Dual-specificity phosphatase 1: a critical regulator of innate immune responses

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Abstract
Innate immune responses are critically dependent on MAPK (mitogen-activated protein kinase) signalling pathways, in particular JNK (c-Jun N-terminal kinase) and p38 MAPK. Both of these kinases are negatively regulated via their dephosphorylation by DUSP1 (dual-specificity phosphatase 1). Several pro- and anti-inflammatory stimuli converge to regulate the DUSP1 gene and to modulate the time course of its expression. In turn, the pattern of expression of DUSP1 dictates the kinetics of activation of JNK and p38 MAPK, and this influences the expression of several mediators of innate immunity. DUSP1 is therefore a central regulator of innate immunity, and its expression can profoundly affect the outcome of inflammatory challenges. We discuss possible implications for immune-mediated inflammatory diseases and their treatment.

Introduction: MAPKs (mitogen-activated protein kinases) and DUSPs (dual-specificity phosphatases)
The MAPKs are components of intracellular signalling pathways that control a vast array of cellular processes, one of which is the innate immune response to infection or tissue injury. The ERK (extracellular-signal-regulated kinase), JNK (c-Jun N-terminal kinase) and p38 MAPK pathways are phosphorylation cascades, in which the activation of each MAPK is mediated by the phosphorylation of specific threonine and tyrosine residues. JNK and p38 MAPK are consistently activated by pro-inflammatory agonists, and contribute to subsequent inflammatory or innate immune responses by regulating the expression of numerous effector genes at transcriptional or post-transcriptional levels. Often, but not invariably, the ERK pathway is also activated by pro-inflammatory agonists, and may contribute to subsequent changes in gene expression and/or cellular proliferation.

The innate immune system must be regulated exceptionally tightly because inadequate responses cause increased susceptibility to infectious pathogens, whereas exaggerated responses may lead to chronic inflammatory diseases or potentially lethal acute inflammatory conditions such as septic shock. The homoeostatic balance of the innate immune system is maintained partly by a network of counter-regulatory, pro- and anti-inflammatory cytokines. At the intracellular level, proportionate responses require tight control of the strength and duration of signalling events; hence the termination of MAPK signalling is a crucial process. The inactivation of MAPKs is partly dependent on MKPs (MAPK phosphatases) or DUSPs. The DUSPs comprise a family of approximately ten phosphatases, differing in expression, subcellular localization and substrate specificity. DUSP1 (otherwise known as MKP-1) is the founder member of this family and the focus of our review. It was originally thought to dephosphorylate and inactivate ERK, but was later shown to preferentially target p38 MAPK and JNK. Consistent with this, cells from a DUSP1−/− mouse showed no abnormality in the kinetics of ERK activation, but did show dysregulation of both JNK and p38 pathways.

Pro- and anti-inflammatory signals converge on DUSP1
TLRs (Toll-like receptors)
DUSP1 was first described as an immediate early gene induced by heat shock and oxidative stress. It has since been...
Several pro- and anti-inflammatory stimuli converge to regulate expression of DUSP1

Broken arrows represent synergistic interactions. Not all regulatory interactions are shown. ANP, atrial natriuretic peptide; CTB, cholera toxin B subunit; GC, glucocorticoid.

shown to be up-regulated by other pro-inflammatory stress stimuli such as UV light and the pro-inflammatory cytokine IL-1 (interleukin 1) [16–18] (Figure 1). In the context of innate immune responses, its induction by the TLRs is particularly significant. The TLRs comprise a large family of transmembrane proteins that act as homo- or hetero-dimers to recognize lipid, peptide and nucleic acid molecules associated with micro-organisms [19,20]. For example, TLR4 recognizes LPS (lipopolysaccharide), a component of the cell wall of Gram-negative bacteria. When bound by appropriate ligands, TLRs engage signalling pathways that culminate in the activation of MAPKs, NF-κB (nuclear factor κB) and (in some cases) members of the IFN (interferon) response factor family of transcription factors [19,20]. Through these pathways, the TLRs initiate and co-ordinate expression of several effector genes, enabling a rapid and efficient innate immune response. In macrophages or myeloid cell lines, rapid and transient DUSP1 expression was induced by LPS [3,6–8,11,21,22] and by various other TLR ligands [7,21,23]. In contrast, both LPS and heat shock caused prolonged expression of DUSP1 in THP-1, an immature myeloid leukaemia cell line [24,25]. This was suggested to contribute to tolerization, a phenomenon in which prior exposure to a pro-inflammatory stimulus impairs the response to a second stimulus [26]. Consistent with this hypothesis, DUSP1−/− macrophages were inefficiently tolerized by either heat shock or LPS [24,25]. However, it is not yet clear whether heat shock- or LPS-induced expression of DUSP1 in primary, non-leukaemic myeloid cells is sufficiently sustained to account for LPS tolerance or whether indirect effects of DUSP1 ablation may play a significant role.

