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Mechanisms of glucocorticoid-mediated anti-inflammatory and immunosuppressive action

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Glucocorticoids (GCs) exert their potent anti-inflammatory and immunosuppressive effects through an intricate combination of mechanisms. Through both pre- and post-transcriptional means, GCs modify gene regulation in target cells by interacting with the cytosolic glucocorticoid receptor (GR). Among their actions, GCs inhibit the production of pro-inflammatory cytokines. This inhibition is accomplished by antagonism of pro-inflammatory transcription factors by either the GR itself or by *de novo*-synthesised antagonists such as glucocorticoid-induced leucine zipper and inhibitor of κB (IκB). Preferential inhibition of type-1 cytokines leads to an eventual shift towards a Th2 profile among CD4+ T-lymphocytes, reducing the pro-inflammatory Th1 population. Not only are pro-inflammatory cytokines inhibited, but the effects of these cytokines upon target cells are diminished due to GC-mediated interference with cytokine receptor signalling. This leads to increases in T-cell, thymocyte, and eosinophil apoptosis, reduced T-cell activation, and decreased production of nitric oxide. Further to these effects, GCs inhibit prostaglandin and leukotriene production by inducing synthesis of lipocortin-1. GCs also up-regulate expression of the anti-inflammatory cytokine transforming growth factor-β (TGF-β) in certain cells. A further mechanism by which GCs suppress normal inflammatory responses is by down-modulating adhesion molecules on antigen-presenting cells (APCs). It is this synergy of many effects that accounts for the potency of GC action and therefore for the utility of these drugs, although it is this very complexity that hampers study in this area. Consideration of GC mechanisms of action is important in order to develop an understanding of the long-term effects of use of these heavily prescribed but poorly understood drugs.

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Introduction

Glucocorticoids (GCs) are among the most prescribed drugs in clinical medicine and are used to treat a variety of inflammatory disorders. GCs are also used in immunosuppressive regimens, as in inhibition of allograft rejection. Despite their use as potent anti-inflammatory and immunosuppressive agents for more than 50 years, GCs exert their effects through mechanisms that

remain only marginally understood. Although it has been possible to determine a number of the key modes by which GCs function, the complex interplay between GCs and target cells remains unclear, although many advances have been made in the last 20 years. This review focuses on the current state of knowledge regarding the specific mechanisms of GC-mediated immunosuppressive and anti-inflammatory action.

As nearly every cell in the body expresses the GC-specific glucocorticoid receptor (GR), endogenous and synthetic GCs profoundly affect virtually every major organ system. The activated GR participates in gene regulation at the level of DNA transcription. The GR is itself a transcription factor, capable of binding directly with target DNA or with other transcription factors to excite or inhibit transcription. Glucocorticoid response elements (GREs) are specific 15 base pair DNA sequences to which the GC receptor can bind to positively or negatively regulate transcription[1,2] (Figure 1).

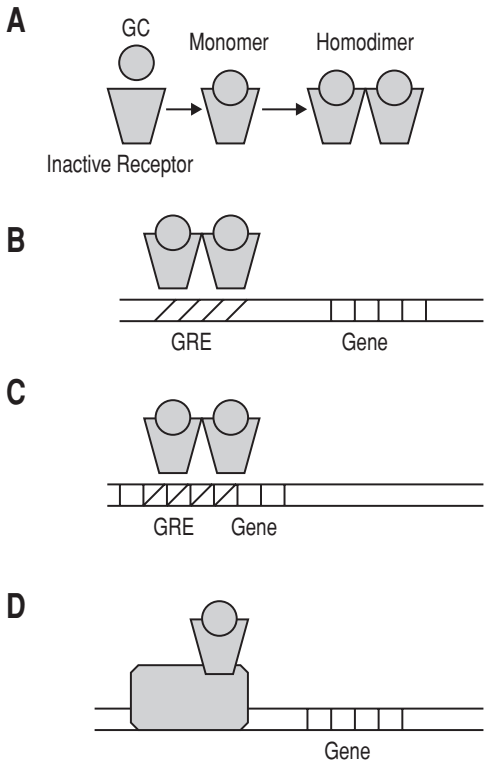

Figure 1 Mechanisms of transcriptional regulation by glucocorticoids: A: The cytosolic glucocorticoid receptor (GR) binds to the GC and migrates to the nucleus as either a monomer or homodimer. B: Dimerised GR directly binds to the GC response element (GRE) upstream of the target segment of DNA. Assemblage of the transcription initiation complex ensues, beginning transcription of the target gene. In some cases, GR does not bind directly to the DNA but will still participate in the transcription initiation complex. C: Activated GR may bind to a GRE within a target gene sequence or promoter, thereby blocking transcription. D: The GR monomer antagonises transcription factors, often by 'tethering' to the transcription initiation complex. This prevents expression of downstream genes, and is believed to be most important mechanism of transcriptional regulation in GC anti-inflammatory activity.

