Minireview

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The glucocorticoid receptor in inflammatory processes: transrepression is not enough

Abstract: Glucocorticoids (GCs) are the most commonly used anti-inflammatory agents to treat inflammatory and immune diseases. However, steroid therapies are accompanied by severe side-effects during long-term treatment. The dogma that transrepression of genes, by tethering of the glucocorticoid receptor (GR) to DNA-bound pro-inflammatory transcription factors, is the main anti-inflammatory mechanism, is now challenged. Recent discoveries using conditional GR mutant mice and genomic approaches reveal that transactivation of anti-inflammatory acting genes is essential to suppress many inflammatory disease models. This novel view radically changes the concept to design selective acting GR ligands with a reduced side-effect profile.

Keywords: ChIP-Seq; glucocorticoid receptor; inflammation; transactivation; transgenic mice; transrepression.

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Introduction

Glucocorticoids (GCs) are among the most potent and most effective anti-inflammatory drugs. Therefore, synthetic GCs, including dexamethasone and prednisolone, are widely used for the treatment of numerous inflammatory disorders, such as rheumatoid arthritis, ulcerative colitis and asthma. In addition to their potent anti-inflammatory properties, GCs are critical regulators of a wide variety of fundamental processes, including metabolic homeostasis, cell proliferation, development and reproduction. Owing to these metabolic actions of GCs, long-term GC treatment is unfortunately associated with various undesirable effects, such as diabetes, osteoporosis, glaucoma and muscle wasting (Kleiman and Tuckermann, 2007; Hübner and Tuckermann, 2012). These unwanted side-effects, together with the occurrence of unresponsiveness to the beneficial effects of GCs, called GC resistance (GCR), constrain the therapeutic success of GCs (Vandevyver et al., 2014). Thus, there is a conceivable unmet clinical need for safer and more efficacious GCs.

Transactivation and transrepression of gene regulation by the GR

GCs are steroid hormones that, owing to their lipophilic nature, can freely diffuse across the cell membrane. Intracellular, GCs exert their actions by binding to their cognate receptor, the glucocorticoid receptor (GR). GR is a member of the nuclear receptor superfamily and is a modular protein composed of three major functional domains: the N-terminal transactivation domain, the central DNA-binding domain (DBD) and the C-terminal ligand-binding domain (LBD). The DBD consists of two zinc fingers, needed for GR dimerisation and DNA binding. The LBD comprises another dimerisation interface and is involved in the interaction with other transcriptional regulators, thus comprising a C-terminal transactivation domain. Furthermore, alternative splicing of the GR generates several splice variants, of which the GRα splice form is most widely and highly expressed. The GRβ form results from an alternative splice acceptor site in exon 9, leading to a smaller LBD, which is unable to bind GCs. Nevertheless, GRβ has an intrinsic transcriptional profile, independent of GRα (Kino et al., 2009). In addition, at least eight proteins are translated from the GRα transcript because of alternative translation initiation...
of the mRNA. These protein variants all have unique tissue distribution patterns and transcriptional regulatory profiles (Oakley and Cidlowski, 2011).

GR predominantly resides in the cytoplasm, sequestered in a multimeric chaperone complex (Vandevyver et al., 2012a,b). Upon ligand binding, the GR translocates to the nucleus where the activated GR positively or negatively regulates gene expression in a coordinated manner, referred to as transactivation and transrepression, respectively. In a classical view, transcriptional induction by GR is mainly mediated by binding of GR dimers to so-called glucocorticoid response elements (GRE), consisting of a variant of the inverted, hexameric, palindromic motif 5′-AGAACAnnnTGTTCT-3′, in which ‘n’ symbolizes any nucleotide (Vandevyver et al., 2013) (Figure 1A). Recent genome-wide ChIP-Seq datasets reveal that a fraction of GR molecules bind DNA via GRE-related sequences (Reddy et al., 2009; Voss et al., 2011). However, not all GRE-like sequences are bound by GR and thus these sequences are not a predictor for binding (John et al., 2011). GR-binding sequences are very versatile, cell-type-specific and are often associated with binding sequences of other transcription factors in a cell-type-specific manner (Biddie et al., 2011; John et al., 2011; Grontved et al., 2013; Uhlenhaut et al., 2013; Starick et al., 2015). A number of GR-binding sites seem to reside in accessible chromatin, as revealed by DNAase I hypersensitivity sequencing (Biddie et al., 2011; John et al., 2011). This open chromatin landscape varies between cell types due to activity of cell-lineage-specific pioneering transcription factors (Siersbaek et al.,

