MECHANISMS OF STEROID ACTION AND RESISTANCE IN INFLAMMATION

MAP kinase phosphatase 1: a novel mediator of biological effects of glucocorticoids?

A R Clark

Kennedy Institute of Rheumatology Division, Faculty of Medicine, Imperial College London, 1 Aspenlea Road, Hammersmith, London W6 8LH, United Kingdom

(Requests for offprints should be addressed to A R Clark; Email: andy.clark@ic.ac.uk)

Abstract

Synthetic glucocorticoids (GCs) potently inhibit the expression of pro-inflammatory genes and are widely used in the treatment of inflammatory diseases. However, some patients are resistant to the therapeutic effects of GCs, and many suffer deleterious side effects from these drugs. Furthermore, the precise mechanisms by which GCs inhibit pro-inflammatory gene expression remain unclear.

A number of recent papers report that GCs induce the sustained expression of MAP kinase (MAPK) phosphatase 1 (MKP-1), a negative regulator of MAPK signal transduction pathways. The potential relevance of MKP-1 to some of the biological effects of GCs is discussed.

Journal of Endocrinology (2003) **178**, 5–12

Introduction

Endogenous glucocorticoids (GCs) are chiefly synthesised in the adrenal gland, under the regulation of the hypothalamic–pituitary–adrenal (HPA) axis. GC synthesis is initiated by stimulation of the hypothalamus to secrete corticotrophin releasing hormone (CRH). This acts upon the anterior pituitary gland to induce release of adrenocorticotrophic hormone (ACTH). ACTH, in turn, induces the release of GCs such as cortisol from the adrenal cortex (Newton 2000). One of the major functions of GCs is the suppression of the immune system, for example by inhibiting the expression of numerous pro-inflammatory genes. Since the production of CRH can be induced by pro-inflammatory cytokines (Besedovsky *et al.* 1986, Del Rey *et al.* 1987), the HPA axis serves as a negative feedback mechanism to limit inflammatory responses to infection. Hence the experimental perturbation of the HPA axis (for example by adrenalectomy) impairs the ability of animals to effectively control inflammation (Masferrer *et al.* 1992, Green *et al.* 1995, Goujon *et al.* 1996, Ruzek *et al.* 1999). The HPA axis is also thought to function abnormally in some chronic inflammatory diseases, and in strains of experimental animals which are prone to auto-immunity (Jafarian-Tehrani & Sternberg 2000, Sternberg 2001, Crofford 2002). However, cause

and effect can be difficult to disentangle because of the multiple levels of cross-talk between the HPA axis and the immune system. The ability of GCs to inhibit expression of a wide variety of pro-inflammatory genes underlies their use in the treatment of chronic inflammatory diseases such as asthma, Crohn's disease, systemic lupus erythematosus and rheumatoid arthritis. Two poorly understood biological effects limit their clinical use. First, GCs are associated with a number of side effects of varying severity, such as osteoporosis, diabetes, hypertension and Cushing's syndrome. Secondly, a small number of patients are resistant to the therapeutic effects of GCs, and may consequently be difficult to treat (DeRijk & Sternberg 1997, Loke *et al.* 2002). The anti-inflammatory mechanisms of action of corticosteroids have been extensively studied for decades, with a view to understanding and overcoming these clinical problems, for example through the design of novel synthetic GCs.

Positive and negative regulation of gene expression by glucocorticoids

The effects of GCs are mediated by a 777 amino acid receptor, which is a member of the nuclear hormone receptor superfamily (Newton 2000). In the absence of

ligand the glucocorticoid receptor (GR) is retained in the cytoplasm in a complex with a number of proteins, including the large heat shock protein hsp90. Upon ligand binding this complex is disrupted and the GR migrates to the nucleus. The transcriptional induction of genes such as tyrosine amino transferase (TAT) and phosphoenolpyruvate carboxykinase (PEPCK) is dependent upon dimerisation of GR and binding to a palindromic promoter sequence, the glucocorticoid response element (GRE). As described in several recent reviews, GR negatively regulates the expression of pro-inflammatory genes by means of transrepression (Gottlicher *et al.* 1998, De Bosscher *et al.* 2000, Adcock & Caramori 2001, Karin & Chang 2001). In this mechanism, ligand-bound, nuclear GR directly interacts with transcription factors such as nuclear factor kappa B (NFκB) and AP1, impairing their ability to induce gene expression. The activation of transcription is dependent upon the recruitment of enzyme complexes which mediate localised chromatin modification such as histone acetylation (Naar *et al.* 2001, Roth *et al.* 2001, Berger 2002, Rahman 2002), and this process appears to be influenced by GR (Ito *et al.* 2000, 2001). Because NFκB is activated by pro-inflammatory stimuli and required for transcriptional activation of very many pro-inflammatory genes (Barnes & Karin 1997, Caamano & Hunter 2002), the transrepression mechanism may account for many of the anti-inflammatory effects of GCs. A single amino acid mutation of GR has been described, which impairs GR dimerisation and activation of transcription through GREs. In transfected cells the dimerisation defective mutant (GR^{dim}) is capable of inhibiting NFκB function (Heck *et al.* 1994, 1997). More significantly, mice which express only GR^{dim} show no impairment in their anti-inflammatory responses to GCs (Reichardt *et al.* 1998, 2001, Tuckermann *et al.* 1999). To some extent transrepression and transactivation mechanisms of GR can also be uncoupled using novel 'dissociated' GCs, which are selectively impaired in one or other function (Vayssiere *et al.* 1997, Vanden Berghe *et al.* 1999, Belvisi *et al.* 2001). For example GCs which poorly activate transcription through palindromic GREs retain some ability to transrepress NFκB and AP1.

