Regulation of cytokine and cytokine receptor expression by glucocorticoids

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Abstract: Glucocorticoids (GCS) profoundly inhibit several aspects of T cell immunity largely through inhibition of cytokine expression at the transcriptional and posttranscriptional levels. GCS were also reported to act indirectly by inducing transforming growth factor-β expression, which in turn blocks T cell immunity. In exerting their antiproliferative effects, GCS diffuse into target cells where they bind their cytoplasmic receptor, which in turn translocates to the nucleus where it inhibits transcription of cytokine genes through direct binding to the glucocorticoid response elements (GRE), which are located in the promoter region of cytokine genes or, alternatively, through antagonism of the action of transcription factors required for optimal transcriptional activation. In contrast to their inhibitory effects on cytokine expression, GCS upregulate cytokine receptor expression that correlates with enhanced cytokine effects on target cells. In this review, we summarize the current state of knowledge of the mechanism of action of GCS, including the phenomenon of steroid-induced rebound, which ensues upon GCS withdrawal. *J. Leukoc. Biol.* 60: 563–572; 1996.

Key Words: *interleukins · transcription factors · gene expression · mRNA · T cells*

INTRODUCTION

Glucocorticoids (GCS) have been successfully used in the management of several disorders associated with heightened immunity, such as transplant rejection [1], autoimmune diseases [2, 3], and inflammatory disorders [4, 5]. It remains unknown as to the precise locus of GCS action because there is evidence of inhibition by GCS of T cell immunity at several stages of T cell activation. The mechanisms postulated for GCS suppression of T cell proliferation include the following: (1) induction of the phospholipid and calcium-binding proteins, lipocortins, which in turn inhibit arachidonic acid release from membrane-bound stores [6]; (2) interference with transmembrane ionic fluxes [7]; (3) modulation of activation-induced cell death [8, 9]; and (4) inhibition of recruitment of neutrophils and monocytes-macrophages into inflammatory sites [10, 11], largely through inhibition of the expression of leukocyte adhesion molecules induced by interferon-γ (IFN-γ) [12], tumor necrosis factor-α (TNF-α) [13], lipopolysaccharide (LPS) [14], and interleukin-1 (IL-1) [11]; and (5) suppression of cytokine production [15–18] and action [19, 20]. However, it is likely that GCS may exert their effects by a multitude of mechanisms depending on the cell type and activation state.

Over the past few years our understanding of the mode of action of GCS and the divergent pathways of T cell activation, including a molecular and cellular investigation into the biology of T cell-derived cytokines, has provided an opportunity for assessment of the mode of action of the GCS at the molecular level. In this review we present an overview of the current state of knowledge at the cellular and molecular levels pertaining to the mechanism of action of the GCS as immunosuppressive agents. In addition, we shall provide an immunological basis for the GCS-induced rebound phenomenon, which ensues upon acute/short-term GCS withdrawal.

T CELL ACTIVATION

T cell activation is a tightly regulated process that involves interaction of the multimeric T cell receptor (TcR)/CD3 complex with antigenic fragments expressed on the surface of professional antigen-presenting cells (APC) and other cells [21, 22]. Optimal T cell activation also requires the participation of accessory signals imparted by APC, including interaction of T cell-associated CD2 [23–25] and/or CD28/CTLA-4 [26, 27] with APC-expressed LFA-3/CD58 and/or B7/BB1, respectively. In addition, co-stimulatory signals imparted in part by APC-derived cytokines, namely IL-1 [28–30] and IL-6 [31, 32] augment T cell activation,

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Abbreviations: GCS, glucocorticoids; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α; LPS, lipopolysaccharide; IL-1, interleukin-1; APC, antigen-presenting cells; PTK, protein tyrosine kinase; PLC, phospholipase C; IP_3, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; PKC, protein kinase C; GR, GCS receptor; GM-CSF, granulocyte-macrophage colony-stimulating factor; TGF-β, transforming growth factor-β; PBMC, peripheral blood mononuclear cells; iNOS, inducible nitric oxide synthase; GRE, glucocorticoid response elements.

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leading to the induction of the IL-2 autocrine pathway, and the expression of several T cell-derived cytokines [26, 30, 31, 33]. In the absence of appropriate costimulatory signals the T cell is driven into clonal anergy [34].

