Glucocorticoid receptor: implications for rheumatic diseases

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ABSTRACT

The glucocorticoid receptor (GR), a member of the nuclear receptor superfamily, mediates most of the known biologic effects of glucocorticoids. The human GR gene consists of 9 exons and expresses 2 alternative splicing isoforms, the GRα and GRβ. GRα is the classic receptor that binds to glucocorticoids and mediates most of the known actions of glucocorticoids, while GRβ does not bind to these hormones and exerts a dominant negative effect upon the GRα-induced transcriptional activity. Each of the two GR splice isoforms has 8 translational variants with specific transcriptional activity and tissue distribution. GRα consists of three subdomains, translocates from the cytoplasm into the nucleus upon binding to glucocorticoids, and regulates the transcriptional activity of numerous glucocorticoid-responsive genes either by binding to its cognate DNA sequences or by interacting with other transcription factors. In addition to these genomic actions, the GR also exerts rapid, non-genomic effects, which are possibly mediated by membrane-localised receptors or by translocation into the mitochondria. All these actions of the GR appear to play an important role in the regulation of the immune system. Specifically, the splicing variant GRβ may be involved in the pathogenesis of rheumatic diseases, while the circadian regulation of the GR activity via acetylation by the Clock transcription factor may have therapeutic implications for the preferential timing of glucocorticoid administration in autoimmune inflammatory disorders.

Introduction

Glucocorticoids, steroid hormones secreted by the *zona fasciculata* of the adrenal cortex, are essential for the maintenance of internal homeostasis challenged by numerous internal and/or external environmental changes, the "stressors". These steroids regulate a variety of biologic processes and exert profound influences on virtually all physiological organ systems (1). Because the immune system is particularly sensitive to glucocorticoids, these hormones are used extensively as potent anti-inflammatory and immunosuppressive agents in the management of many autoimmune, allergic and inflammatory disorders, including rheumatoid arthritis (2).

The diverse actions of glucocorticoids are mediated by an intracellular receptor molecule, the glucocorticoid receptor (GR), which belongs to the steroid/sterol/thyroid/retinoid/orphan receptor superfamily of nuclear transcription factors, with over 150 members currently cloned and characterised across species (3). This complex modular receptor protein functions as a hormone-dependent transcription factor by changing its molecular structure upon binding to its ligand glucocorticoid, and by translocating from the cytoplasm into the nucleus (4).

Inside the nucleus, GRα regulates positively and negatively the transcriptional activity of up to 5–20% of the human expressed genome (5, 6). The prototype human GRα is a single polypeptide chain of 777 amino acid residues, and is ubiquitously expressed in almost all human tissues and organs, including the constituent cells of the immune system (4).

Structure of the human GR gene and protein

The GR cDNA was isolated by expression cloning in 1985 (7). The human GR gene is located in the chromosome 5 (5q 31.3) and spans almost 160 kilo-base pairs. It consists of 9 exons and produces 2 transcripts by alternative use of specific exon 9α and 9β (4) (Fig. 1). These two mRNAs subsequently generate two highly homologous receptor isoforms, termed GRα and GRβ. The human GRα encodes for 777 amino acids, while the human

GRβ for 742. The two molecules are identical through amino acid 727, but then diverge, with GRα having an additional 50 amino acids and GRβ having an additional, 15 non-homologous amino acids. The molecular weights of these receptor isoforms are 97 and 94 kilo-Dalton, respectively.

GRα, the classic glucocorticoid receptor, is expressed virtually in all organs and tissues and mediates most of the known actions of glucocorticoids. GRβ is also expressed ubiquitously, does not bind glucocorticoids and has a dominant negative activity upon the GRα-induced transcriptional activity (8, 9). The GRα mRNA expresses multiple N-terminal isoform proteins by using at least 8 alternative translation initiation sites (10) (Fig. 2). Since GRβ shares a common mRNA domain that contains the same translation initiation sites with GRα (7), the GRβ variant mRNA may quite likely be translated through the same initiation sites to a similar host of β isoforms. Given that these shorter isoforms of GRβ appear to have variable dominant negative effect upon the GRα transcriptional activity, they may play a crucial role in determining tissue sensitivity to glucocorticoids by differentially influencing the GRα-induced transcriptional activity.

Translational GRα, and possibly, GRβ isoforms are differentially expressed in various cell lines (10). They are produced by ribosomal leaky scanning and/or ribosomal shunting from their alternative translation initiation sites located at amino acids 27 (GRα/β-B), 86 (GRα/β-C1), 90 (GRα/β-C2), 98 (GRα/β-C3), 316 (GRα/β-D1), 331 (GRα/β-D2) and 336 (GRα/β-D3), C-terminally from the classic translation start site (1: for the GRα/β-A) (10).

The GR protein consists of three major subdomains in a modular structure comprised of five regions (A-E), similarly to all steroid hormone receptors (Fig. 1): the immunogenic or N-terminal domain consisting of A and B regions, the DNA-binding domain corresponding to the C region, and the ligand-binding domain formed by the E region. Between the DNA-binding domain and the ligand-binding domain, there is the hinge domain encoded by the D region.