These observations lead to the hypothesis (sometimes stated as fact) that deleterious side effects of GCs are mediated by dimerisation-dependent transcriptional activation by GR, whilst anti-inflammatory effects are mediated by dimerisation-independent transrepression by GR. However, this hypothesis remains open to challenge for a number of reasons. A number of broadly anti-inflammatory genes, including the NFκB inhibitor IκBα, lipocortin and interleukin (IL)-1 type II receptor are transcriptionally induced by GCs (Newton 2000), although their relevance to the anti-inflammatory effects of GCs is disputed. Some GC inducible genes do not possess palindromic GREs, and may be regulated in a manner which does not depend upon GR dimerisation

(Diamond *et al.* 1990, Cella *et al.* 1998, Cha *et al.* 1998). Dimerisation-independent GC inducible genes could include important anti-inflammatory mediators, a possibility which might be assessed by expression profiling of GC responses in wild type and GR^{dim}/GR^{dim} mice. Such a study has not yet been reported. Studies using dissociated GCs focus upon well characterised, palindromic GRE-dependent genes such as TAT and PEPCK, and may likewise fail to address atypical GC inducible genes. In any case the selectivity of impairment of transactivation or transrepression achieved with dissociated GCs is often imperfect, cell type- and species-specific. Experiments employing such compounds must be interpreted with caution. Finally, many pro-inflammatory genes are repressed by GCs at a post-transcriptional level, via mRNA destabilisation or inhibition of translation (references in Clark *et al.* 2003). These phenomena cannot be accounted for by transrepression, and suggest the existence of an additional anti-inflammatory mechanism of GCs.

Post-transcriptional regulation of pro-inflammatory gene expression

The mRNAs encoding many immune mediators contain adenosine/uridine rich elements (AREs) within their 3' untranslated regions (UTRs) (Caput *et al.* 1986, Shaw & Kamen 1986, Chen & Shyu 1995). These sequences were initially characterised as destabilising elements which conferred a short mRNA half life, contributing to the rapid responsiveness of gene expression in the immune system. It has subsequently become clear that AREs can also be involved in the dynamic regulation of mRNA stability, notably by the mitogen activated protein kinase (MAPK) p38 signalling pathway (Clark *et al.* 2003). This pathway (Fig. 1) is activated by pro-inflammatory stimuli such as IL-1 and tumour necrosis factor α (TNFα), bacterial lipopolysaccharide (LPS) and ultraviolet irradiation (Ono & Han 2000). MAPK p38 itself is activated by phosphorylation of both threonine and tyrosine residues within a Thr-Gly-Tyr motif, catalysed by the dual specificity MAPK kinases, MKK6 or MKK3. The activation of p38 is terminated by removal of one or both of the activating phosphate groups, catalysed by serine/threonine-specific phosphatases, tyrosine-specific phosphatases or dual specificity phosphatases (which are able to dephosphorylate both the phospho-threonine and the phospho-tyrosine residues). Members of each class of phosphatase are capable of inactivating p38 (Saxena *et al.* 1998, Takekawa *et al.* 1998, 2000, Camps *et al.* 2000, Keyse 2000). MAPK p38 activates the kinase MAPKAPK-2 which, in turn, targets the AREs of certain pro-inflammatory mRNAs to bring about their stabilisation (Winzen *et al.* 1999, Lasa *et al.* 2000, Clark *et al.* 2003).

Figure 1 Organisation of the MAPK p38 pathway. Pro-inflammatory stimuli result in the activation of MKK6, a dual specificity kinase. MKK6 activates p38 by phosphorylating threonine and tyrosine residues within the TGY activation motif. In turn, p38 phosphorylates and activates a number of substrates, including MK2, which is implicated in the post-transcriptional regulation of several pro-inflammatory genes. Because both threonine and tyrosine phosphorylations are required for its full activation, p38 can be inactivated by serine/threonine-specific phosphatases, tyrosine-specific phosphatases or dual specificity phosphatases. MKK6, MAPK kinase 6; MK2, MAPK activated protein kinase 2; S/T P-ase, serine/threonine specific phosphatase; Y P-ase, tyrosine specific phosphatase; DUSP, dual specificity phosphatase; P, phosphate group.

Induction of MAPK phosphatase 1 by glucocorticoids

It is striking that many genes that are positively regulated at a post-transcriptional level by p38 are negatively regulated at the same level by GCs (Table 1). Prompted by this observation, we investigated the effect of GCs upon the p38 pathway, and showed that dexamethasone destabilised cyclooxygenase 2 (Cox-2) mRNA by inhibiting the function, but not the expression of MAPK p38 (Lasa *et al.* 2001). The inhibition of p38 was then shown to be mediated by a phosphatase (Lasa *et al.* 2002). We and others demonstrated that dexamethasone induces the expression of MAPK phosphatase 1 (MKP-1), a dual specificity phosphatase which potently inactivates p38 (Kassel *et al.* 2001, Chen *et al.* 2002, Lasa *et al.* 2002). This induction was mediated by the GR and dependent upon ongoing transcription. No other known p38-inactivating phosphatases were significantly induced by dexamethasone, and cells which failed to express MKP-1 also failed to down-regulate p38 activity in response to dexamethasone (Lasa *et al.* 2002). In mast cells an additional level of regulation was described, in which GCs inhibited the degradation of MKP-1 (Kassel *et al.* 2001).