Although the TcR/CD3 complex lacks the endogenous protein tyrosine kinase (PTK) moiety, T cell activation via TcR/CD3 involves rapid phosphorylation of several cellular proteins on tyrosine residues, including TcR itself [35]. This phosphorylation ensues upon physical association of TcR/CD3 with src PTK $p59^{fyn}$ and ZAP70, and CD4 interaction with the nonreceptor PTK $p56^{lck}$ [36, 37]. TcR/CD3-associated kinase activity leads to the phosphorylation of downstream target molecules, including phospholipase C (PLC) [38, 39]. Phosphorylated PLC in turn induces the hydrolysis of phosphatidyl inositol 4,5-bisphosphate and the generation of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) [40–42].

IP_3 in turn mobilizes calcium from intracellular stores, resulting in the activation of calmodulin-regulated kinases, including the serine/threonine phosphatase calcineurin [43, 44]. On the other hand, DAG in the presence of high intracellular calcium concentrations, binds to and translocates protein kinase C (PKC) from cytosolic to membrane-bound compartments where it expresses its enzymatically active conformation. In addition, elevation in intracellular calcium, coupled with sustained PKC activation, induces the expression of nuclear factors such as NF-AT, NF-κB, and AP-1 required for efficient transcription of cytokine genes [45, 46].

Targeting of any of the T cell activation events results in inhibition of T cell activation. In these regards, immunosuppressive agents such as GCS, cyclosporin A, FK506, rapamycin, RS-61443, and brequinar sodium exert their immunosuppressive effects largely through blockade of proximal or distal events of T cell activation (**Table 1**) [47–49]. Because of the limited scope of this review, we shall limit our discussion to the effect of GCS in inhibiting T cell proliferation through blockade of cytokine expression. For discussion about the mechanism of action of other immunosuppressive/antiproliferative agents the reader is referred to excellent reviews published previously [47, 48, 50].

TABLE 1. Principal Immunosuppressive Drugs

1. Immunophilin-binding drugs:
   a. CsA & FK506: inhibit Ca^{2+}-dependent T cell activation by antagonizing calcineurin.
   b. Rapamycin: binds FKBP and blocks IL-2-driven proliferation.
2. Anti-metabolites:
   a. General (nonselective) inhibitors: azathioprine
   b. Selective inhibitors:
      i. Purine synthesis inhibitors: RS-61443 & mizorbine.
      ii. Pyrimidine synthesis inhibitors: brequinar sodium.
3. Anti-mitotic agents: cyclophosphamide.
4. Glucocorticoids:
   a. Anti-inflammatory: reduce production of pro-inflammatory mediators, cellular infiltration, and expression of cell adhesion molecules.
   b. Immunosuppressive: inhibit cytokine gene expression.

TABLE 2. Inhibition of Cytokine Expression by GCS

Cytokine	Cell type	Mechanism of suppression
IL-1α	Peritoneal macrophages, monocyte-like cell lines	GRE binding
IL-1β	Human monocytes, promonocytic cell line	mRNA destabilization GRE binding
IL-2	T cells	Antagonism of transcription factors GRE binding Signaling via IL-2R complex
IL-3	Helper T cell lines	ND[a]
IL-4	T cells	Earlier inhibition of IL-2[b] Direct DNA binding[c]
IL-5	PBMC, T cells	ND
IL-6	T cells, macrophages, monocyte-like cell lines, fibroblasts	Antagonism of transcription factors GRE binding
IL-8	Fibroblasts, epithelial cells, fibrosarcoma cell lines	GRE binding
IL-12	T cells, monocytes, moncytic cell lines	ND
IFN-γ	T cells, fibroblasts	GRE binding
TNF-α	Monocyte-like cell lines, macrophages, fibroblasts	GRE binding Antagonism of transcription factors

[a]ND not determined. [b]Proposed mechanism of action. [c]Not established whether this involves a GRE-like binding.