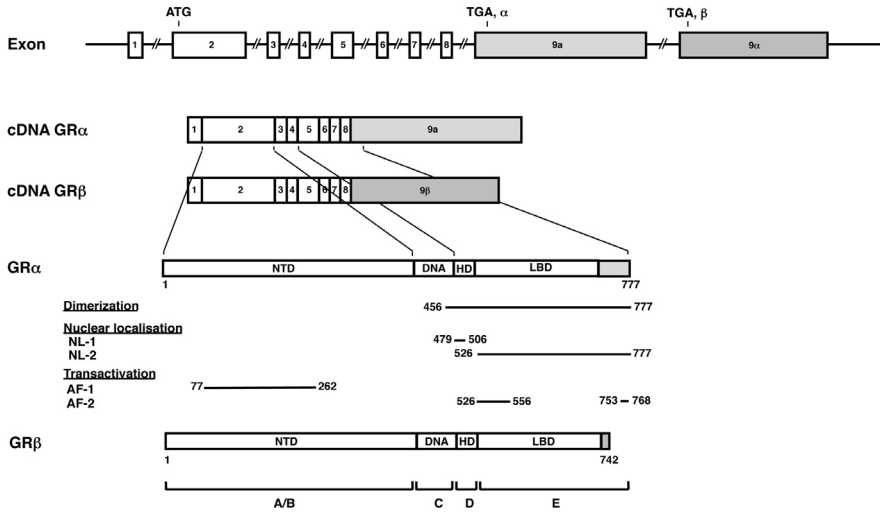

Fig. 1. Genomic and complementary DNA, and protein structures of the human (h) GR, functional distribution of the GRα, and the isoforms produced through alternative splicing.

The hGR gene consists of 9 exons. Exon 1 is untranslated region, exon 2 codes for the immunogenic domain (A/B), exon 3 and 4 for the DNA-binding domain (C), and exons 5-9 for the hinge region (D) and the ligand-binding domain (E). The GR gene contains two terminal exon 9s (exon 9α and 9β) alternatively spliced to produce the classic GRα and the non-ligand-binding GRβ. C-terminal gray colored domains in GRα and GRβ show their specific portions. Locations of several functional domains are also indicated. (modified from ref. 97)

AF-1 and -2: activation function 1 and 2; DBD; DNA-binding domain; HD: hinge region; LBD: Ligand-binding domain; NTD: N-terminal domain, NL1 and 2: Nuclear translocation signal -1 and -2.

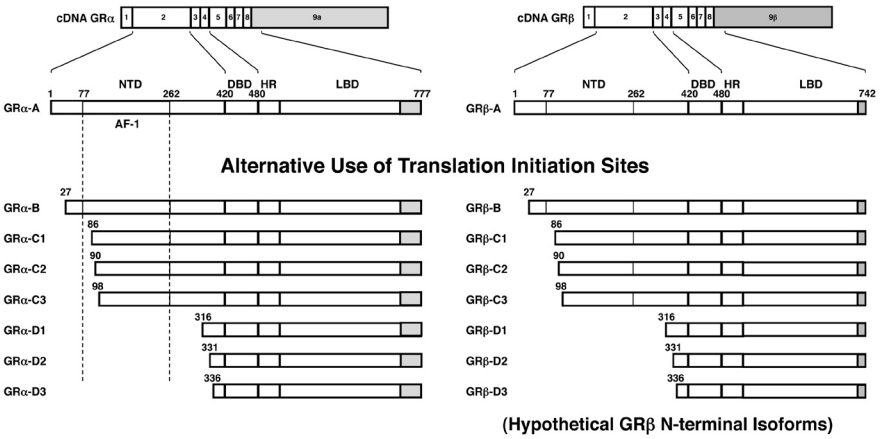

Fig. 2. GR isoforms produced through alternative splicing or use of different translational initiation sites.

Using at least 8 different translation initiation sites located in N-terminal domain, the GR gene produces multiple GRα isoforms termed A through D (A, B, C1-C3 and D1-D3) with distinct transcriptional activities on glucocorticoid-responsive genes. Since GRα and GRβ share a common mRNA domain that contains the same translation initiation sites, the GRβ variant mRNA appears to be also translated through the same initiation sites and to produce 8 β isoforms with different lengths N-terminal domain (modified from refs. 21, 98)

AF-1 and -2: activation function 1 and 2; DBD; DNA-binding domain; HD: hinge region; LBD: Ligand-binding domain.

The GR does not have an F region. The N-terminal domain is encoded by exon 2, while the DNA-binding domain is formed by two exons, 3 and 4. The remaining exons (exons 5 to 9) constitute the ligand-binding domain. Exon 1 does not contain a coding sequence, and there are at least 11 alternative first exons with their specific promoters that enable tissue-specific expression of the same GR proteins (11, 12).