The precise mechanisms of regulation of MKP-1 expression and the impact of this additional, non-genomic pathway in cells other than mast cells are not yet clear. Nevertheless, it is likely that MKP-1 plays a role in the inhibition of p38 and the consequent destabilisation of pro-inflammatory mRNAs by GCs.

Transcriptional and post-transcriptional mechanisms of action of glucocorticoids

An important distinction between transcriptional and post-transcriptional mechanisms of inhibition of pro-inflammatory gene expression is their time dependence. Transrepression can be effective only during the period when the transcription of the pro-inflammatory gene is active. For many pro-inflammatory genes this transcriptional window may be relatively brief. In contrast, the destabilisation of a pro-inflammatory mRNA or the inhibition of its translation may have a profound effect on gene expression even if it occurs some time after the pro-inflammatory stimulus and the period of active transcription. This distinction is illustrated by the example of a lung epithelial cell line, which expresses the pro-inflammatory mediator Cox-2 in response to IL-1. The synthetic GC, dexamethasone, destabilises Cox-2 mRNA and inhibits Cox-2 expression even if added 10 h after the stimulus. In contrast, the transcriptional inhibitor, actinomycin D, is able to inhibit Cox-2 expression only if added within an hour of the IL-1 stimulus (Newton *et al.* 1998). Post-transcriptional repression allows cells to rapidly and specifically switch off gene expression in response to extracellular signals, a property which is invaluable in the context of the immune system (Clark 2000). In a physiological context cells will be recruited to sites of inflammation, exposed to pro-inflammatory stimuli, endogenous or exogenous GCs at different stages. It is arguable that the efficient inhibition of an inflammatory response may require both transcriptional and post-transcriptional mechanisms to block the induction of expression of pro-inflammatory mRNAs, and to rapidly clear pre-existing transcripts. Experimental systems have typically been designed to address either transcriptional or post-transcriptional suppression of pro-inflammatory gene expression, and do not make clear the relative contributions of these processes *in vivo*. In fact changes in steady state mRNA have often been ascribed to transcriptional regulation without assessing possible changes in mRNA stability.

Physiological significance of MAPK phosphatase 1 gene expression

The phosphatase MKP-1 preferentially inactivates MAPK p38 and c-Jun N-terminal kinase (JNK) (Franklin & Kraft 1997), but under some circumstances may also

Table 1 Positive and negative post-transcriptional regulation of gene expression by GCs and the stress activated protein kinases p38 and JNK

Gene	Positive post-transcriptional regulation by p38 or JNK	Negative post-transcriptional regulation by GCs
Cox-2	Dean <i>et al.</i> (1999), Lasa <i>et al.</i> (2000), Ridley <i>et al.</i> (1998)	Lasa <i>et al.</i> (2001), Newton <i>et al.</i> (1998)
IL-1	Wang <i>et al.</i> (1999)	Amano <i>et al.</i> (1993)
IL-2	Chen <i>et al.</i> (1998)	Fessler <i>et al.</i> (1996)
IL-6	Miyazawa <i>et al.</i> (1998), Winzen <i>et al.</i> (1999)	Amano <i>et al.</i> (1993), Tobler <i>et al.</i> (1992)
IL-8	Winzen <i>et al.</i> (1999)	Chang <i>et al.</i> (2001), Tobler <i>et al.</i> (1992)
TNF α	Brook <i>et al.</i> (2000), Rutault <i>et al.</i> (2001), Wang <i>et al.</i> (1999)	Han <i>et al.</i> (1990), Kontoyiannis <i>et al.</i> (1999), Swantek <i>et al.</i> (1997)
GM-CSF	Winzen <i>et al.</i> (1999)	Tobler <i>et al.</i> (1992)
VEGF	Pages <i>et al.</i> (2000)	Gille <i>et al.</i> (2001)
MMP-1 and -3	Reunanen <i>et al.</i> (2002)	Delany & Brinckerhoff (1992)

GM-CSF, granulocyte-macrophage colony stimulating factor; VEGF, vascular endothelial growth factor; MMP, matrix metalloproteinase.

dephosphorylate extracellular signal regulated kinase (Erk) (Camps *et al.* 2000, Keyse 2000). Its expression is induced by a remarkable variety of stimuli, including cellular stresses, LPS, pro-inflammatory cytokines and agonists with anti-inflammatory effects, including transforming growth factor (TGF)- β , cholera toxin B subunit and cAMP elevating agents (Keyse & Emslie 1992, Guo *et al.* 1998, Burgun *et al.* 2000, Valledor *et al.* 2000, Chen *et al.* 2002, Lasa *et al.* 2002, Xiao *et al.* 2002). Sustained GC-induced expression of MKP-1 has been demonstrated in HeLa cells (Lasa *et al.* 2002), a rat mast cell line (Kassel *et al.* 2001) and a mouse macrophage cell line (Chen *et al.* 2002), in all of which it appears to mediate inhibition of MAPK signalling and pro-inflammatory gene expression in response to cell stimulation. As the MAPK pathways are pleiotrophic regulators of gene expression in the immune system (Kracht & Saklatvala 2002), MKP-1 may be an important negative regulator of many aspects of the inflammatory response. MAPK p38 regulates transcription via factors that include MEF2C, ATF2 and NF κ B (Treisman 1996, Ono & Han 2000, Schmitz *et al.* 2001, Vermeulen *et al.* 2003), whereas JNK is an activator of AP1 and other transcription factors (Whitmarsh & Davis 1996, Ip & Davis 1998), suggesting a potential role for MKP-1 in the inhibition of transcription by GR. However, GCs may suppress JNK activity in the absence of ongoing transcription (Caelles *et al.* 1997, Gonzalez *et al.* 2000), and the dissociated GC RU38486, which does not induce MKP-1 gene expression (Lasa *et al.* 2002), retains some ability to transrepress AP1 (Heck *et al.* 1994, Vayssiere *et al.* 1997). Induction of MKP-1, therefore, appears to be dispensible for transrepression, yet may provide an additional mechanism for inhibition of transcription by GCs.