Other members of the DUSP family may also be expressed by macrophages in response to TLR engagement, including DUSP10 [27], DUSP2 and DUSP16 [21,28]. DUSP10−/− macrophages overexpress the inflammatory cytokines TNF (tumour necrosis factor) and IL-6 in response to LPS [27], suggesting that this phosphatase may overlap in function with DUSP1 (see below). Surprisingly, LPS-activated DUSP2−/− macrophages express less TNF and IL-6 than wild-type controls [29]; therefore DUSP2 may serve a distinct, positive regulatory role in innate immunity.

Generally consistent conclusions on the role of DUSP1 have been drawn from studies using macrophages from a DUSP1−/− mouse strain [6–11], RNA interference-mediated knockdown of DUSP1 expression [23] or pharmacological blockade of DUSP1 expression using triptolide, a component of a Chinese traditional medicine, whose specificity and mechanism of action are not known [3,10,23]. In the absence of DUSP1 expression, the LPS-induced activation of JNK and p38 MAPK was prolonged, but the time course of ERK activation was not affected, in agreement with the putative JNK/p38 MAPK specificity of DUSP1 [4,5]. Conversely, adenovirus-mediated overexpression of DUSP1 accelerated the inactivation of JNK and p38 MAPK but did not affect ERK [3,10,24]. Early peaks of LPS-induced JNK and p38 MAPK activity were not markedly enhanced in DUSP1−/− cells, suggesting that induction of phosphatase expression is primarily a negative feedback mechanism to regulate the off-phase of JNK and p38 MAPK signalling. It should be noted that levels of phosphorylated JNK and p38 MAPK did decline, albeit slowly, in cells lacking DUSP1. Either dephosphorylation and inactivation are mediated by other phosphatase(s) or turnover of the phosphorylated MAPKs contributes to eventual termination of the signal.

Most of the effects of DUSP1 ablation have been functionally linked to dysregulation of p38 MAPK. Although alterations in JNK signalling are also very likely to be significant, this is less easy to demonstrate because of a lack of highly specific inhibitors. The p38 MAPK pathway positively regulates expression of many pro-inflammatory cytokines [1,30], but is also required for the expression of IL-10 (see [6,7,11] and references cited therein). A product of activated macrophages and other haemapoietic cells, IL-10 has powerful but incompletely understood immunomodulatory and anti-inflammatory effects, including suppression of TNF production [31,32]. In response to engagement of TLRs, macrophages typically express IL-10 more slowly than TNF. The use of a common signalling pathway to regulate early and late expression of pro- and anti-inflammatory mediators may serve as a failsafe mechanism to prevent exaggerated responses to inflammatory agonists [7,11]. Consistent with such a dual role, dysregulation of the p38 MAPK (and JNK) pathway(s) in DUSP1−/− macrophages has complex consequences, which include overexpression of both pro- and anti-inflammatory effectors. For example, LPS challenge of DUSP1−/− macrophages, splenocytes, dendritic cells or whole animals caused overexpression of both TNF and IL-10 at early times. The sustained overexpression of IL-10 inhibited TNF biosynthesis, so that the amounts of TNF expressed by DUSP1−/− and DUSP1−/− macrophages were similar at later time points. Other inflammatory mediators that were overexpressed at the protein or mRNA level by DUSP1−/− cells or animals included CSF2 (colony-stimulating factor 2), COX-2 (cyclo-oxygenase 2), IL-1β, IL-6...
and the chemokines CCL2 [chemokine (CC motif) ligand 2], CCL3, CCL4, CXCL1 [chemokine (CXC motif) 1 ligand] and CXCL2. Unlike TNF, some chemokines continued to be overexpressed by DUSP1−/− macrophages up to 20 h after LPS challenge [8]. Conflicting reports on the expression of IL-12 [8,9,11] or IFN-γ [7,9,11] in DUSP1−/− cells or animals remain to be resolved. Other inflammatory mediators may be found to be overexpressed by DUSP1−/− cells, depending on the time point selected for detailed analysis. In summary, DUSP1 deficiency results in overexpression of several pro-inflammatory genes in response to LPS. Such overexpression may be transient or sustained, presumably depending (at least in part) on whether these genes are sensitive to inhibition by IL-10. In vivo, the outcome is an elevated sensitivity to LPS-induced mortality [7–9,11], associated with pulmonary, renal and hepatic damage that is consistent with lethal endotoxic shock [11]. Either the transient overexpression of TNF is sufficient to drive this response, or other mediators that are expressed in a more sustained fashion play a crucial role. LPS-induced lethality occurred despite the presence of elevated IL-10.

