Published in final edited form as:
*J Investig Med*. 2010 January ; 58(1): 19–22. doi:10.231/JIM.0b013e3181b91654.

# Glucocorticoid Efficacy in Asthma: Is Improved Tissue Remodeling Upstream of Anti-Inflammation

**Robert J. Freishtat**[1,2,3,*], **Kanneboyina Nagaraju**[2,3], **William Jusko**[4], and **Eric P. Hoffman**[2,3]

[1]Division of Emergency Medicine, Children's National Medical Center, 111 Michigan Avenue, NW, Washington, DC 20010, United States

[2]Research Center for Genetic Medicine Children's National Medical Center, 111 Michigan Avenue, NW, Washington, DC 20010, United States

[3]School of Medicine and Health Sciences, The George Washington University, 2300 I Street, NW, Washington, DC 20037, United States

[4]Department of Pharmaceutical Sciences, University at Buffalo, Buffalo, NY 14260.

## Abstract

Synthetic glucocorticoids, such as prednisone, are among the most widely prescribed drugs worldwide and are used to treat many acute and chronic inflammatory conditions. The current paradigm of glucocorticoid efficacy is that they are potent anti-inflammatory agents. Decreased inflammation in many disorders is thought to lead to decreased pathological tissue remodeling. However, this model has never been validated. In particular, improvements in inflammation have not been shown to improve the rate of lung function decline in asthma. Herein, we present an alternative paradigm, where GC efficacy is mediated through more successful tissue remodeling, with reduction in inflammation secondary to successful regeneration.

### Keywords

Glucocorticoid; Asthma; Tissue Remodeling; Lung; Inflammation

## Introduction

Synthetic glucocorticoids (GCs), such as prednisone, are among the most widely prescribed drugs worldwide and are used to treat many acute and chronic inflammatory conditions.[1] GCs are very old drugs, yet they remain the standard of care for treatment of a variety of diseases including asthma, muscular dystrophy, autoimmune disorders, and arthritis. Other newer and more potent or targeted immunosuppressants have been tried in many of these disorders, but they have not shown the same efficacy as prednisone.[2-4] Additionally, chronic use of prednisone is associated with significant negative side effects. It is clear that a greater understanding of the molecular action of GCs could lead to more optimized drugs and dosing regimens that improve efficacy and reduce side effects.

GCs exert anti-inflammatory effects in large part through inhibition of nuclear factor (NF) κB and NFκB downstream inflammatory targets.[5] It is important to note that GCs have both acute effects on protein-protein signaling occurring in minutes, as well as longer acting effects on mRNA transcription via GC receptor (GR) binding to target promoters (hours).

*Corresponding Author (RJF); rfreishtat@cnmcresearch.org; Phone: (202) 476-2971; Fax: (202) 476-6014.

The acute non-transcriptional activities via NFκB appear responsible for the majority of anti-inflammatory effects of GCs, while the receptor-mediated transcriptional activities account for most side effects.[5]

The current paradigm of GC efficacy is that efficacy is mediated through a direct anti-inflammatory role. Decreased inflammation in these disorders is thought to lead to decreased pathological tissue remodeling. However, this model has never been validated. In particular, improvements in inflammation have not been shown to improve the rate of lung function decline in asthma. Herein, we present an alternative paradigm, where GC efficacy is mediated through more successful tissue remodeling, with reduction in inflammation secondary to successful regeneration.

## Mechanisms of GC action

Molecular mechanistic studies of GCs in lung inflammation have focused on their impact on transcriptional targets of the hormone/receptor complex.[6–8] However, it is widely recognized that the anti-inflammatory activities are not a direct consequence of GC/GR complex binding to gene targets, but rather GC effects on NFκB protein signaling (either with or without receptor involvement). Importantly, GCs are known to have both transcriptional and signaling effects in the lung. They endogenously mediate physiological processes such as cortisol suppression, intracellular signaling and trafficking.[9]