SCOPE AND CONSEQUENCES OF BLOCKADE OF CYTOKINE EXPRESSION BY GCS

T cell activation by mitogens, alloantigens, and receptor cross-linking antibodies (as surrogates for antigenic stimulation) is a preprogrammed process that results in the orderly expression of several cytokine genes and induction of T cell proliferation [21, 32, 35]. GCS-mediated antiproliferative effect is largely attributed to inhibition of cytokine gene expression (**Table 2**). In these regards, GCS were shown to inhibit mRNA expression of IL-1 [16, 51–53], IL-2 [15, 54–56], IL-3 [57, 58], IL-4 [59–61], IL-5 [17, 62, 63], IL-6 [51, 64], IL-8 [18, 65, 66], IL-12 [67], IFN-γ [59, 68], TNF-α [69, 70], and the colony-stimulating factors (CSF) macrophage (M)-CSF [71], granulocyte (G)-CSF [72], CSF-1 [73], and granulocyte-macrophage (GM)-CSF [66, 74].

Target of GCS-suppressive effects

The primary target of GCS effects appeared to be accessory cells; GCS-mediated inhibition of cytokine expression was attributed to their suppression of IL-1 and IL-6 expression. In light of reports demonstrating the central role of both IL-1 and IL-6 as accessory signals in the induction of the IL-2 autocrine pathway [32, 75, 76], it was postulated that inhibition of IL-2, IL-3, IL-8, and IFN-γ and other cytokine gene expression is secondary to earlier inhibition of IL-1 and IL-6 expression. This concept was challenged by several reports demonstrating direct suppression

by GCS of IL-2 [54, 56, 77], IL-8 [65, 78], c-Jun [79], and other cytokine gene expression via a mechanism involving direct binding of the activated GCS receptor (GR) to select sites on DNA and, in turn, repression of transcription via steric hindrance (see below). Discussed in a physiological context, it remains to be established whether and to which extent earlier inhibition of IL-1 and IL-6 gene expression by GCS contributes to downstream inhibition of cytokine gene expression.

Transcriptional repression by GCS

Inhibition of cytokine expression by GCS occurred at the transcriptional level, resulting in a concentration-dependent inhibition of cytokine mRNA expression and a parallel decrease in cytokine secretion [18, 51, 54]. Repression of cytokine expression and inhibition of cellular activation was demonstrated to be specific for the GCS because: (1) non-GCS steroids failed to affect cytokine expression [54] and T cell proliferation [6] and (2) RU-486, a GR antagonist, abrogated GCS-mediated repression of cytokine expression and cellular activation [16, 51, 55, 69]. It should be noted here that RU-486 has a mixed agonist/antagonist property. Although it clearly antagonizes dexamethasone (DEX)-induced inhibition of cytokine release by mitogen-stimulated human PBMC, RU-486 inhibited cytokine (IL-1, IL-6, IL-8, TNF-α) release when tested in the absence of GCS [80].

That GCS exert their antiproliferative/immunosuppressive effects by targeting cytokine expression was verified along several lines of evidence. First, antiproliferative concentrations of GCS parallel those required for inhibition of cytokine mRNA accumulation and protein secretion [15, 52, 81]. Second, GCS-mediated antiproliferative effects could be abrogated by the combination of rIL-1, rIL-6, and rIFN-γ, although not by each cytokine tested individually [15].

Posttranscriptional effects of GCS

GCS were also described to inhibit cytokine expression at a posttranscriptional level through reduction in cytokine mRNA stability. In these regards, GCS were shown to decrease mRNA half-life of c-myc [76], IL-1 [16, 51, 53], IL-2 [54], IL-6 [66], IL-8 [66], MCAF [83], TNF-α [84], and GM-CSF [66]. GCS-induced decrease in cytokine mRNA half-life is a rapid event, with 50% of the mRNA degraded 1–2 h post-GCS treatment [54]. It also requires de novo synthesis of an mRNA destabilizer protein, as revealed by the capacity of cycloheximide [51, 66] and actinomycin D [66, 82] to abrogate GCS effects, resulting in significant increase in cytokine mRNA half-life.

GCS were also described to exert their antiproliferative effects by attenuating cytokine effects on target cells such as cytokine-driven cellular proliferation. In these regards, GCS were shown to inhibit the IL-2-driven proliferation of human T cells [20, 85] and IL-2-dependent CTLL-2 cells [86], and of PMA-preactivated T cell blasts [87], as well as IL-1α/IL-1β-driven proliferation of the murine Th2 cells, D10.G4.1 [88]. GCS inhibition of cytokine-driven proliferation was suggested to involve induction of an inhibitory protein [such as transforming growth factor-β (TGF-β), see below], as GCS anti-proliferative effects were blocked by cycloheximide [85, 86].

