



**HAL**  
open science

## Glucocorticoid receptor action in beneficial and side effects of steroid therapy: Lessons from conditional knockout mice

Anna Kleiman, Jan P. Tuckermann

► **To cite this version:**

Anna Kleiman, Jan P. Tuckermann. Glucocorticoid receptor action in beneficial and side effects of steroid therapy: Lessons from conditional knockout mice. *Molecular and Cellular Endocrinology*, 2007, 275 (1-2), pp.98. 10.1016/j.mce.2007.05.009 . hal-00531928

**HAL Id: hal-00531928**

**<https://hal.science/hal-00531928>**

Submitted on 4 Nov 2010

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Accepted Manuscript

Title: Glucocorticoid receptor action in beneficial and side effects of steroid therapy: Lessons from conditional knockout mice

Authors: Anna Kleiman, Jan P. Tuckermann

PII: S0303-7207(07)00193-1  
DOI: doi:10.1016/j.mce.2007.05.009  
Reference: MCE 6658

To appear in: *Molecular and Cellular Endocrinology*

Received date: 13-3-2007  
Revised date: 12-5-2007  
Accepted date: 12-5-2007

Please cite this article as: Kleiman, A., Tuckermann, J.P., Glucocorticoid receptor action in beneficial and side effects of steroid therapy: Lessons from conditional knockout mice, *Molecular and Cellular Endocrinology* (2007), doi:10.1016/j.mce.2007.05.009

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



**Glucocorticoid receptor action in beneficial and side effects of steroid therapy: lessons from conditional knockout mice**

Anna Kleiman and Jan P Tuckermann

Leibniz Institute for Age Research – Fritz-Lipmann-Institute, Group of Tissue specific Hormone Action<sup>1</sup>

---

<sup>1</sup> Address: Beutenberg Str. 11, D-07745 Jena, Germany; phone: +49-3641-656134, fax: +49-3641-656133, e-mail: [jan@fli-leibniz.de](mailto:jan@fli-leibniz.de), (J.T., corresponding author)

**Keywords**

glucocorticoid receptor, transcription, inflammation, skeleton

**Summary**

Glucocorticoids (GCs) are potent immune suppressive drugs with unfortunately severe side effects. Different molecular modes of actions of the GC receptor (GR) have been identified. Transcriptional transactivation by binding of a dimerized GR protein complex to the promoter of GC regulated genes or interference with activity of pro-inflammatory transcription factors by GR monomers are considered as the two major mechanisms. It has been hypothesized that selective GR agonists (SEGRAs) addressing dimer-independent function would reveal potent steroid therapeutic activity with reduced side effects. Recent studies of a mouse knock-in strain with a dimerization-deficient GR demonstrate that some inflammatory processes can be suppressed by GCs, while others can not. Also side effects of GCs occur in these mice. Thus, depending on the process that is treated, SEGRA could be therapeutically more or less effective and not all side effects of steroid therapy may be reduced.

## ***Introduction***

When cortisol related substances are prescribed to treat an allergic skin reaction, or a chronic ulcerative disease, medical doctors as well as patients are left with a feeling that the “evil is combated by the devil”. Why are these powerful anti-inflammatory drugs so double sided as the schizophrenic entities living in one person called Dr. Jekyll and Mr. Hyde? Are there possibilities to strengthen Dr. Jekyll and to weaken Mr. Hyde’s properties of cortisone like substances? Obviously a better understanding of the molecular nature of these factors and the contribution to the phenotypes of both gentlemen will allow us to speculate, if intelligent drugs will prevent Mr. Hyde in steroid therapy.

Glucocorticoids (GCs), such the synthetic drugs prednisolone, bethamethasone, or the potent compound dexamethasone, belong to the class of steroids, which bind to the glucocorticoid receptor (GR). The endogenous ligands of the GR, cortisol, corticosterone and aldosterone – the latter at least acting glucocorticoid like in the brain – are synthesized in the adrenal cortex. Their production is diurnal in a pulsatile manner. The release of GCs is regulated by a hierarchy of endocrine organs. The hypothalamus secretes corticotropin-releasing hormone (CRH) controlling the pituitary, which in turn triggers the release of adrenocorticotrophic hormone (ACTH) that stimulates the steroid synthesis in the adrenal cortex. The hormonal network between these endocrine tissues is considered as the HPA (hypothalamus-pituitary-adrenal)-axis. The HPA axis is subject to intense negative feedback regulation by GCs levels themselves, finally leading to a circadian pulsatile behaviour of GC production. The input to the hypothalamus is either derived from the central circadian clock or – from psychological and physiological stress. The latter can be starvation, but also systemic inflammatory reactions.

As their name tells us, one of the primary functions of GCs is to increase serum glucose levels to allow rapid energy supply for the brain, which consumes preferably glucose. Anabolic processes in the liver achieve this by inducing gluconeogenesis and catabolic actions in peripheral organs by triggering protein degradation and increased lipolysis. One can envisage that these catabolic actions contribute much to the side effects, when GCs are applied at pharmacological doses. In particular the adverse effects manifest in numerous features such as brittle skin (Schoepe et al., 2006), muscle weaknesses, osteoporosis (Canalis and Delany, 2002), fat redistribution (Peeke and Chrousos, 1995), diabetes (Peeke and Chrousos, 1995), but also leads to neurodegeneration or suppressed neurogenesis in the brain (De Kloet et al., 1998). Why are GCs still the therapeutic standard for the treatment of allergic diseases such as asthma, and of chronic inflammation such as rheumatoid arthritis and inflammatory bowel diseases? Dependent on the severity of the inflammatory disease the beneficial effects of GCs still balance out some of the adverse effects. In some cases non steroidal anti-inflammatory drugs (NSAIDs) can replace GC, whereas in other conditions GC therapy is combined with acute treatment of the side effects, e.g. to avoid GC induced osteoporosis by application of bisphosphonates (Canalis and Delany, 2002). However, there is still the ambitious goal in the pharmaceutical industry to produce steroidal analogs that avoid the side effects and maintain the therapeutic efficacy of steroid treatment.

The understanding of the molecular basis that underlie the therapeutic and side effects have been tremendously increased in the last years by the combination of molecular biology and the analysis of transgenic mice with cell type specific or function selective glucocorticoid receptor mutations. Based on these findings the first attempts of the design of selective GR agonists (SEGRA) have been performed and tested for

their therapeutic efficacy and side effects (Belvisi et al., 2001; Lin et al., 2002; Schacke et al., 2004; Vayssiere et al., 1997).

In this review we will summarize how molecular actions of the GC receptor (GR) contribute to certain therapeutic and certain adverse effects and finally we discuss how much hope lies in the pharmaceutical attempts to create potent anti-inflammatory GR ligands without side effects.

### ***Functional domains of the GR protein***

The gene NR3C1 encodes the nuclear receptor “glucocorticoid receptor”, which was cloned in the beginning of 1980ies in mammalian species (Evans, 1988). The GR was among the first described bona fide enhancer activating transcription factors in mammals (Miesfeld et al., 1984). The arrangement of functional domains of the GR protein – ligand-binding (LBD), DNA-binding (DBD) and transactivation domains - is common with most of the other nuclear receptors. The transactivation domain AF-1 is located in the N-terminus and its activity is subject to modulation by posttranscriptional modifications (Schaaf and Cidlowski, 2002), whereas the AF-2 transactivation domain is close to the C-terminus of the LBD in the C-terminus and changes to an active conformation after binding of a ligand. The DBD contains two so called Zn finger motifs. Each of this motif consists of two protein loops that are coordinated by a Zn atom. Both Zn fingers are followed by an amphipathic alpha helix (Luisi et al., 1991). The LBD is build up by a three-layer helical sandwich that embeds a hydrophobic pocket for ligand binding, which is followed by the C-terminal AF-2 containing helix 12 (Bledsoe et al., 2002; Shiau et al., 1998). In the absence of hormone the GR is localized in the cytoplasm, where it is in a complex with chaperonic molecules, hsp90, hsp70, hsp40, the co-chaperone p23 and the immunophilins FKBP52 and Cyp40 (Pratt et al., 1996). Binding of the hormone leads

to disruption of the cytoplasmic complex and NLS sequences become unmasked and the GR molecules shuttles into the nucleus with the help of importin proteins (Freedman and Yamamoto, 2004).

The GR controls gene expression by several mechanisms. Some of them imply interaction with signal transduction components, such as PI3K, JNK, 14-3-3 proteins (Caelles et al., 1997; Kino et al., 2003; Limbourg and Liao, 2003) and take place partially in the cytoplasm. Some events even occur at the membrane as reviewed elsewhere in this issue [Buttgereit]. However, the best characterized actions of the GR are on the transcriptional level and employ two major mechanisms: i) binding to DNA and ii) interaction with other transcription factors by tethering mechanisms (Figure 1).