The human GR N-terminal domain, which corresponds to amino acids 1 to 419 of the human GR, contains a major transactivation domain, termed activation function-1, which is located

between amino acids 77 and 262 (13, 14). Activation function-1 belongs to a group of acidic activators, contains four α-helices, and functions as a hormone-independent transactivation domain by communicating/interacting with molecules necessary for the initiation of transcription, such as coactivators, chromatin remodelling factors and basal transcription factors, including the RNA polymerase II and its associated proteins (15). This domain also contains several serine residues known to be phosphorylated by some proline-directed serine/threonine kinases, such as the cyclin-dependent kinases and the mitogen-activated protein kinases (MAPKs), including p38 MAPK and the cJun N-terminal kinase (JNK) (16), thereby mediating input from other signalling pathways that employ these kinases.

The DNA-binding domain corresponds to amino acids 420-480 of the human GR and contains two zinc finger motifs through which the GR binds to specific DNA sequences, the glucocorticoid-response elements (GREs) (17). It has two similar zinc finger modules encoded by exon 3 and 4, respectively. Each zinc finger is nucleated by a Zn ion coordination center held by four cysteine residues and is followed by an α-helix. The first α-helix found in the N-terminally located zinc finger lies in the major groove of the double-stranded DNA, while the second α-helix formed by the C-terminal zinc finger is positioned over the minor groove.

The ligand-binding domain of the human GRα corresponds to amino acids 520-777, binds to glucocorticoids and plays a critical role in the ligand-induced activation of the receptor. The crystal structure of the GRα ligand-binding domain revealed that this receptor domain consists of 12 α-helices and 4 small β-strands that fold into a three-layer helical domain (18). Helices 1 and 3 form one side of a helical sandwich, while helices 7 and 10 form the other side. The middle layer of the helices (helices 4, 5, 8, and 9) is present in the top but not the bottom half of the protein.

This arrangement of helices creates a cavity in the bottom half of the ligand-binding domain, which is surrounded by helices 3, 4, 11 and 12, and functions as a ligand-binding pocket (18-20). Interaction of the ligand-binding domain with the heat shock protein 90 contributes to maintenance of the protein structure that allows the ligand-binding domain to associate with the ligand. Ligand-binding induces a conformational change in the ligand-binding domain and allows the GR to communicate with several molecules, such as importin α, cofactors, components of the transcription initiation complex and other transcription factors that mediate the ligand-dependent actions of the GR (21). The ligand-binding domain also contains another transactivation domain, termed activation function-2, which is formed by helices 3, 4 and 12. Among these helices, helix 12 plays a central role in the formation of activation function-2 by dramatically changing its localisation inside the molecule upon binding to the ligand (18). The transactivational activity of the activation function-2 is ligand-dependent.

Although the crystallographic analysis has not been performed on the ligand-binding domain of GRβ, this receptor isoform appears to preserve helices 1 to 10 and the first 3 β-sheets, but does not have helices 11 and 12, and the last fourth β-sheet, as it shares the same amino acid sequence with GRα up to the position 727. The computer-based simulation of the ligand-binding domain of this isoform indicated that the C-terminal peptide unique to GRβ destroys the ligand-binding pocket by altering localisation of helix 10 and by replacing the helix 12 and the fourth β-sheets, and thus, makes the receptor unable to bind to glucocorticoids (22).

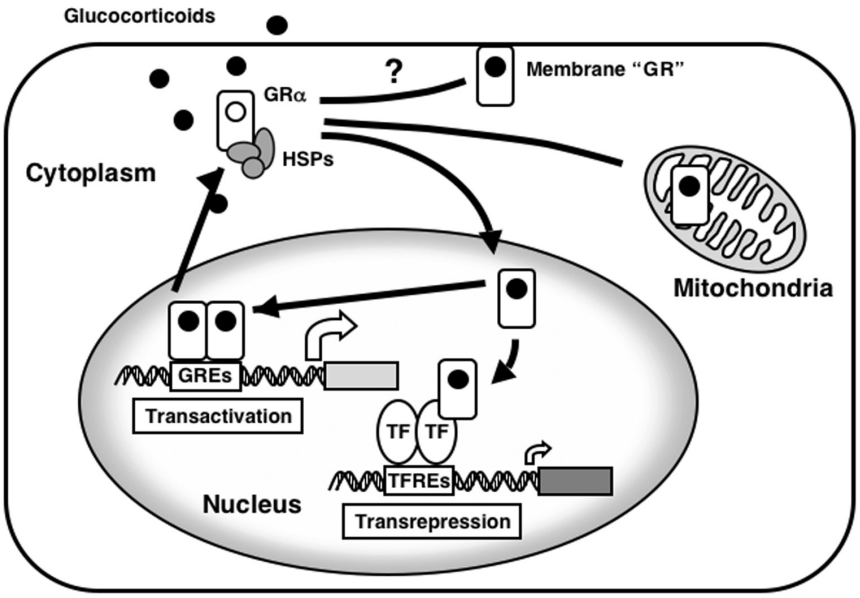

Fig. 3. Intracellular circulation of the GR.
Circulation of GRα between the cytoplasm and the nucleus, and its transactivation or transrepressive activities (from ref. 67).
GR: glucocorticoid receptor; GRE: glucocorticoid responsive element; TFREs: transcription factor responsive elements; HSPs: heat shock proteins; TF: transcription factor.