As determined in tissue culture systems, the properties of MKP-1 suggest a versatile role for this phosphatase in the negative regulation of immune responses (Chen *et al.* 2002). However, several questions remain to be answered before the physiological significance of this phosphatase can be understood. Do GCs inhibit MAPK function and induce MKP-1 expression *in vivo*, particularly in physiologically relevant cell types such as macrophages, mast cells, gut and lung epithelia? Are other phosphatases induced by GCs *in vivo*? Is the induction of MKP-1 dependent on GR dimerisation, for example does it occur in mouse cells which express only the dimerisation defective form of the receptor? How does its expression respond to novel, dissociated GCs? Finally, does the absence of MKP-1 significantly impair the anti-inflammatory effects of GCs? The latter question will be most easily addressed by means of antisense or RNA interference technology, or using an MKP-1 knock out mouse line which was described several years ago (Dorfman *et al.* 1996). The MKP-1 null mouse develops normally and shows no abnormalities in the regulation of Erk function; however the regulation of p38 and JNK was not examined, nor were the responses to pro-inflammatory stimuli or GCs.

If MKP-1 plays a significant role in the suppression of inflammation by GCs, it follows that GC resistance in some inflammatory disease states could be related to defects in the expression or function of MKP-1. Elevated JNK and p38 activities have been described in inflammatory diseases, and are possible targets for clinical intervention (Badger *et al.* 1996, Hallsworth *et al.* 2001, Kumar *et al.* 2001, Hommes *et al.* 2002, Waetzig *et al.* 2002). GC resistance in asthma and inflammatory bowel disease may be associated with a failure of GCs to inhibit JNK and p38 (Sousa *et al.* 1999, Bantel *et al.* 2002). Because these

Figure 2 A regulatory loop for the control of pro-inflammatory mediator synthesis. The strength of regulatory interaction is indicated by the thickness of the line connecting two effectors. (A) Normal homeostatic regulation. (B) Hypothetical breakdown of homeostatic regulation due to a defect in GC-induced expression or activity of MKP-1. GR, glucocorticoid receptor; MKP-1, MAPK phosphatase 1; SAPK, stress activated protein kinase (JNK or p38); TNF α , tumour necrosis factor α (a representative pro-inflammatory mediator).

kinases negatively regulate GR function (Rogatsky *et al.* 1998, Irusen *et al.* 2002), the elevation of MAPK activity could be self-perpetuating. In other words an initial defect in GC-induced MKP-1 expression or activity might increase MAPK activity, impairing GR function and further inhibiting the induction of MKP-1 expression (Fig. 2). Consequences of the fracture of this regulatory loop might include increases in transcription of pro-inflammatory genes, or in the stability of the mRNAs produced.

Several mechanisms are employed by GCs to inhibit the expression of pro-inflammatory genes, hence there are likely to be several paths to GC insensitivity. The involvement of MKP-1 in these phenomena should become clearer over the next few years. Interestingly, GC-induced osteoporosis in the rat is prevented by sodium orthovanadate, an efficient inhibitor of tyrosine phosphatases, including MKP-1 (Hulley *et al.* 1998, 2002). The involvement of MKP-1 in the side effects of GCs may also be worth further investigation.

Note added in proof

It was recently reported that GCs also induce the expression of MKP-1 in osteoblasts (Engelbrecht *et al.* 2003).