![Figure 1](image-url)

**Figure 1:** Genomic actions of the glucocorticoid receptor (GR).
(A) The GR can act as a dimer, by binding to glucocorticoid response elements (GRE), thereby recruiting coactivators and activating gene expression. Transrepression of genes by GR dimerisation and GR monomers occur through binding to negative acting GREs (nGREs) and recently discovered inverted repeats (IR) nGREs (fewer than three nucleotides between the GRE half sites) and subsequent recruitment of co-repressors NCoR and Smrt. The GR can act as a monomer and interact with pro-inflammatory transcription factors (AP-1, NF-kB, IRF3), thereby repressing and/or modulating gene activity with the help of co-integrators. The binding of the GR as a dimer to GREs in close proximity to NF-kB and AP-1 binding sites can also occur, and in this way induce or repress target gene expression. Furthermore, the GR monomer is also able to bind to half GRE sites that are in the vicinity of motifs of lineage-specific TFs and modulate transcription. (B) In addition to transrepression of pro-inflammatory transcription factors – such as AP1, CREB, NF-kB, NFAT, IRF3/5, T-bet, GATA3 – GR also represses inflammation through transactivation of protein-coding target genes, such as DUSP1 (MKP1), KLF2, TSC22D3 (GILZ), SLPI, ANXA1 (annexin A1), TTP, IkBa, IL10, and the IncRNA Lethe.
DNA binding by GR is followed by recruitment of transcriptional coregulators, which enable highly coordinated regulation of gene transcription by modifying and remodeling the chromatin structure. Coactivators like histone acetylases p300 and homologous cAMP-responsive element-binding protein (CREB)-binding protein (CBP) (p300/CBP), p300/CBP associated factor (p/CAF), ATPase BRG1 [an ATP-dependent chromatin remodeling complex that is a central unit of switch/sucrose non-fermenting (SWI/SNF) proteins] and p160 family members (e.g., SRC-1, TIF2/GRIP1/SRC-2, SRC-3) lead to chromatin decondensation and initiate and promote transcription via RNA Polymerase II (Rosenfeld et al., 2006; Nicolaides et al., 2010). Subsequently, secondary coactivators such as methyltransferase CARM1 are then recruited by p160 family members to enhance the transcription process (Chen et al., 1999; Ma et al., 2001). The transcriptional activity of GR can be modified by posttranslational modifications of the receptor protein, including phosphorylation, acetylation, ubiquitination and sumoylation and is reviewed elsewhere (Vandevyver et al., 2014).