GR as a shuttling protein between the cytoplasm and the nucleus

In the absence of ligand, the GR resides in the cytoplasm of cells as part of a large multiprotein complex, which consists of one receptor molecule, two hsp90s and several smaller hsps and other proteins (23-26) (Fig. 3). After ligand binding, the receptor dissociates from the heat shock proteins and translocates into the nucleus. The GR contains two nuclear translocation signals, NL-1 and NL-2: Nuclear translocation signal-1 contains a classic basic-type nuclear localisation signal structure that spans from the C-terminal portion of DNA-binding domain to the hinge region of the GR (27). The function of nuclear translocation signal-1 is dependent on importin α, a protein component of the nuclear translocation system, which is energy-dependent and

Table I. Mechanisms through which GR mediates anti-inflammatory activity of glucocorticoid hormones.

Mechanisms of action	Target molecules	Comments	References
Transactivation of gene expression through binding to GREs	Annexin A1 DUSP1 GILZ IL-10 I κB MAPK-1		(38, 99, 100)
Suppression of transcriptional activity of transcription factors important for inflammation	AP-1 FLASH GATA-3 IRFs NF-κB NR4A nuclear receptors Smads STATs T-bet	Through these transcription factors, the GR suppresses the expression of numerous cytokines and mediators for inflammation, including TNFα, iNOS, IL-1β, IL-6, IL-12 p40, GMCSF and TGFβ	(38, 45-48, 53, 101)
Regulation through membrane GR	CDKs ERK Fyn IKKα JNK Lck p38 MAPK Protein kinase B Protein kinase C	This action of membrane GR may be important for the treatment of rheumatoid arthritis and and systemic lupus erythematosus	(29, 54, 55, 60-63, 100)

facilitates the translocation of the activated receptor into the nucleus through the nuclear pore (28). Nuclear translocation signal-2 spans almost over the entire ligand-binding domain (27). In the absence of ligand, binding of heat shock proteins with the ligand-binding domain of GR masks/inactivates nuclear translocation signal-1 and -2, thereby maintaining the receptor in the cytoplasm. In contrast, upon binding to the ligand, the nuclear translocation signals become available to interact with importin α, which facilitates GR entry into the nuclear compartment. Some fractions of GR may be associated with plasma membrane (membrane GR) or translocate into the mitochondria after binding to the ligand (29). The biological significance of the GRs located in these subcellular compartments will be discussed below.

Inside the nucleus, the GRα modulates the transcriptional activity of glucocorticoid-responsive genes either by binding to GREs located in their promoter regions of target genes or by physically interacting with other transcription factors (21). After modulating the transcription of its responsive genes, the GR dissociates from the ligand and is slowly exported to the cytoplasm as a component of heterocomplexes with heat shock proteins (30-32). The Ca^{2+}-binding protein calreticulin plays a role in the nuclear export of GR by physically interacting with the DNA-binding domain of this receptor (33-35). The GR also interacts with protein 14-3-3, which has a nuclear export signal in its C-terminal portion and functions as "attached nuclear export signal", shifting the localisation of the GR toward the cytoplasm (36).

Transcriptional regulation by GR

The GR exerts its classic transcriptional activity on glucocorticoid-responsive promoters by binding as a homodimer to the tandem GREs, whose optimal sequence is an inverted hexameric palindrome separated by 3 base pairs, PuGNACANNNTGTNCPy, with each GR molecule binding to one of the palindromes (37). This GRE-mediated activation of transcription is particularly important for the up-regulation of several genes important for the regulation of immune function. For example, glucocorticoids activate anti-inflammatory activity by stimulating the transcription of genes encoding annexin A1, interleukin (IL)-10, inhibitor of nuclear factor of κB (IκB), glucocorticoid-inducible leucine zipper protein (GILZ) and mitogen-activated protein kinase-1 (MAPK-1), while they also stimulate proinflammatory immunity by increasing the transcription of chemokine receptors (CCR1 and 2), complement components (C1q, C3 and C5), complement receptors (C3aR1, CR2 and C5aR1), interferon-γ receptors (IFNγRI and II), IL receptors (IL1R1 and IL8R), Toll-like receptors 2 and 4, macrophage migration inhibitory factor, thrombospondins 1, 2, and 4, and matrix metalloproteinases 7, 10, 16 and 19 (38) (Table I). The GRE-bound GR stimulates the transcription rate of responsive genes by facilitating the formation of the transcription initiation complex, including the RNA polymerase II and its ancillary components via its activation function-1 and -2, located in the N-terminal domain and ligand-binding domain, respectively (21). In addition to these basal transcriptional components, the GR attracts via these transactivation domains protein complexes, called coactivators, that bridge the DNA-bound GR with the transcription initiation complex (39). Recent research revealed that these coactivators function also as multi-protein enzyme complexes for modifying chromatin-bound histones and other molecules [40]. For example, p300 and the homologous cAMP-responsive element-binding protein (CREB)-binding protein (CBP), the p300/CBP-associated factor (p/CAF) and the p160 family of coactivators are histone acetyltransferases forming a complex through their mutual interacting domains.