Furthermore, GCS were described to repress cytokine-induced cytokine release. For example, GCS inhibited IL-6 and IL-8 expression by IL-1- and TNF-α-stimulated human fibroblasts [70], IL-5 production by IL-2-stimulated human peripheral blood mononuclear cells (PBMC) [17], and M-CSF production by IL-1-stimulated cartilage fibroblasts [71]. Together with their reported effects on cytokine-stimulated cellular proliferation, this suggests that GCS inhibit cytokine production at two stages. First, GCS act proximally by reducing cytokine availability through blockade of cytokine mRNA expression either via transcriptional repression and/or through destabilization of cytokine mRNA. Second, GCS may exert their antiproliferative effects distally by inhibiting cytokine-mediated signaling events, hence attenuating further cytokine expression and induction of T cell proliferation.

Other reports have suggested that GCS do not alter cytokine mRNA stability or affect cytokine-mediated cellular activation (cytokine-stimulated proliferation and/or cytokine release) [18, 89, 90], thus raising concerns about the universality of such conclusions. In light of the multiplicity of GCS effects, coupled with the differential requirements for induction of cytokine genes, and the diverse experimental settings employed in assessing GCS effects, one cannot negate any of the conclusions reached. Accordingly, results regarding posttranscriptional effects of GCS must be viewed in the context of the cell type investigated, stimulus used, and experimental conditions/readout system ultilized.

Mediation of GCS effects through TGF-β induction

Of interest was the recent demonstration of a novel mechanism by which GCS inhibit cytokine expression and T cell proliferation: induction of TGF-β expression [91–93]. In light of the similarities in scope and nature between GCS- and TGF-β-induced antiproliferative effects, as both GCS and TGF-β are potent inhibitors of the activation of T cells [91], macrophages [96], and several tumor cell lines [92, 93], it was of interest to test whether GCS-mediated antiproliferative effect is, at least in part, TGF-β-dependent. This hypothesis was verified by the following findings. First, GCS enhance accumulation of TGF-β steady-state mRNA in unstimulated and mitogen-stimulated T cell cultures [91], fibroblasts [94], osteoblasts [95], and several other cell lines [92, 93]. Second, blockade of TGF-β activity by neutralizing anti-TGF-β monoclonal antibody (mAb) abrogated GCS-mediated antiproliferative effects [91, 93, 97].

TGF-β expression is constitutive and is up-regulated upon stimulation with mitogens, growth factors, and GCS [91]. GCS induction of TGF-β expression was described in some reports to be at the transcriptional [93, 94] and at the posttranscriptional [92, 95] levels involving stabilization of TGF-β mRNA. Furthermore, GCS induction of

TGF-β was described to be an indirect event, being preceded by the induction of select lysosomal proteases, which in turn results in enhanced TGF-β secretion [95].

Other reports provided evidence arguing against involvement of TGF-β in GCS-mediated antiproliferative effects. For example, GCS were described to: (1) be ineffective in altering TGF-β mRNA expression [98]; (2) inhibit TGF-β secretion by fibroblasts and lung carcinomas [99]; (3) antagonize TGF-β-stimulated effects (stimulation of prostaglandin synthesis) [100]; (4) differentially regulate induced nitric oxide synthase (iNOS) activity [TGF-β, but not GCS (DEX), inhibited IL-1-stimulated iNOS mRNA expression] [101]; (5) block EGF-induced c-fos mRNA accumulation in contrast to TGF-β, which was described to be ineffective [102]; and (6) inhibit procollagen synthesis in contrast to TGF-β, which was shown to stimulate procollagen synthesis [103]. Accordingly, mediation of GCS effects by TGF-β appear to be cell type- and stimulus-specific, and warrants further scrutiny in assigning a future role for TGF-β in mediating GCS-associated antiproliferative effects.