Transrepression of genes may occur by a couple of mechanisms involving GR dimerisation and GR monomer molecules. GR suppresses genes by binding to negative acting GREs (nGREs) and to recently discovered inverted repeats (IR) nGREs with fewer than three nucleotides between the GRE half sites, followed by recruitment of co-repressors NCoR and Smrt (Surjit et al., 2011) (Figure 1A). A prominent mechanism of transrepression of genes encoding pro-inflammatory mediators such as major cytokines, matrix metalloproteases and others is attributed to the monomer form of the GR, independent of direct DNA contact. A tethering of the monomer GR to regulatory transcription factors (TFs) of inflammatory genes, such as AP1, NF-xB, IRF3, STAT5, CREB, NFAT, T-bet and GATA3 (Ratman et al., 2013) leads to a repression of their activity and subsequently reduction of gene expression (Figure 1A and B). Here, the GR co-activator GRIP1 can act as a corepressor to mediate inhibition of NF-xB, AP1 and STAT1 activity by GR (Flammer et al., 2010) through chromatin modification (Uhlenhaut et al., 2013). Genome-wide studies discovered indeed co-occupancies of DNA by GR and p65/NF-κB (Rao et al., 2011). Whether the majority are tethered sites is not yet clear, as Uhlenhaut and colleagues also observed GREs presumably bound by GR dimers in the vicinity of p65 binding sites in the region of GC repressed genes in macrophages (Figure 1A).

Conversely, direct binding of GR monomers to DNA seems to occur more often than previously anticipated. Recent genome-wide ChIP-Seq analysis and ChIP exo Seq analysis demonstrated that the GR monomer binds to half GRE-like sites (Schiller et al., 2014; Lim et al., 2015; Starick et al., 2015). The half site motifs are in vicinity of binding to motifs of lineage-specific TFs observed in distinct cell lines and in liver and macrophages (Lim et al., 2015; Starick et al., 2015) (Figure 1A). These half sites are also occupied by a GR with attenuated dimerisation from GR<sup>Δ</sup> mice (discussed in detail below) that fail to occupy classical GREs in liver in vivo (Lim et al., 2015). Intriguingly, pharmacological GC treatment of mice leads to an increase of dimeric binding of the GR on the genome at least in the liver (Lim et al. 2015). This complex binding pattern of GR dimers and monomers in the genome together with functional studies of animals with an attenuated GR dimerisation interface lead to a challenge of a long standing strategy to develop GR selective ligands to alleviate side-effects.

**The generation of GR selective ligands to avoid GR dimerisation**

For decades, it was generally believed that the unwanted metabolic side-effects, associated with GC therapy, were attributed to the GR dimer-dependent transactivation effects, as this leads to the induction of several enzymes involved in carbohydrate, glucose and lipid metabolism, such as fatty acid synthase, PEPCK (phosphoenolpyruvate carboxykinase), TAT (tyrosine aminotransferase) and G6P (glucose-6-phosphatase). On the contrary, it was thought that the beneficial anti-inflammatory effects were mainly mediated by the GR monomer through transrepression of pro-inflammatory TFs (Schacke et al., 2007). Therefore, research was directed towards the development of dissociated ligands that favour the GR monomer activities of GR, but without inducing the dysmetabolic effects. Multiple selective GR agonists (SEGRAs), such as RU24858 and RU24782, were screened for their anti-inflammatory power associated ligands that favour the GR monomer activities of GR, and reduced side-effects. Later, the focus shifted from steroidal scaffolds to non-steroidal ligands, such as LDG552, ZK216348 and Compound A, with the advantage that these do not bind other steroid receptors, i.e., the mineralocorticoid receptor and the progesterone receptor, thereby preventing side-effects derived from activation of other hormonal receptors (De Bosscher et al., 2010). However, so far, only a few compounds have made it to clinical trials for topical application (Vandevyver et al., 2013). The limited success of translation to the in vivo situation can be, in part, attributed to the oversimplified transactivation/transrepression model, which is commonly used. The
ability to dissociate between transrepression and gene activation (transactivation) was tested on a limited number of simple promoter constructs in vitro, e.g., transactivation of TAT promoter vs. transrepression of IL8 (interleukin 8) promoter. However, with the current notion of the role of the endogenous more complex GRE sequences as allosteric GR ligands, determining the transcriptional outcome (Meijser et al., 2009), simple assays based on the conserved GRE sequence, likely do not suffice. Moreover, most were selected for their capacity to inhibit the pro-inflammatory GR dimers of GRx and AP1 (Schacke et al., 2007), despite the growing list of immune-regulating TFs that are inhibited by GR, including NFAT, IRF3, CREB, T-bet and GATA3 (Hübner and Tuckermann, 2012).