The GRE-bound GR also attracts to the glucocorticoid-responsive promoters the mating-type switching/sucrose nonfermenting (SWI/SNF) complex and components of the vitamin D receptor-interacting protein/thyroid hormone receptor-associated protein (DRIP/TRAP) complex (40). The former is an ATP-dependent chromatin remodelling factor with a multi-subunit structure in which an ATPase functions as the catalytic center, while the latter complex is also a multi-protein conglomerate, which consists of over 10 different proteins, including DRIP205/TRAP220/PBP and

components of the SRB/MED-containing cofactor complex (SMCC) (40). Several coactivators with histone methyltransferase activity also have been reported (40). The coactivator complexes alter the accessibility of the chromatin and DNA of the GR-bound regulatory regions to other transcription factors and the RNA polymerase II holo-complex by chemically modifying the associated histones.

Modulation of other transcription factor activity through protein-protein interactions

Glucocorticoids exert many of their effects through mutual protein-protein interactions of the GR with transcription factors of other signal transduction cascades by influencing their ability to stimulate or inhibit the transcription rates of their respective target genes (21). This activity may be more important than the GRE-mediated one, granted that mice harbouring a mutant GR, which is active in terms of protein-protein interactions but inactive in terms of transactivation via DNA, survive and procreate, in contrast to mice with a deletion of the entire GR gene that die immediately after birth from severe respiratory distress syndrome (41, 42). The former mouse model and additional *in vitro* results indicate that GR may interact with and influences other transcription factors as a monomer (41, 43).

The protein-protein interactions of the GR with other transcription factors may take place on promoters that do not contain GREs, as well as on promoters that have both GRE(s) and response element(s) of transcription factors that interact with the GR (44). Repression of transactivation of other transcription factors through protein-protein interactions may be particularly important in the suppression of immune function and inflammation by glucocorticoids (41, 43). Indeed, a substantial part of the effects of glucocorticoids on the immune system may be explained by the interaction between the GR and NF-κB, AP-1, STATs and probably Smads (45-48) (Table I). For example, glucocorticoids suppress the expression of the tumour necrosis factor α (TNF-α), inducible nitric oxide synthase (iNOS), IL-1β, IL-6, IL-12 p40, granulocyte/monocytes colony-stimulating factor (GMCSF) and transforming growth factor β (TGFβ) by inhibiting the transcriptional activity of these transcription factors at the regulatory regions of such cytokines (38).

Recent reports also indicate that GR directly interacts with the transcription factors "T-box expressed in T-cells" (T-bet) and GATA-3, which play key roles, respectively, in the differentiation of T helper-1 and T helper-2 lymphocytes (49, 50). The GR influences indirectly the actions of the interferon regulatory factor-3 (IRF-3) through the p160 nuclear receptor glucocorticoid receptor-interacting protein 1, by competing with this factor for binding to the coactivator (51). In contrast, the TNF-α and Fas-associated protein FLASH inhibits the transcriptional activity of the GR by binding to the nuclear receptor-binding domain of the p160-type nuclear receptor coactivators, interfering with its interaction with these proteins (52, 53).

Non-genomic actions of GR

Glucocorticoids have long been known to regulate several cellular biologic actions independently of their effects on transcriptional regulation promoted inside the nucleus and these actions were characterised as non-genomic. Such actions of glucocorticoids are rapid and take place within seconds to minutes in contrast to the genomic actions that usually require 20 minutes to many hours. Several cellular mediators have been postulated to play a role in mediating the non-genomic actions of glucocorticoids. These include: (1) putative membrane receptors for glucocorticoids (membrane GRs), (2) direct membrane effects of glucocorticoids, (3) classic GRs that target signalling proteins associated with the plasma membrane and (4) classic GRs that translocate into the mitochondria (29) (Fig. 3).

Several putative membrane GRs distinct from the classic GRs were reported in some lymphoid cell lines and the amphibian brain. The numbers of membrane GR-positive monocytes detected with specific antibodies are correlated with the incidence of rheumatois arthritis and systemic lupus erythematosus, but not with disease activity of systemic lupus erythematosus (29, 54, 55). B-cells also expressed membrane GR but its frequency did not correlated with the incidence of these conditions (55). While the membrane GR reported in the amphibian brain may be one of the κ-opioid receptors in the ligand competition study (56), neither amino acid sequences nor molecular characterisations for such membrane GRs have been reported. Since a specific G protein-coupled receptor having 7 transmembrane domains was identified for progestins (57), glucocorticoids might also employ a similar G protein-coupled receptor-type receptor expressed on the plasma membrane to exert their non-genomic actions.

