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The kinases MSK1 and MSK2 act as negative regulators of Toll-like receptor signaling

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The kinases MSK1 and MSK2 are activated 'downstream' of the p38 and Erk1/2 mitogen-activated protein kinases. Here we found that MSK1 and MSK2 were needed to limit the production of proinflammatory cytokines in response to stimulation of primary macrophages with lipopolysaccharide. By inducing transcription of the mitogen-activated protein kinase phosphatase DUSP1 and the anti-inflammatory cytokine interleukin 10, MSK1 and MSK2 exerted many negative feedback mechanisms. Deficiency in MSK1 and MSK2 prevented the binding of phosphorylated transcription factors CREB and ATF1 to the promoters of the genes encoding interleukin 10 and DUSP1. Mice doubly deficient in MSK1 and MSK2 were hypersensitive to lipopolysaccharide-induced endotoxic shock and showed prolonged inflammation in a model of toxic contact eczema induced by phorbol 12-myristate 13-acetate. Our results establish MSK1 and MSK2 as key components of negative feedback mechanisms needed to limit Toll-like receptor–driven inflammation.

The innate immune system recognizes pathogens through the detection of specific fungal, microbial or viral molecules called 'pathogen-associated molecular patterns'. Several groups of receptors for these molecules have been described, including Toll-like receptors (TLRs), Nod-like receptors and C-type lectins, which recognize distinct sets of pathogen-associated molecular patterns[1–3]. In macrophages, a chief consequence of TLR activation is the production of proinflammatory cytokines, including tumor necrosis factor (TNF), interleukin 1 (IL-1), IL-6 and IL-12. The production of these cytokines is tightly controlled at the level of transcription, translation and secretion and requires the activation of several signaling pathways, including those involving the transcription factor NF-κB and the mitogen-activated protein kinases (MAPKs) Erk1/2, p38 and Jnk[1–3]. The p38 pathway has attracted considerable interest as a possible target for anti-inflammatory drugs. Originally described as a MAPK activated by lipopolysaccharide (LPS), stress or IL-1 (refs. 4–6), p38 is the cellular target of a group of pyridinyl imidazole compounds that inhibit the production of IL-1 and TNF by monocytes[7]. Four p38 isoforms (p38α, p38β, p38γ and p38δ) have been described, but only p38α (A001717) and p38β (A001718) are inhibited by widely used p38 inhibitors such as SB203580 (ref. 8). The use of gene-targeted mice has shown that p38α, not p38β, is critical in the innate immune system[9–11]. Although many p38 inhibitors have been developed as potential drugs for autoimmune disease, none of these have progressed through clinical trials because of problems with toxicity. Although it is possible that this toxicity is due to 'off-target' activity or inhibition of p38 outside the immune system, it is also possible that p38 inhibition could have unforeseen deleterious effects relating to the immune system[12,13].

In addition to their functions in promoting inflammation, TLRs can also activate negative feedback mechanisms and anti-inflammatory signals, which are critical in preventing excessive inflammation and may also be involved in the resolution of inflammation[12,14]. Several MAPKs, including p38 and Erk1/2, are required for some of these negative feedback mechanisms, and it is possible that some of the toxicity of p38 inhibitors could be due to the inhibition of these negative feedback pathways. For example, p38 activation results in the phosphorylation of TAB1, the regulatory subunit of the complex containing TAK1, a MAPK kinase kinase involved in the activation of p38, Jnk and NF-κB that results in the inhibition of TAK1; thus, p38 inhibition can result in enhanced Jnk and NF-κB activity[15]. Erk1/2 and p38 also induce the transcription of DUSP1 (A000025; also called MKP-1), a MAPK phosphatase that dephosphorylates and thus inactivates p38 and Jnk *in vivo*[16]. Mice lacking DUSP1 show prolonged activation of p38 and Jnk in response to LPS, as well as augmented TNF production and greater sensitivity to endotoxic shock[17–20].

MSK1 (A001562) and MSK2 (A001563) are two related kinases that are activated *in vivo* 'downstream' of p38α and Erk1/2 (ref. 21). Mice lacking both MSK1 and MSK2 are viable and do not develop any obviously altered phenotype when maintained in specific pathogen–free conditions[22]. MSK1 and MSK2 can phosphorylate the transcription factors CREB and ATF1, as well as the chromatin protein histone H3, in response to mitogens and cellular stress[22,23]. As a result,

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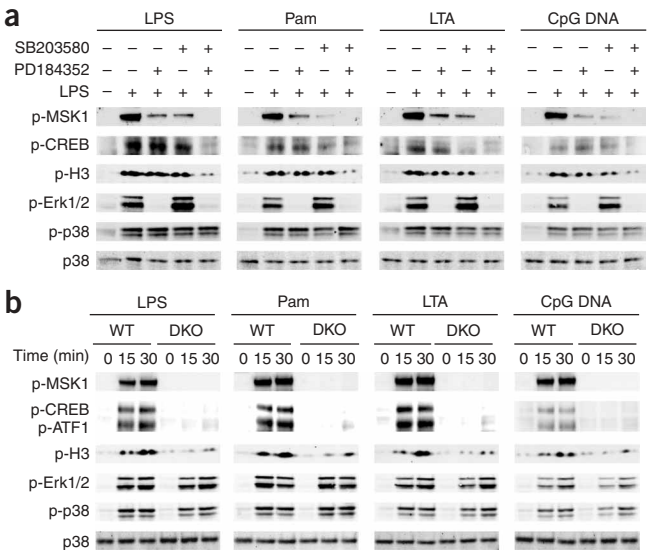