**IL-10**

It was recently shown that, in the presence of LPS, IL-10 prolongs the expression of DUSP1, accelerating the inactivation of JNK and p38 MAPK without diminishing peak activity [21]. IL-10 had previously been suggested to block TNF biosynthesis by inhibiting p38 MAPK function [33]. To date, this putative mechanism has remained controversial (see [21,30] and references cited therein), several investigators failing to detect inhibition of p38 MAPK function by IL-10. This might be partly because investigators have naturally focused on the early peak of kinase activity, whereas IL-10 may exert its effects during the late phase of relatively weak activity. An additional consideration is the abundant expression of endogenous IL-10 by activated macrophages, potentially masking effects of exogenous addition [21]. At any rate the link between IL-10, p38 MAPK and DUSP1 now appears rather complex. The anti-inflammatory cytokine lies both upstream of DUSP1 (modulating its expression in response to LPS) and downstream of DUSP1 (as a target of the p38 MAPK signalling pathway). It has been suggested that DUSP1 mediates anti-inflammatory effects of IL-10 [21], but this is difficult to test directly because of the strong overexpression of IL-10 by DUSP1−/− macrophages. The nature of this complex interaction might be best explored by studying DUSP1−/− IL-10−/− mice.

**Other cytokines and pathogen gene products**

The immunosuppressive cytokine TGF-β (transforming growth factor β) can also induce DUSP1 expression [34,34a]. In the murine macrophage cell line RAW264.7, TGF-β co-operated with LPS to induce DUSP1 expression and decreased the activation of p38 MAPK [34]. Whether DUSP1 is relevant to the anti-inflammatory or immunosuppressive effects of TGF-β on myeloid and other cell types [35,36] remains to be determined.

Atrial natriuretic peptide exerts anti-inflammatory and anti-atherogenic effects on vascular endothelial cells. These effects have been linked to the induction of DUSP1 gene expression [37–39]. Antisense or siRNA (small interfering RNA)-mediated inhibition of DUSP1 expression prolonged the activation of p38 MAPK by TNF in vascular endothelial cells, enhancing the activation of an E-selectin promoter construct or the expression of the chemokine CCL2 [38–40]. These observations make a case for studying vascular inflammatory responses in DUSP1−/− mice.

IFN-γ is a potent ‘priming’ cytokine, which enhances the responsiveness of macrophages to pro-inflammatory agonists such as TLR ligands [41]. In contrast with the factors discussed above, IFN-γ partially inhibits the induction of DUSP1 by LPS, and consequently prolongs the activation of JNK and p38 MAPK [11]. At least in theory, this provides a mechanism for the synergistic regulation of pro-inflammatory gene expression by LPS and IFN-γ [14].

Both the cholera toxin B polyepitope [3] and the HIV nef protein [42] can up-regulate DUSP1 and inhibit expression of innate immune mediators. These observations suggest that DUSP1 may be targeted by pathogens to prevent activation of MAPK pathways and evade the innate immune system.