Direct membrane effects of glucocorticoids have been observed especially at high doses of glucocorticoids in red blood cells, leukocytes, several cancer cell lines and some neuronal cells (29). Very high concentrations of glucocorticoids appear to alter cell membrane fluidity and other physico-chemical properties possibly though intercalation in the lipid bilayer of cell membranes (29). In immune cells, this effect of glucocorticoids alters the circulation of Na^+ and K^+ across the plasma membrane, leading to suppression of the inflammatory reaction (58, 59).

An example of the classic nuclear receptor GR-mediated modulation of the activity of signalling molecules associated with the plasma membrane is the inhibition of lymphocyte-specific protein tyrosine kinase (Lck) and the proto-oncogene tyrosine kinase Fyn in CD4+ lymphocytes, blocking the recruitment of these molecules to the T-cell receptor (TCR), and, finally inactivating TCR-mediated signalling events, such as the stimulation of downstream kinases MAPK, JNK, and protein kinase B and C (60-62). Glucocorticoids also inhibit TCR signalling further downstream at the level of 1,4,5-triphosphate (IP3)-mediated release of Ca^{2+} from the endoplasmic reticulum, possibly through inactivation of Lck (63).

The GR also translocates into the mitochondria and regulates their activities possibly in a non-genomic fashion (29, 64-66). This action of GR is particu-

larly important for induction of apoptosis by activating the caspase pathway in thymocytes and lymphoid cells (64). The mechanisms of GR-mediated apoptosis in the mitochondria remain poorly understood, but the reduction of mitochondrial membrane potential, the interaction with pro-apoptotic Bcl-2 family proteins, and the stimulation of the Bax/Bak assembly may play a role (29). Furthermore, the GR might influence the induction of apoptosis by changing directly the transcription of the mitochondrial genes via interacting with their GRE-like sequences (29).

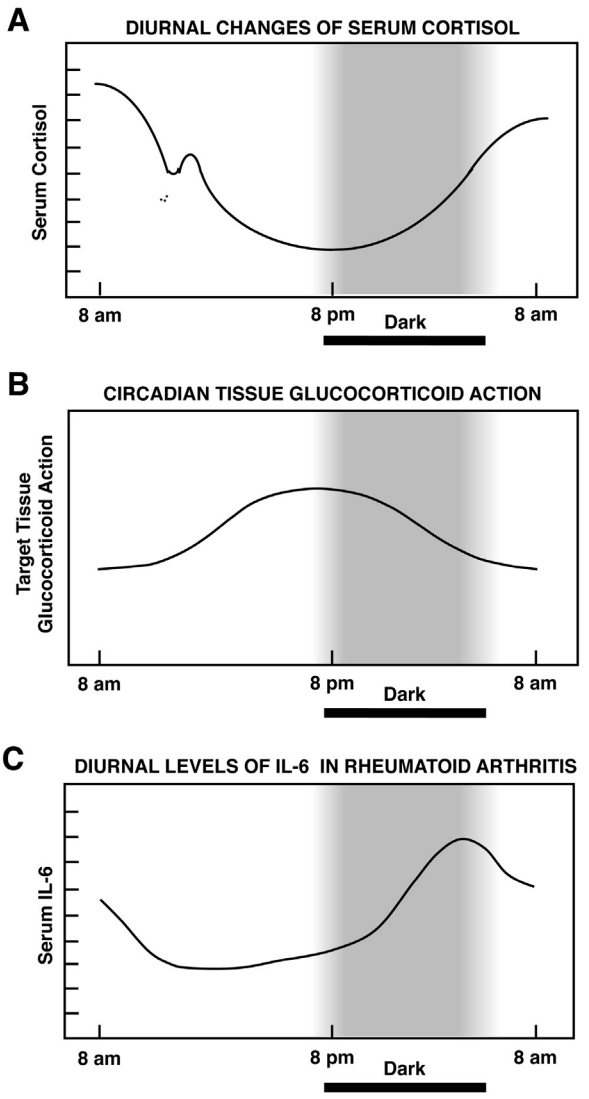

Fig. 4. Circadian fluctuation of serum cortisol and target tissue sensitivity to glucocorticoids, as well as levels of IL-6 in patients with rheumatoid arthritis. Schematic diurnal changes of serum levels of cortisol (**A**) and target tissue glucocorticoid sensitivity (**B**) as well as of IL-6 (**C**) are shown (90). Note that night-directed glucocorticoid treatment (grey areas) targets the period with high sensitivity to glucocorticoids in target tissues, which may explain the pronounced effect of these hormones administered in the evening.