GC effects include inhibition of pro-inflammatory cytokine production, which is regarded as the most pronounced effect of these drugs on the immune system[3]. Pro-inflammatory cytokines can be either directly or indirectly inhibited by GCs. Direct inhibition may entail GR-mediated transcriptional repression as outlined above. GCs also regulate cytokine expression by augmenting production of proteins that destabilise cytokine mRNA, which in turn diminishes expression of these cytokines[4]. Indirect means through which GCs inhibit cytokine production include excitation of synthesis of glucocorticoid-induced leukine zipper, inhibitor of κB (IκB), and lipocortin-1, all of which reduce synthesis of inflammatory compounds. Both IκB and glucocorticoid-induced leukine zipper inhibit the activity of pro-inflammatory transcription factors, thereby reducing expression of targeted genes. Although GC up-regulation of lipocortin-1 does not affect cytokine production *per se*, lipocortin-1 action inhibits synthesis of prostaglandins and leukotrienes, two important classes of inflammatory mediators[5].

Cells targeted by GCs in the suppression of normal immune function include T-lymphocytes, monocyte-macrophages, eosinophils, mast cells, dendritic cells, and endothelial cells. Cytokine down-regulation is not the only effect of GCs on normal immune and inflammatory responses. GCs are believed to excite production of the anti-inflammatory cytokine TGF-β in target cells through pre- and post-transcriptional mechanisms[6,7]. GCs interfere with the expression of adhesion molecules on antigen-presenting cells (APCs) through a mechanism requiring GR activation[8,9]; and GCs induce apoptosis in mature T-lymphocytes, monocytes, and eosinophils, but paradoxically interfere with the normal destruction of developing thymocytes that exhibit self-affinity[10-13]. An *in vivo* study conducted on mice has confirmed that glucocorticoids can therefore play a role in autoimmune disorders[14].

In addition to these effects, GCs inhibit T-cell response to activating stimuli through interference with T-cell receptor-mediated signalling pathways[15-17]. Lastly, it is now widely believed that preferential inhibition of T helper cells (Th) and in particular Th1 cytokines causes a long-lasting shift towards a predominantly Th2 profile among CD4+ T-lymphocytes[18,19]. It has been postulated that this shift results in a persisting anti-inflammatory profile, although preliminary *in vivo* studies have not fully supported this hypothesis[20-22].

Further to the anti-inflammatory effects of GCs, there are paradoxical pro-inflammatory effects on T-lymphocytes. Unexpectedly, high affinity IL-2 and IL-7 receptors are up-regulated in T-cells treated with GCs[23-25]. Subsequent mitogen stimulation of these GC-treated cells leads to increased T-cell effector function versus untreated cells, as measured by increased cellular proliferation[23,24]. It is possible that this rebound phenomenon could account for increases in inflammatory disorder following withdrawal of GCs in patients, but there remains uncertainty regarding the significance of this phenomenon.

Prior to reflecting upon the net effects of GC action, it is first necessary to investigate the mechanisms in more detail. Table 1 gives an overview of cellular processes affected by glucocorticoid treatment in the management of immune disorders.

Inhibition of cytokine and inflammatory mediator production

There are numerous implications of decreased cytokine production resulting from GC treatment. Cytokines play important roles in cell activation and survival, as well as in the production of certain important inflammatory enzymes. GC-mediated inhibition of cytokine production may account for the observed apoptosis of eosinophils and T-lymphocytes, and for inhibition of the production of the pro-inflammatory enzyme nitric oxide synthase in bronchial epithelial cells[14,27-29]. However, the best documented effects of decreased cytokine production are inhibition of T-cell and macrophage activation and reduction in T-cell proliferation.