Many of the effects of GCs occur through binding and activation of the GC receptor (GR) that is expressed in all tissues. The GR has many isoforms, each with different ligand and binding affinities and properties, tissue-specific effects, and non-receptor (signaling) activities. The best characterized effect of GCs is their ability to bind soluble steroid hormone receptors, and then move to the nucleus where the receptor complex directly binds to promoter elements and mediates gene transcription. In the case of GCs, there are well studied steroid response *cis*-elements (sRE) in target gene promoters that are responsible for binding the ligand/receptor complex, with downstream modulation of gene transcription.[10, 11] (Figure 1) Ligand-activated GR dimers bind directly to sRE *cis*-elements and directly or indirectly recruit molecules with histone acetyl transferase (HAT) activity, resulting in acetylation of lysines on H4 or H3, which opens up chromatin to facilitate gene transcription. Coactivators with HAT activity include the CREB binding protein (CBP) and the p300/CBP-associated factor (PCAF).[12, 13] These coactivators can also recruit other HATs.[14] As an example, an *in vivo* temporal microarray study looking at the effects of a bolus of methylprednisolone in adrenalectomized rats showed approximately 200 genes differentially regulated at two hours.[15]

A *second* mechanism of GC action focuses on inflammatory genes that lack sRE *cis*-sites in their promoters, but have functional NF-κB or AP-1 *cis*-sites that bind inflammatory mediator-induced transcription factors.[16, 17] Ligand-activated GR binds to these activated transcription factors, thereby blocking their binding to target *cis*-sites and up-regulation of target genes by GR-mediated *trans*-repression. Ligand activated GR also binds to and inhibits coactivators with intrinsic HAT activity, resulting in inhibition of HAT activity, recruitment of histone deacetylases (HDACs), a decrease in histone acetylation, and a reduction in gene expression.

Additionally, GCs have a less characterized non-transcriptional (signaling) response. Prednisone has been shown to acutely activate the PKC-dependent mitogen activated protein kinase (MAPK) pathway via putative G-protein coupled receptors in immune, brain, and lung cells.[18, 19] Other than the well characterized translocation of the GR from the cytosol to the nucleus, a few proteins, such as annexin A1, 5-lipoxygenase, and S100A11 have been shown to translocate in response to GCs.[18, 20] Synthetic GCs have also been shown in

multiple tissues to decrease membrane fluidity by altering the cholesterol/phospholipids ratio.[21, 22]

## The current paradigm: Inflammation leads to remodeling in asthma

Asthma is a complex, multifactorial disease comprising multiple different subtypes, rather than a single disease entity.[23] Asthma is a chronic disease of the lower respiratory tract characterized by inflammation, airway hyperresponsiveness, and mucus obstruction in the airways. It reflects airway remodeling by goblet cell hyperplasia, mucus hypersecretion, smooth muscle hyperplasia, fibroblast proliferation, angiogenesis, and increased collagen deposition.[24–27]

Oral and inhaled GC's are the mainstay of acute and chronic asthma therapy. GC's, such as prednisone and fluticasone, are commonly used for their potent ability to modulate the inflammation that leads to acute exacerbations of asthma symptoms. For example, daily inhaled fluticasone reduces the frequency of acute asthma symptoms.[28] As a result, depending on the severity and frequency of asthma symptoms, daily inhaled GC's are the standard of asthma care.[29]

Under the current paradigm, where asthmatic inflammation is assumed to lead to pathological tissue remodeling, it follows that anti-inflammatory treatment with GCs would diminish the typical accelerated loss of lung function over time in individuals with asthma.[30] However, this is not the case. The Childhood Asthma Management Program (CAMP) studied the lung function of 5–12 year olds with mild-moderate asthma being treated with budesonide or placebo. Although the budesonide group showed an initial improvement in lung function, this difference disappeared by the end of the four year study period.[31] This finding has been confirmed repeatedly, most recently in the Inhaled Treatment as Regular Therapy in Early Asthma (START) Study.[32] These investigators showed the same disappearance of lung function benefit from inhaled GCs (i.e. budesonide) in children and adults after a five year study period. Further, the bronchial reticular layer thickness in asthmatics treated with inhaled GCs is not diminished unless high doses are used.[33]