### ***Control of Gene expression by DNA binding of the GR***

The binding to DNA occurs on imperfect palindromic sequences present in the promoters and enhancer regions of GC responsive genes, designated as GC responsive elements (GRE). FRAP technology and chromatin-immunoprecipitation experiments exhibited a kinetic view for the DNA binding of the GR and other nuclear receptors. These experiments showed that the interaction with DNA is a dynamic process during which the GR and coactivator complexes cycle between DNA and nucleoplasm within seconds (McNally et al., 2000) or minutes (Metivier et al., 2003). The GR binds to GREs as a homodimer. Both half sites of the GRE are bound by one molecule of the dimer. The dimerization occurs via distinct hydrophobic motifs present in the ligand binding domain (Bledsoe et al., 2004) and is further stabilized by interaction in the DNA binding domain itself. Directly at the GRE half site the N-terminal of two Zn fingers contact the DNA whereas the second Zn finger contacts the partner molecule via an amino acid stretch called the dimerization box (Luisi et al., 1991). Thus, the dimerization is essential for binding of

the GR to GRE controlled genes. The mechanism of the binding of the GR to negative acting GREs (nGRE) so far identified only in a limited amount of genes, is less well understood, but seems also to require dimerization (Dostert and Heinzl, 2004).

Bound to the GRE the transactivation domains of the GR serve as docking platform for the recruitment of transcriptional coactivators that either remodel chromatin directly or facilitate the initiation of transcription. The AF-1 domain recruits in a ligand independent manner the multimeric chromatin-remodeling ATPase BRG1, a homologue of yeast SWI/SNF (Cairns et al., 1996; Yoshinaga et al., 1992) that in turn assemble the histone acetylases P/CAF and CBP/p300. The AF-2 domain upon binding of hormone recruits members of the p160 family of coactivators, which share a nuclear receptor interaction motif called the NR box (LxxLL). A number of these p160-factors, such as SRC-1, SRC-2/GRIP1/TIF2 and RAC3/ACTR/p/CIP possess histone acetylase (HAT) activity, leading to disruption of nucleosomes. Recently it could be demonstrated that an additional factor called STAMP seems to be part of the SRC-2/GRIP1/TIF2-GR complex and enhances its transcription-inducing activity (He and Simons, 2007). Furthermore additional chromatin-modifying enzymes such as the aforementioned CBP/p300 and the histone arginine methylase CARM1 (Chen et al., 1999; Ma et al., 2001) interact with the GR.

However, which co-activator/chromatin complexes are finally present on the promoters of genes depends on availability of coactivators in a certain cellular context, the abundance of other transcription factors competing for the same coactivators, packed stage of the chromatin and the phosphorylation status of the AF-1 domain. The mechanisms of positive regulation of gene transcription by the GR has been understood in detail only for a few well characterized promoters such as the MMTV, the tyrosine amino transferase gene (Schmid et al., 1987) and the

phosphoenolpyruvate carboxykinase (Hanson and Reshef, 1997). For a number of known GC induced genes, such as MAPK phosphatase-1 (Kassel et al., 2001) and GTR (Nocentini et al., 1997) knowledge of the precise mechanisms for up-regulation is poor. For the growing number of novel recognized GC induced genes by gene expression profiling (James et al., 2007; Rogatsky et al., 2003) a comprehensive mechanistic analysis is challenging. Recent advances with chromatin IP scanning of GC regulated promoters gave first insights of GR occupancy in sequences of some GC induced genes (Wang et al., 2004). The requirements of coactivator composition on individual promoters of GC regulated genes could be addressed by a combination of genome wide chromatin-immunoprecipitation approaches and traditional promoter sequence deletion analysis.

### ***Cross-talk of the GR with Transcription Factors***

Since 1990 a DNA binding independent mechanisms of GR action on transcription were discovered. Pro-inflammatory transcription factors such as AP-1 (Jonat et al., 1990; Schüle et al., 1990; Yang Yen et al., 1990), NF- $\kappa$ B (De Bosscher et al., 1997; Heck et al., 1997; Ray and Prefontaine, 1994; Scheinman et al., 1995b), IRF-3 (Ogawa et al., 2005; Reily et al., 2006) and STAT proteins (Stöcklin et al., 1996; Zhang et al., 1997) can be influenced in their activity to control transcription by GCs in the absence of a GRE, but in the presence of the binding sites of the interacting transcription factors. This protein-protein interaction is designated as “tethering mechanism” (Beato et al., 1995; De Bosscher et al., 2003). Mutational analysis of the GR revealed that the DNA binding domain was required for the interaction, even when the dimerization box of the second Zn-Finger was disrupted (Heck et al., 1994).

Whereas interaction of the GR with STAT5 leads to an increase of gene expression in cells (Stoeklin et al., 1997) and in mice (Tronche et al., 2004), for AP-1, NF- $\kappa$ B and IRF-3 a suppression of gene regulatory activity for most of the respective target genes was demonstrated (Göttlicher et al., 1998; Ogawa et al., 2005; Reichardt et al., 2001; Tuckermann et al., 1999). Numerous mechanisms have been proposed for the molecular nature of the inhibition of transcription factors by the GR. One involves a squelching model of limiting amounts of CBP/p300 (Kamei et al., 1996), which are competed out by the GR, and thereby attenuate AP-1 and NF- $\kappa$ B activity. However, De Bosscher and colleagues demonstrated that repression of AP-1 and NF- $\kappa$ B is independent on CBP/p300 levels (De Bosscher et al., 2001). But the competing model seems to hold true for the inhibition of IRF-3 by the GR. In macrophages overexpression of the coactivator GRIP-1 abolished GC-inhibition of IRF-3 whereas knock down of GRIP-1 diminished IRF-3 activity to the same extent than GR activation by GC (Reily et al., 2006). NF- $\kappa$ B could be also inhibited indirectly by GC induced synthesis of its inhibitor I $\kappa$ B $\alpha$  (Auphan et al., 1995; Scheinman et al., 1995a) or by the GC induced leucine Zipper protein GILZ (D'Adamio et al., 1997), which was also demonstrated to inhibit AP-1 (Mittelstadt and Ashwell, 2001). As an additional mechanism GC induced expression of histone deacetylase 2 was shown to blunt NF- $\kappa$ B activity by recruitment of this HDAC to the p65-HAT-CBP complex (Ito et al., 2000). Direct interaction of the monomeric GR and pro-inflammatory transcription factors within a tethering complex that prevent their recruitment of co-activators (De Bosscher et al., 1997) or co-repressors or by interfering with interaction of RNA polymerase II (Nissen and Yamamoto, 2000) seems to be the best characterized mechanism of transrepression by the GR. The interaction between the GR and the transcription factors often requires integrator proteins such as the nuclear

isoform of the LIM-domain protein TRIP-6 (Kassel et al., 2004) and a recently identified factor STAMP (He and Simons, 2007).

### ***Disrupting dimerization-induced DNA-Binding in vivo: GR<sup>dim</sup> mice***

In order to discriminate between the tethering mechanism and dimerization induced DNA binding of the GR the mutant GR-version described by Cato and colleagues (Heck et al., 1994) was knocked into mice (Reichardt et al., 1998). These GR<sup>dim</sup> mice with a dimerization deficient receptor exhibit an absence of GRE regulated genes (Reichardt et al., 1998), but a preserved suppression of AP-1 (Tuckermann et al., 1999) and NF- $\kappa$ B-activity (Reichardt et al., 2001). Interestingly, these mice overcame the lethal lung phenotype of GR-knockout mice, stressing the importance of DNA binding independent activities of the GR for certain physiological processes (Reichardt et al., 1998). Furthermore irritant contact dermatitis by phorbol esters could be suppressed by GC in GR<sup>dim</sup> mice (Reichardt et al., 2001). These findings lead to the promising concept that pharmaceuticals that would act as a SEGRA addressing the ability of the GR monomer by avoiding dimerization would promote Dr. Jekyll's beneficial side and would omit Mr. Hyde's deleterious side effects (Fig. 1). Is this promise realistic? We will come back to this question after we discussed in the remaining sections the tissue specific requirement of the GR and the involvement of dimerization induced DNA binding in therapeutic and side effects of GC.