**GR induced anti-inflammatory acting genes**

An even more important reason to nuance the dissociation hypothesis is that GR dimers, in addition to metabolic genes, also induce the expression of anti-inflammatory genes, such as DUSP1 (coding for MKP1, a regulator of MAPK signalling), TSC22D3 (coding for GILZ), and ANXA1 (Figure 1B). Endogenous GCs protect mice from TNF-induced lethal shock in a GR dimerisation-dependent manner by enhancing MKP1 expression (Vandevyver et al., 2012a,b). Furthermore, the GR in macrophages is essential for limiting mortality and cytokine production via MKP1-mediated p38 inhibition (Bhattacharyya et al., 2007, 2010). Mice lacking MKP1 were more sensitive to sepsis owing to the inability to reduce inflammatory cytokines (Salojin et al., 2006; Zhao et al., 2006; Hammer et al., 2010). In conclusion, GC-induced MKP1 gene expression was shown to be an essential anti-inflammatory mechanism in vitro and in vivo (Abraham et al., 2006; Vandevyver et al., 2013). Furthermore, some GC-mediated anti-inflammatory effects are shown to be mediated by GILZ via modulation of MAPK pathways resulting in repression of inflammation (Clark and Lasa, 2003; Ayrolldi and Riccardi, 2009). In detail, in macrophages and T-cells GILZ inhibits NF-κB function by binding to the p65 subunit and thereby preventing transcription of NF-κB dependent genes (Ayrolldi et al., 2001; Pinheiro et al., 2013). Another GR dimer-dependent anti-inflammatory acting gene is ANXA1, coding for Annexin1 (Hannon et al., 2003; Clark, 2007). Inflammation induced by LPS in Annexin1-deficient mice resulted in an impaired reduction of pro-inflammatory cytokines based on the failed up-regulation of GC-induced GILZ (Yang et al., 2009).

In addition to the well-known GC-induced anti-inflammatory genes, such as TSC22D3 and DUSP1, many others are positively regulated by GR and play a putative, yet undiscovered, role in the defence against inflammation. Also, long non-coding RNAs (lncRNAs), induced by GCs, might be involved in the anti-inflammatory actions of GCs, as it was recently documented that the lncRNA Lethe as a GC-induced gene represses NF-κB (Rapicavoli et al., 2013). As the identification of these genes and their functionality is still in its infancy, it is clear that the full complexity of the anti-inflammatory nature of the GR is far from fully understood. This indicates that not only interference of pro-inflammatory TFs is of importance for the beneficial effects of GCs.

**Lessons from GRdim mice**

The importance of the GR-mediated transactivation actions for the anti-inflammatory functions of GCs became evident by in vivo studies with so-called GRdim mice. These mice express a point mutant version of GR, where Alanine 465 is changed to a Threonine (A465T and in human GR A458T), located in the second zinc finger motif of the DBD, leading to reduced homodimerisation of the GR and subsequent reduced binding to GRE elements (Heck et al., 1994; Reichardt et al., 1998; Lim et al., 2015). Interfering with the dimerisation interface strongly abrogates the anti-inflammatory actions of endogenous GCs, as GRdim mice are highly susceptible for several inflammatory disease models (Nixon et al., 2013; Vandevyver et al., 2013), such as TNF-and LPS-induced acute inflammation (Kleiman and Tuckermann 2007; Tuckermann et al., 2007; Hübner and Tuckermann, 2012; Kleiman et al., 2012; Vandevyver et al., 2012a,b; Silverman et al., 2013) and CLP (cecal ligation and puncture)-induced sepsis (Kleiman and Tuckermann, 2007; Kleiman et al., 2012) (Table 1). In addition, GR dimerisation-dependent actions are also indispensable in the protection by exogenous GCs during rheumatoid arthritis (Baschant et al., 2011; Baschant et al., 2012) and allergic contact dermatitis (Kleiman and Tuckermann 2007; Tuckermann et al., 2007) (Table 1). Only a few types of inflammation can be reduced by GCs in GRdimmice, such as skin irritation (Reichardt et al., 2001) and experimental autoimmune encephalomyelitis, a mouse model for multiple sclerosis (Schweingruber et al., 2014) (Table 1). Therefore, most of inflammatory paradigms tested are refractory to GCs in these mice. Furthermore, GRdim mice still suffer from GC-induced osteoporosis (Rauch et al., 2010) and muscle atrophy (Waddell et al., 2008), indicating that GR monomers also contribute to the adverse
side-effects. In addition, not all metabolic genes that are positively regulated by GR, are affected by the GRdim mutation, for example, genes activated by composite elements bound by GR-STAT5 heterodimers, such as the growth hormone and IGF1 (Tronche et al., 2004).