## GC regulation of the cell cycle

Endogenous GC expression is tightly controlled by a system of regulatory loops known as the hypothalamic-pituitary-adrenal (HPA) axis. Corticotropin-releasing hormone (CRH) is secreted by the hypothalamus and stimulates secretion of adrenocorticotropic hormone (ACTH) by the anterior pituitary. ACTH in turn stimulates the adrenal cortex to synthesize and secrete GCs, most notably cortisol and corticosterone. These secretions are pulsatile and occur as circadian rhythms entrained by the internal clock, the suprachiasmatic nucleus (SCN), and the light-dark cycle. This circadian rhythm of GCs further serves to reset the internal and peripheral clocks of the organism, including lung and lung-relevant cytokines such as IL-5.[34] The HPA axis is regulated by a negative feedback inhibition loop where GCs directly down-regulate secretion of CRH and ACTH, and by a positive feedback excitation loop where epinephrine and norepinephrine increase ACTH synthesis after being secreted in response to fear or stress. GCs are highly expressed around the start of the organism's period of activity/waking (morning for diurnal, evening for nocturnal), and decrease over the course of this period.

The normal cell cycle consists of two mitotic stages separated by two "gap" stages where DNA is assessed for damage and can be repaired. (Figure 2) In peripheral tissues, such as lung, mitosis is regulated by a negative feedback loop among circadian oscillator gene products. Bmal1-Clock protein heterodimers enhance the expression of the Period genes, PER1, PER2, and PER3. These negatively feedback to inhibit the formation of Bmal1-Clock

heterodimers.[35] In addition, circadian oscillator gene expression is also regulated by endogenous GCs directly or indirectly through multiple signaling pathways.[15, 36–38] In particular, PER1 has a GRE in its promoter sequence[39–41] which increases PER1 transcription.[42]

Ironically, the circadian peak (i.e. pre-waking hours) in endogenous cortisol corresponds to an asthmatic clinical trough: minimum lung function[43] and maximum inflammation.[34, 44, 45] Interestingly, chronotherapeutic trials of exogenous GCs in nocturnal asthma have found improved lung function, symptoms, and inflammation with a single late afternoon dose compared to the usual AM or even later PM dosing.[46–48] Knowledge is sparse regarding how circadian oscillators may contribute to asthma pathobiology. However, there is evidence of a GC-regulated circadian clock in the lung mediated by Clara cells.[49] Further, there is evidence of circadian variation in airway caliber, inflammation, and hyperreactivity.[50]

It should be noted that there is mounting evidence that many drug classes, including GCs, should be administered with respect to either the circadian rhythm of the disease's symptoms or of the endogenous analogues. Chronopharmacology and chronotherapy are currently being applied to diseases such as asthma, hypertension, rheumatoid arthritis, cancer, and rhinitis.[50–52]

### Future Directions

Pharmacologic analogues of cortisol have been the standard treatment to generate and maintain a state of anti-inflammation in asthmatic patients for decades. As described, control of inflammation does not equate to restoration of normal lung remodeling. Studies need to be designed to test the hypothesis described herein, that GC efficacy is mediated through more successful tissue remodeling, with reduction in inflammation secondary to successful regeneration. In vitro experiments of pulse GC dosing of primary human airway epithelial cells from normal and asthmatic donors can be a useful first step. Animal models of chronotherapeutic dosing of GCs in asthma can provide complementary data. Additionally, the relationships between GCs, circadian oscillator gene expression, and lung fibrosis/inflammation needs to be studied. The results of these investigations would likely lead to optimized treatment regimens for asthma.

## Acknowledgments

**Grants**

Funding support provided by the National Institutes of Health, Bethesda, Maryland, USA via grant K23-RR-020069 to RJF and institutional funding from Children's National Medical Center.