## ***Endogenous GCs in inflammatory responses: The example of Septic shock***

In a variety of conditions the anti-inflammatory actions of endogenous GC are required to control massive immune responses. One example is sepsis. Sepsis is viewed as a complex dysregulation of inflammation arising when the host is unable to successfully defeat an infection. This exaggerated immune response that damages the organism, can lead to septic shock, frequently with lethal outcome. In rodents septic shock can be induced by a bolus injection of toll-like receptor (TLR) agonists such as lipopolysaccharides (LPS) and DNA (polyI:C), which bind to TLR4 and TLR3, respectively (Aderem and Ulevitch, 2000; Akira et al., 2006). In macrophages for example TLR4 activation leads to the recruitment of the intracellular adaptor molecules MyD88/IRAK/TRAF6 or TRIF/RIP-1 which causes the activation of MAP-kinases and IKKs (Akira et al., 2006). This results in the activation of the transcription factors AP-1, NF- $\kappa$ B, IRF-3 and others to trigger the induction of pro-inflammatory genes including cytokines associated with sepsis, such as TNF- $\alpha$ , IL-1 and IL-6. This finally converges on a systemic inflammatory response syndrome (SIRS). During this systemic inflammation GCs are synthesized in the adrenal gland in response to stress or systemic cytokine release following exposure to bacterial endotoxin (Yeager et al., 2004). Although treatment of septic shock with high GC doses in humans seems not to show a reduction of mortality (Meade, 2005), there are several lines of evidence that GCs indeed participate in control of this process. In patients that exhibit severe sepsis, adrenal insufficiency is accompanied. Furthermore, adrenalectomized rodents have difficulty in surviving mild septic shock reviewed in (Yeager et al., 2004). Recent studies with conditional GR mutant mice by us and others (Bhattacharyya et al., 2007), showed that the GR in myeloid cells is required to

survive sub-lethal LPS doses and cecal ligation and puncture (CLP). More important, to survive septic shock the regulation of GRE dependent gene expression is required, since knock-in mice with a dimerization deficient GR also do not survive lethal sepsis (Kleiman, unpublished). Thus, a transrepression mechanism, i.e. the down regulation of NF- $\kappa$ B, AP-1 or IRF-3 is not sufficient to lead to a successful management for sepsis. Genes that are induced by GC, such as the MAPK inhibitor DUSP-1 (MPK-1) have been hypothesized to be implicated in the immune suppressive effects of sepsis (Bhattacharyya et al., 2007). However, normal DUSP-1 protein levels in GR<sup>dim</sup> macrophages have been observed (Abraham et al., 2006), although GR<sup>dim</sup> animals do not survive sepsis. Which GC regulated genes could mediate the survival of a sub-lethal sepsis conditions? The answer of this question is complex, since numerous genes were identified to be regulated by GC in murine macrophages as revealed by gene expression profiling studies (Ogawa et al., 2005). A recent study of global gene expression in GC treated human monocytes (Ehrchen et al., 2007) revealed that GC not only suppress inflammatory functions of monocytes but rather shift them to an “anti-inflammatory” activated phenotype that includes enhanced phagocytotic properties and migratory behaviour and a protection from apoptosis. These findings together with previous observations confirm that GC-treatment could stimulate monocytes/macrophages to optimize the clearance of pro-inflammatory complexes, dying neutrophils and finally to a resolution of the inflammation (Yona and Gordon, 2007).

Also the requirement of the GR in other cell types for the survival of sepsis cannot be excluded. The conditional GR mutant GR<sup>lysMCre</sup> mouse lacks the GR not only in macrophages also in some other myeloid cell populations, in particular neutrophils. Whereas animals lacking the GR in T-cells show no difference in sepsis responses

(Kleiman, unpublished) animals that have no GC response in other compartments, such as endothelial cells have not been analyzed, yet. In summary, for the beneficial effect of GC in sepsis the presence of the GR at least in myeloid cells is required and dimerization induced DNA binding is needed.

### ***Exogenous GC action in skin inflammation***

Inflammatory skin diseases, such as contact and atopic dermatitis are frequently prescribed with GCs with the long term risk of side effects, namely atrophy of the skin (Schoepe et al., 2006). The phorbol ester induced edema formation is a commonly used model for assessing unspecific and irritant skin inflammation (Gschwendt et al., 1984) and GCs and analogous compounds have been extensively tested for their anti-inflammatory action. For the suppression of this type of inflammation the dimerization of the GR is not required, since GR<sup>dim</sup> mice show an efficient reduction of phorbol ester inflammatory response (Reichardt et al., 2001). In this model also function-selective GR ligands have been tested for their anti-suppressive efficacy (Schacke et al., 2004; Vayssiere et al., 1997). From the croton oil experiments one could conclude that dimerized-induced DNA binding can be omitted for suppression of inflammatory skin diseases. However, recent studies in contact hypersensitivity, a model for contact dermatitis and thus an inflammatory type of a different mechanism revealed that more than transrepression of the GR is required for suppression of inflammation (Tuckermann et al., 2007).

Contact dermatitis is represented by an experimental rodent model, contact hypersensitivity (CHS) and has been extensively studied over decades. Once seen as a rather simple allergic reaction independent on a humoral response, only dependent on cell-cell-interactions with three main players, antigen presenting cells, T cells and

effector cells, recently the picture has become more complex. The CHS reaction is divided into two phases, the sensitization phase and the challenge phase (Figure 2). The current view (Askenase, 2001) tells us that small molecular compounds called haptens when applied the first time to the body are covalently bound to epidermal proteins. This irritates keratinocytes, which subsequently activate antigen presenting cells (APCs). The APCs – notably dermal DCs triggered by cytokines such as TNF- $\alpha$  and IL1 $\beta$  – take up the hapten loaded proteins, migrate to draining lymph nodes and present the processed haptens to naïve T cells on MHC II molecules. In turn, T cells differentiate into antigen-specific Th1 and cytotoxic T cells and are designated as sensitized. Sensitized T cells proliferate, leave the local lymph node and patrol through the body. In the challenge phase, initiated by the re-exposure of the same hapten, activated endothelium allow the penetration of a first wave of lymphocytes, including the surveying sensitized T cells. These T cells become restimulated by APCs in the skin and subsequently release pro-inflammatory mediators, which trigger resident myeloid cells to secrete chemokines, finally leading to a fully inflammatory reaction including a second wave of leukocyte infiltration and edema. So there are numerous potential targets for GC action in this system to suppress the inflammatory response. We could show recently (Tuckermann et al., 2007), that anti-inflammatory action of GC is dispensable in keratinocytes, T-cells and presumably antigen presenting cells: Whereas conditional GR-knockout mice for Keratinocytes (GR<sup>K14Cre</sup>) and T cells (GR<sup>kkCre</sup>) mount a perfect immune suppressive response by GCs, GR<sup>lysMCre</sup>-mice, lacking the GR in macrophages and neutrophils were refractory to GC treatment. Thus, the GR in macrophages and neutrophils seems to be critical for steroid therapy. Interestingly, in contrast to irritant contact dermatitis mice with a dimerization defective GR (GR<sup>dim</sup>) were largely resistant to GC treatment and

exhibited a persistent leukocyte infiltration after steroid therapy. Macrophages isolated from GR<sup>dim</sup> mice showed impaired suppression of IL-1 $\beta$ , MIP-2, MCP-1 and IP-10 by GC (Tuckermann et al., 2007), all cytokines that functionally counteracted GC effects on CHS. TNF- $\alpha$  showed only a minor effect. Thus, depending on the type of skin inflammation different cell types and different molecular mechanisms can be involved.

This has consequences for therapeutic strategies, for example one would expect that function selective GR ligands (SEGRAs) that address the transrepression activity with the hope to reduce atrophy of the skin (Schoepe et al., 2006) would be potent in the treatment of irritant dermatitis, but less in contact allergy.

### ***Side effects: GCs and Glucose homeostasis***

The most predominant side effects of GCs in the organism are of catabolic nature i.e. remodeling of tissue, such as the aforementioned skin, but also in bone leading to osteoporosis, redistribution of fat and insulin resistance leading to diabetes. These catabolic actions are due to the capability of glucocorticoids to enhance glucose levels by stimulating gluconeogenesis, which in part relies on the degradation of proteins and modulating fatty acid metabolism. The central role of GCs to maintain glucose levels is evident by the findings that patients deprived from GC have low glucose levels (Addison's disease), patients with GC excess (Cushing syndrome) exhibit glucose intolerance (Andrews and Walker, 1999). The clinical features are corroborated by findings in CRH knock-out mice devoid of GCs that fail to counteract hypoglycemia (Jacobson et al., 2006). The maintenance of glucose by GC could be explained by several potential mechanisms. First the induction of enzymes involved in gluconeogenesis in the liver could be crucial. Mice deficient for the GR in

hepatocytes (GR<sup>alfcre</sup>) suffer from hypoglycemia after starvation and do not upregulate gluconeogenic enzymes (Opherk et al., 2004). Prednisolone administration stimulates expression of the tyrosine aminotransferase gene in contrast to the SEGRA compound ZK216348 that fail to elevate both TAT mRNA and glucose levels in the blood (Schacke et al., 2004). Second, decreased glucose uptake in peripheral organs could be prevented by GC by inhibition of glucose transporter (GLUT4) translocation to the cell membrane (Horner et al., 1987), and stimulation of lipolysis by GC could counteract insulin mediated reduction of blood glucose levels (Andrews and Walker, 1999). Third, GC could prevent insulin production. Mice over expressing the GR in pancreatic beta-cells show impaired insulin production (Delaunay et al., 1997). If this directly occurs with normal GR levels in the excess of hormone has not been addressed so far. Fourth, GC-induced biosynthesis of ceramides, which inhibit Akt/PKB signaling could thereby lead to insulin resistance (Zierath, 2007). Recently, in DES1 knockout mice, which exhibit impaired ceramide synthesis, GC induced insulin resistance could be diminished (Holland et al., 2007). How much these potential mechanisms contribute to the GC-regulation of glucose levels and the GC-induced insulin resistance requires in depth analysis of GR cell type specific and GR function selective mutant mice.