MECHANISM OF REPRESSION OF CYTOKINE GENE EXPRESSION BY GCS

One of the distinct features of the GR is its unusual capacity as a transcription factor to both up-regulate and down-regulate gene expression, depending on the target gene and experimental conditions tested. GCS gain access to their target cells by diffusing through the lipid bilayer and bind their cytoplasmic receptor. The receptor, in turn, binds DNA and hence modulates transcriptional activation of target genes. Loss or mutation as well as antagonism of GR (for example by RU-486) severely curtails the transcription-modulating capacity of the GR.

The GCS receptor

Similar to other steroids, GCS exert their immunoregulatory effects by binding their intracellular receptor, which directly regulates transcriptional rate. Owing to their hydrophobic nature, GCS diffuse into the cytoplasm and bind the GR, which, in the absence of GCS, is tightly bound to the 90-kDa heat-shock protein (HSP90) and thus is prevented from translocating to the nuclear compartment [104, 105]. Upon binding its ligand (GCS), activated GR complex dissociates from HSP90 and undergoes structural modifications that enable it to bind DNA [106, 107]. The GR contains a single DNA binding domain of the following form: Cys-X_2-Cys-X_{13}-Cys-X_2-Cys-X_{15-17}-Cys-X_5-Cys-X_9-Cys-X_2-Cys-X_4-Cys, where X denotes a variable amino acid [108]. Each four cysteine residues, arranged tetrahedrally, bind one Zn^{2+} [106] leading to the formation of two zinc fingers that interact directly with DNA [107]. Such an interaction initiates [109, 110] or blocks [107, 111] gene expression depending on cell type and experimental conditions used.

GCS response elements (GRE)

The DNA binding sites for GCS, the GRE, have a conserved palindromic sequence, GGTACAnnnTGTTCT; each half-GRE palindrome binds one subunit of GR [106, 112]. GRE elements have been localized in the 5′ flanking regions of GCS-responsive genes, including cytokine genes [61, 113]. Depending on the physiological context, binding of the activated GR complex to GRE alters transcriptional activation both in *cis*-acting [61, 111] and *trans*-acting [55, 77] fashions. Furthermore, activated GR may directly interact with GRE DNA elements [65, 111] or alternatively, may require the cooperation of other transcription factors for acquisition of full transcriptional activity [114, 115].

Models of transcriptional repression by GCS

Several models have been proposed for GCS-mediated inhibition of cytokine gene expression. According to the GR-GRE model, activated GR translocates to the nucleus where it binds GRE on the promoter of cytokine genes leading to blockade of access of transcription-enhancing factors to their putative DNA binding sites. On the other hand, the GR-transcription factor(s) model proposes that GR first binds to basal and/or induced transcription factors, hence abolishing their capacity to interact with DNA and thus initiating transcription. Both models are not mutually exclusive; whether GCS inhibit transcription through interaction with DNA or with transcription factor(s) depends on the gene studied, stimulus utilized, and experimental model used [79, 87].

Transcriptional repression through GR-GRE interaction

In addition to basal transcriptional machinery, activation of cytokine gene requires binding of class-specific and gene-specific transcription factors to select sites in their 5′ flanking region [116–118]. Due to their close proximity of transcription factors DNA binding sites to GRE sites, GCS were postulated to inhibit cytokine gene expression in a *cis*-acting fashion through binding to GRE and, accordingly, blocking access of transcription factors to their putative DNA sites. This was best exemplified by the findings of Mukaida and co-workers [61] who, using deletion constructs of the IL-8 promoter ligated to a CAT expression vector, demonstrated that deletion of a GRE-like element located at −325 (relative to the start site) from the IL-8 promoter region abolishes DEX-mediated repression of the IL-8 gene. DEX did not alter CAT activity in constructs lacking the putative GRE elements but retaining κB and AP-1 binding sites, thereby supporting the notion that DEX-mediated inhibition of IL-8 gene expression: (1) required the presence of a GRE copy in the promoter region and, (2) does not interfere with the DNA binding of NF-κB and AP-1. Although multiple copies of GRE are present in the promoter region of cytokine genes [113], one copy is sufficient to transduce GCS-mediated effects [114].