**Glucocorticoids and MIF (macrophage migration inhibitory factor)**

Glucocorticoids are powerful anti-inflammatory agents which reduce the expression of countless inflammatory mediators and have been successfully used for more than 50 years in the treatment of immune-mediated inflammatory diseases [43]. Glucocorticoids are able to inhibit function of MAPKs [44], and induce sustained expression of DUSP1 in HeLa cells [17,45], mast cells [12,46], osteoblasts [48], breast and lung epithelial cells [49,50], T-lymphocytes [51], rheumatoid synovial fibroblasts [18] and myeloid cells [3,6,10,21,51]. A variety of commonly prescribed glucocorticoids induced DUSP1 expression in alveolar macrophages [10]. In a murine model of acute inflammation, the synthetic glucocorticoid dexamethasone exerted significant anti-inflammatory effects in DUSP1+/+ mice but not in DUSP1−/− mice [6], implicating DUSP1 as an important mediator of anti-inflammatory actions of glucocorticoids in vivo. In DUSP1+/+ macrophages but not in DUSP1−/− macrophages, dexamethasone impaired late phase JNK and p38 MAPK signalling, and down-regulated the expression of several inflammatory mediators, notably COX-2, TNF, IL-1α, IL-1β, CXCL1 and IFN-γ [6]. All of these are encoded by labile transcripts and can be negatively regulated by glucocorticoids at a post-transcriptional level. Their efficient expression requires prolonged activation of p38 MAPK to maintain mRNA stability (references cited in [30]). We suggest that an important mechanism of action of glucocorticoids is to destabilize a subset of pro-inflammatory mRNAs by inducing DUSP1 and impairing the prolonged phase of p38 MAPK activation. On the other hand, some
inflammatory mediators were equally inhibited by dexamethasone in both DUSP1+/+ and DUSP1−/− macrophages [6]; therefore DUSP1-independent inhibitory mechanisms of glucocorticoids must also exist.

MIF is a widely expressed cytokine that has profound effects on innate immune responses and has been implicated in a wide variety of inflammatory pathologies, such as rheumatoid arthritis, sepsis and colitis [52,53]. Although induced by glucocorticoids, MIF overrides their anti-inflammatory effects [54–56], and has been described as a physiological antagonist of glucocorticoid activity [52]. A number of mechanisms have been suggested for this phenomenon (references cited in [52,53]). Most recently MIF was shown to inhibit glucocorticoid-induced DUSP1 expression, which was proposed to enhance inflammatory mediator production by modulating MAPK signalling pathways [57,58]. Effects of MIF that are independent of glucocorticoids or DUSP1 have not yet been ruled out.

**Regulation of DUSP1 gene expression**

The expression of DUSP1 is co-ordinately regulated by a very wide variety of different agonists, but is still quite poorly understood. Reactive oxygen species were suggested to play an important role in the induction of DUSP1 [16,37,59] although, paradoxically, these also impair the catalytic activity of DUSPs [60]. The p38 MAPK, ERK and PKCε (protein kinase Cε) pathways have been implicated in the regulation of DUSP1 expression by pro-inflammatory or stress stimuli [3,16,17,22,34], although transcription factors that transduce these signals have not been identified.

Glucocorticoids regulate gene expression via the GR (glucocorticoid receptor), a member of the nuclear hormone receptor family of transcription factors [43]. GR can homodimerize and bind to palindromic GREs (glucocorticoid response elements), or can interact with other transcription factors to control gene expression [43]. A 1.7 kb DUSP1 promoter fragment responded to dexamethasone in Cos-7 cells transfected with wild-type GR but not a dimerization defective mutant of GR [12]. Nevertheless, dexamethasone was able to up-regulate DUSP1 expression in primary murine macrophages expressing only the dimerization defective GR [6]. The DUSP1 promoter does not contain obvious homologies to the canonical GRE sequence, and the mechanisms of regulation of DUSP1 by glucocorticoids, alone or in synergy with pro-inflammatory stimuli, remain to be resolved. Similarly, sequences that mediate the regulation of DUSP1 expression by IL-10 have so far proved elusive [21].

Stably integrated DUSP1 promoter constructs responded to stress stimuli, whereas transiently transfected constructs did not [16], suggesting that chromatin modifications may play a critical role. Consistent with this, the activation of DUSP1 transcription was accompanied by acetylation and phosphorylation of histone H3 at the locus, and was acutely sensitive to inhibitors of histone deacetylase [16,61]. Growth factors controlled DUSP1 expression at the level of transcriptional processivity [62], but the relevance of this mechanism to the inflammatory response has not been demonstrated.

The highly unstable DUSP1 transcript was stabilized by heat shock [63] but not LPS, IL-10, H2O2 or IL-1 [21,59] (C. Boucheron and A.R. Clark, unpublished work). The 3′-untranslated region contains four copies of the AUUUA destabilizing motif, but was not sufficient to confer instability on a reporter mRNA (C. Boucheron and A.R. Clark, unpublished work). At the post-translational level, DUSP1 protein is subject to proteasome-mediated degradation, and its levels were increased by proteasome inhibitors [12,64–66]. There is disagreement over how signalling pathways influence DUSP1 protein turnover [12,64,67,68]. In summary, almost every aspect of the regulation of DUSP1 gene expression remains puzzling.