References

- Adcock IM & Caramori G 2001 Cross-talk between pro-inflammatory transcription factors and glucocorticoids. *Immunology and Cell Biology* **79** 376–384.
- Amano Y, Lee SW & Allison AC 1993 Inhibition by glucocorticoids of the formation of interleukin-1 α , interleukin-1 β , and interleukin-6: mediation by decreased mRNA stability. *Molecular Pharmacology* **43** 176–182.
- Badger AM, Bradbeer JN, Votta B, Lee JC, Adams JL & Griswold DE 1996 Pharmacological profile of SB 203580, a selective inhibitor of cytokine suppressive binding protein/p38 kinase, in animal models of arthritis, bone resorption, endotoxin shock and immune function. *Journal of Pharmacology and Experimental Therapeutics* **279** 1453–1461.
- Bantel H, Schmitz ML, Raible A, Gregor M & Schulze-Osthoff K 2002 Critical role of NF- κ B and stress-activated protein kinases in steroid unresponsiveness. *FASEB Journal* **16** 1832–1834.
- Barnes PJ & Karin M 1997 Nuclear factor- κ B: a pivotal transcription factor in chronic inflammatory diseases. *New England Journal of Medicine* **336** 1066–1071.
- Belvisi MG, Wicks SL, Battram CH, Bottoms SE, Redford JE, Woodman P, Brown TJ, Webber SE & Foster ML 2001 Therapeutic benefit of a dissociated glucocorticoid and the relevance of *in vitro* separation of transrepression from transactivation activity. *Journal of Immunology* **166** 1975–1982.
- Berger SL 2002 Histone modifications in transcriptional regulation. *Current Opinion in Genetics and Development* **12** 142–148.
- Besedovsky H, del Rey A, Sorkin E & Dinarello CA 1986 Immunoregulatory feedback between interleukin-1 and glucocorticoid hormones. *Science* **233** 652–654.
- Brook M, Sully G, Clark AR & Saklatvala J 2000 Regulation of tumour necrosis factor α mRNA stability by the mitogen-activated protein kinase p38 signalling cascade. *FEBS Letters* **483** 57–61.
- Burgun C, Esteve L, Humblot N, Aunis D & Zwiller J 2000 Cyclic AMP-elevating agents induce the expression of MAP kinase phosphatase-1 in PC12 cells. *FEBS Letters* **484** 189–193.
- Caamano J & Hunter CA 2002 NF- κ B family of transcription factors: central regulators of innate and adaptive immune functions. *Clinical Microbiology Reviews* **15** 414–429.
- Caelles C, Gonzalez-Sancho JM & Munoz A 1997 Nuclear hormone receptor antagonism with AP-1 by inhibition of the JNK pathway. *Genes and Development* **11** 3351–3364.
- Camps M, Nichols A & Arkinstall S 2000 Dual specificity phosphatases: a gene family for control of MAP kinase function. *FASEB Journal* **14** 6–16.
- Caput D, Beutler B, Hartog K, Thayer R, Brown-Shimer S & Cerami A 1986 Identification of a common nucleotide sequence in the 3'-untranslated region of mRNA molecules specifying inflammatory mediators. *PNAS* **83** 1670–1674.
- Cella N, Groner B & Hynes NE 1998 Characterization of Stat5a and Stat5b homodimers and heterodimers and their association with the glucocorticoid receptor in mammary cells. *Molecular and Cellular Biology* **18** 1783–1792.
- Cha HH, Cram EJ, Wang EC, Huang AJ, Kasler HG & Firestone GL 1998 Glucocorticoids stimulate p21 gene expression by targeting multiple transcriptional elements within a steroid responsive region of the p21 waf1/cip1 promoter in rat hepatoma cells. *Journal of Biological Chemistry* **273** 1998–2007.
- Chang MM, Juarez M, Hyde DM & Wu R 2001 Mechanism of dexamethasone-mediated interleukin-8 gene suppression in cultured airway epithelial cells. *American Journal of Physiology* **280** L107–L115.
- Chen CY & Shyu AB 1995 AU-rich elements: characterization and importance in mRNA degradation. *Trends in Biochemical Sciences* **20** 465–470.