### ***Side effects: GC actions on the skeleton***

GC long-term therapy has a strong impact on the skeletal system. Prolonged GC treatment of children with juvenile rheumatoid arthritis, chronic asthma and post renal transplantation strongly impairs longitudinal growth in children that receive steroid therapy (De Luca, 2006). Longitudinal growth in prepubertal vertebrates is occurring in the cartilaginous growth plate at the epiphysis of bones. In the growth plate chondrocytes undergo the serial events of proliferation, subsequent differentiation and

apoptosis which results in the replacement of cartilage by bone (Karsenty and Wagner, 2002). Apoptosis of terminal differentiated (hypertrophic) chondrocytes, osteoclastic resorption and the formation of bone matrix (mainly constituent collagen type I) by the osteoblasts lead to the replacement of cartilage by mineralized bone. GCs seem to influence the activity of the growth plate on several levels finally leading to reduced growth. First, GC impair proliferation of growth plate chondrocytes, by simultaneously enhancing apoptosis of hypertrophic chondrocytes (Smink et al., 2003b). Second, in vitro studies in cultured chondrocytes suggest that GCs induce apoptosis by activation of caspase-3 (Chrysis et al., 2003) and Bax (Mocetti et al., 2001), and decreased expression of Bcl-2 and Bcl-x (Mocetti et al., 2001). Third, GC seem to affect the GH/IGF-1 axis, which is important for proliferation and survival of chondrocytes on multiple levels. GC inhibit GH secretion in the pituitary (Devesa et al., 1995), decrease IGF-1 expression in the growth plate (Chrysis et al., 2003; Smink et al., 2003b), and down regulate GH receptor and GH binding protein (Gevers et al., 2002). In addition, GC impair IGF-1 signaling by inhibition of PI3K (Chrysis et al., 2005). Finally, the reduction of VEGF in chondrocytes points to a possible prevention of vascularigenesis leading to an impairment of growth by inhibition of mineralization (Koedam et al., 2002). The complex effects of GC on chondrocyte functions were recently reflected by results from expression profiling of GC treated chondrocytic micro mass cultures that recapitulate chondrocyte differentiation in vitro (James et al., 2005; James et al., 2007). GC treatment suppressed genes favoring proliferation such as growth factors, and enriched metabolic associated genes and genes encoding matrix genes, indicating that GC enhance chondrocyte differentiation. On the other hand factors promoting vascularization and mineralization were inhibited by GC (James et al., 2007). All

these changes of gene expression probably result in a reduced growth plate activity and a reduction of bone growth. However, studies with cartilage specific deletion of the GR are required to unequivocally dissect the GR function in cartilage cells versus effects on osteoblasts, osteoclasts and endothelial cells in the process of bone growth.

The other prominent side effect on the skeleton, GC induced osteoporosis seems now to become better understood. GC excess leads to a rapid bone loss that is followed by a longer lasting decline of bone formation and increasing the fracture risk to 50% for patients with more than 7.5 mg/kg prednisolone treatment (Van Staa et al., 2000). The effect of GC on bone homeostasis could be systemic or local. Systemic effects include a decreased calcium-absorption in the gut and decreased calcium-reabsorption in the kidney (Mazziotti et al., 2006). Lowering calcium-levels in the blood enhance PTH secretion from the parathyroid glands, which act on osteoblasts to stimulate osteoclast formation by inducing cortical bone loss. However, GC induced osteoporosis is accompanied by trabecular bone loss and reduced bone turnover. Hyperparathyroidism leads instead to increased bone turnover (Mazziotti et al., 2006), making it unlikely to be the cause. Furthermore the secretion of osteotropic hormones such as sex steroids and growth hormone secretion is attenuated (Mazziotti et al., 2006). Decrease of sex steroids by GC enhances bone loss and increases the risk for fractures. As for the actions in the growth plate inhibition of GH activity by decrease of IGF-I, GH receptor and IGF receptor 1 expression in osteoblasts as well as abrogating the release of GH from the pituitary could lead to a reduced bone strength (Delany et al., 2001; Devesa et al., 1995; Itagane et al., 1991; Smink et al., 2003a; Wehrenberg and Giustina, 1992).

In contrast to evidence for systemic actions, the direct effects on bone cells by GC leading to osteoporosis could be recently demonstrated with cell type specific

mutations of the GR. High dose GC treatment rapidly leads to impairment of bone formation that includes induction of apoptosis of osteoblasts and osteocytes in wild type mice (Weinstein et al., 1998). But apoptosis seems not to be the only mechanism. Osteoblast numbers are controlled by the canonical Wnt pathway (Hartmann, 2006) and recently GCs could be shown to impair Wnt signaling in osteosarcoma cell lines by direct binding of the GR to  $\beta$ -catenin (Takayama et al., 2006). Whereas low dose GC-treatment seems to stimulate osteoblast differentiation (Shalhoub et al., 1992), high dose GC treatment leads to inhibition of differentiation of primary mouse osteoblasts (Smith et al., 2000). The suppression of osteoblast function could be due to decreased expression of genes directly involved in bone formation, such as collagen 1 or runx2 (Pereira et al., 2001), but also due to antagonizing BMP pathways (Luppen et al., 2003). Also osteoclast-activity modulation by GCs participates in GC induced osteoporosis. GCs induce RANKL in osteoblasts, a potent stimulator of osteoclastogenesis (Hofbauer et al., 1999). GCs prolong longevity of osteoclasts in vivo (Jia et al., 2006; Kim et al., 2006). But bone degrading activity was also found to be suppressed by steroids (Kim et al., 2006). Furthermore in mice lacking the GR in osteoclasts a defect of bone formation activity by the osteoblasts was detected, linking osteoclast GC reactivity to osteoblast function (Kim et al., 2006). However, deleting the GR in osteoblasts themselves (Tuckermann, unpublished) also strongly ameliorated osteoblastic bone formation, while osteoclast numbers and activity remained intact. This is in agreement with studies of Manolagas and colleagues (Jia et al., 2006), which demonstrate that inhibition of GC action in osteoclasts in TRAP-11b-HSD2 transgenic mice still exhibit a decrease of osteoblast numbers and a decrease of bone formation. Thus, it seems so that inhibition of bone formation requires the cell autonomous GR of the osteoblasts, whereas actions of GCs in

osteoclasts contribute also to the decrease of bone mineral density occurring in glucocorticoid induced osteoporosis. Finally we subjected GR<sup>dim</sup> mice lacking the dimerization function of the receptor to high dose GC treatment and observed a similar inhibition of bone formation, osteoblast numbers and bone mineral density as in wild type mice (Tuckermann, unpublished). Interestingly, dimerization induced DNA binding is dispensable for this type of side effect and we have to hypothesize that protein-protein interactions with other transcription factors could be sufficient to mediate GC induced bone loss.

### **Conclusion**

The analysis of the GR<sup>dim</sup> mice in steroid therapy (Table 1) so far revealed that GC could successfully treat irritant dermatitis. GCs could efficiently suppress inflammatory regulators, such as TNF or MMP-1 and MMP-13 in these mutant mice, indicating that for therapeutic action of GC dimer-independent mechanisms of the GR are sufficient, such as tethering or interaction with MAPK-pathways. However, when the analysis of GR<sup>dim</sup> mice was expanded to other inflammatory processes, we observed a failure of GCs to exert a full anti-inflammatory response. This was the case for exogenous actions of GCs in contact allergy and for the modulatory role of endogenous GCs in septic shock and for the early response during wound healing exhibiting elevated cytokine and chemokine expression (Grose et al., 2002; Tuckermann et al., 2007). As a side effect GC inhibition of bone formation occur in these mice. Keeping the findings in GR<sup>dim</sup> mice in mind, what is the prediction for dissociating ligands, addressing the monomer function of the GR, the SEGRAs? Are they really potent to maintain therapeutic efficacy and reduce side effects? Although we do not know if the selective GR agonists (SEGRAs) do exactly mimic the GR<sup>dim</sup> mutation on the molecular level one could speculate that their success – dissociating

side effects from beneficial effects - depends on which conditions they are used for (Table 2). Whereas for contact allergy they may be less efficient, irritant dermatitis evoked by croton oil could be successfully cured as demonstrated (Schacke et al., 2004; Vayssiere et al., 1997) (Table 2). We would expect that effects on glucose homeostasis could be avoided with these compounds, but we would not assume that glucocorticoid induced osteoporosis would be prevented. However, some important inflammatory processes that are classically cured with steroids have not yet been analyzed with a SEGRA application or in the GR<sup>dim</sup> mice (Table 1 and 2). Also not all possible side effects have been extensively studied. Most importantly, the molecular mechanisms of GC suppression of asthma and rheumatoid arthritis have to be unraveled. Future research on these GC affected processes will tell if dissociating GR ligands are the solution for a low side-effect-therapy of these severe diseases affecting a major fraction of the population.