## References

1. Larj M, Bleecker E. Therapeutic responses in asthma and COPD. Corticosteroids. Chest. 2004; 126:138S–149S.
2. Kissel JTM, Lynn DJM, Rammohan KWM, et al. Mononuclear cell analysis of muscle biopsies in prednisone- and azathioprine-treated Duchenne muscular dystrophy. Neurology. 1993; 43:532–536. [PubMed: 8450996]
3. Griggs RCM, Moxley RTIM, Mendell JRM, et al. Duchenne dystrophy: Randomized, controlled trial of prednisone (18 months) and azathioprine (12 months). Neurology. 1993; 43:520–527. [PubMed: 8450994]
4. Weller B, Massa R, Karpati G, Carpenter S. Glucocorticoids and immunosup pressants do not change the prevalence of necrosis and regeneration in mdx skeletal muscles. Muscle & Nerve. 1991; 14:771–774. [PubMed: 1891001]

5. Clark AR. Anti-inflammatory functions of glucocorticoid-induced genes. Mol Cell Endocrinol. 2007; 275:79–97. [PubMed: 17561338]
6. Adcock IM, Ito K. Glucocorticoid pathways in chronic obstructive pulmonary disease therapy. Proc Am Thorac Soc. 2005; 2:313–319. discussion 40-1. [PubMed: 16267355]
7. Ito K, Barnes PJ, Adcock IM. Glucocorticoid receptor recruitment of histone deacetylase 2 inhibits interleukin-1beta-induced histone H4 acetylation on lysines 8 and 12. Mol Cell Biol. 2000; 20:6891–6903. [PubMed: 10958685]
8. Kagoshima M, Wilcke T, Ito K, et al. Glucocorticoid-mediated transrepression is regulated by histone acetylation and DNA methylation. Eur J Pharmacol. 2001; 429:327–334. [PubMed: 11698053]
9. Heitzer MD, Wolf IM, Sanchez ER, Witchel SF, DeFranco DB. Glucocorticoid receptor physiology. Rev Endocr Metab Disord. 2007; 8:321–330. [PubMed: 18049904]
10. Herrlich P. Cross-talk between glucocorticoid receptor and AP-1. Oncogene. 2001; 20:2465–2475. [PubMed: 11402341]
11. Coghlan MJ, Elmore SW, Kym PR, Kort ME. The pursuit of differentiated ligands for the glucocorticoid receptor. Curr Top Med Chem. 2003; 3:1617–1635. [PubMed: 14683518]
12. Bannister AJ, Kouzarides T. The CBP co-activator is a histone acetyltransferase. Nature. 1996; 384:641–643. [PubMed: 8967953]
13. Ogryzko VV, Schiltz RL, Russanova V, Howard BH, Nakatani Y. The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell. 1996; 87:953–959. [PubMed: 8945521]
14. Yang XJ, Ogryzko VV, Nishikawa J, Howard BH, Nakatani Y. A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. Nature. 1996; 382:319–324. [PubMed: 8684459]
15. Almon RR, DuBois DC, Yao Z, Hoffman EP, Ghimbovschi S, Jusko WJ. Microarray analysis of the temporal response of skeletal muscle to methylprednisolone: comparative analysis of two dosing regimens. Physiol Genomics. 2007; 30:282–299. [PubMed: 17473217]
16. Schoneveld OJ, Gaemers IC, Lamers WH. Mechanisms of glucocorticoid signalling. Biochim Biophys Acta. 2004; 1680:114–128. [PubMed: 15488991]
17. Pelaia G, Vatrella A, Cuda G, Maselli R, Marsico SA. Molecular mechanisms of corticosteroid actions in chronic inflammatory airway diseases. Life Sci. 2003; 72:1549–1561. [PubMed: 12551744]
18. Solito E, Mulla A, Morris JF, Christian HC, Flower RJ, Buckingham JC. Dexamethasone induces rapid serine-phosphorylation and membrane translocation of annexin 1 in a human folliculostellate cell line via a novel nongenomic mechanism involving the glucocorticoid receptor, protein kinase C, phosphatidylinositol 3-kinase, and mitogen-activated protein kinase. Endocrinology. 2003; 144:1164–1174. [PubMed: 12639897]
19. ten Hove W, Houben LA, Raaijmakers JA, Koenderman L, Bracke M. Rapid selective priming of FcalphaR on eosinophils by corticosteroids. J Immunol. 2006; 177:6108–6114. [PubMed: 17056537]
20. Dezitter X, Hammoudi F, Belverge N, et al. Proteomics unveil corticoid-induced S100A11 shuttling in keratinocyte differentiation. Biochem Biophys Res Commun. 2007; 360:627–632. [PubMed: 17624315]
21. Gerritsen ME, Schwarz SM, Medow MS. Glucocorticoid-mediated alterations in fluidity of rabbit cardiac muscle microvessel endothelial cell membranes: influences on eicosanoid release. Biochim Biophys Acta. 1991; 1065:63–68. [PubMed: 2043652]
22. Dudeja PK, Dahiya R, Brown MD, Brasitus TA. Dexamethasone influences the lipid fluidity, lipid composition and glycosphingolipid glycosyltransferase activities of rat proximal-small-intestinal Golgi membranes. Biochem J. 1988; 253:401–408. [PubMed: 3140778]
23. Martinez FD. Definition of pediatric asthma and associated risk factors. Pediatr Pulmonol Suppl. 1997; 15:9–12. [PubMed: 9316095]
24. Busse W, Elias J, Sheppard D, Banks-Schlegel S. Airway remodeling and repair. Am J Respir Crit Care Med. 1999; 160:1035–1042. [PubMed: 10471638]
25. Fahy JV, Corry DB, Boushey HA. Airway inflammation and remodeling in asthma. Curr Opin Pulm Med. 2000; 6:15–20. [PubMed: 10608420]