- Chen CY, Del Gatto-Konczak F, Wu Z & Karin M 1998 Stabilization of interleukin-2 mRNA by the c-Jun NH2-terminal kinase pathway. *Science* **280** 1945–1949.
- Chen P, Li J, Barnes J, Kokkonen GC, Lee JC & Liu Y 2002 Restraint of proinflammatory cytokine biosynthesis by mitogen-activated protein kinase phosphatase-1 in lipopolysaccharide-stimulated macrophages. *Journal of Immunology* **169** 6408–6416.
- Clark A 2000 Post-transcriptional regulation of pro-inflammatory gene expression. *Arthritis Research* **2** 172–174.
- Clark AR, Dean JL & Saklatvala J 2003 Post-transcriptional regulation of gene expression by mitogen-activated protein kinase p38. *FEBS Letters* (In Press).
- Crofford LJ 2002 The hypothalamic–pituitary–adrenal axis in the pathogenesis of rheumatic diseases. *Endocrinology and Metabolism Clinics of North America* **31** 1–13.
- De Bosscher K, Vanden Berghe W & Haegeman G 2000 Mechanisms of anti-inflammatory action and of immunosuppression by glucocorticoids: negative interference of activated glucocorticoid receptor with transcription factors. *Journal of Neuroimmunology* **109** 16–22.
- Dean JL, Brook M, Clark AR & Saklatvala J 1999 p38 mitogen-activated protein kinase regulates cyclooxygenase-2 mRNA stability and transcription in lipopolysaccharide-treated human monocytes. *Journal of Biological Chemistry* **274** 264–269.
- Del Rey A, Besedovsky H, Sorkin E & Dinarello CA 1987 Interleukin-1 and glucocorticoid hormones integrate an immunoregulatory feedback circuit. *Annals of the New York Academy of Sciences* **496** 85–90.
- Delany AM & Brinckerhoff CE 1992 Post-transcriptional regulation of collagenase and stromelysin gene expression by epidermal growth factor and dexamethasone in cultured human fibroblasts. *Journal of Cellular Biochemistry* **50** 400–410.
- DeRijk R & Sternberg EM 1997 Corticosteroid resistance and disease. *Annals of Medicine* **29** 79–82.
- Diamond MI, Miner JN, Yoshinaga SK & Yamamoto KR 1990 Transcription factor interactions: selectors of positive or negative regulation from a single DNA element. *Science* **249** 1266–1272.
- Dorfman K, Carrasco D, Gruda M, Ryan C, Lira SA & Bravo R 1996 Disruption of the erp/mkp-1 gene does not affect mouse development: normal MAP kinase activity in ERP/MKP-1-deficient fibroblasts. *Oncogene* **13** 925–931.
- Engelbrecht Y, de Wet H, Horsch K, Langeveldt CR, Hough FS & Hulley PA 2003 Glucocorticoids induce rapid up-regulation of mitogen-activated protein kinase phosphatase-1 and dephosphorylation of extracellular signal-regulated kinase and impair proliferation in human and mouse osteoblast cell lines. *Endocrinology* **144** 412–422.
- Fessler BJ, Paliogianni F, Hama N, Balow JE & Boumpas DT 1996 Glucocorticoids modulate CD28 mediated pathways for interleukin 2 production in human T cells: evidence for posttranscriptional regulation. *Transplantation* **62** 1113–1118.
- Franklin CC & Kraft AS 1997 Conditional expression of the mitogen-activated protein kinase (MAPK) phosphatase MKP-1 preferentially inhibits p38 MAPK and stress-activated protein kinase in U937 cells. *Journal of Biological Chemistry* **272** 16917–16923.
- Gille J, Reisinger K, Westphal-Varghese B & Kaufmann R 2001 Decreased mRNA stability as a mechanism of glucocorticoid-mediated inhibition of vascular endothelial growth factor gene expression by cultured keratinocytes. *Journal of Investigative Dermatology* **117** 1581–1587.
- Gonzalez MV, Jimenez B, Berciano MT, Gonzalez-Sancho JM, Caelles C, Lafarga M & Munoz A 2000 Glucocorticoids antagonize AP-1 by inhibiting the activation/phosphorylation of JNK without affecting its subcellular distribution. *Journal of Cell Biology* **150** 1199–1208.
- Gottlicher M, Heck S & Herrlich P 1998 Transcriptional cross-talk, the second mode of steroid hormone receptor action. *Journal of Molecular Medicine* **76** 480–489.
- Goujon E, Parnet P, Laye S, Combe C & Dantzer R 1996 Adrenalectomy enhances pro-inflammatory cytokines gene expression, in the spleen, pituitary and brain of mice in response to lipopolysaccharide. *Brain Research. Molecular Brain Research* **36** 53–62.
- Green PG, Miao FJ, Janig W & Levine JD 1995 Negative feedback neuroendocrine control of the inflammatory response in rats. *Journal of Neuroscience* **15** 4678–4686.
- Guo YL, Kang B & Williamson JR 1998 Inhibition of the expression of mitogen-activated protein phosphatase-1 potentiates apoptosis induced by tumor necrosis factor- α in rat mesangial cells. *Journal of Biological Chemistry* **273** 10362–10366.
- Hallsworth MP, Moir LM, Lai D & Hirst SJ 2001 Inhibitors of mitogen-activated protein kinases differentially regulate eosinophil-activating cytokine release from human airway smooth muscle. *American Journal of Respiratory and Critical Care Medicine* **164** 688–697.
- Han J, Thompson P & Beutler B 1990 Dexamethasone and pentoxifylline inhibit endotoxin-induced cachectin/tumor necrosis factor synthesis at separate points in the signaling pathway. *Journal of Experimental Medicine* **172** 391–394.
- Heck S, Kullmann M, Gast A, Ponta H, Rahmsdorf HJ, Herrlich P & Cato AC 1994 A distinct modulating domain in glucocorticoid receptor monomers in the repression of activity of the transcription factor AP-1. *EMBO Journal* **13** 4087–4095.
- Heck S, Bender K, Kullmann M, Gottlicher M, Herrlich P & Cato AC 1997 I kappaB α -independent downregulation of NF-kappaB activity by glucocorticoid receptor. *EMBO Journal* **16** 4698–4707.
- Hommes D, van den Blink B, Plasse T, Bartelsman J, Xu C, Macpherson B, Tytgat G, Peppelenbosch M & Van Deventer S 2002 Inhibition of stress-activated MAP kinases induces clinical improvement in moderate to severe Crohn's disease. *Gastroenterology* **122** 7–14.
- Hulley PA, Gordon F & Hough FS 1998 Inhibition of mitogen-activated protein kinase activity and proliferation of an early osteoblast cell line (MBA 15.4) by dexamethasone: role of protein phosphatases. *Endocrinology* **139** 2423–2431.
- Hulley PA, Conradi MM, Langeveldt CR & Hough FS 2002 Glucocorticoid-induced osteoporosis in the rat is prevented by the tyrosine phosphatase inhibitor, sodium orthovanadate. *Bone* **31** 220–229.
- Ip YT & Davis RJ 1998 Signal transduction by the c-Jun N-terminal kinase (JNK) – from inflammation to development. *Current Opinion in Cell Biology* **10** 205–219.
- Irusen E, Matthews JG, Takahashi A, Barnes PJ, Chung KF & Adcock IM 2002 p38 mitogen-activated protein kinase-induced glucocorticoid receptor phosphorylation reduces its activity: role in steroid-insensitive asthma. *Journal of Allergy and Clinical Immunology* **109** 649–657.
- Ito K, Barnes PJ & Adcock IM 2000 Glucocorticoid receptor recruitment of histone deacetylase 2 inhibits interleukin-1 β -induced histone H4 acetylation on lysines 8 and 12. *Molecular and Cellular Biology* **20** 6891–6903.
- Ito K, Jazrawi E, Cosio B, Barnes PJ & Adcock IM 2001 p65-activated histone acetyltransferase activity is repressed by glucocorticoids: mifepristone fails to recruit HDAC2 to the p65-HAT complex. *Journal of Biological Chemistry* **276** 30208–30215.
- Jafarian-Tehrani M & Sternberg EM 2000 Neuroendocrine and other factors in the regulation of inflammation. Animal models. *Annals of the New York Academy of Sciences* **917** 819–824.
- Karin M & Chang L 2001 AP-1–glucocorticoid receptor crosstalk taken to a higher level. *Journal of Endocrinology* **169** 447–451.
- Kassel O, Sancono A, Kratzschmar J, Kreft B, Stassen M & Cato AC 2001 Glucocorticoids inhibit MAP kinase via increased expression and decreased degradation of MKP-1. *EMBO Journal* **20** 7108–7116.