That GCS alter transcription by binding of activated GR

to GRE was supported by several findings. First, resistance to GCS was associated with significant reduction in the capacity of GR to bind GRE, largely stemming from a reduction in GR numbers [119]. Second, transcriptional repression by GCS was blocked by the GR antagonist RU-486 [120]. Third, deletion or mutation of either half of the GRE palindromic sequence failed to bind GR [112] and abolished DEX-mediated inhibition of (insulin) gene expression [121]. Fourth, insertion of a GRE-like DNA element in the promoter region of an otherwise GCS-nonresponsive gene confers GCS sensitivity [108, 122]. Fifth, mutation in the DNA binding domain of the GR blocks its capacity to bind DNA, thus rendering the receptor transcriptionally inactive [77, 123, 124].

Accordingly, inhibition of transcription induced by GR-GRE binding was postulated to be due to a masking effect involving binding of the GR to functional DNA-binding sites overlapping the binding sites of basal [111, 125] and other [126, 127] transcription factors, resulting in blockade of access of transcription factors to their putative DNA binding sites. Here, the DNA binding site of the GR must be intact because deletion or mutation of that site abolishes GCS effects [120, 126, 127]. Accordingly, the inhibitory effects of GCS may result from steric interference with binding of enhancing transcription factors (e.g., NF-κB and NF-AT) to their putative DNA sites by direct binding of GR to target DNA.

ALTERNATE MECHANISMS FOR GCS EFFECTS

Antagonism of transcription factor(s)

A number of reports have suggested alternate mechanisms for GCS inhibition of cytokine gene expression: functional antagonism between GR and transcription factors responsible for mediating cytokine expression such as AP-1 [128–132], NF-κB [133], and NF-AT [77, 87, 134], without ruling out the requirement for direct DNA binding (via GRE?). This was viewed as a direct protein-protein interaction between the GR and the transcription factor, which in turn prevents binding of the transcription factor to its DNA-binding site [120, 129, 135]. Evidence supporting direct GR-transcription factor interactions include: (1) physical association of GR with AP-1 (c-Fos and c-Jun) [114, 135], (2) swapping of the DNA binding domain of the GR with those of related receptors (RAR, TR) did not affect GCS effects [130], (3) disruption of GR-GRE interaction by excess c-Jun [130], and (4) antagonism of GR effect by both c-Jun and c-Fos [129, 135].

Cooperation of GR with other transcription factors

Insofar as GCS do not block the appearance of the transcription factors NF-κB, AP-1, AP-3, Oct-1, and NF-AT levels [77, 133, 136], a protein-protein interaction model involving a complex of transcription factors and GR was proposed as the mechanism by which GCS repress cytokine gene expression. According to this protein-protein interaction model, a functional GR may not necessarily contain an intact hormone-binding domain [77, 134] but must retain an intact DNA binding domain because deletion of that domain abolishes GCS effects (zinc finger motif) [134, 137]. The DNA-binding domain of the GR need not be GCS-specific and can be swapped with the DNA-binding domain of receptors of the same (steroid) family without significantly affecting its overall transcriptional activity [130].

The association of the GR with transcription factors leads to inhibition of IL-2 [77, 134] and IL-6 [137] promoter activity, as well as stimulation of other GCS-sensitive gene promoters (α1-acid glycoprotein) [138]. Transcription factors must then cooperate to impart functional capacity on the GR. This was demonstrated to be a true assumption as c-Jun and c-Fos (but not the c-Jun homodimer) [114, 115], NF-AT and AP-1 [77, 87], and ANF-1 and ANF-2 [115] were all described to be required for efficient transcriptional repression by GR.

The molecular mechanism by which transcription factors enhance GR effects remains to be elucidated, although it does not appear to be likely due to altered expression of *trans*-activating factors as was initially suggested [120, 129], and later ruled out [77, 133]. The association of transcription factors with GR may, in principle, stabilize GR binding to DNA, which in turn results in steric inhibition of the assembly of the transcriptional machinery needed to initiate proper transcription. In addition, the spatial arrangement of the DNA binding sites of transcription factors (AP-1 and NF-AT) was shown to be crucial for transcriptional repression by GCS, since introduction of tandem repeats of either factor abolished GCS effects [134].

Whether GCS-mediated antiproliferative effect is the result of a direct protein-DNA (GR-GRE) or alternatively, protein-protein (GR-transcription factor) interactions remains the subject of intense research. The multitude of biological effects mediated by the GCS, coupled with the diverse target cells and experimental conditions employed in assessing GCS effects (primary cultures vs. cell lines, transfectants, etc.) warrants careful scrutiny in assigning a mechanism by which GCS inhibits cytokine transcription. And, although the protein-DNA model is favored by most over the protein-protein model, prior modification of the GR involving protein-protein interaction (for example through complexing with other factors) cannot be dismissed at present.