Alteration of GR activity in inflammatory diseases and its underlying mechanisms

Tissue-specific alteration of local glucocorticoid sensitivity is reported in various disorders associated with dysregulation of immune function (67). For example, increased (hyper-) sensitivity to glucocorticoids can be seen in several viral infections including that of the human immunodeficiency virus type-1 (HIV-1). Viral encoding molecules, such as HIV-1 Vpr and Tat, may explain the increased sensitivity to glucocorticoids by modulating the transcriptional effect of histone acetyltransferase coactivators on GR via physical interaction with these two components (68). Kaposi's sarcoma, caused by infection with the human herpesvirus 8 in HIV-1-infected patients, demonstrates acceleration of growth in the presence of glucocorticoids (68). On the other hand, patients chronically treated with these hormones have a high prevalence of this neoplasm (69). These findings are consistent with a pathogenetic link between the two viruses and glucocorticoids.

Insensitivity or resistance to glucocorticoids is often recognised as resistance to pharmacologic doses of glucocorticoids, and is observed in patients with glucocorticoid-resistant asthma, rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, chronic lymphocytic leukaemia and nasal polyps (69-75). Several types of clinical evidence indicate that the splicing isoform GRβ is highly expressed in these patients, and thus, might explain in part such resistance to glucocorticoids through its dominant negative effect on GRα. Elevated levels of pro-inflammatory cytokines, such as IL-1, -2, -4, -7, -8 and -18, TNF-α, and IFN-α and γ, might be responsible for the increased GRβ expression in cells from patients with these pathologic conditions, as these cytokines experimentally stimulate the expression of GRβ in lymphocytes, neutrophils or airway smooth muscle cells (76-81). Furthermore, the presence of a single nucleotide polymorphism in the 3' untranslated region of the hGRβ mRNA (rs6198G allele), which increases its stability causing increased expression of the GRβ protein, is associated with increased incidence of rheumatoid arthritis and systemic lupus erythematosus (70, 82).

Several mechanisms explaining the dominant negative effect of GRβ on GRα-induced transcriptional activity have been reported, including (1) competition for GRE-binding through their shared DNA-binding domain, (2) heterodimerisation with GRα and (3) coactivator squelching through the preserved activation function-1 domain (8, 83, 84). All these different mechanisms of action appear to be functional, depending on the promoters and tissues affected by this GR isoform. In addition to the dominant negative effect of GRβ on GRα-mediated activities of glucocorticoids, this GR isoform was recently shown to possess intrinsic, GRα-independent transcriptional activity, modulating the expression of a certain groups of human genes (85-87). It also remains unclear whether GRβ might modulate the immune function through this newly discovered activity.

Implication of circadian clock-mediated negative regulation of GR activity to time-directed treatment of rheumatic diseases

Cortisol is secreted in a circadian fashion, with early morning elevations peaking around 8:00 am and reaching a nadir in the late evening, around 8:00 pm as depicted in Figure 4A. Manifestations of autoimmune inflammatory diseases, such as rheumatoid arthritis with stiffness of the joints, pain and functional disability, all fluctuate in a circadian fashion, becoming worse in the morning, while being relatively milder in the afternoon or evening (88, 89). Circulating levels of several cytokines relevant to these diseases, such as TNF-α, IL-6, IL-2 and IFN-γ, also fluctuate in a circadian fashion, demonstrating their peaks between midnight and early morning period, in part explaining the flare of the above symptoms in the morning (Fig. 4C) (88-90). To improve the severity of morning manifestations, prednisolone or a modified release (MR) prednisone tablet, which releases the steroid several hours after oral administration, was administered in the evening and resulted in significant improvement of several symptoms in the morning (91, 92).

The circadian rhythm of glucocorticoid secretion is maintained by a ubiquitous molecular ''clock'', the CLOCK system, which creates internal circadian periodicity under the influence of light/dark information (see *Prednisone chronotherapy* p. S-42) (93). The circadian CLOCK system consists of central and peripheral components, which, respectively, are located in the suprachiasmatic nuclei of the hypothalamus acting as a "master" CLOCK and in the rest of the brain and all organs and peripheral tissues behaving as "slave" CLOCKs under synchronisation to the central CLOCK (94). Their circadian rhythm is maintained by the common transcriptional regulatory machinery with coordinated activation/inactivation of a set of transcription factors, such as the circadian locomotor output cycle kaput (Clock) and its heterodimer partner brain-muscle-arnt-like protein 1 (Bmal1) (93).

The morning flare of patient symptoms such as morning stiffness in patients with rheumatoid arthritis, may be under the regulation of this circadian CLOCK system, in part though the diurnal fluctuation of circulating cortisol, which is also strictly regulated under the control of the central CLOCK located in the hypothalamic SCN and the downstream hypothalamic-pituitary-adrenal axis. The peripheral component of the circadian CLOCK system negatively regulates the transcriptional activity of GR by interacting with it and acetylating a lysine cluster located in its hinge region through acetyltransferase activity of the Clock transcription factor (94, 95) (Fig. 5). Since acetylation of the GR fluctuates in a circadian fashion, being higher in the morning and lower in the evening via oscillation of local Clock expression in human peripheral mononuclear cells, tissue sensitivity to glucocorticoids also demonstrates diurnal changes reaching a nadir in the early morning and a zenith in the late evening, mirroring the fluctuation of circulating cortisol (Fig. 4B) (95, 96).