Figure 1 MSK is activated by TLR signaling. (**a**) Immunoblot analysis of MSK1 phosphorylated (p-) at Thr581, CREB phosphorylated at Ser133, histone H3 phosphorylated at Ser10, phosphorylated Erk1/2 and p38, and total p38 in lysates of wild-type BMDMs preincubated for 1 h with PD184352 (2 μM), SB203580 (5 μM) or a combination of both inhibitors and then stimulated for 30 min with LPS (100 ng/ml), Pam_3CSK_4 (Pam; 1 μg/ml), LTA (5 μg/ml) or CpG DNA (2.5 μM). Results are representative of three independent experiments. (**b**) Immunoblot analysis as described in **a** of BMDMs isolated from wild-type (WT) and double-knockout (DKO) mice and stimulated for 0, 15 or 30 min with LPS (100 ng/ml), Pam_3CSK_4 (1 μg/ml), LTA (5 μg/ml) or CpG DNA (2.5 μM). Results are representative of three independent experiments.

MSK1 and MSK2 are involved in the transcriptional induction of CREB-dependent immediate-early genes[24,25]. Neurons lacking MSK1 have less DUSP1 transcription after stimulation with neurotrophins[24]. If MSK1 and MSK2 also regulate DUSP1 transcription 'downstream' of TLR signaling, they may have an anti-inflammatory function. Although TLR-induced MSK activation has been demonstrated in cell lines[21], the function of MSK1 and MSK2 in inflammation *in vivo* has not been addressed before, to our knowledge. We therefore used mice doubly deficient in both MSK1 and MSK2 (double-knockout mice) to examine the function of MSKs 'downstream' of TLR signaling both *in vivo* and in primary macrophages *in vitro*.

RESULTS

MSKs in TLR-induced signaling

TLRs activate Erk1/2 and p38 in macrophages, which suggests that TLR signaling should also activate MSKs. LPS, through TLR4, activated Erk1/2 and p38α in bone marrow–derived macrophages (BMDMs; **Fig. 1a**). LPS also activated MSK1, as assessed by phosphorylation of the threoine residue at position 581 (Thr581), a site important for MSK1 activation[21]. MSK1 activation was partially blocked by preincubation of cells with inhibitors of the kinases MEK1 and MEK2 (PD184352, which blocks Erk1/2 activation[8]) or p38 (SB203580). A combination of both inhibitors was required for full blockade of MSK activation (**Fig. 1a**), which indicated that both p38 and Erk1/2 contribute to TLR4-driven MSK activation. LPS also induced phosphorylation of CREB and histone H3.

To determine if MSKs were responsible for the TLR4-induced phosphorylation of CREB and histone H3, we analyzed BMDMs from mice lacking MSK1 and MSK2. Deficiency of both MSK1 and MSK2 did not seem to affect macrophage differentiation, as we obtained equivalent numbers of BMDMs from wild-type control mice and double-knockout mice, and wild-type and double-knockout BMDMs expressed the macrophage-specific marker F4/80 and lacked the dendritic cell marker CD83 (**Supplementary Fig. 1** online). However, LPS-driven phosphorylation of CREB and histone H3 was much lower in double-knockout BMDMs (**Fig. 1b**). This impaired phosphorylation was not due to an effect on 'upstream' signaling, as double-knockout BMDMs had normal phosphorylation of Erk1/2 and p38α in response to LPS (**Fig. 1b**). We obtained similar results with the TLR ligands lipoteichoic acid (LTA; TLR2 ligand), tripalmitoyl cysteinyl seryl tetralysine lipopeptide (Pam_3CSK_4; a ligand of TLR2, TLR1 and TLR6) and CpG DNA (TLR9 ligand; **Fig. 1**), which indicated that MSK activation is not restricted to TLR4.

MSKs suppress proinflammatory cytokine production

As MSKs are activated by TLRs, we next used a cytokine multiplex array to examine the function of MSKs in the production of proinflammatory cytokines by BMDMs. Notably, deficiency in both MSK1 and MSK2 resulted in much more production of TNF, IL-6 and IL-12 (both IL-12p70 and IL-12p40) by LPS-stimulated BMDMs (**Fig. 2a**). Flow cytometry and immunoblot analysis of 'pre-TNF' also showed the enhancement in TNF (**Fig. 2b**). We found no difference in wild-type and double-knockout cells in the production of other cytokines (IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-9, IL-13 and IL-17) in this array (**Supplementary Fig. 2** online).

Cytokine production in macrophages is regulated at several levels, including transcription, mRNA stability, translation and secretion. We found that deficiency in both MSK1 and MSK2 resulted in more transcription of genes encoding the p35 and p40 subunits of IL-12 at 6 h and 8 h after LPS stimulation (**Fig. 2c**). Deficiency in both MSK1 and MSK2 exerted more moderate influence on TNF transcription and influenced IL-6 transcription only after 8 h of stimulation (**Fig. 2c**). The effects of deficiency in both MSK1 and MSK2 were in contrast to the effect of inhibiting p38 and Erk1/2 which, as expected, resulted in less production of TNF and IL-6 in response to LPS (**Supplementary Fig. 3** online). This finding suggests that MSKs might be specifically involved in the activation of negative feedback pathways 'downstream' of Erk1/2 and p38.