### **References:**

- Abraham, S.M., Lawrence, T., Kleiman, A., Warden, P., Medghalchi, M., Tuckermann, J., Saklatvala, J., Clark, A.R. 2006. Antiinflammatory effects of dexamethasone are partly dependent on induction of dual specificity phosphatase 1. *J Exp Med* 203, 1883-1889.
- Aderem, A., Ulevitch, R.J. 2000. Toll-like receptors in the induction of the innate immune response. *Nature* 406, 782-787.
- Akira, S., Uematsu, S., Takeuchi, O. 2006. Pathogen recognition and innate immunity. *Cell* 124, 783-801.
- Andrews, R.C., Walker, B.R. 1999. Glucocorticoids and insulin resistance: old hormones, new targets. *Clin Sci (Lond)* 96, 513-523.
- Askenase, P.W. 2001. Yes T cells, but three different T cells (alphabeta, gammadelta and NK T cells), and also B-1 cells mediate contact sensitivity. *Clin Exp Immunol* 125, 345-350.
- Auphan, N., DiDonato, J.A., Rosette, C., Helmberg, A., Karin, M. 1995. Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. *Science* 270, 286-290.

- Beato, M., Herrlich, P., Schütz, G. 1995. Steroid hormone receptors: many actors in search of a plot. *Cell* 83, 851-857.
- Belvisi, M.G., Wicks, S.L., Battram, C.H., Bottoms, S.E., Redford, J.E., Woodman, P., Brown, T.J., Webber, S.E., Foster, M.L. 2001. Therapeutic benefit of a dissociated glucocorticoid and the relevance of in vitro separation of transrepression from transactivation activity. *J Immunol* 166, 1975-1982.
- Bhattacharyya, S., Brown, D.E., Brewer, J.A., Vogt, S.K., Muglia, L.J. 2007. Macrophage glucocorticoid receptors regulate Toll-like receptor-4-mediated inflammatory responses by selective inhibition of p38 MAP kinase. *Blood*.
- Bledsoe, R.K., Montana, V.G., Stanley, T.B., Delves, C.J., Apolito, C.J., McKee, D.D., Consler, T.G., Parks, D.J., Stewart, E.L., Willson, T.M., et al. 2002. Crystal structure of the glucocorticoid receptor ligand binding domain reveals a novel mode of receptor dimerization and coactivator recognition. *Cell* 110, 93-105.
- Bledsoe, R.K., Stewart, E.L., Pearce, K.H. 2004. Structure and function of the glucocorticoid receptor ligand binding domain. *Vitam Horm* 68, 49-91.
- Caelles, C., Gonzalez-Sancho, J.M., Munoz, A. 1997. Nuclear hormone receptor antagonism with AP-1 by inhibition of the JNK pathway. *Genes Dev* 11, 3351-3364.
- Cairns, B.R., Levinson, R.S., Yamamoto, K.R., Kornberg, R.D. 1996. Essential role of Swp73p in the function of yeast Swi/Snf complex. *Genes Dev* 10, 2131-2144.
- Canalis, E., Delany, A.M. 2002. Mechanisms of glucocorticoid action in bone. *Ann N Y Acad Sci* 966, 73-81.
- Chen, D., Ma, H., Hong, H., Koh, S.S., Huang, S.M., Schurter, B.T., Aswad, D.W., Stallcup, M.R. 1999. Regulation of transcription by a protein methyltransferase. *Science* 284, 2174-2177.
- Chrysis, D., Ritzen, E.M., Savendahl, L. 2003. Growth retardation induced by dexamethasone is associated with increased apoptosis of the growth plate chondrocytes. *J Endocrinol* 176, 331-337.
- Chrysis, D., Zaman, F., Chagin, A.S., Takigawa, M., Savendahl, L. 2005. Dexamethasone induces apoptosis in proliferative chondrocytes through activation of caspases and suppression of the Akt-phosphatidylinositol 3'-kinase signaling pathway. *Endocrinology* 146, 1391-1397.
- Coghlan, M.J., Jacobson, P.B., Lane, B., Nakane, M., Lin, C.W., Elmore, S.W., Kym, P.R., Luly, J.R., Carter, G.W., Turner, R., et al. 2003. A novel antiinflammatory maintains glucocorticoid efficacy with reduced side effects. *Mol Endocrinol* 17, 860-869.
- D'Adamio, F., Zollo, O., Moraca, R., Ayroldi, E., Bruscoli, S., Bartoli, A., Cannarile, L., Migliorati, G., Riccardi, C. 1997. A new dexamethasone-induced gene of the leucine zipper family protects T lymphocytes from TCR/CD3-activated cell death. *Immunity* 7, 803-812.
- De Bosscher, K., Schmitz, M.L., Vanden Berghe, W., Plaisance, S., Fiers, W., Haegeman, G. 1997. Glucocorticoid-mediated repression of nuclear factor-kappaB-dependent transcription involves direct interference with transactivation. *Proc Natl Acad Sci U S A* 94, 13504-13509.

- De Bosscher, K., Vanden Berghe, W., Haegeman, G. 2001. Glucocorticoid repression of AP-1 is not mediated by competition for nuclear coactivators. *Mol Endocrinol* 15, 219-227.
- De Bosscher, K., Vanden Berghe, W., Haegeman, G. 2003. The interplay between the glucocorticoid receptor and nuclear factor-kappaB or activator protein-1: molecular mechanisms for gene repression. *Endocr Rev* 24, 488-522.
- De Kloet, E.R., Vreugdenhil, E., Oitzl, M.S., Joels, M. 1998. Brain corticosteroid receptor balance in health and disease. *Endocr Rev* 19, 269-301.
- De Luca, F. 2006. Impaired growth plate chondrogenesis in children with chronic illnesses. *Pediatric research* 59, 625-629.
- Delany, A.M., Durant, D., Canalis, E. 2001. Glucocorticoid suppression of IGF I transcription in osteoblasts. *Mol Endocrinol* 15, 1781-1789.
- Delaunay, F., Khan, A., Cintra, A., Davani, B., Ling, Z.C., Andersson, A., Ostenson, C.G., Gustafsson, J., Efendic, S., Okret, S. 1997. Pancreatic beta cells are important targets for the diabetogenic effects of glucocorticoids. *J Clin Invest* 100, 2094-2098.
- Devesa, J., Barros, M.G., Gondar, M., Tresguerres, J.A., Arce, V. 1995. Regulation of hypothalamic somatostatin by glucocorticoids. *The Journal of steroid biochemistry and molecular biology* 53, 277-282.
- Dostert, A., Heinzl, T. 2004. Negative glucocorticoid receptor response elements and their role in glucocorticoid action. *Current pharmaceutical design* 10, 2807-2816.
- Ehrchen, J., Steinmuller, L., Barczyk, K., Tenbrock, K., Nacken, W., Eisenacher, M., Nordhues, U., Sorg, C., Sunderkotter, C., Roth, J. 2007. Glucocorticoids induce differentiation of a specifically activated, anti-inflammatory subtype of human monocytes. *Blood* 109, 1265-1274.
- Evans, R.M. 1988. The steroid and thyroid hormone receptor superfamily. *Science* 240, 889-895.
- Freedman, N.D., Yamamoto, K.R. 2004. Importin 7 and importin alpha/importin beta are nuclear import receptors for the glucocorticoid receptor. *Mol Biol Cell* 15, 2276-2286.
- Gevers, E.F., van der Eerden, B.C., Karperien, M., Raap, A.K., Robinson, I.C., Wit, J.M. 2002. Localization and regulation of the growth hormone receptor and growth hormone-binding protein in the rat growth plate. *J Bone Miner Res* 17, 1408-1419.
- Göttlicher, M., Heck, S., Herrlich, P. 1998. Transcriptional cross-talk, the second mode of steroid hormone receptor. *J Mol Med* 76, 480-489.
- Grose, R., Werner, S., Kessler, D., Tuckermann, J., Huggel, K., Durka, S., Reichardt, H.M. 2002. A role for endogenous glucocorticoids in wound repair. *EMBO Rep* 3, 575-582.
- Gschwendt, M., Kittstein, W., Furstenberger, G., Marks, F. 1984. The mouse ear edema: a quantitatively evaluable assay for tumor promoting compounds and for inhibitors of tumor promotion. *Cancer Lett* 25, 177-185.
- Hanson, R.W., Reshef, L. 1997. Regulation of phosphoenolpyruvate carboxykinase (GTP) gene expression. *Annu Rev Biochem* 66, 581-611.