26. Rogers DF. Airway goblet cell hyperplasia in asthma: hypersecretory and anti-inflammatory? Clin Exp Allergy. 2002; 32:1124–1127. [PubMed: 12190646]
27. Blyth DI. The homeostatic role of bronchoconstriction. Respiration. 2001; 68:217–223. [PubMed: 11287841]
28. Guilbert TW, Morgan WJ, Zeiger RS, et al. Long-term inhaled corticosteroids in preschool children at high risk for asthma. N Engl J Med. 2006; 354:1985–1997. [PubMed: 16687711]
29. Expert Panel Report 3 (EPR-3): Guidelines for the Diagnosis and Management of Asthma-Summary Report 2007. J Allergy Clin Immunol. 2007; 120:S94–S138. [PubMed: 17983880]
30. Lange P, Parner J, Vestbo J, Schnohr P, Jensen G. A 15-year follow-up study of ventilatory function in adults with asthma. N Engl J Med. 1998; 339:1194–1200. [PubMed: 9780339]
31. Long-term effects of budesonide or nedocromil in children with asthma. The Childhood Asthma Management Program Research Group. N Engl J Med. 2000; 343:1054–1063. [PubMed: 11027739]
32. Busse WW, Pedersen S, Pauwels RA, et al. The Inhaled Steroid Treatment As Regular Therapy in Early Asthma (START) study 5-year follow-up: effectiveness of early intervention with budesonide in mild persistent asthma. J Allergy Clin Immunol. 2008; 121:1167–1174. [PubMed: 18405951]
33. Sont JK, Willems LN, Bel EH, van Krieken JH, Vandenbroucke JP, Sterk PJ. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment. The AMPUL Study Group. Am J Respir Crit Care Med. 1999; 159:1043–1051. [PubMed: 10194144]
34. Kelly EA, Houtman JJ, Jarjour NN. Inflammatory changes associated with circadian variation in pulmonary function in subjects with mild asthma. Clin Exp Allergy. 2004; 34:227–233. [PubMed: 14987302]
35. Shearman LP, Sriram S, Weaver DR, et al. Interacting molecular loops in the mammalian circadian clock. Science. 2000; 288:1013–1019. [PubMed: 10807566]
36. Almon RR, Chen J, Snyder G, DuBois DC, Jusko WJ, Hoffman EP. In vivo multi-tissue corticosteroid microarray time series available online at Public Expression Profile Resource (PEPR). Pharmacogenomics. 2003; 4:791–799. [PubMed: 14596642]
37. Almon RR, DuBois DC, Jusko WJ. A microarray analysis of the temporal response of liver to methylprednisolone: a comparative analysis of two dosing regimens. Endocrinology. 2007; 148:2209–2225. [PubMed: 17303664]
38. Almon RR, Yang E, Lai W, et al. Relationships between circadian rhythms and modulation of gene expression by glucocorticoids in skeletal muscle. Am J Physiol Regul Integr Comp Physiol. 2008; 295:R1031–R1047. [PubMed: 18667713]
39. Le Minh N, Damiola F, Tronche F, Schutz G, Schibler U. Glucocorticoid hormones inhibit food-induced phase-shifting of peripheral circadian oscillators. EMBO J. 2001; 20:7128–7136. [PubMed: 11742989]
40. Hida A, Koike N, Hirose M, Hattori M, Sakaki Y, Tei H. The human and mouse Period1 genes: five well-conserved E-boxes additively contribute to the enhancement of mPer1 transcription. Genomics. 2000; 65:224–233. [PubMed: 10857746]
41. Koyanagi S, Okazawa S, Kuramoto Y, et al. Chronic treatment with prednisolone represses the circadian oscillation of clock gene expression in mouse peripheral tissues. Mol Endocrinol. 2006; 20:573–583. [PubMed: 16269518]
42. Burioka N, Takata M, Okano Y, et al. Dexamethasone influences human clock gene expression in bronchial epithelium and peripheral blood mononuclear cells in vitro. Chronobiol Int. 2005; 22:585–590. [PubMed: 16076656]
43. Martin RJ, Cicutto LC, Ballard RD. Factors related to the nocturnal worsening of asthma. Am Rev Respir Dis. 1990; 141:33–38. [PubMed: 2404440]
44. Kraft M, Djukanovic R, Wilson S, Holgate ST, Martin RJ. Alveolar tissue inflammation in asthma. Am J Respir Crit Care Med. 1996; 154:1505–1510. [PubMed: 8912772]
45. Kraft M, Martin RJ, Wilson S, Djukanovic R, Holgate ST. Lymphocyte and eosinophil influx into alveolar tissue in nocturnal asthma. Am J Respir Crit Care Med. 1999; 159:228–234. [PubMed: 9872843]