MSKs promote DUSP1 transcription in macrophages

Dusp1 is an immediate-early gene encoding the MAPK phosphatase DUSP1, which promotes p38 deactivation after LPS stimulation; DUSP1 deficiency results in more TNF production after TLR stimulation. In neurons, *Dusp1* transcription is regulated by MSKs through CREB phosphorylation[24]. Consistent with the finding that MSKs controlled the phosphorylation of CREB and ATF1 'downstream' of TLRs (**Fig. 1b**), we found that MSKs also promoted *Dusp1* transcription in macrophages. DUSP1 mRNA was rapidly and transiently upregulated in response to LPS in wild-type BMDMs; this upregulation was much lower in double-knockout cells (**Fig. 3a**). Consistent with involvement of MSK1, *Dusp1* transcription in BMDMs after LPS stimulation was not affected much by preincubation with either PD184352 or SB203580 alone; however, a combination of both PD184352 and SB203580 was able to inhibit the induction of DUSP1 mRNA (**Fig. 3b**). LPS stimulation also increased DUSP1 protein expression more in wild-type than in double-knockout BMDMs (**Fig. 3c**).

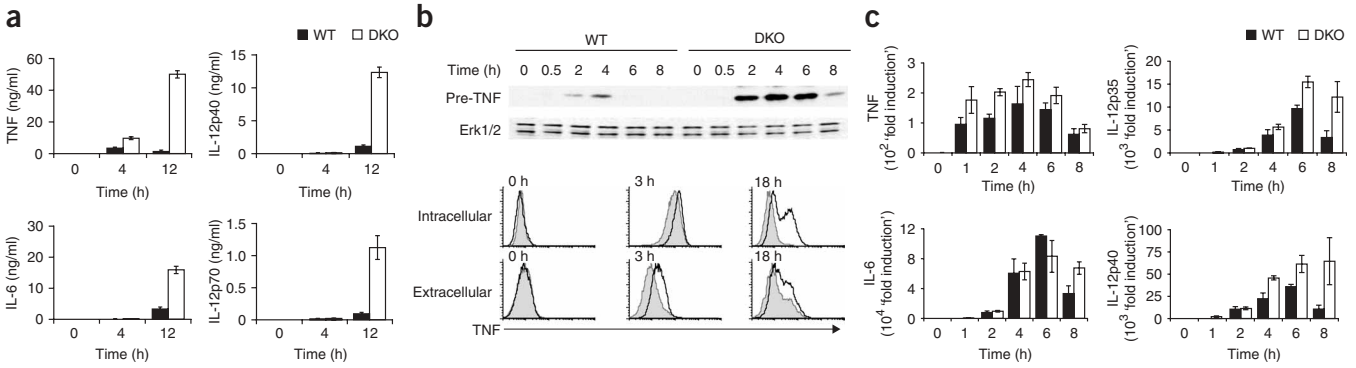

Figure 2 Deficiency in MSK1 and MSK2 results in more production of proinflammatory cytokines in response to LPS. (**a**) TNF, IL-6, IL-12p40 and IL-12p70 in culture media of wild-type or double-knockout BMDMs stimulated with LPS (100 ng/ml), assessed at 0, 4 and 12 h of stimulation. (**b**) Immunoblot analysis of cellular 'pre-TNF' in wild-type or double-knockout BMDMs stimulated for various times (above lanes) with LPS (100 ng/ml). Total Erk1/2, loading control. Below, flow cytometry of plasma membrane–bound (extracellular) and intracellular TNF in wild-type BMDMs (gray shaded histograms) and double-knockout BMDMs (black lines). Stimulated for 0, 3 or 18 h with LPS (100 ng/ml). (**c**) Quantitative PCR of total RNA from wild-type or double-knockout BMDMs stimulated for various times (horizontal axes) with LPS (100 ng/ml). Results were normalized to expression of 18s RNA and 'fold induction' was calculated relative to wild-type expression at 0 h. Data are from one experiment of three with similar results (error bars (**a**,**c**), s.e.m. of independent cultures from three mice).

In macrophages, DUSP1 'preferentially' dephosphorylates p38 rather than Erk1/2 (refs. 17–20). To determine whether the lower DUSP1 protein expression in double-knockout cells affected MAPK signaling, we quantified the phosphorylation of Erk1/2 and p38 over an extended time course of LPS stimulation. Deficiency in both MSK1 and MSK2 had no effect on the time course of LPS-induced Erk1/2 phosphorylation (**Fig. 3c**,**d**). In contrast, double-knockout cells had slower dephosphorylation of p38 than did wild-type cells (**Fig. 3c**,**d**). The partial nature of this effect probably stems from the fact that DUSP1 is not the only phosphatase for p38; overall, the pattern and magnitude of our results were in line with what has been reported before for DUSP1-deficient macrophages[17–20].