- Hartmann, C. 2006. A Wnt canon orchestrating osteoblastogenesis. *Trends in cell biology* 16, 151-158.
- He, Y., Simons, S.S., Jr. 2007. STAMP, a Novel Predicted Factor Assisting TIF2 Actions in Glucocorticoid Receptor-Mediated Induction and Repression. *Mol Cell Biol* 27, 1467-1485.
- Heck, S., Bender, K., Kullmann, M., Gottlicher, M., Herrlich, P., Cato, A.C. 1997. I kappaB alpha-independent downregulation of NF-kappaB activity by glucocorticoid receptor. *Embo J* 16, 4698-4707.
- Heck, S., Kullmann, M., Gast, A., Ponta, H., Rahmsdorf, H.J., Herrlich, P., Cato, A.C. 1994. A distinct modulating domain in glucocorticoid receptor monomers in the repression of activity of the transcription factor AP-1. *Embo J* 13, 4087-4095.
- Hofbauer, L.C., Gori, F., Riggs, B.L., Lacey, D.L., Dunstan, C.R., Spelsberg, T.C., Khosla, S. 1999. Stimulation of osteoprotegerin ligand and inhibition of osteoprotegerin production by glucocorticoids in human osteoblastic lineage cells: potential paracrine mechanisms of glucocorticoid-induced osteoporosis. *Endocrinology* 140, 4382-4389.
- Holland, W.L., Brozinick, J.T., Wang, L.P., Hawkins, E.D., Sargent, K.M., Liu, Y., Narra, K., Hoehn, K.L., Knotts, T.A., Siesky, A., et al. 2007. Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. *Cell metabolism* 5, 167-179.
- Horner, H.C., Munck, A., Lienhard, G.E. 1987. Dexamethasone causes translocation of glucose transporters from the plasma membrane to an intracellular site in human fibroblasts. *J Biol Chem* 262, 17696-17702.
- Itagane, Y., Inada, H., Fujita, K., Isshiki, G. 1991. Interactions between steroid hormones and insulin-like growth factor-I in rabbit chondrocytes. *Endocrinology* 128, 1419-1424.
- Ito, K., Barnes, P.J., Adcock, I.M. 2000. Glucocorticoid receptor recruitment of histone deacetylase 2 inhibits interleukin-1beta-induced histone H4 acetylation on lysines 8 and 12. *Mol Cell Biol* 20, 6891-6903.
- Jacobson, L., Ansari, T., Potts, J., McGuinness, O.P. 2006. Glucocorticoid-deficient corticotropin-releasing hormone knockout mice maintain glucose requirements but not autonomic responses during repeated hypoglycemia. *Am J Physiol Endocrinol Metab* 291, E15-22.
- James, C.G., Appleton, C.T., Ulici, V., Underhill, T.M., Beier, F. 2005. Microarray analyses of gene expression during chondrocyte differentiation identifies novel regulators of hypertrophy. *Mol Biol Cell* 16, 5316-5333.
- Jia, D., O'Brien, C.A., Stewart, S.A., Manolagas, S.C., Weinstein, R.S. 2006. Glucocorticoids act directly on osteoclasts to increase their life span and reduce bone density. *Endocrinology* 147, 5592-5599.
- Jonat, C., Rahmsdorf, H.J., Park, K.K., Cato, A.C., Gebel, S., Ponta, H., Herrlich, P. 1990. Antitumor promotion and antiinflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. *Cell* 62, 1189-1204.
- Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S.C., Heyman, R.A., Rose, D.W., Glass, C.K., et al. 1996. A CBP integrator complex

mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell* 85, 403-414.

Karsenty, G., Wagner, E.F. 2002. Reaching a genetic and molecular understanding of skeletal development. *Developmental cell* 2, 389-406.

Kassel, O., Sancono, A., Kratzschmar, J., Kreft, B., Stassen, M., Cato, A.C. 2001. Glucocorticoids inhibit MAP kinase via increased expression and decreased degradation of MKP-1. *Embo J* 20, 7108-7116.

Kassel, O., Schneider, S., Heilbock, C., Litfin, M., Gottlicher, M., Herrlich, P. 2004. A nuclear isoform of the focal adhesion LIM-domain protein Trip6 integrates activating and repressing signals at AP-1- and NF-kappaB-regulated promoters. *Genes Dev* 18, 2518-2528.

Kim, H.J., Zhao, H., Kitaura, H., Bhattacharyya, S., Brewer, J.A., Muglia, L.J., Ross, F.P., Teitelbaum, S.L. 2006. Glucocorticoids suppress bone formation via the osteoclast. *J Clin Invest* 116, 2152-2160.

Kino, T., Souvatzoglou, E., De Martino, M.U., Tsopanomihalu, M., Wan, Y., Chrousos, G.P. 2003. Protein 14-3-3sigma interacts with and favors cytoplasmic subcellular localization of the glucocorticoid receptor, acting as a negative regulator of the glucocorticoid signaling pathway. *J Biol Chem* 278, 25651-25656.

Koedam, J.A., Smink, J.J., van Buul-Offers, S.C. 2002. Glucocorticoids inhibit vascular endothelial growth factor expression in growth plate chondrocytes. *Mol Cell Endocrinol* 197, 35-44.

Limbourg, F.P., Huang, Z., Plumier, J.C., Simoncini, T., Fujioka, M., Tuckermann, J., Schutz, G., Moskowitz, M.A., Liao, J.K. 2002. Rapid nontranscriptional activation of endothelial nitric oxide synthase mediates increased cerebral blood flow and stroke protection by corticosteroids. *J Clin Invest* 110, 1729-1738.

Limbourg, F.P., Liao, J.K. 2003. Nontranscriptional actions of the glucocorticoid receptor. *J Mol Med* 81, 168-174.

Lin, C.W., Nakane, M., Stashko, M., Falls, D., Kuk, J., Miller, L., Huang, R., Tyree, C., Miner, J.N., Rosen, J., et al. 2002. trans-Activation and repression properties of the novel nonsteroid glucocorticoid receptor ligand 2,5-dihydro-9-hydroxy-10-methoxy-2,2,4-trimethyl-5-(1-methylcyclohexen-3-yl)-1H-[1]benzopyranof[3,4-f]quinoline (A276575) and its four stereoisomers. *Mol Pharmacol* 62, 297-303.

Luisi, B.F., Xu, W.X., Otwinowski, Z., Freedman, L.P., Yamamoto, K.R., Sigler, P.B. 1991. Crystallographic analysis of the interaction of the glucocorticoid receptor with DNA. *Nature* 352, 497-505.

Luppen, C.A., Leclerc, N., Noh, T., Barski, A., Khokhar, A., Boskey, A.L., Smith, E., Frenkel, B. 2003. Brief bone morphogenetic protein 2 treatment of glucocorticoid-inhibited MC3T3-E1 osteoblasts rescues commitment-associated cell cycle and mineralization without alteration of Runx2. *J Biol Chem* 278, 44995-45003.

Ma, H., Baumann, C.T., Li, H., Strahl, B.D., Rice, R., Jelinek, M.A., Aswad, D.W., Allis, C.D., Hager, G.L., Stallcup, M.R. 2001. Hormone-dependent, CARM1-directed, arginine-specific methylation of histone H3 on a steroid-regulated promoter. *Curr Biol* 11, 1981-1985.