GCs inhibit IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-11, IL-12, IFN-γ, TNF-α, and GM-CSF[3,4,30-40]. Conversely, the anti-inflammatory cytokine TGF-

Table 1 Mechanisms of glucocorticoid-induced immunosuppression

Targeted cellular process	Result of GC treatment	Cells affected	Mechanism
Cytokine production	↓ Expression of IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-11, IL-12, IFN-γ, TNF-α, and GM-CSF	CD4+ T-lymphocytes, macrophages, epithelial cells, fibroblasts	Antagonism of transcription factors NF-κB, NF-AT, and AP-1 mRNA de-stabilisation (IL-1, IL-2, IL-6, IL-8, and GM-CSF)
Nitric oxide production	↓	Bronchial epithelial cells	Caused by cytokine down-regulation and a corresponding decrease in nitric oxide synthase levels
Production of prostaglandins, leukotrienes	↓	T-lymphocytes	Up-regulation of lipocortin-1 synthesis, which inhibits prostaglandin and leukotriene production
TGF-β production	↑	T-lymphocytes	Increased transcription, -stabilisation of mRNA
Cellular viability	↓	T-lymphocytes, monocytes, and eosinophils	Stimulation of apoptosis by: Decreased cytokine production Induction of DNA cleavage[26]
Adhesion molecule expression	Decreased expression of ELAM-1, E-selectin, ICAM-1, and VCAM-1	Monocytes, endothelial cells, bronchial epithelial cells, neutrophils	Interference with NF-κB action Cytokine down-regulation TGF-β up-regulation 'Non-genomic' mechanisms
1L-2 and IFN-γ -induced cellular activation	↓	T-lymphocytes	Synthesis of antagonistic proteins Signalling enzyme repression
Mitogen-activated protein kinase activity	↓	endothelial cells	Non-genomic' mechanisms
Primary APC stimulation	↓	T-lymphocytes	
Th1/Th2 differentiation	Th1 → Th2 (anti-inflammatory) profile	T-lymphocytes	Caused by altered balance of pro- versus anti-inflammatory cytokines in environment of naive Th cells

β is up-regulated in T-lymphocytes. The prevailing mechanism of cytokine inhibition depends on the specific cytokine and type of cell being affected. GCs may reduce the synthesis of inflammatory mediators by three main mechanisms: direct disruptive GR-GRE binding, antagonism of transcription factors, and *de novo* synthesis of antagonistic products. Among these *de novo* synthesised inhibitory products are proteins that destabilise cytokine mRNA, as well as lipocortin-1.

Direct inhibition of transcription by GR-GRE binding

Upon cytosolic binding with a GC, the activated GR dimerises, migrates to the nucleus and uses its zinc fingers to bind with high affinity the GRE segments in the DNA. The GRE locus is the critical factor influencing the effect of the GCs on target DNA. Activated GR can act in a manner either inhibitory or excitatory of transcription depending upon where in a gene it binds to DNA. In the case of the osteocalcin gene, GR-GRE binding occurs in the promoter region, blocking the binding of important transcription factors to the DNA, thus preventing gene transcription[41,42]. Recent evidence suggests that although GR-GRE binding negatively regulates a multitude of genes, cytokine inhibition is achieved through antagonism of transcription factors without a requirement for GR-GRE binding[43]. It is not clear at this time which, if any, cytokines are directly down-regulated by interaction between the glucocorticoid receptor and its DNA response element.

Antagonism of transcription factors

NF-κB, NF-AT, and AP-1 are three of the most important transcription factors in the synthesis of pro-inflammatory cytokines. They are proteins that bind to promoters of target genes to begin transcription, and GC action negatively regulates the function of all three[43]. Macrophages produce glucocorticoid-induced leucine zipper upon GC stimulation, which attenuates NF-κB- and AP-1-mediated transcription in T-cells[44,45]. Furthermore, activated GR is itself an antagonist of pro-inflammatory transcription factors, binding directly to AP-1 to inhibit IL-2 production in T-cells[46,47]. In T-lymphocytes, activated GR also acts as a transcription factor for IκB, a known antagonist of NF-κB[48,49]. By binding to and thus segregating NF-κB in the cytosol, IκB reduces the action of this important transcription factor, suggesting a corresponding decrease in synthesis of pro-inflammatory products[48-50]. However, IκB action on its own is not sufficient to fully abrogate NF-κB activity[51,52]. The mechanism also likely involves direct GR inactivation of NF-κB through the tethering mechanism shown in Figure 1d[53]. Inhibition of the majority of cytokines is accomplished by preventing transcription of these cytokines by reducing efficacy of their transcription factors.