MSKs drive IL-10 production

In addition to triggering the production of proinflammatory cytokines, LPS also triggers production of the anti-inflammatory cytokine IL-10 in macrophages. Studies have linked both Erk1/2 and p38 to IL-10 production[26]; consistent with those findings, DUSP1 deficiency results in more IL-10 production in response to LPS[18]. In our studies, IL-10 mRNA quantities increased after LPS stimulation; however, the magnitude of IL-10 mRNA induction was much lower in double-knockout BMDMs than in wild-type BMDMs (**Fig. 4a**). Consistent with involvement of MSKs in *Il10* transcription, inhibition of p38 inhibited LPS-mediated induction of both IL-10 mRNA expression and IL-10 secretion (**Supplementary Fig. 4** online). Notably, inhibition of Erk1/2 caused an increase in IL-10 mRNA expression after LPS stimulation (**Supplementary Fig. 4**), which suggested that MAPK signaling can also influence *Il10* transcription independently of MSKs. Concentrations of secreted IL-10 steadily increased over an 8-hour time course after LPS stimulation of wild-type BMDMs (**Fig. 4b**). IL-10 secretion from LPS-stimulated double-knockout BMDMs was consistently lower than that of wild-type BMDMs. This discrepancy was greatest at early time points; by 8 h, the amount of IL-10 released by double-knockout cells reached 60% of the production by their wild-type counterparts (**Fig. 4b**).

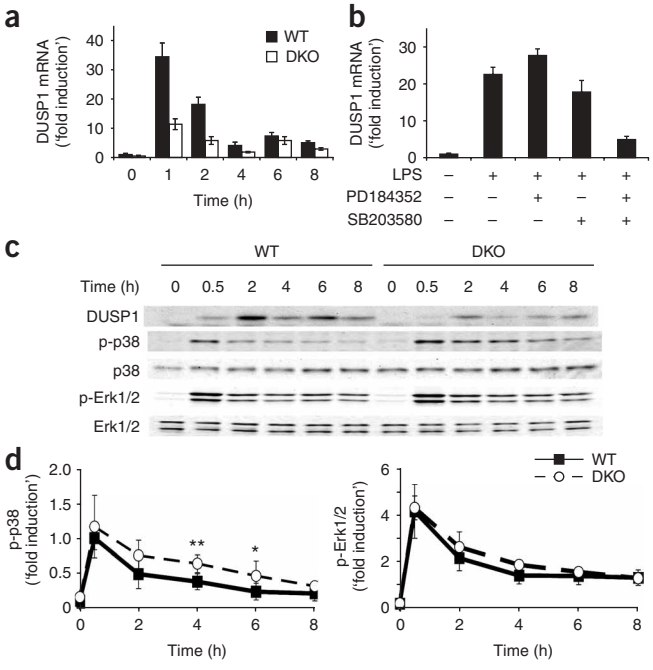

Figure 3 MSKs regulate *Dusp1* transcription in macrophages. (**a**) Quantitative PCR of DUSP1 mRNA in total RNA from wild-type or double-knockout BMDMs stimulated for various times (horizontal axis) with LPS (100 ng/ml). Results were normalized to 18s RNA expression and 'fold induction' was calculated relative to wild-type expression at 0 h. (**b**) Quantitative PCR of DUSP1 mRNA in total RNA from wild-type BMDMs preincubated for 1 h with PD184352 (2 μM), SB203580 (5 μM) or a combination of both inhibitors, then stimulated for 60 min with LPS (100 ng/ml). Results are normalized to 18s RNA expression. (**c**) Immunoblot analysis of DUSP1, phosphorylated p38 and Erk1/2, and total p38 and Erk1/2 in lysates of wild-type or double-knockout BMDMs stimulated with LPS (time, above lanes). (**d**) Quantification of phosphorylated p38 and Erk1/2 in the immunoblots in **c**, normalized to total p38 and Erk1/2, respectively. 'Fold induction' is relative to wild-type expression at 0 h. *, $P < 0.05$; **, $P < 0.01$, wild-type versus double-knockout. Data are from one experiment of three with similar results (**a**,**b**; error bars, s.e.m. of independent cultures from three mice) or are representative of two experiments with cultures from a total of six mice per genotype (**c**,**d**; error bars, s.e.m.).