- Mazziotti, G., Angeli, A., Bilezikian, J.P., Canalis, E., Giustina, A. 2006. Glucocorticoid-induced osteoporosis: an update. *Trends Endocrinol Metab* 17, 144-149.
- McNally, J.G., Muller, W.G., Walker, D., Wolford, R., Hager, G.L. 2000. The glucocorticoid receptor: rapid exchange with regulatory sites in living cells. *Science* 287, 1262-1265.
- Meade, M. 2005. Review: glucocorticoids reduced mortality in sepsis in recent (post-1997) but not previous (pre-1989) trials, or all trials combined. *ACP J Club* 142, 31.
- Metivier, R., Penot, G., Hubner, M.R., Reid, G., Brand, H., Kos, M., Gannon, F. 2003. Estrogen receptor-alpha directs ordered, cyclical, and combinatorial recruitment of cofactors on a natural target promoter. *Cell* 115, 751-763.
- Miesfeld, R., Okret, S., Wikstrom, A.C., Wrangé, O., Gustafsson, J.A., Yamamoto, K.R. 1984. Characterization of a steroid hormone receptor gene and mRNA in wild-type and mutant cells. *Nature* 312, 779-781.
- Mittelstadt, P.R., Ashwell, J.D. 2001. Inhibition of AP-1 by the glucocorticoid-inducible protein GILZ. *J Biol Chem* 276, 29603-29610.
- Mocetti, P., Silvestrini, G., Ballanti, P., Patacchioli, F.R., Di Grezia, R., Angelucci, L., Bonucci, E. 2001. Bcl-2 and Bax expression in cartilage and bone cells after high-dose corticosterone treatment in rats. *Tissue & cell* 33, 1-7.
- Nissen, R.M., Yamamoto, K.R. 2000. The glucocorticoid receptor inhibits NFkappaB by interfering with serine-2 phosphorylation of the RNA polymerase II carboxy-terminal domain. *Genes Dev* 14, 2314-2329.
- Nocentini, G., Giunchi, L., Ronchetti, S., Krausz, L.T., Bartoli, A., Moraca, R., Migliorati, G., Riccardi, C. 1997. A new member of the tumor necrosis factor/nerve growth factor receptor family inhibits T cell receptor-induced apoptosis. *Proc Natl Acad Sci U S A* 94, 6216-6221.
- Ogawa, S., Lozach, J., Benner, C., Pascual, G., Tangirala, R.K., Westin, S., Hoffmann, A., Subramaniam, S., David, M., Rosenfeld, M.G., et al. 2005. Molecular determinants of crosstalk between nuclear receptors and toll-like receptors. *Cell* 122, 707-721.
- Opherck, C., Tronche, F., Kellendonk, C., Kohlmuller, D., Schulze, A., Schmid, W., Schutz, G. 2004. Inactivation of the glucocorticoid receptor in hepatocytes leads to fasting hypoglycemia and ameliorates hyperglycemia in streptozotocin-induced diabetes mellitus. *Mol Endocrinol* 18, 1346-1353.
- Peeke, P.M., Chrousos, G.P. 1995. Hypercortisolism and obesity. *Ann N Y Acad Sci* 771, 665-676.
- Pereira, R.M., Delany, A.M., Canalis, E. 2001. Cortisol inhibits the differentiation and apoptosis of osteoblasts in culture. *Bone* 28, 484-490.
- Pratt, W.B., Gehring, U., Toft, D.O. 1996. Molecular chaperoning of steroid hormone receptors. *Stress-Inducible Cellular Responses*, 79-95.
- Ray, A., Prefontaine, K.E. 1994. Physical association and functional antagonism between the p65 subunit of transcription factor NF-kappa B and the glucocorticoid receptor. *Proc Natl Acad Sci U S A* 91, 752-756.

- Reichardt, H.M., Kaestner, K.H., Tuckermann, J., Kretz, O., Wessely, O., Bock, R., Gass, P., Schmid, W., Herrlich, P., Angel, P., et al. 1998. DNA binding of the glucocorticoid receptor is not essential for survival. *Cell* 93, 531-541.
- Reichardt, H.M., Tuckermann, J.P., Göttlicher, M., Vujic, M., Weih, F., Angel, P., Herrlich, P., Schütz, G. 2001. Repression of inflammatory responses in the absence of DNA binding by the glucocorticoid receptor. *Embo J* 20, 7168-7173.
- Reily, M.M., Pantoja, C., Hu, X., Chinenov, Y., Rogatsky, I. 2006. The GRIP1:IRF3 interaction as a target for glucocorticoid receptor-mediated immunosuppression. *Embo J* 25, 108-117.
- Rogatsky, I., Wang, J.C., Derynck, M.K., Nonaka, D.F., Khodabakhsh, D.B., Haqq, C.M., Darimont, B.D., Garabedian, M.J., Yamamoto, K.R. 2003. Target-specific utilization of transcriptional regulatory surfaces by the glucocorticoid receptor. *Proc Natl Acad Sci U S A* 100, 13845-13850.
- Schaaf, M.J., Cidlowski, J.A. 2002. Molecular mechanisms of glucocorticoid action and resistance. *J Steroid Biochem Mol Biol* 83, 37-48.
- Schacke, H., Schottelius, A., Docke, W.D., Strehlke, P., Jaroch, S., Schmees, N., Rehwinkel, H., Hennekes, H., Asadullah, K. 2004. Dissociation of transactivation from transrepression by a selective glucocorticoid receptor agonist leads to separation of therapeutic effects from side effects. *Proc Natl Acad Sci U S A* 101, 227-232.
- Scheinman, R.I., Cogswell, P.C., Lofquist, A.K., Baldwin, A.S., Jr. 1995a. Role of transcriptional activation of I kappa B alpha in mediation of immunosuppression by glucocorticoids. *Science* 270, 283-286.
- Scheinman, R.I., Gualberto, A., Jewell, C.M., Cidlowski, J.A., Baldwin, A.S., Jr. 1995b. Characterization of mechanisms involved in transrepression of NF-kappa B by activated glucocorticoid receptors. *Mol Cell Biol* 15, 943-953.
- Schmid, E., Schmid, W., Jantzen, M., Mayer, D., Jastorff, B., Schutz, G. 1987. Transcription activation of the tyrosine aminotransferase gene by glucocorticoids and cAMP in primary hepatocytes. *Eur J Biochem* 165, 499-506.
- Schoepe, S., Schacke, H., May, E., Asadullah, K. 2006. Glucocorticoid therapy-induced skin atrophy. *Exp Dermatol* 15, 406-420.
- Schüle, R., Rangarajan, P., Kliewer, S., Ransone, L.J., Bolado, J., Yang, N., Verma, I.M., Evans, R.M. 1990. Functional antagonism between oncoprotein c-Jun and the glucocorticoid receptor. *Cell* 62, 1217-1226.
- Shalhoub, V., Conlon, D., Tassinari, M., Quinn, C., Partridge, N., Stein, G.S., Lian, J.B. 1992. Glucocorticoids promote development of the osteoblast phenotype by selectively modulating expression of cell growth and differentiation associated genes. *J Cell Biochem* 50, 425-440.
- Shiau, A.K., Barstad, D., Loria, P.M., Cheng, L., Kushner, P.J., Agard, D.A., Greene, G.L. 1998. The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell* 95, 927-937.
- Smink, J.J., Buchholz, I.M., Hamers, N., van Tilburg, C.M., Christis, C., Sackers, R.J., de Meer, K., van Buul-Offers, S.C., Koedam, J.A. 2003a. Short-term glucocorticoid treatment of piglets causes changes in growth plate morphology and angiogenesis. *Osteoarthritis Cartilage* 11, 864-871.

- Smink, J.J., Gresnigt, M.G., Hamers, N., Koedam, J.A., Berger, R., Van Buul-Offers, S.C. 2003b. Short-term glucocorticoid treatment of prepubertal mice decreases growth and IGF-I expression in the growth plate. *J Endocrinol* 177, 381-388.
- Smith, E., Redman, R.A., Logg, C.R., Coetzee, G.A., Kasahara, N., Frenkel, B. 2000. Glucocorticoids inhibit developmental stage-specific osteoblast cell cycle. Dissociation of cyclin A-cyclin-dependent kinase 2 from E2F4-p130 complexes. *J Biol Chem* 275, 19992-20001.
- Stöcklin, E., Wissler, M., Gouilleux, F., Groner, B. 1996. Functional interactions between Stat5 and the glucocorticoid receptor. *Nature* 383, 726-728.
- Stoecklin, E., Wissler, M., Moriggl, R., Groner, B. 1997. Specific DNA binding of Stat5, but not of glucocorticoid receptor, is required for their functional cooperation in the regulation of gene transcription. *Mol Cell Biol* 17, 6708-6716.
- Takayama, S., Rogatsky, I., Schwarcz, L.E., Darimont, B.D. 2006. The glucocorticoid receptor represses cyclin D1 by targeting the Tcf-beta-catenin complex. *J Biol Chem* 281, 17856-17863.
- Tronche, F., Opherck, C., Moriggl, R., Kellendonk, C., Reimann, A., Schwake, L., Reichardt, H.M., Stangl, K., Gau, D., Hoeflich, A., et al. 2004. Glucocorticoid receptor function in hepatocytes is essential to promote postnatal body growth. *Genes Dev* 18, 492-497.
- Tuckermann, J.P., Kleiman, A., Moriggl, R., Spanbroek, R., Neumann, A., Illing, A., Clausen, B.E., Stride, B., Förster, I., Habenicht, A.J.R., et al. 2007. Macrophages and neutrophils are the targets for immune suppression by glucocorticoids in contact allerg. *J Clin Invest* 117, 1381-1390.
- Tuckermann, J.P., Reichardt, H.M., Arribas, R., Richter, K.H., Schutz, G., Angel, P. 1999. The DNA binding-independent function of the glucocorticoid receptor mediates repression of AP-1-dependent genes in skin. *J Cell Biol* 147, 1365-1370.
- Van Staa, T.P., Leufkens, H.G., Abenhaim, L., Zhang, B., Cooper, C. 2000. Use of oral corticosteroids and risk of fractures. *J Bone Miner Res* 15, 993-1000.
- Vayssiere, B.M., Dupont, S., Choquart, A., Petit, F., Garcia, T., Marchandeu, C., Gronemeyer, H., Resche-Rigon, M. 1997. Synthetic glucocorticoids that dissociate transactivation and AP-1 transrepression exhibit antiinflammatory activity in vivo. *Mol Endocrinol* 11, 1245-1255.
- Wang, J.C., Derynck, M.K., Nonaka, D.F., Khodabakhsh, D.B., Haqq, C., Yamamoto, K.R. 2004. Chromatin immunoprecipitation (ChIP) scanning identifies primary glucocorticoid receptor target genes. *Proc Natl Acad Sci U S A* 101, 15603-15608.
- Wehrenberg, W.B., Giustina, A. 1992. Basic counterpoint: mechanisms and pathways of gonadal steroid modulation of growth hormone secretion. *Endocr Rev* 13, 299-308.
- Weinstein, R.S., Jilka, R.L., Parfitt, A.M., Manolagas, S.C. 1998. Inhibition of osteoblastogenesis and promotion of apoptosis of osteoblasts and osteocytes by glucocorticoids. Potential mechanisms of their deleterious effects on bone. *J Clin Invest* 102, 274-282.
- Yang Yen, H.F., Chambard, J.C., Sun, Y.L., Smeal, T., Schmidt, T.J., Drouin, J., Karin, M. 1990. Transcriptional interference between c-Jun and the glucocorticoid