- Keyse SM 2000 Protein phosphatases and the regulation of mitogen-activated protein kinase signalling. *Current Opinion in Cell Biology* **12** 186–192.
- Keyse SM & Emslie EA 1992 Oxidative stress and heat shock induce a human gene encoding a protein-tyrosine phosphatase. *Nature* **359** 644–647.
- Kontoyiannis D, Pasparakis M, Pizarro TT, Cominelli F & Kollias G 1999 Impaired on/off regulation of TNF biosynthesis in mice lacking TNF AU-rich elements: implications for joint and gut-associated immunopathologies. *Immunity* **10** 387–398.
- Kracht M & Saklatvala J 2002 Transcriptional and post-transcriptional control of gene expression in inflammation. *Cytokine* **20** 91–106.
- Kumar S, Votta BJ, Rieman DJ, Badger AM, Gowen M & Lee JC 2001 IL-1- and TNF-induced bone resorption is mediated by p38 mitogen activated protein kinase. *Journal of Cellular Physiology* **187** 294–303.
- Lasa M, Mahtani KR, Finch A, Brewer G, Saklatvala J & Clark AR 2000 Regulation of cyclooxygenase 2 mRNA stability by the mitogen-activated protein kinase p38 signaling cascade. *Molecular and Cellular Biology* **20** 4265–4274.
- Lasa M, Brook M, Saklatvala J & Clark AR 2001 Dexamethasone destabilizes cyclooxygenase 2 mRNA by inhibiting mitogen-activated protein kinase p38. *Molecular and Cellular Biology* **21** 771–780.
- Lasa M, Abraham SM, Boucheron C, Saklatvala J & Clark AR 2002 Dexamethasone causes sustained expression of mitogen-activated protein kinase (MAPK) phosphatase 1 and phosphatase-mediated inhibition of MAPK p38. *Molecular and Cellular Biology* **22** 7802–7811.
- Loke TK, Sousa AR, Corrigan CJ & Lee TH 2002 Glucocorticoid-resistant asthma. *Current Allergy and Asthma Reports* **2** 144–150.
- Masferrer JL, Seibert K, Zweifel B & Needleman P 1992 Endogenous glucocorticoids regulate an inducible cyclooxygenase enzyme. *PNAS* **89** 3917–3921.
- Miyazawa K, Mori A, Miyata H, Akahane M, Ajisawa Y & Okudaira H 1998 Regulation of interleukin-1 beta-induced interleukin-6 gene expression in human fibroblast-like synoviocytes by p38 mitogen-activated protein kinase. *Journal of Biological Chemistry* **273** 24832–24838.
- Naar AM, Lemon BD & Tjian R 2001 Transcriptional coactivator complexes. *Annual Review of Biochemistry* **70** 475–501.
- Newton R 2000 Molecular mechanisms of glucocorticoid action: what is important? *Thorax* **55** 603–613.
- Newton R, Seybold J, Kuitert LM, Bergmann M & Barnes PJ 1998 Repression of cyclooxygenase-2 and prostaglandin E2 release by dexamethasone occurs by transcriptional and post-transcriptional mechanisms involving loss of polyadenylated mRNA. *Journal of Biological Chemistry* **273** 32312–32321.
- Ono K & Han J 2000 The p38 signal transduction pathway: activation and function. *Cell Signal* **12** 1–13.
- Pages G, Berra E, Milanini J, Levy AP & Pouyssegur J 2000 Stress-activated protein kinases (JNK and p38/HOG) are essential for vascular endothelial growth factor mRNA stability. *Journal of Biological Chemistry* **275** 26484–26491.
- Rahman I 2002 Oxidative stress, transcription factors and chromatin remodelling in lung inflammation. *Biochemical Pharmacology* **64** 935–942.
- Reichardt HM, Kaestner KH, Tuckermann J, Kretz O, Wessely O, Bock R, Gass P, Schmid W, Herrlich P, Angel P & Schutz G 1998 DNA binding of the glucocorticoid receptor is not essential for survival. *Cell* **93** 531–541.
- Reichardt HM, Tuckermann JP, Gottlicher M, Vujic M, Weih F, Angel P, Herrlich P & Schutz G 2001 Repression of inflammatory responses in the absence of DNA binding by the glucocorticoid receptor. *EMBO Journal* **20** 7168–7173.
- Reunanen N, Li SP, Ahonen M, Foschi M, Han J & Kahari VM 2002 Activation of p38 alpha MAPK enhances collagenase-1 (matrix metalloproteinase (MMP)-1) and stromelysin-1 (MMP-3) expression by mRNA stabilization. *Journal of Biological Chemistry* **277** 32360–32368.
- Ridley SH, Dean JL, Sarsfield SJ, Brook M, Clark AR & Saklatvala J 1998 A p38 MAP kinase inhibitor regulates stability of interleukin-1-induced cyclooxygenase-2 mRNA. *FEBS Letters* **439** 75–80.
- Rogatsky I, Logan SK & Garabedian MJ 1998 Antagonism of glucocorticoid receptor transcriptional activation by the c-Jun N-terminal kinase. *PNAS* **95** 2050–2055.
- Roth SY, Denu JM & Allis CD 2001 Histone acetyltransferases. *Annual Review of Biochemistry* **70** 81–120.
- Rutault K, Hazzalin CA & Mahadevan LC 2001 Combinations of ERK and p38 MAPK inhibitors ablate tumor necrosis factor-alpha (TNF-alpha) mRNA induction. Evidence for selective destabilization of TNF-alpha transcripts. *Journal of Biological Chemistry* **276** 6666–6674.
- Ruzek MC, Pearce BD, Miller AH & Biron CA 1999 Endogenous glucocorticoids protect against cytokine-mediated lethality during viral infection. *Journal of Immunology* **162** 3527–3533.
- Saxena M, Williams S, Gilman J & Mustelin T 1998 Negative regulation of T cell antigen receptor signal transduction by hematopoietic tyrosine phosphatase (HePTP). *Journal of Biological Chemistry* **273** 15340–15344.
- Schmitz ML, Bacher S & Kracht M 2001 I kappa B-independent control of NF-kappa B activity by modulatory phosphorylations. *Trends in Biochemical Sciences* **26** 186–190.
- Shaw G & Kamen R 1986 A conserved AU sequence from the 3' untranslated region of GM-CSF mRNA mediates selective mRNA degradation. *Cell* **46** 659–667.
- Sousa AR, Lane SJ, Soh C & Lee TH 1999 *In vivo* resistance to corticosteroids in bronchial asthma is associated with enhanced phosphorylation of JUN N-terminal kinase and failure of prednisolone to inhibit JUN N-terminal kinase phosphorylation. *Journal of Allergy and Clinical Immunology* **104** 565–574.
- Sternberg EM 2001 Neuroendocrine regulation of autoimmune/inflammatory disease. *Journal of Endocrinology* **169** 429–435.
- Swanek JL, Cobb MH & Geppert TD 1997 Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) is required for lipopolysaccharide stimulation of tumor necrosis factor alpha (TNF-alpha) translation: glucocorticoids inhibit TNF-alpha translation by blocking JNK/SAPK. *Molecular and Cellular Biology* **17** 6274–6282.
- Takekawa M, Maeda T & Saito H 1998 Protein phosphatase 2 Calpha inhibits the human stress-responsive p38 and JNK MAPK pathways. *EMBO Journal* **17** 4744–4752.
- Takekawa M, Adachi M, Nakahata A, Nakayama I, Itoh F, Tsukuda H, Taya Y & Imai K 2000 p53-inducible wip1 phosphatase mediates a negative feedback regulation of p38 MAPK-p38 signaling in response to UV radiation. *EMBO Journal* **19** 6517–6526.
- Tobler A, Meier R, Seitz M, Dewald B, Baggiolini M & Fey MF 1992 Glucocorticoids downregulate gene expression of GM-CSF, NAP-1/IL-8, and IL-6, but not of M-CSF in human fibroblasts. *Blood* **79** 45–51.
- Treisman R 1996 Regulation of transcription by MAP kinase cascades. *Current Opinion in Cell Biology* **8** 205–215.
- Tuckermann JP, Reichardt HM, Arribas R, Richter KH, Schutz G & Angel P 1999 The DNA binding-independent function of the glucocorticoid receptor mediates repression of AP-1-dependent genes in skin. *Journal of Cell Biology* **147** 1365–1370.
- Valledor AF, Xaus J, Comalada M, Soler C & Celada A 2000 Protein kinase C epsilon is required for the induction of mitogen-activated protein kinase phosphatase-1 in lipopolysaccharide-stimulated macrophages. *Journal of Immunology* **164** 29–37.
- Vanden Berghe W, Francesconi E, De Bosscher K, Resche-Rigon M & Haegeman G 1999 Dissociated glucocorticoids with