The successful outcome associated with the evening administration of glucocorticoids in rheumatic patients may be supported in part by the elevated sensitivity of immune cells to glucocorticoids that effectively suppresses cytokine levels and consequent inflammation this time of the day. It should, however, be mentioned that this time-directed treatment with glucocorticoids might increase various side effects of these hormones even though the doses employed are relatively low.

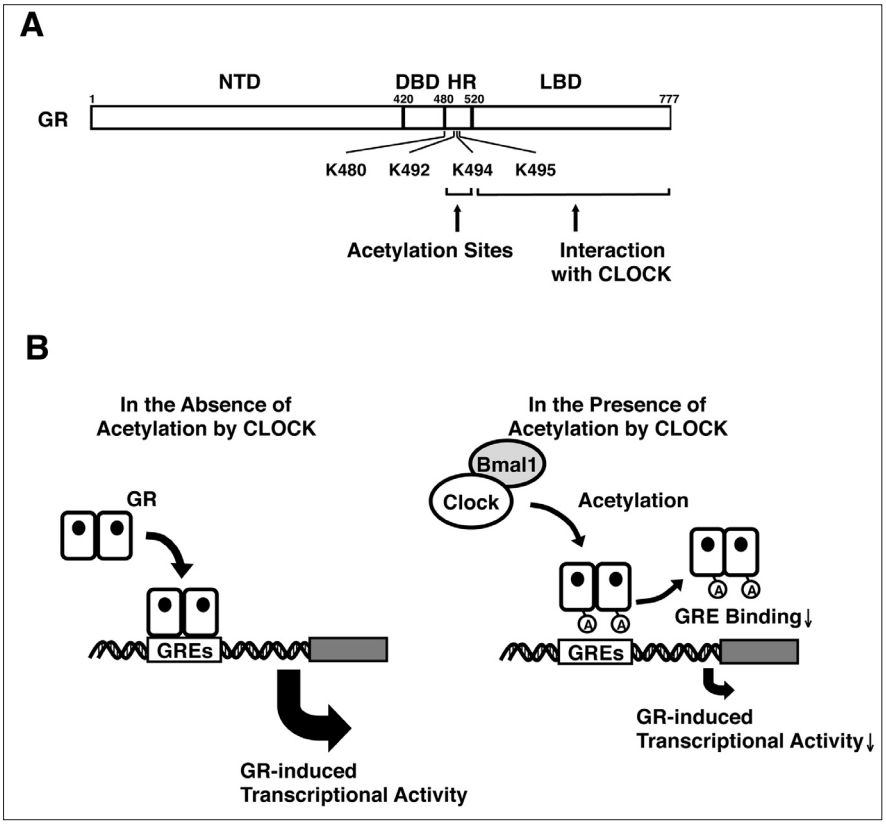

Fig. 5. Acetylation sites of the GR and regulation of GR transcriptional activity by Clock.
A: Multiple lysines acetylated by Clock in the human GR.
The human GR has 4 acetylation sites in its hinge region; lysines, 480, 492, 494 and 495, acetylated by Clock. Clock physically interacts with GR ligand-binding domain through the domain enclosed in its C-terminal part and acetylates GR at all lysine residues located in a lysine cluster of the hinge region. (modified from ref. 95).
B: A heuristic model of the physiologic implications of this study.
Clock/Bmal1 acetylates GR via its intrinsic histone acetyltransferase activity through physical interaction with GR ligand-binding domain, reduces affinity of GR to its cognate DNA GREs and ultimately suppresses GR-induced transcriptional activity. (modified from ref. 95).
A: acetylation, Bmal1: brain-muscle-arnt-like protein 1, Clock: circadian locomotor output cycle kaput, DBD: DNA-binding domain, GR: glucocorticoid receptor, GRE: glucocorticoid response element, HD: hinge region, LBD: ligand-binding domain, NTD: N-terminal domain.

Concluding remarks

The GR exerts numerous and diverse cellular functions primarily by altering the transcriptional activity of glucocorticoid-responsive genes either through binding to DNA GREs or through interaction with other transcription factors. The GR gene expresses two splicing isoforms and multiple translational variants with specific transcriptional activity and tissue distribution. Chemical modifications, such as phosphorylation and acetylation, also modulate the transcriptional activity of the GR, the latter of which is particularly important for the circadian adjustment of local glucocorticoid activities. In addition to these genomic actions of GR, this GR protein might also alter the cellular functions non-genomically, possibly by targeting plasma membrane or cytosolic signalling molecules of other biologic pathways, and by translocating into the mitochondria and changing their activities. All these actions of GR play important roles in the regulation of immune function. Thus, elucidation of specific GR actions in the pathophysiology of rheumatic diseases would warrant the development of more rational and efficient glucocorticoid therapies for these autoimmune and inflammatory disorders.

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