Of note, in transient transfection studies it was shown that by the GR dim mutation, dimerisation is not entirely absent (Jewell et al., 2012; Watson et al., 2013; Presman et al., 2014). The residual dimerisation of the GRdim mutant is due to the fact that promoters may engage several dimerisation interfaces of the GR that are still present in the GRdim mutation (e.g., in the LBD) (Savory et al., 2001; Bledsoe et al., 2002; Jewell et al., 2012; Presman et al., 2014). Indeed, single-cell measurements revealed that transfected GRdim mutant, bound to the GRE type response elements with reduced residence time on the DNA (Gebhardt et al., 2013). Eventually the reduced stability of GR-DNA binding of the GRdim mutant is the result of reduced allosteric communication between the DNA and the GR, in which the particular Alanine 458 (A465 in mouse) is involved (Watson et al., 2013). Accordingly, reduced transcriptional activity of gene expression was observed in liver in a genome-wide manner (Frijters et al., 2010) and recent ChIP Exonuclease Sequencing from the livers of GRdim mice show a strongly diminished occupancy by GR dimers, whereas GR binding to GRE half sites was not affected (Lim et al., 2015). This indicates that despite certain limitations GRdim mice are a useful tool to discover GR target genes that require a full dimerisation competent GR and transactivation essential for suppression of inflammation. In particular, GRdim mice have contributed to the knowledge that future development of GC-based therapeutic strategies should also rely on GR transcriptional induction of downstream anti-inflammatory molecules.

### Future directions

Despite our increasing knowledge on the mechanisms of action of GCs by using conditional mouse mutants and genome-wide analysis, the precise mechanisms on the full anti-inflammatory profile of the GR are only just unfolding.

One additional complexity is certainly provided by cell-type-specific effects of gene regulation by the GR and the distinct requirement of certain cell types to mediate anti-inflammatory effects. Analysis of conditional knockout mice revealed that for models in septic shock myeloid cells are decisive mediators of the protective function of endogenous released GCs (Bhattacharyya et al., 2007; Kleiman and Tuckermann, 2007; Kleiman et al., 2012), whereas in antigen-induced arthritis the GR in T-cells is decisive (Baschant et al., 2011). In some types of inflammation not only one, but also several cell types might contribute to protective effects of encapsulated GCs, such as myeloid cells and T-cells in experimental autoimmune encephalomyelitis (EAE) (Schweingruber et al., 2011). Given the plethora of cell types that contribute to inflammatory processes including immune cells, but also cells of the inflamed tissue, for a comprehensive understanding of regulatory actions of the GR, the genome-wide analysis of GR actions has to be expanded to various cell types in the future. In combination with functional studies, by knocking out cooperating factors of GR or GR-binding sites using CRISPR/Cas9 system a biological picture of GR action will emerge and aid in shaping anti-inflammatory responses.