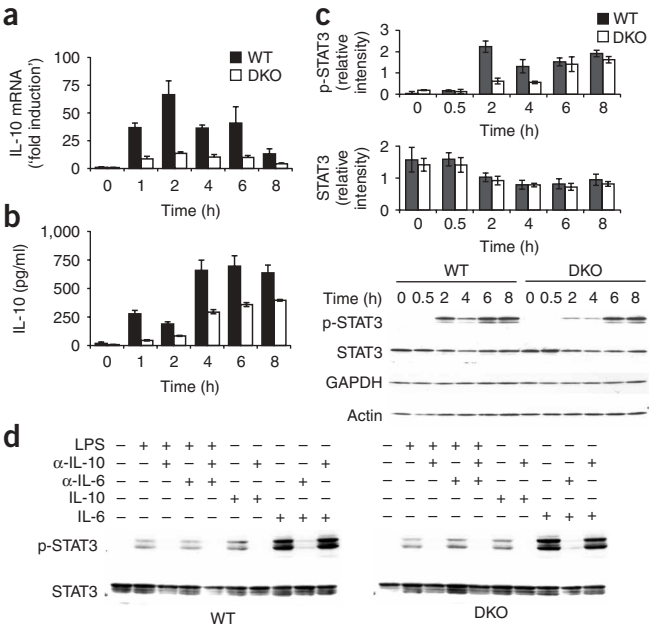

Figure 4 MSK regulates LPS-induced IL-10 production. (**a**,**b**) Quantitative PCR of IL-10 mRNA in total mRNA (**a**) and assay of secreted IL-10 (**b**) from wild-type or double-knockout BMDMs stimulated for various times (horizontal axes) with LPS (100 ng/ml). (**a**) Results normalized to expression of 18s RNA and 'fold induction' calculated relative to wild-type expression at 0 h. (**c**) Immunoblot analysis (bottom) and quantification (top) of STAT3 phosphorylated at Tyr705 and total STAT3 in wild-type or double-knockout BMDMs stimulated for various times with LPS (100 ng/ml); quantification is relative to an average of GAPDH (glyceraldehyde phosphate dehydrogenase) and actin (loading controls). (**d**) Immunoblot analysis of STAT3 phosphorylated at Tyr705 and total STAT3 in wild-type or double-knockout BMDMs given no pretreatment or pretreated for 15 min with antibodies neutralizing IL-10 (α-IL-10) or IL-6 (α-IL-6; 2.5 μg/ml), then stimulated with LPS (100 ng/ml; 8 h), recombinant mouse IL-10 (10 ng/ml; 30 min) or recombinant mouse IL-6 (10 ng/ml; 30 min). Data are from one experiment of three with similar results (error bars (**a**–**c**), s.e.m. of independent cultures from three mice).

IL-10 inhibits the production of proinflammatory cytokines in macrophages through the activation of Janus kinase–STAT transcription factor signaling. The importance of STAT3 in this process is emphasized by the finding that IL-10 does not effectively inhibit cytokine production in the absence of STAT3 (ref. 27). Stimulation of BMDMs with LPS for 2–8 h induces phosphorylation of STAT3 at Tyr705. This phosphorylation is probably the result of autocrine IL-10 production, as it does not occur in IL-10-deficient mice[28]. Consistent with those results, we found that STAT3 phosphorylation induced by either 2 h or 6 h of LPS stimulation was blocked by an IL-10-neutralizing antibody (**Supplementary Fig. 5** online). In line with the lower IL-10 production by double-knockout BMDMs (**Fig. 4b**), LPS-induced STAT3 phosphorylation was lower in double-knockout BMDMs than in wild-type BMDMs after 2 h of LPS stimulation; however, double-knockout and wild-type cells had similar STAT3 phosphorylation at 6 and 8 h after LPS stimulation (**Fig. 4c**). The effect of deficiency in both MSK1 and MSK2 on early LPS-triggered STAT3 phosphorylation was not due to involvement of MSKs 'downstream' of IL-10, as stimulation of BMDMs with IL-10 resulted in similar amounts of STAT3 phosphorylation as well as induction of SOCS3 mRNA, an established target of IL-10 signaling, in double-knockout and wild-type BMDMs (**Supplementary Fig. 5**). The normal STAT3 phosphorylation noted at later time points after LPS stimulation in the double-knockout BMDMs could be explained by the induction of STAT3 phosphorylation by another cytokine, such as IL-6, which had higher production in the double-knockout cells (**Fig. 2a**). Alternatively, it could also be explained by the finding that by 8 h, the double-knockout BMDMs produced 60% as much IL-10 as wild-type cells did (**Fig. 4b**); perhaps this amount of IL-10 was sufficient to induce full STAT3 phosphorylation. To test those hypotheses, we used neutralizing antibodies to IL-10 and IL-6. STAT3 phosphorylation induced by IL-10 and IL-6 was blocked by IL-10- and IL-6-neutralizing antibodies, respectively (**Fig. 4d**). In both double-knockout and wild-type BMDMs, the IL-10-neutralizing antibody, but not the IL-6-neutralizing antibody, blocked STAT3 phosphorylation induced by 8 h of LPS stimulation. These findings suggest that the amount of IL-10 released by the double-knockout macrophages was sufficient to trigger full STAT3 phosphorylation at later time points after LPS stimulation.

The addition of exogenous IL-10 to macrophages inhibits the induction of proinflammatory cytokines in response to LPS[27]. We therefore used an IL-10-neutralizing antibody to block the action of endogenous IL-10 to determine if the difference in TNF, IL-6 and IL-12 production by wild-type and double-knockout BMDMs could be explained by the lower IL-10 production by the double-knockout cells. In the absence of the IL-10-neutralizing antibody, the double-knockout BMDMs produced more TNF, IL-6 and IL-12 mRNA and protein than wild-type BMDMs did after LPS treatment. The addition of antibody to IL-10 resulted in only moderately more LPS-driven production of TNF, IL-6 and IL-12 in double-knockout BMDMs but a substantial augmentation of LPS-driven IL-6 and IL-12 cytokine production in wild-type BMDMs (**Fig. 5a**,**b**). TNF secretion was also increased by antibody to IL-10 in wild-type BMDMs; however, unlike IL-6 and IL-12, the increased TNF did not approach the quantity secreted by double-knockout macrophages (**Fig. 5a**,**b**). We found no effect when we used an isotope control antibody (**Fig. 5a**,**b**), and treatment with antibody to IL-10 did not substantially affect LPS-induced IL-10 production by either wild-type or double-knockout BMDMs (**Supplementary Fig. 6** online).

To confirm the results with the IL-10-neutralizing antibody, we crossed the double-knockout mice onto an IL-10-deficient background. As expected, BMDMs deficient in IL-10 alone secreted more IL-12, IL-6 and TNF than wild-type cells did in response to LPS (**Fig. 5c**). IL-10-deficient BMDMs and 'triple-knockout' BMDMs deficient in MSK1, MSK2 and IL-10 showed little difference in LPS-driven production of IL-6 and IL-12 (**Fig. 5c**). These results collectively indicate that the lower IL-10 production by double-knockout BMDMs contributed to their higher production of IL-6 and IL-12. In contrast to IL-6 and IL-12, more TNF was produced by the triple-knockout BMDMs than by IL-10-deficient BMDMs (**Fig. 5c**), consistent with the results obtained with IL-10-neutralizing antibody. These findings collectively indicate that MSKs regulate TNF production by IL-10-dependent and IL-10-independent mechanisms.