receptor: mutual inhibition of DNA binding due to direct protein-protein interaction. *Cell* 62, 1205-1215.

Yeager, M.P., Guyre, P.M., Munck, A.U. 2004. Glucocorticoid regulation of the inflammatory response to injury. *Acta Anaesthesiol Scand* 48, 799-813.

Yona, S., Gordon, S. 2007. Inflammation: Glucocorticoids turn the monocyte switch. *Immunol Cell Biol*.

Yoshinaga, S.K., Peterson, C.L., Herskowitz, I., Yamamoto, K.R. 1992. Roles of SWI1, SWI2, and SWI3 proteins for transcriptional enhancement by steroid receptors. *Science* 258, 1598-1604.

Zhang, Z., Jones, S., Hagood, J.S., Fuentes, N.L., Fuller, G.M. 1997. STAT3 acts as a co-activator of glucocorticoid receptor signaling. *J Biol Chem* 272, 30607-30610.

Zierath, J.R. 2007. The path to insulin resistance: paved with ceramides? *Cell metabolism* 5, 161-163.

**Figure Legends****Figure 1: Dimer dependent and independent control of gene expression by the**

**GR.** Schematic representation of the two major mechanisms for the regulation of gene expression by the ligand bound GR. On the left the cartoon shows the GR dimer bound to the palindromic GRE. This complex transactivates genes by interaction of the helix 12 of the GR with the LXXL motif present in the NR interaction box of co-activators of the p160 family. Interaction and recruitment of CBP/p300 coactivator/histone acetyl transferase (HAT) and numerous other coactivator complexes and HATs finally leads to decondensation of chromatin and enhanced transcriptional synthesis of mRNA molecules of the GR target gene. On the right the dimerization and DNA binding independent mechanism is shown. The GR molecule can interfere either with the activity of signal transduction components by direct or indirect mechanisms resulting in an inhibition of p38 and JNK activity or an enhancement of PI3K activity. In the nucleus the monomeric GR can influence the activity of pro-inflammatory transcription factors NF- $\kappa$ B, AP-1, IRF-3 and other factors. Co-integrators mediate this interaction as the nuclear isoform of the Trip-6 protein and STAMP or by competing out co-activators, such as the p160 family protein GRIP1. Traditionally interference of pro-inflammatory transcription factors is regarded as the molecular basis of anti-inflammatory effects of GC (Dr. Jekyll), whereas DNA binding contributes to the side effects of GC (Mr. Hyde). Recent studies show that for side effects such as glucocorticoid induced osteoporosis tethering might be sufficient and for full therapeutic efficacy of GC in contact dermatitis and for the endogenous actions of GC also dimerization induced DNA binding is required.

**Figure 2: Contact hypersensitivity (CHS) responses are suppressed by GC via the GR in macrophages, require dimerization of the GR and the suppression of cytokines and chemokines.** CHS is evoked by haptens, which are at least two times applied to the skin and therefore involve two phases: sensitization and elicitation. After the first exposure to the hapten “immature” dendritic cells of the dermis (DC) take up the hapten-protein complexes (1), and migrate to the draining lymph node (2). There the mature DC present the hapten by their surface MHC type II molecules to naïve T cells that recognize a matching hapten/MHCII complex with their T cell receptor and are *sensitized* by costimulation via B7 molecules from DCs by interaction of CD28 molecules (3). Sensitized T cells proliferate and repopulate the body (4). The elicitation phase starts by the second exposure of the same hapten to the skin (5). The endothelium (Endothel) becomes activated and allows the rapid entry of sensitized T cells (6), which in the skin are potently activated by hapten presenting DC (7). Those T cells trigger a massive activation of macrophages in the skin, which in turn release inflammatory mediators (8). These mediators (IL-1b, MCP-2, MIP-1 IP-10) lead to an influx of leukocytes, such as neutrophils (PMN) and monocytes (Mono) and manifest an inflammatory edema (9). The suppressive effect of GCs (10) is critical in macrophages and requires dimerization of the GR, since GR<sup>lysMCre</sup> mice and GR<sup>dim</sup> mice are resistant against GC suppression of CHS. GC action in keratinocytes and T cells is not sufficient, because mice lacking the GR in those cells (GR<sup>lckCre</sup>, GR<sup>K14Cre</sup>) can be cured with GCs.

**Phenotypes of pharmacological GC administration in GR<sup>dim</sup> mice**

Process	Phenotype
<b>Beneficial Effects of GC</b>	
Treatment of irritant dermatitis	like in wild type (Reichardt et al., 2001)
Treatment of CHS	impaired (Tuckermann et al., 2007)
Suppression of inflammatory mediators	like in wild type: TNF $\alpha$ (Tuckermann et al., 2007), MMP-13, MMP-9 (Tuckermann et al., 1999) impaired: MCP-1, IP-10, IL-1b (Grose et al., 2002; Tuckermann et al., 2007)
PI3K coactivation by high dose GC, important for steroid protection from stroke	like in wild type: PI3K activity normal in GR <sup>dim</sup> MEFs (Limbourg et al., 2002)
<b>Side Effects</b>	
Induction of catabolic enzymes	impaired: TAT, PEPCK (Reichardt et al., 1998), (unpubl.)
GC induced osteoporosis	like in wild type (Tuckermann, unpubl.)
<b>Endogenous Actions of GC</b>	
Survival of Sepsis	impaired (Kleyman et al., unpubl.)
Wound healing	like in wild type; but delayed kinetics (Grose et al., 2002)
Hepatic GH activity controlling body growth	like in wild type (Tronche et al., 2004)

Table 1: Phenotypes of pharmacological GC administration in GR<sup>dim</sup> mice

**Examples of beneficial and side effects of SEGRAs in vivo**

SEGRA-Compound	Anti-inflammatory effect	Adverse effect
<b>RU24858</b> (Belvisi et al., 2001; Vayssiere et al., 1997)	croton-oil induced ear edema <i>efficiently reduced</i> , cotton-pellet granuloma model <i>efficiently reduced</i> , Sevhadex-model of lung edema <i>efficiently reduced</i>	body weight loss <i>not changed</i> , thymus involution <i>not changed</i> , osteopenia <i>not changed</i>
<b>ZK216348</b> (Schacke et al., 2004)	croton-oil induced ear edema <i>efficiently reduced</i>	body weight reduction <i>decreased</i> , blood glucose elevation <i>decreased</i> , spleen involution <i>decreased</i> , skin atrophy slightly <i>decreased</i> , adrenocorticotrophic hormone suppression <i>not changed</i>
<b>AL-438</b> (Coghlan et al., 2003)	carageenan-induced arthritis, adjuvant induced arthritis <i>efficiently reduced</i>	hyperglycemia <i>decreased</i> , inhibition of bone apposition <i>decreased</i>

Table 2: Examples of SEGRAs tested in vivo for anti-inflammatory activities and adverse effects in comparisons with conventional GCs

**Acknowledgements:**

This work was generally supported by the Deutsche Forschungsgemeinschaft (DFG Tu-220/3).

Dimerization-induced  
DNA Binding

Dimerization-independent  
Gene regulation