MODULATION OF CYTOKINE RECEPTOR EXPRESSION BY GCS

GCS up-regulate cytokine receptor expression

Although GCS have been described to inhibit cytokine gene expression at the transcriptional and/or posttranscriptional levels, several reports have described a paradoxical effect of GCS in inducing/enhancing cytokine receptor expression. In this regard, GCS were shown to up-regulate

TABLE 3. Up-regulation of Cytokine Receptor Expression by GCS

Receptor	Cell type	Stimulus
IL-1R	PMNL	DEX
	Bone marrow cells	DEX + GM-CSF
	B cells	DEX
	Monocytes	DEX + PMA
	Glioblastoma cells	DEX, corticosterone
IL-2R	T cells	DEX
IL-6R	Epithelial cells	DEX, PRED
	B cells	DEX + TPA
	Hepatoma cell lines	DEX + TPA, DEX + TGF-β
IFN-γR	T cells	DEX
	Monocytes	DEX
CSF-1R	HL-60 (monocytic)	TPA + DEX
GM-CSFR	Monocytes	DEX

the expression of IL-1R [139–143], IL-6R [144, 145], IFN-γR [146, 147], GM-CSFR [148], and CSF-1R [73]. GCS-mediated up-regulation of cytokine receptor expression was described to be either a direct effect of GCS on target cells [140, 145, 146] or an indirect effect requiring the participation of growth factors [139, 148, 149] or other stimuli [141, 144] (**Table 3**). Although GCS clearly up-regulate receptor density on target cells, they apparently do not affect receptors' affinity for their respective ligands [145, 146].

Whereas GCS clearly up-regulated pro-inflammatory cytokine receptor (IL-1R, IL-6R, IFN-γR) expression (see above), their effects on IL-2R are controversial. For example, GCS were shown to up-regulate high-affinity IL-2R expression directly [149], or indirectly in association with IL-2 [150]. GCS were also shown to inhibit the expression of IL-2Rα, but not IL-2Rβ, by mitogen-stimulated human PBMC [54, 85]. In the quoted studies, transcription of the IL-2Rα was not affected by high concentrations of DEX, thus raising the possibility that the reduction in IL-2Rα expression seen is merely due to blockade of IL-2 secretion, described to be required for induction of the high-affinity IL-2R complex [151, 152]. This was supported by the finding that IL-2 abrogated DEX-induced reduction in IL-2Rα expression [54, 85], although not DEX-mediated antiproliferative effect [15], suggesting that GCS-mediated antiproliferative effect is not merely due to blockade of the IL-2 autocrine pathway, but rather a complex one involving inhibition of T cell activation at several stages of the activation pathway.

Mechanism and specificity of GCS-induced up-regulation of cytokine receptor expression

GCS-mediated up-regulation of cytokine receptor was specific for GCS as non-GCS failed to alter IL-6R [145)], IL-1R [140, 142], and GM-CSFR [148] expression on target cells. GCS-mediated up-regulation of cytokine receptor expression was receptor-mediated as it was completely abolished by the GR antagonist RU-486 [142, 153]. In addition, it required de novo receptor synthesis because: (1) GCS enhance the accumulation of cytokine receptor mRNA transcripts [140, 144, 145, 154], and (2) GCS-mediated enhancement in cytokine receptor expression was blocked by the anti-metabolites actinomycin D [140, 143] and cycloheximide [140, 143, 146, 155].

Furthermore, GCS-mediated up-regulation of cytokine expression was associated with enhanced cytokine effect on target cells [140, 147, 148, 156, 157]. This was supported by an earlier report from our group indicating that GCS-mediated antiproliferative effects could be abrogated, in a concentration-dependent fashion, by a combination of rIL-1, rIL-6, and rIFN-γ, but not by each cytokine tested individually [15], further demonstrating that GCS exert their antiproliferative effects by inhibiting cytokine expression. It was of interest to note that neither rIL-2 nor rIL-4, even if individually tested at concentrations as high as 1000 U/mL, failed to abrogate GCS-mediated antiproliferative effects [15], thereby indicating that cytokine-mediated abrogation of GCS effects is not exacted at the level of the IL-2 autocrine pathway as previously suggested [30, 75, 76], but may involve other as yet unknown mechanisms.

