

Mitogen-activated protein kinase pathways: Therapeutic targets in steroid resistance?

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Immune activation and tissue inflammation play a central role in the pathogenesis of chronic allergic and autoimmune diseases. The anti-inflammatory glucocorticoids are currently the most effective agents for the treatment of these diseases. Although the majority of patients respond to glucocorticoid therapy, some demonstrate persistent tissue inflammation despite treatment with high doses of glucocorticoids. Glucocorticoid resistance has been widely recognized as complicating the management of chronic inflammatory diseases, such as asthma, inflammatory bowel disease, autoimmune diseases, and transplantation rejection. Knowledge of the molecular mechanisms responsible for glucocorticoid insensitivity is crucial for the development of effective therapies.

The anti-inflammatory effects of glucocorticoids are mediated through a specific glucocorticoid receptor (GR) that modulates inflammatory gene expression. The GR is a transcriptional regulatory protein characterized by 3 distinct functional domains (Fig 1). The amino-terminal region harbors a hormone-independent transcriptional activation function (AF-1). The central part of the protein contains a highly conserved zinc finger DNA-binding domain. The C-terminus includes a hormone-binding domain with an additional transcriptional activation function (AF-2). Of these 3 functional domains, the amino-terminal AF-1 region is the major target for phosphorylation at multiple serine residues. There is mounting evidence that posttranslational modification of GRs by means of phosphorylation modulates receptor function.¹

Before ligand binding, GRs exist as a large multiunit complex in the cytoplasm that includes 2 molecules of heat shock protein 90. After activation by binding of glucocorticoid hormone, the GR dissociates from the chaperone proteins and rapidly translocates to the nucleus (Fig 2). Within the nucleus, the GRs bind as a dimer to specific glucocorticoid response elements on DNA within the promoter region of glucocorticoid-responsive genes to

Abbreviations used

ERK: Extracellular signal-regulated kinase
GR: Glucocorticoid receptor
JNK: c-Jun N-terminal kinase
MAPK: Mitogen-activated protein kinase
MEK: Mitogen-activated protein kinase kinase

enhance transcription of anti-inflammatory genes. GRs can also repress transcription of proinflammatory genes by means of interaction with inflammatory transcription factors, such as activator protein 1 and nuclear factor κ B.

In this issue of the Journal, Li et al² report studies of a mechanism of staphylococcal superantigen-induced glucocorticoid resistance in T cells from healthy subjects. T-cell proliferation induced by anti-CD3 activation of the T-cell receptor (TCR) was sensitive to inhibition by the glucocorticoid dexamethasone, but T-cell proliferation induced by superantigen was resistant to dexamethasone inhibition. The development of glucocorticoid resistance after superantigen stimulation required the presence of antigen-presenting cells or additional stimulation of CD28. Superantigen, compared with anti-CD3, produced a more rapid and sustained phosphorylation of the mitogen-activated protein kinase (MAPK) extracellular signal-regulated kinase (ERK), and ERK was able to phosphorylate GR in an *in vitro* kinase assay. Specific inhibitors of the MAPK kinase (MEK)/ERK pathway, but not other kinase inhibitors, blocked the development of superantigen-induced steroid resistance in the proliferation assay. Interestingly, glucocorticoid resistance was associated with a decrease in GR nuclear translocation after dexamethasone treatment, and this effect was reversed with MEK/ERK pathway inhibitors. These findings suggest that the development of superantigen-induced glucocorticoid resistance in T cells in this system is mediated by means of phosphorylation of GRs (or proteins that interact with GRs) and is associated with decreased translocation of GRs to the nucleus. Although the authors do not directly demonstrate the mechanism for the development of resistance, blockade of MEK/ERK signaling in their system reversed resistance and restored GR translocation.

A recent report by Tsitoura and Rothman³ complements the findings of Li et al.² These investigators examined murine and human T cells for responsiveness to glucocorticoids *in vitro* and found that dexamethasone

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Received for publication August 30, 2004; revised September 3, 2004; accepted for publication September 3, 2004.