- anti-inflammatory potential repress interleukin-6 gene expression by a nuclear factor-kappaB-dependent mechanism. *Molecular Pharmacology* **56** 797–806.
- Vayssiere BM, 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*. *Molecular Endocrinology* **11** 1245–1255.
- Vermeulen L, De Wilde G, Damme PV, Vanden Berghe W & Haegeman G 2003 Transcriptional activation of the NF-kappaB p65 subunit by mitogen- and stress-activated protein kinase-1 (MSK1). *EMBO Journal* **22** 1313–1324.
- Waetzig GH, Seegert D, Rosenstiel P, Nikolaus S & Schreiber S 2002 p38 mitogen-activated protein kinase is activated and linked to TNF-alpha signaling in inflammatory bowel disease. *Journal of Immunology* **168** 5342–5351.
- Wang SW, Pawlowski J, Wathen ST, Kinney SD, Lichenstein HS & Manthey CL 1999 Cytokine mRNA decay is accelerated by an inhibitor of p38-mitogen-activated protein kinase. *Inflammation Research* **48** 533–538.
- Whitmarsh AJ & Davis RJ 1996 Transcription factor AP-1 regulation by mitogen-activated protein kinase signal transduction pathways. *Journal of Molecular Medicine* **74** 589–607.
- Winzen R, Kracht M, Ritter B, Wilhelm A, Chen CY, Shyu AB, Muller M, Gaestel M, Resch K & Holtmann H 1999 The p38 MAP kinase pathway signals for cytokine-induced mRNA stabilization via MAP kinase-activated protein kinase 2 and an AU-rich region-targeted mechanism. *EMBO Journal* **18** 4969–4980.
- Xiao YQ, Malcolm K, Worthen GS, Gardai S, Schiemann WP, Fadok VA, Bratton DL & Henson PM 2002 Cross-talk between ERK and p38 MAPK mediates selective suppression of pro-inflammatory cytokines by transforming growth factor-beta. *Journal of Biological Chemistry* **277** 14884–14893.

Received 3 December 2002

Accepted 10 April 2003

Made available online as an

Accepted Preprint 23 April 2003