CONSEQUENCES OF GCS WITHDRAWAL

GCS are potent immunosuppressive and anti-inflammatory agents and exert their effects by targeting several aspects of cellular activation. Whereas short-term high-dose GCS may be administered without precipitating any unwanted effects, prolonged GCS is frequently associated with numerous side effects that can involve virtually any organ system [158, 159], and that can be reversed upon cessation of treatment. Complete withdrawal was investigated as an alternative to avoiding toxicity in long-term/chronic GCS-treated patients. While early results were encouraging, rebound episodes, characterized by increased morbidity and requirement for brief pulses of high-dose GCS, were frequent in all reported cases [1, 160]. An alternate approach consisted of a later and gradual (tapering off) GCS withdrawal. Here, the incidence of increased morbidity was significantly less [161] than early/abrupt withdrawal [162], and it would appear that the duration of GCS withdrawal (abrupt vs. gradual) is more important than the timing (early vs. late) post-GCS initiation.

The reason for the high incidence of rebound episodes associated with acute/abrupt GCS withdrawal is not completely understood. Using the proliferation of human T cells stimulated with mitogens, alloantigens, and receptor cross-linking antibodies as biological readout, we demonstrated that abrupt GCS withdrawal is associated with enhanced accumulation of IL-2, IL-7, IL-12, and IFN-γ steady-state mRNA, parallel increase in IL-1R, IL-2R (CD25), and IFN-γR expression, and vigorous T cell proliferation [163]. Other investigators have reported on similar effects of GCS pretreatment on subsequent T cell-proliferative responses and cytokine expression. For example, pretreatment with hydrocortisone was found to significantly augment LPS-stimulated IL-6 and TNF-α expression [164]. In addition, pretreatment with GCS was also associated with enhanced T cell effector function (increase

in DTH responses) upon subsequent antigenic stimulation [165]. It remains to be determined as to the sequence of events associated with GCS pretreatment on cytokine expression and T cell function upon subsequent stimulation. It is possible that it may involve eradication of a GCS-sensitive suppressor cell population [165] or alternatively, frank transcriptional up-regulation.

GCS withdrawal has been associated with premature and sustained activation of the rat α_{2a}-globulin gene (RUG), normally repressed by GCS [122, 166]. An elegant model postulated by Hess and co-workers suggests that GR in conjunction with an ancillary factor (AP-1?) binds two classes of GRE: negative GRE (nGRE) and secondary GRE (sGRE). Binding of GR to nGRE represses RUG transcription in a manner analogous to repression of the murine proliferin gene [114] or to the human IL-2 gene [77]. Binding of GR to sGRE, in turn, stimulates induction of an auxiliary factor, which, upon accumulation to a threshold, binds GR and induces its dissociation from nGRE sites. Withdrawal of GCS results in the dissociation of the auxiliary factor from GR leading to direct DNA binding and stimulation of transcription and resulting in an exaggerated and sustained gene activation.

This, coupled with earlier reports documenting that: (1) GCS-mediated inhibition of cytokine expression is a reversible event requiring the continued presence of the GCS and (2) GCS up-regulate the expression of several cytokine receptors offers an explanation for the accelerated allograft rejection rates seen upon sudden GCS withdrawal and explains the beneficial effects of high-dose GCS pulse or rapamycin (which inhibits signaling through cytokine receptors) in controlling GCS-induced rebound.

CONCLUSIONS

During the last two decades, substantial advances toward understanding the precise mode of action of the GCS have been made, especially with regard to their paradoxical regulation of cytokine and cytokine receptor expression. Several questions need to be addressed including, although not restricted to, molecular events associated with GCS-induced up-regulation of cytokine expression, target of GCS in inhibiting cytokine-stimulated cellular activation, and the mode of action of recombinant cytokines in abrogating GCS-mediated antiproliferative effects. A thorough understanding of the mode of action of the GCS is of paramount importance in better management of GCS toxicity, and in development of future immunosuppressive regimens.

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