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J Allergy Clin Immunol 2004;114:1055-8.
0091-6749/\$30.00

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doi:10.1016/j.jaci.2004.09.001

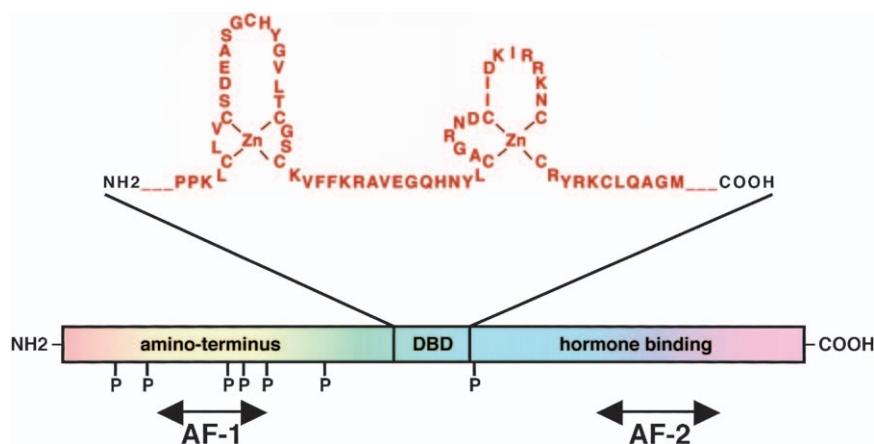


FIG 1. Structure-function map of the glucocorticoid receptor. The receptor protein consists of 3 domains: amino-terminus domain, DNA-binding domain (DBD), and hormone-binding domain. For the DNA-binding domain, the amino acid sequence and zinc finger structure are shown. Potential sites of phosphorylation and the locations of the ligand-independent (AF-1) and ligand-dependent (AF-2) transcriptional activation functions are depicted. Reprinted from *The Journal of Allergy and Clinical Immunology*, Vol. 111, Leung DY and Bloom JW, "Update on glucocorticoid action and resistance," p. 3-22, Copyright 2003, with permission from the American Academy of Allergy, Asthma and Immunology.

suppressed TCR-induced proliferation of naive CD4⁺ T cells through a mechanism involving inhibition of the proinflammatory transcription factors activator protein 1, nuclear factor of activated T cells, and nuclear factor κ B. Enhancement of TCR signaling by CD28 or IL-2-mediated costimulation produced resistance to the suppressive effect of dexamethasone and restored cellular proliferation. As in the study by Li et al,² MEK/ERK signaling was essential for the development of T-cell glucocorticoid resistance. Thus these studies demonstrate that MEK/ERK signaling is a potential therapeutic target for patients with glucocorticoid resistance.

The MEK/ERK signaling pathway is a constituent of the MAPK family.⁴ MAPKs integrate and process various extracellular signals and are primary components of intracellular signaling. A series of 3 protein kinases constitutes a MAPK cascade (Fig 3). Thus far, 3 distinct MAPK pathways have been described in mammalian cells that seem to be conserved from yeast to human subjects. The extracellular signal-regulated kinases ERK1 and ERK2 were the first of these MAPKs to be discovered. Subsequently, c-Jun N-terminal kinase (JNK) and p38 MAPK were identified. Each MAPK subfamily consists of several isoforms, which often have distinct functions. These kinases are activated by the dual phosphorylation of threonine and tyrosine residues by a dual-specificity MEK (also termed MAPKK). The MEK, in turn, is phosphorylated by a serine-threonine kinase termed MEKK (or MAPKKK). In general, the ERKs are activated by mitogenic and proliferative stimuli, whereas the JNKs and p38 MAPKs respond to environmental stress, such as ultraviolet heat, osmotic shock, and inflammatory cytokines.

In patients with steroid-resistant asthma, there is evidence that resistance to the anti-inflammatory effects of glucocorticoids can be induced by several mecha-

nisms.⁵ The reduction in glucocorticoid responsiveness observed in cells from these patients has been attributed to altered affinity of ligand binding to GRs, a reduced number of GRs, a reduced ability of GRs to bind to DNA, increased expression of inflammatory transcription factors, increased expression of the β -isoform of GRs that binds to DNA but not glucocorticoids, and decreased translocation of the GR from the cytoplasm to the nucleus after activation.⁶⁻⁸ Because the initial step in the classical glucocorticoid signaling pathway is translocation of the GR from the cytoplasm to the nucleus, decreased nuclear translocation is a particularly plausible molecular mechanism of resistance. Although inhibition of GR nuclear translocation has been associated with factors such as excessive expression of cytokines and enhanced GR nitrosylation,^{9,10} it has been most closely linked with altered GR phosphorylation status. There is mounting evidence that posttranslational modification of GRs by means of phosphorylation modulates receptor function, including target promoter specificity, cofactor interaction, subcellular localization, and receptor stability.¹ The GR is a phosphoprotein and is phosphorylated in the absence of ligand, but further phosphorylation occurs with glucocorticoid binding. Data from *in vitro* studies suggest that phosphorylation sites in the amino-terminal region of the GR (at serines 203, 211, and 226) play a critical role in GR function.¹¹⁻¹³ In the absence of glucocorticoid, the GR is phosphorylated predominantly at the serine 203 site. Treatment with the GR agonist results in increased phosphorylation at serine 211 relative to that seen at serine 203. Interestingly, localization studies showed that after glucocorticoid treatment, the serine 203-phosphorylated GR is predominantly in the cytoplasm, whereas the serine 211-phosphorylated form is found in the nucleus. Of particular importance, the transcriptional activity of the GR correlated with the amount of phosphorylation at