Lipocortin synthesis

GCs directly induce transcription of lipocortin-1, providing the basis of another mechanism of anti-inflammatory action[5]. Lipocortin-1 action prevents arachidonic acid liberation, the result of which is decreased levels of prostaglandins and leukotrienes[54-56]. Lipocortin-1 has been shown in this way to inhibit proliferation of mitogen-stimulated T-cells and mimic many GC immunosuppressive effects *in vitro*[5].

Degradation of cytokine mRNA

GCs reduce the half-life of mRNA encoding IL-1, IL-2, IL-6, IL-8, and GM-CSF, a mechanism independent of pre-transcriptional cytokine down-regulation[4,31,34,57,58]. This reduction in mRNA stability results in decreased synthesis of the aforementioned cytokines and is directly caused by the GC-induced synthesis of a protein that targets cytokine mRNA for degradation[4].

Synthesis of transforming growth factor-β

Transforming growth factor-β is an immuno-suppressive cytokine that reduces macrophage activation and is produced upon GC stimulation of resting or activated T-lymphocytes[6,59]. GCs augment production of TGF-β through increased transcription as well as through stabilisation of TGF-β mRNA; the latter is achieved through inhibition of an mRNA-degrading protein[6,7,60,61]. Whereas TGF-β production is not increased in monocyte-macrophages, TGF-β treatment increases glucocorticoid receptor expression in these cells[62,63]. As macrophages are targeted by GCs in cytokine down-regulation, this suggests that TGF-β augment the degree of suppression of pro-inflammatory cytokines. TGF-β up-regulation therefore suggests an important way in which GCs attenuate the inflammatory response.

The above mechanisms outline ways in which GCs affect cytokine and inflammatory-mediator levels in target cells. However, modulating cytokine production is far from the only mode of GC immunosuppressive and anti-inflammatory action. Other mechanisms of GC action are detailed in the remainder of the paper, followed by a discussion of future directions for research.

Adhesion molecule down-modulation

Interaction between cell surface molecules of lymphocytes and primary antigen-presenting cells is critical to both the immune and inflammatory responses. GCs are capable of down-modulating adhesion molecules both *in vivo* and *in vitro*[64,65]. *In vitro* studies show GC-mediated decreases in expression of ELAM-1, L-selectin, E-selectin, ICAM-1, and VCAM-1[9,64,66]. Cells affected by reduced adhesion molecule expression include monocytes, neutrophils, endothelial cells, and bronchial epithelial cells. The latter suggests one important way in which GCs are useful in the treatment of asthma[67]. Adhesion molecule expression is mediated by the transcription factor NF-κB[68]. GC inhibition of NF-κB reduces adhesion molecule expression in endothelial cells[69]. In neutrophils, adhesion molecules are inhibited due to GC-mediated decreases in extracellular concentrations of cytokines such as TNF-α which normally stimulate adhesion molecule expression[70,71]. TGF-β, up-regulated by GCs, also inhibits endothelial expression of VCAM-1[72]. Overall, down-modulation of adhesion molecules reduces leukocyte adhesion to target tissue in the inflammatory response, supporting the notion that adhesion molecule down-regulation is a critical *in vivo* mode by which GCs function in suppressing inflammation and the immune response[73].

Interference with cellular signalling events

The reduced ability of immune cells to react to stimuli is an important characteristic of GC action. The specific synergistic action of Il-1, IL-6, and IFN-γ has been shown to abrogate GC-mediated inhibition of T-cell proliferation *in vitro*, but the general inability of most cytokines to stimulate proliferation of GC-treated cells suggests that GCs interfere with cytokine effects on target cells[30]. GC treatment of T-lymphocytes initially reduces cell responsiveness to IL-2 and IFN-γ through a mechanism that does not simply involve decreased cytokine receptor expression[74,75]. This disruption of cytokine-mediated T-cell proliferation may operate through GC-induced synthesis of antagonistic proteins and/or inhibition of enzymes necessary for the function of cytokine receptor signalling pathways[15,17]. Interference with T-cell receptor signalling is also responsible for the decrease in cytokine-induced cytokine production documented in GC-treated T-cells[37,39]. Disruption of mitogen-activated protein kinases in endothelial cells and of primary APC stimulation in T-cells offer further mechanisms through which GC-mediated disruption of cell signalling events results in decreased target cell activity[16,76,77]. A recent hypothesis proposes that T-cell activation via intercellular adhesion is down-regulated by fast-acting 'non-genomic' mechanisms[78]. This hypothesis aims to explain the rapidity of GC action in inhibiting T-cell activation, and suggests that GCs physically interact with cell membrane components to block signalling pathways important to cellular activation[78,79].