**Implications for inflammatory diseases and their treatment**

**DUSP1 in inflammatory disease**

The function of DUSP1 in the regulation of innate immune responses has been studied mainly using myeloid cell lines and a knockout mouse strain; therefore conclusions about human pathologies must be tentative. In the mouse, DUSP1 serves as a central regulator of innate immune responses by integrating pro- and anti-inflammatory inputs and modulating the strength and duration of MAPK signalling. There are clear differences in the LPS responses of DUSP1+/+ and DUSP1−/− macrophages [7], although in vivo responses of DUSP1+/+ and DUSP1−/− animals have not yet been compared. A possible implication of this haplo-insufficiency is that relatively minor differences in the expression of DUSP1 may strongly influence reactions to infectious pathogens. In the DUSP1−/− mouse, collagen-induced arthritis (a model of rheumatoid arthritis) is exacerbated [9]. It will be fascinating to determine whether other complex, immune-mediated inflammatory diseases are also exacerbated in DUSP1−/− or DUSP1+/− animals. It may also be informative to investigate whether genetic or epigenetic differences at the human DUSP1 locus are associated with differences in susceptibility to, or severity of, inflammatory conditions such as rheumatoid arthritis, Crohn’s disease, sepsis and atherosclerosis.

**Glucocorticoids in the treatment of inflammation**

The use of glucocorticoids in the treatment of inflammation is hampered by the severe side effects of these drugs [69], and by their poor efficacy in some individuals [70]. Anti-inflammatory effects are generally believed to be mediated by transrepression, a mechanism in which activated GR impairs the activity of the transcription factors NF-κB and AP-1 (activating protein 1) and hence blocks transcription of many pro-inflammatory genes [71]. Side effects are thought to be largely dependent on transcriptional induction by GR [69]. Current efforts to reduce the side effects
of glucocorticoids are based on the premise that desired and undesired functions of the GR might be uncoupled using a new class of ‘dissociated’ GR agonists, which cause transrepression but do not strongly activate gene expression [72,73]. This model of discrete and separable glucocorticoid actions will be weakened, and the challenge of improving the therapeutic profile of glucocorticoids will become yet more complex if the anti-inflammatory effects of glucocorticoids are significantly dependent on the induction of DUSP1 gene expression (which remains to be proved in humans). At the same time, if DUSP1 contributes to the anti-inflammatory effects of glucocorticoids, then defects in DUSP1 expression or activity could result in glucocorticoid insensitivity. In asthma and inflammatory bowel disease, glucocorticoid insensitivity has previously been linked to elevated JNK and p38 MAPK activities, which failed to be suppressed by glucocorticoids [74,75]. It may be worth closer examination of the effects of glucocorticoids upon DUSP1 expression and MAPK activities in the context of glucocorticoid-insensitive inflammatory disease. As an endogenous cytokine that limits glucocorticoid efficacy, MIF is another potential target in the treatment of inflammation [53]. For example, neutralization of MIF might enable lower doses of glucocorticoids to be used, reducing side effects and perhaps even overcoming glucocorticoid resistance.

**DUSP1 as a therapeutic target**

DUSP1 has recently gained interest in the field of oncology, since it is overexpressed by several tumours and has been implicated in enhancing cell survival by inhibiting pro-apoptotic signalling pathways. Specific inhibitors of DUSP1 are in development [76,77], and have been mooted as chemotherapeutic agents. In the context of chronic inflammation, physicians might have the opposite aspiration: to increase the expression or activity of DUSP1. To increase endogenous DUSP1 expression, a much more detailed understanding of the transcriptional, post-transcriptional and post-translational control of this gene is required. Another possible approach would be gene therapy, for example using a promoter construct that is transiently activated by pro-inflammatory diseases.

**Concluding remarks**

The DUSP1 story provides an object lesson on how the phenotype of a knockout can depend on the questions that are asked of it. DUSP1 was originally considered a regulator of the ERK pathway, and defects in cellular proliferation were anticipated (but not found) when the knockout was first generated [5]. As more has been learned about the role of the p38 MAPK and JNK pathways and their regulation by DUSP1, different questions have been asked using the original strain or a second, independently derived strain [9]. From these studies, it has emerged that DUSP1 plays a vital role in the regulation of innate immune responses. An important question is whether human DUSP1 plays a similar, seemingly non-redundant role. If this proves to be the case, there may be extremely interesting implications for susceptibility to, treatment of, and outcomes in immune-mediated inflammatory diseases.

**References**