Thus, the previous approach to screen for selective ligands to transactivate one promoter and to repress another promoter to obtain GR ligands with reduced side-effects has to be revised. The induction of potent anti-inflammatory acting genes cannot be ignored and readouts should be developed that represent the entire response of a certain cell as the sum of gene regulation. This will be certainly a challenge, but recent progress in high content screening using automatic microscopes to determine the adaption of anti-inflammatory phenotypes of immune cells vs. ‘side-effect’-like effects, could be exploited for novel GR ligands with reduced side-effects. Once such ligands are isolated, their gene regulatory
actions should be validated including ChIP- and RNA-seq analyses to determine their impact on GR-DNA binding and transcriptional induction of GR-dependent genes.

Therapeutic advancement can also be achieved by targeted therapy to the diseased tissue, thereby limiting the systemic side-effects. For example, an anti-CD163 antibody drug conjugate that specifically targets Dex to the haemoglobin scavenger receptor CD163 in macrophages allows using lower doses, leading to reduced metabolic side-effects, while maintaining the therapeutic potential (Granfeldt et al., 2013). Another possibility for tissue-specific delivery is linkage of glucocorticoids to tissue-selective peptide-ligands as it was demonstrated for the delivery for oestrogens linked to glucagon-like peptide-1 (Finan et al., 2012). Future research on a cell-specific genome-wide understanding of transcriptional regulation by the GR and decisive protein interactions will give rationales for novel readouts in drug screenings to favour certain GR-protein-protein interactions. The identification of factor and tissue-specific GR agonists will benefit healthcare, directly by reducing patient suffering, and indirectly by decreasing economical costs.

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References


Bionotes

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Sabine Hübner studied Biology at the University of Jena in Germany. She undertook her PhD about tissue-specific hormone-action in the group of Jan Tuckermann at the Fritz Lipmann Institute for Age Research in Jena, Germany. In April 2013 she became a postdoctoral researcher at the university of Ulm in the Institute for Comparative Molecular Endocrinology. She is interested in glucocorticoid action in inflammation and metabolism.

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Lien Dejager finished her PhD in Biotechnology from the University of Ghent in 2010 under the guidance of Prof. Claude Libert, IRC, VIB. After that she became a postdoctoral researcher at FWO-Vlaanderen in the same group. In 2014, she did a research stay in the lab of Prof Jan Tuckermann, University of Ulm. Her major research interests are elucidating the anti-inflammatory mechanisms of glucocorticoids and the mechanisms underlying glucocorticoid resistance, aiming to design more efficient glucocorticoid-based therapies.
Claude Libert (1964) has a Masters degree and PhD in Sciences, both obtained at the University of Ghent under the guidance of Walter Fiers. After a postdoc in Italy, he became a group leader with VIB (Flanders Institute for Biotechnology) in 1997 and a professor at the University of Ghent in 2003. His major research interest is acute inflammation and the cross-talk between several important players in inflammation, with a focus on TNF, IFN, matrix metalloproteinases and glucocorticoids.

Jan P. Tuckermann studied Biology and performed his graduate studies in the labs of Peter Herrlich (Karlsruhe, Germany) and Peter Angel (Heidelberg, Germany) and his postdoc with Günther Schütz (Heidelberg, Germany). He then worked as a group leader at the Fritz Lipmann Institute (Jena, Germany) and was appointed in 2012 as a full professor to head the Institute of Comparative Molecular Endocrinology at the University of Ulm (Germany). Dr. Tuckermann made major contributions to the molecular mechanisms of corticosteroids in beneficial and side-effects of steroid therapy. With the help of conditional and function selective knockout mice for the glucocorticoid receptor (GR) he identified the critical cell types for anti-inflammatory activities of glucocorticoids in different inflammatory disease models. A second focus of his work concerns the effects of glucocorticoids on bone integrity, as glucocorticoid-induced osteoporosis which is a major side effect of long-term corticosteroid therapy.