CREB and ATF1 in *Dusp1* and *Il10* transcription

The finding that the expression of IL-10 and DUSP1 mRNA induced by LPS stimulation was considerably impaired in double-knockout BMDMs suggested that MSKs may directly control the *Il10* and *Dusp1* promoters. MSKs phosphorylate CREB at Ser133 and thus increase its transcriptional activity. MSKs also phosphorylate ATF1, a CREB family member, at Ser63, a site with surrounding

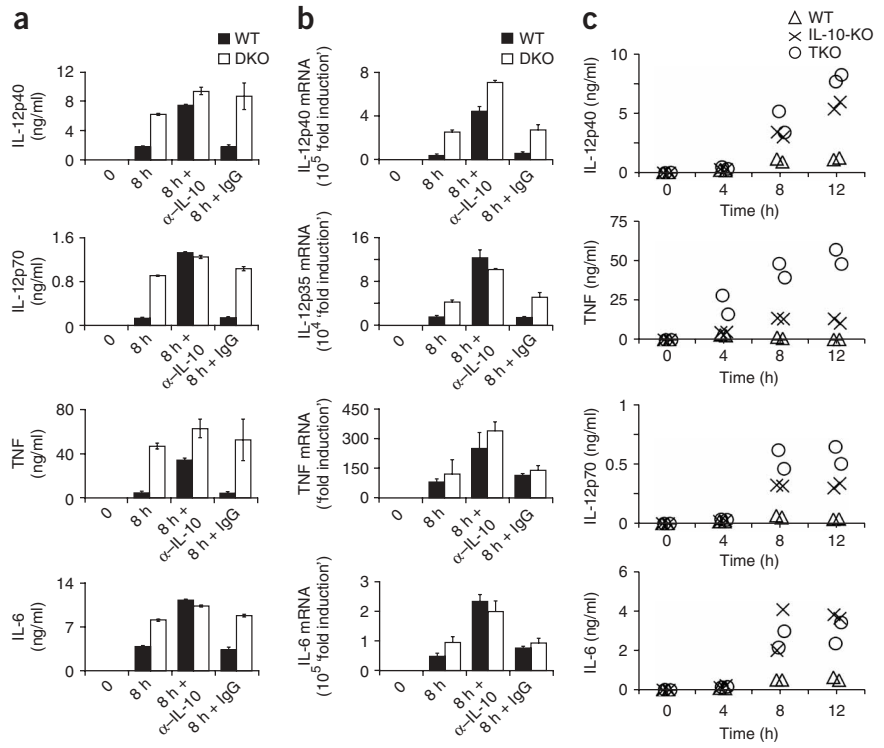

Figure 5 MSK-induced IL-10 production inhibits IL-6 and IL-12 production. (**a**) Cytokines in media of BMDMs given no pretreatment (8 h) or pretreated for 15 min with antibody to IL-10 (2.5 μg/ml; 8 h + α–IL-10) or rat immunoglobulin G (2.5 μg/ml; 8 h + IgG), then left unstimulated (0) or stimulated for 8 h with LPS (100 ng/ml). Data are from one of three experiments with similar results (error bars, s.e.m. of independent cultures from three mice). (**b**) Quantitative PCR of total mRNA from cells treated as described in **a**. Results were normalized to expression of 18s RNA and 'fold induction' was calculated relative to wild-type expression at 0 h time. Data are from one of three experiments with similar results (error bars, s.e.m. of independent cultures from three mice). (**c**) Secreted cytokines in media of wild-type BMDMs, IL-10-deficient BMDMs (IL-10-KO) and BMDMs deficient in IL-10, MSK1 and MSK2 (TKO) stimulated with LPS (100 ng/ml). Each symbol represents an individual mouse. Data are representative of one experiment.

sequences identical to those of Ser133 in CREB. As CREB and ATF1 were phosphorylated by MSK1 and MSK2 after LPS stimulation (**Fig. 1b**), we investigated the influence of CREB and ATF1 on the transcription of *Il10* and *Dusp1*. Chromatin immunoprecipitation with an antibody that recognizes the phosphorylated forms of CREB and ATF1 showed that phosphorylated CREB and/or ATF1 were/was bound to the *Il10* and *Dusp1* promoters after LPS stimulation in wild-type but not double-knockout BMDMs (**Fig. 6a**).