46. Pincus DJ, Szefler SJ, Ackerson LM, Martin RJ. Chronotherapy of asthma with inhaled steroids: the effect of dosage timing on drug efficacy. J Allergy Clin Immunol. 1995; 95:1172–1178. [PubMed: 7797785]

47. Pincus DJ, Humeston TR, Martin RJ. Further studies on the chronotherapy of asthma with inhaled steroids: the effect of dosage timing on drug efficacy. J Allergy Clin Immunol. 1997; 100:771–774. [PubMed: 9438485]

48. Beam WR, Weiner DE, Martin RJ. Timing of prednisone and alterations of airways inflammation in nocturnal asthma. Am Rev Respir Dis. 1992; 146:1524–1530. [PubMed: 1456570]

49. Gibbs JE, Beesley S, Plumb J, et al. Circadian timing in the lung; a specific role for bronchiolar epithelial cells. Endocrinology. 2009; 150:268–276. [PubMed: 18787022]

50. Smolensky MH, Lemmer B, Reinberg AE. Chronobiology and chronotherapy of allergic rhinitis and bronchial asthma. Adv Drug Deliv Rev. 2007; 59:852–882. [PubMed: 17900748]

51. Levi F, Schibler U. Circadian rhythms: mechanisms and therapeutic implications. Annu Rev Pharmacol Toxicol. 2007; 47:593–628. [PubMed: 17209800]