Preferential inhibition of Th1 cells resulting in a predominantly Th2 profile

In the differentiation of a naive (Th0) CD4+ T-cell into a type 1 or type 2 cell, local cytokines introduced into the cellular environment by CD4+ T-lymphocytes and macrophages play a decisive role. Th1 cells, formed under type 1 cytokine conditions, mediate inflammatory response through secretion of pro-inflammatory cytokines such as IFN-γ, whereas Th2 cells are generally viewed as anti-inflammatory due primarily to the immunosuppressive roles of 1L-4 and IL-10, both type 2 cytokines[44,80,81]. GCs strongly inhibit lymphocyte- and macrophage-based production of Th1-stimulating cytokines IL-1, IL-12 and IFN-γ, leading to higher relative concentrations of IL-4 and IL-10 (type 2 cytokines), and therefore to an increase in the Th2 : Th1 ratio[18,19,33]. Although IL-4 production may be suppressed by GCs, this suppression is to a lesser degree than the inhibition of the cytokines which drive Th1 cell lineage[35,40]. Furthermore, cytokines secreted by Th2 cells are inhibitory of Th1 cells, and vice versa[82]. This negative cross-regulation between the cytokines produced by Th1 and Th2 cells suggests that this anti-inflammatory profile could be long-lasting, possibly conferring upon a GC-treated patient sustained resistance to inflammatory disorder[19]. Conversely, this change in T-cell profile could also increase the risk of allergic (type 2) morbidity, since hypersensitivity reactions can be associated with a predominantly Th2 profile[83]. However, limited *in vivo* data suggests only a short-lived increase in type 2 cytokine secretion, and the precise significance of an altered T-cell profile discovery remains to be determined[20-22].

Conclusion

Despite several advances in the understanding of the mechanisms of GC immunosuppressive and anti-inflammatory action, there remains the need for a single comprehensive model in order to significantly improve patient care. This paper has detailed and classified the means by which glucocorticoids are currently believed to exert their powerful effects. Down-regulation of select

pro-inflammatory cytokines, which is mediated by the activated glucocorticoid receptor, is clearly an action of particular importance. However, the multiplicity of GC action also includes interference with activation of cytokine-stimulated T-cells and decreased activation of T-cells stimulated by antigen-presenting cells. *In vivo* cellular activation is also lessened by down-regulating cellular adhesion molecules on APCs, a mechanism particularly important to asthma treatment. The list of cellular processes and inflammatory mediators down-modulated by GCs is substantial. However, the activated glucocorticoid receptor is capable of excitatory activity as well.

GCs up-regulate anti-inflammatory mediators such as TGF-β, lipocortin-1, and at least two known antagonists of pro-inflammatory transcription factors, glucocorticoid-induced leucine zipper and IκB. Proteins that degrade cytokine mRNA are also up-regulated. Overall, any attempt at studying GCs with the intention of improving drug activity requires an in-depth analysis of the drug's positive and negative regulatory capabilities.

Other effects that follow from GC treatment are apoptosis in T-cells, monocytes, and eosinophils, and altered composition of Th lymphocytes by means of the promotion of anti-inflammatory cytokines. Three aspects of GC treatment may prove important in future studies of long-term effects of drug use; up-regulation of (pro-inflammatory) cytokine receptors, interference with negative T-cell selection, and a favoured Th2 (anti-inflammatory) profile. Results of *in vivo* studies described above have not shown conclusive evidence of permanent alterations to a patient's immune system, although research in this area should continue. Due to the multifactorial nature of GC action, *in vitro* studies are not sufficient to fully detail the complex relationship between the effects of these drugs. Despite the level of knowledge that we have now reached in terms of individual effects of GCs, it is the combination of these effects that is of greatest importance. There are many negative aspects of GC treatment, and a better understanding of the mechanisms of GC action may allow for the development of more effective drugs that retain the potency of GCs but lack the deleterious effects. However, our current knowledge of GC mechanisms of action can be used to make more educated decisions in assigning treatment regiments to patients than was possible even ten years ago.

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