To determine if CREB phosphorylation was essential for *Il10* and *Dusp1* transcription, we assessed gene-targeted mice with a conditional point mutation in endogenous *Creb1* resulting in the substitution of alanine for serine at position 133 (S133A) that were crossed with mice transgenic for tamoxifen-inducible Cre recombinase (**Supplementary Fig. 7a** online). The mutation of *Creb1* in BMDMs was induced by excision of a wild-type 'minigene' by the addition of tamoxifen. In the resultant 'CREB-S133A' BMDMs, we noted residual CREB phosphorylation in response to LPS, which suggested that some cells may have escaped excision of the minigene (**Fig. 6b**). Analysis of the phosphorylation of Erk1/2, p38 and MSK1 after LPS stimulation showed that the S133A substitution did not affect the activation of these 'upstream' signaling pathways (**Fig. 6b**). Expression of Egr1 mRNA (a transcript established as being independent of MSK1 and CREB)[22], as well as of TNF and IL-6 mRNA, was not affected by the S133A substitution (**Supplementary Fig. 7b**). In contrast, expression of *nur77*, a known CREB-regulated MSK target gene[25], was lower in CREB-S133A BMDMs (**Fig. 6c**). Consistent with results obtained with double-knockout BMDMs, CREB-S133A BMDMs showed impaired LPS-induced expression of IL-10 mRNA; however, the S133A substitution did not alter DUSP1 mRNA expression (**Fig. 6c**). These results show that MSKs promote *Il10*

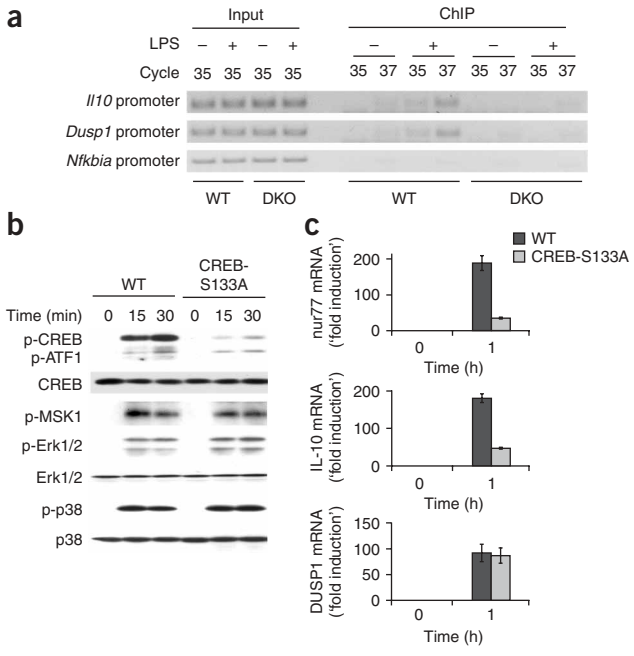

Figure 6 *Il10* and *Dusp1* promoters associate with phosphorylated CREB and/or ATF1. (**a**) Chromatin-immunoprecipitation assay of wild-type and double-knockout BMDMs stimulated with LPS (100 ng/ml); after cells were fixed in formaldehyde, chromatin was immunoprecipitated with antibody to phosphorylated CREB and ATF1, and input DNA and immunoprecipitated chromatin (ChIP) were analyzed by 35 or 37 cycles (above lanes) of PCR with primers specific for promoters of genes encoding IL-10, DUSP1 and the inhibitor of NF-κB (*Nfkbia*). (**b**) Immunoblot analysis of wild-type and CREB-S133A BMDMs treated with 0.1 μM tamoxifen on days 5–7 of differentiation, then replated in tamoxifen-free media and stimulated 24 h later for various times (above lanes) with LPS (100 ng/ml). (**c**) Quantitative PCR of total RNA from cells treated as described in **b**; 18S RNA was used as a loading control and 'fold induction' was calculated relative to wild-type expression at 0 h. Data are representative of one experiment with two mice per genotype (**a**) or are from one experiment of three with similar results (error bars, s.e.m. of four stimulations with two mice per genotype; **b**,**c**).