52. Bruguerolle B, Labrecque G. Rhythmic pattern in pain and their chronotherapy. Adv Drug Deliv Rev. 2007; 59:883–895. [PubMed: 17716777]

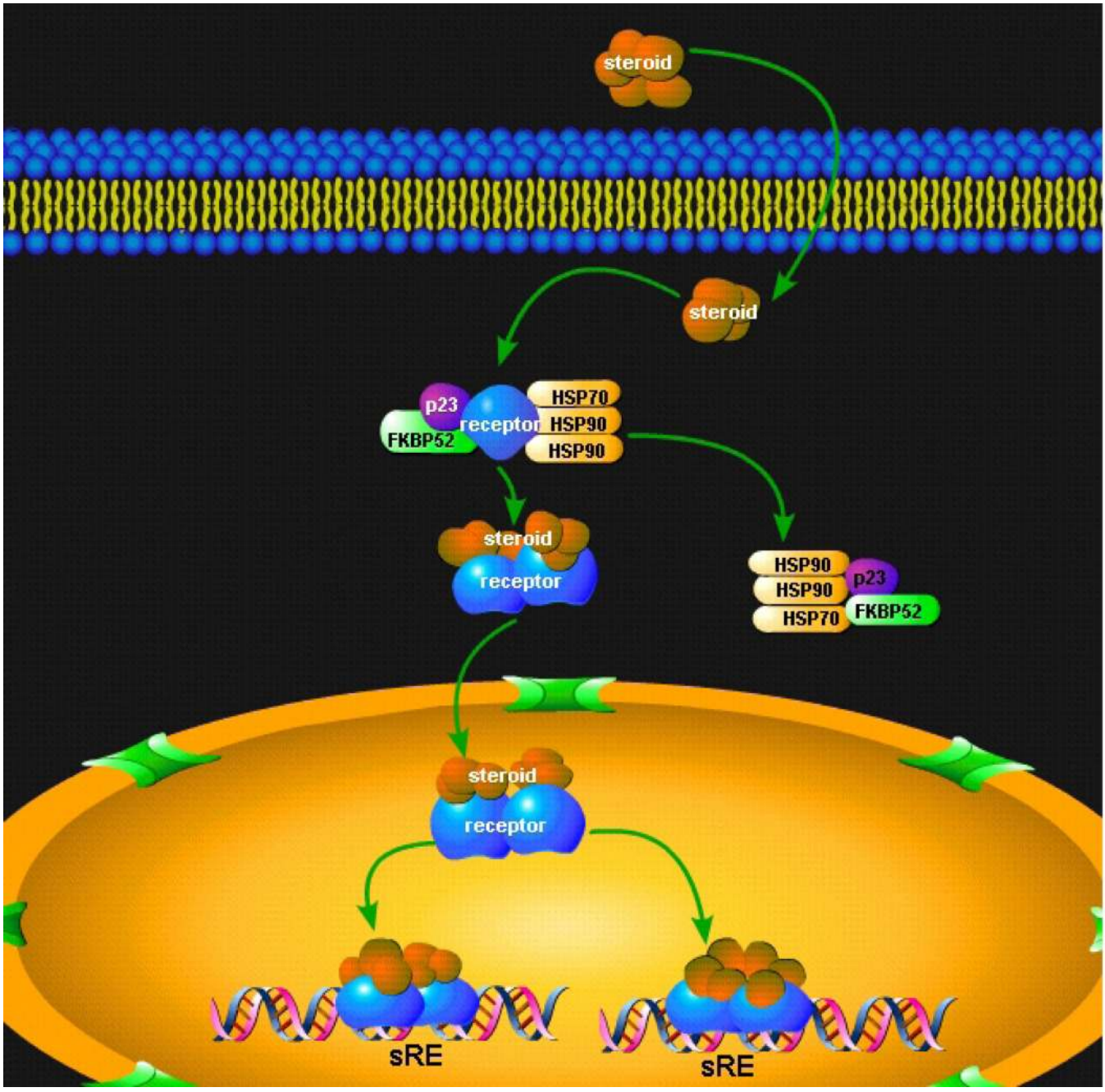


**Figure 1.**

Binding and activation of the glucocorticoid receptor. Circulating steroids diffuse across cell membranes and bind inactive GRs in the cytosol. The heat shock proteins (HSP) bound to the unoccupied GR receptor are displaced by the steroid ligand. The steroid-receptor complex then translocates to the nucleus where ligand-activated GR dimers are shown binding to sRE *cis*-elements. This activates sRE containing genes leading to transactivation or transrepression.