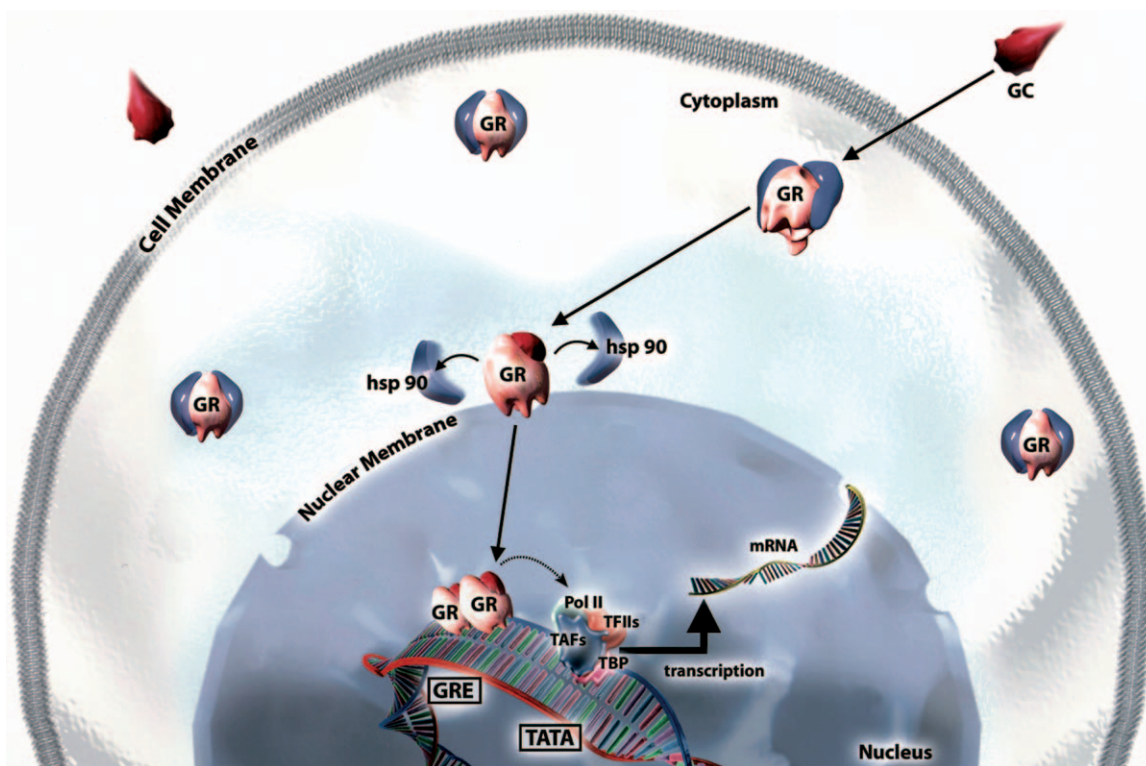


FIG 2. Glucocorticoid-mediated transcriptional activation. Before glucocorticoid binding, the GR exists as a large multiunit complex in the cytoplasm, which includes 2 molecules of heat shock protein 90 (*hsp 90*). After activation by binding of hormone, the GR dissociates from the chaperone proteins and translocates to the nucleus. In the nucleus the GR binds as a homodimer to a specific palindromic DNA sequence located in the regulatory regions of target genes. The bound GR homodimer interacts with the basal transcriptional machinery shown bound to the TATA box. The basal transcription complex includes TATA-binding protein, associated transcription factors (*TAFs* and *TFIIs*), and RNA polymerase II (*Pol II*). The interaction between the GR and the basal transcription complex enhances transcription of the GR target gene. *GC*, Glucocorticoid; *GRE*, glucocorticoid response element. Reprinted from *The Journal of Allergy and Clinical Immunology*, Vol. 111, Leung DY and Bloom JW, "Update on glucocorticoid action and resistance," p. 3-22, Copyright 2003, with permission from the American Academy of Allergy, Asthma and Immunology.

