrophils. Glucocorticoids also induce the expression and secretion of Annexin-1, which is able to induce apoptosis of neutrophils at the site of inflammation. Prolonged glucocorticoid exposure induces tissue resident macrophages (MΦ) to undergo a phenotypic change to become M2-like or anti-inflammatory. These macrophages no longer produce proinflammatory cytokines. Instead they produce interleukin-10 (IL-10), have enhanced phagocytic activity to remove apoptotic cells, and promote tissue healing. Glucocorticoids also act on naïve and differentiated T cells that have been recruited to the inflammatory site by blocking T helper 1 (Th1)- and Th2-derived cytokine production as well as inducing apoptosis.

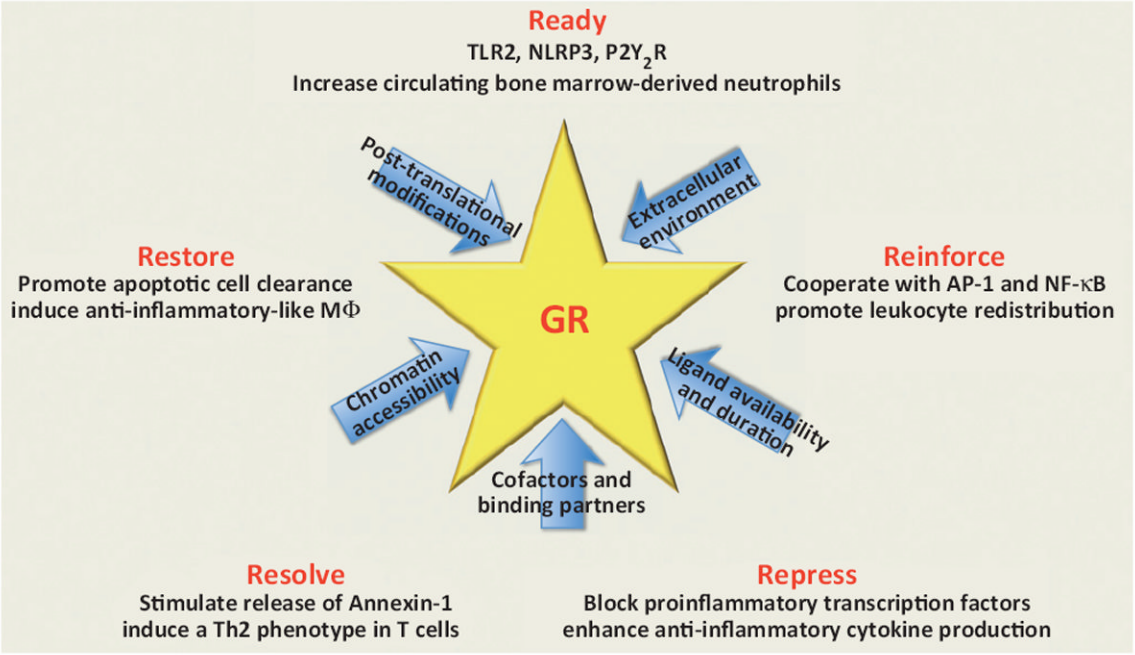

Figure 5.

The glucocorticoid receptor (GR) acts a cellular rheostat to ensure the proper response is elicited by the immune system. Glucocorticoids, acting through GR, affect all phases of the immune response. By enhancing the expression of TLR2, P2Y2R, and NLRP3, glucocorticoids ready the innate immune system to respond to microbial products and tissue injury. Additionally, glucocorticoids increase the levels of circulating bone marrow-derived neutrophils. Glucocorticoids reinforce the immune system by cooperating with the proinflammatory transcription factors AP-1, NF-κB, and STAT3. This is accomplished by repressing their activity at specific promoters, inhibiting the production of proinflammatory cytokines. However, GR can also act synergistically with these transcription factors, enhancing the expression and activation of some proinflammatory responses. Glucocorticoids are able to promote the resolution of inflammation, and restore homeostasis, by stimulating the secretion of proresolving molecules (Annexin-1), shifting T cell signaling towards a Th2 response, inducing neutrophil and T cell apoptosis, promoting a wound-healing and antiinflammatory phenotype in macrophages (MΦ), and promoting the removal of apoptotic cells. The ability of GR to accomplish these pleiotropic actions will depend on several factors including post-translational modifications, extracellular environment, ligand availability and duration of signaling, cell type-specific cofactors and binding partners, and chromatin accessibility.