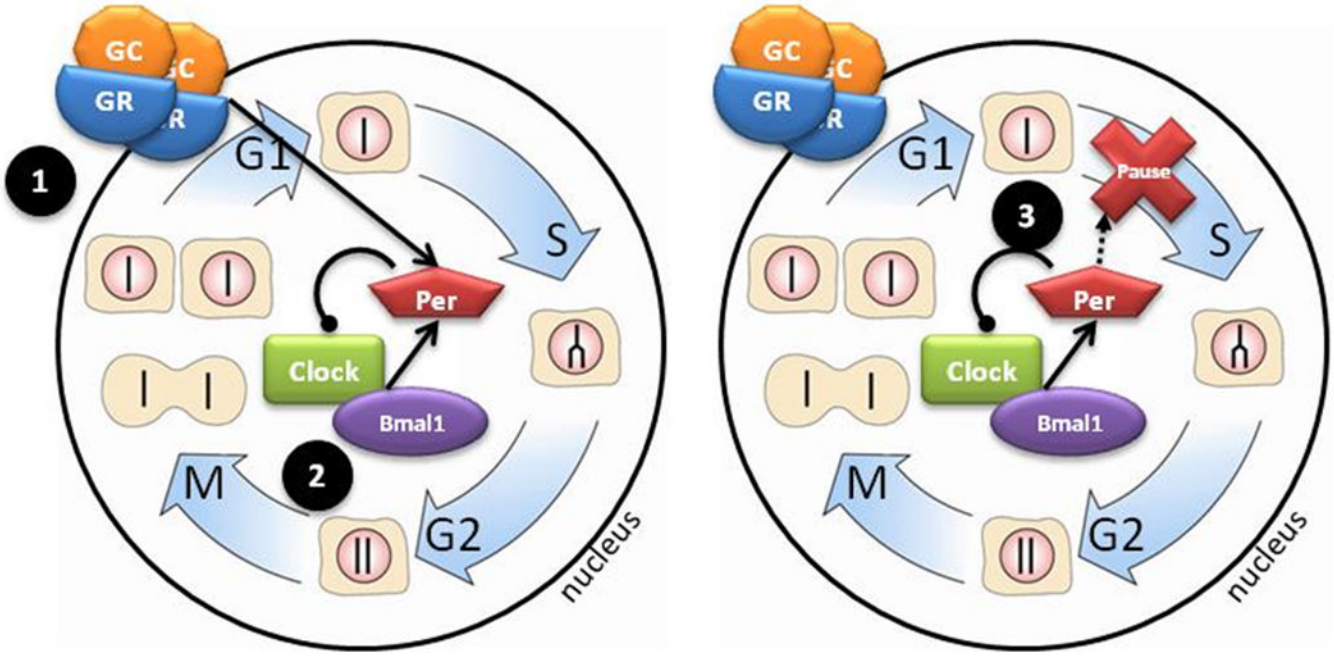


**Figure 2.**

Model of cell cycle progression in normal respiratory epithelium. In normal tissue, circadian peaks in endogenous GC's act via the GC receptor (GR) (1) to induce expression of Period (Per) genes (2). Increased Per expression negatively feeds back to decrease Clock-Bmal1 dimerization (3) resulting in results in a pause of the cell cycle at the G1 phase. Ultimately, less Clock-Bmal1 leads to less Per allowing the cell cycle to progress.