serine 211. Also, phosphorylation at serine 226 has been shown to inhibit GR-mediated transcriptional activation. Phosphorylation of the GR at serine 226 by the MAPK JNK enhances GR nuclear export and likely contributes to termination of GR-mediated transcription.¹⁴ Thus post-translational modification of the GR by means of phosphorylation induces a distinct conformation, influences the association with coregulatory proteins that modulate the GR subcellular location and transcriptional activation, or both.

Irusen et al¹⁵ have demonstrated that phosphorylation of the GR, mediated by p38 MAPK, is associated with reduced responsiveness to dexamethasone in PBMCs from patients with steroid-resistant asthma. Recently, Goleva et al¹⁶ found that when mouse T cells are treated with IL-2, the GR no longer translocated to the nucleus after dexamethasone treatment. Immunoprecipitation experiments demonstrated that interactions between the GR and phosphorylated signal transducer and activator of transcription 5 are important in the failure of the GR to

translocate to the nucleus and suppress proliferation. In these experiments IL-2-induced glucocorticoid resistance could be blocked by an inhibitor of p38 MAPK. These studies demonstrate a potential role for p38 MAPK pathways in the inhibition of GR translocation functions and induction of glucocorticoid resistance. Thus there is evidence that signaling through each of the MAPK pathways (ERK, JNK, and p38) might produce glucocorticoid resistance.

Also, it is intriguing that evidence from several studies supports the hypothesis that enhanced GR translocation to the nucleus is the mechanism responsible for the apparent steroid-sparing effect of long-acting β -agonists when used in combination with inhaled corticosteroids.^{17,18} This finding suggests the possibility that drugs that enhance GR nuclear translocation, possibly long-acting β -agonists, could be of some benefit in patients with glucocorticoid resistance.

Inhibition of specific MAPK pathways would be a more direct approach to the therapy of patients with

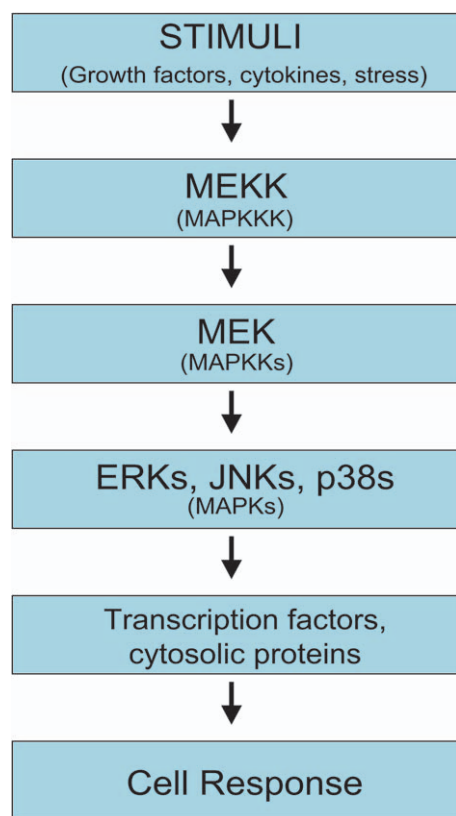


FIG 3. Generic MAPK pathways. The MAPK pathway consists of a cascade of 3 protein kinases: a MAPK and 2 upstream components, a MAPK kinase (MEK or MAPKK) and a MAPKK kinase (MEKK or MAPKKK).

glucocorticoid resistance. Small molecule inhibitors of MEK/ERK, JNK, and p38 pathways have been developed and have reached clinical trials for inflammatory diseases and cancer.¹⁹

Because of the complex cross-talk between the MAPK signaling cascades, as well as the cell-type and response-specific modulation, one might predict that adverse effects would arise from MAPK pathway inhibition. The fact that compounds that inhibit MAPK pathways are progressing in clinical trials suggests that sufficient safety and risk-benefit margins might be possible. Successful development of MAPK inhibitors has major implications for the treatment of glucocorticoid resistance and for therapy of inflammatory disease in general.

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