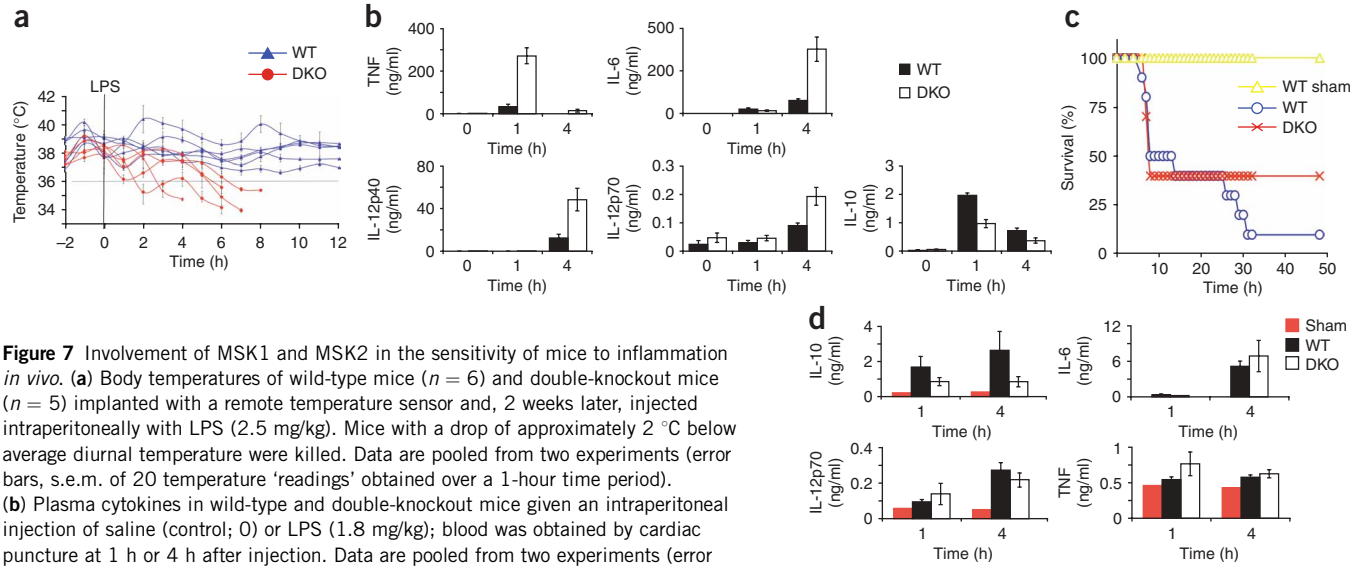

Figure 7 Involvement of MSK1 and MSK2 in the sensitivity of mice to inflammation *in vivo*. (**a**) Body temperatures of wild-type mice ($n = 6$) and double-knockout mice ($n = 5$) implanted with a remote temperature sensor and, 2 weeks later, injected intraperitoneally with LPS (2.5 mg/kg). Mice with a drop of approximately 2 °C below average diurnal temperature were killed. Data are pooled from two experiments (error bars, s.e.m. of 20 temperature 'readings' obtained over a 1-hour time period). (**b**) Plasma cytokines in wild-type and double-knockout mice given an intraperitoneal injection of saline (control; 0) or LPS (1.8 mg/kg); blood was obtained by cardiac puncture at 1 h or 4 h after injection. Data are pooled from two experiments (error bars, s.e.m. of six mice). (**c**) Survival of wild-type mice ($n = 10$) and double-knockout mice ($n = 10$) subjected to CLP and wild-type mice given 'sham' treatment ($n = 5$). Mice were killed if moribound with a body temperature of 30 °C or if their temperature remained below 30 °C for 2 h. Data are pooled from two experiments. (**d**) Plasma cytokine concentrations of wild-type mice ($n = 7$) and double-knockout mice ($n = 7$) subjected to CLP and wild-type mice given 'sham' treatment ($n = 4$), assessed at 0, 1 and 4 h. Data are pooled from two experiments (error bars, s.e.m.).

transcription through CREB phosphorylation and suggest that *Dusp1* transcription may be regulated by the phosphorylation of ATF1 by MSKs.

Influence of MSK1 and MSK2 in inflammatory models

The intraperitoneal injection of LPS in mice can lead to endotoxic shock and mortality. On the basis of the effects of deficiency in both MSK1 and MSK2 on LPS responses in BMDMs described above, we predicted that the double-knockout mice would be more sensitive than wild-type mice to LPS-induced endotoxic shock. To test our hypothesis, we gave mice a sublethal dose of LPS and monitored their body temperature over time. We considered a drop in body temperature of 2 °C as being predictive of the development endotoxic shock and probable mortality[29] and humanely killed the mice at that point. Injection of wild-type mice with 2.5 mg LPS per kg body weight (2.5 mg/kg) did not result in any substantial drop in body temperature over a 24-hour time course, and the mice did not show any severe signs of LPS-induced toxicity. In contrast, injection of double-knockout mice with the same amount of LPS resulted in obvious signs of LPS-induced toxicity, including piloerection, diarrhea, eye inflammation and a substantial drop in body temperature within 4–8 h of LPS injection (**Fig. 7a**); all LPS-injected double-knockout mice developed pathology of a severity that necessitated they be killed during this time interval. In similar experiments, we injected mice with a lower dose of LPS (1.8 mg/kg) and measured plasma cytokine concentrations after 1 h and 4 h. In wild-type mice, plasma TNF concentrations peaked at 1 h after stimulation but decreased toward baseline after 4 h. Notably, TNF concentrations 1 h after LPS injection were approximately sevenfold higher in double-knockout mice than in wild-type mice (**Fig. 7b**). In contrast to TNF, IL-6 and IL-12 were only marginally higher in wild-type mice by 1 h but were much higher at the 4 h time point. Both IL-6 and IL-12 were much higher in double-knockout mice than in wild-type mice at 4 h after LPS injection (**Fig. 7b**). Similar to results obtained with BMDMs, substantially lower concentrations of IL-10 were present in the plasma of double-knockout mice than of wild-type mice after LPS injection (**Fig. 7b**). There were no differences in plasma concentrations of IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-9, IL-13 and IL-17 in wild-type versus double-knockout mice (**Supplementary Fig. 8** online). These results collectively show that MSK deficiency increases the sensitivity of mice to LPS-induced endotoxic shock, and they correlate with the larger amounts of TNF, IL-6 and IL-12 and smaller amount of IL-10 in double-knockout mice.

Although intraperitoneal injection of LPS results in a disorder with some similarities to septic shock, clinical sepsis is a much more complex condition and considerable differences between the two conditions have been described[30]. As the cecal ligation and puncture (CLP) model is more similar to septic shock in humans[30], we also examined the effect of deficiency in both MSK1 and MSK2 in this system. In these experiments, wild-type and double-knockout mice showed similar mortality or morbity within 8 h of CLP (six of ten double-knockout mice died and five of ten wild-type mice died). However, after 48 h, all remaining double-knockout mice survived but only one wild-type mouse survived (**Fig. 7c**). In a separate experiment, we measured plasma cytokine concentrations at 1 h and 4 h after CLP. In contrast to results obtained with intraperitoneal LPS injection, CLP induced little TNF expression at this time point. Similarly, IL-12 expression was only four- to sixfold higher and was not affected much by knockout of MSK1 and MSK2 (**Fig. 7d**). IL-6 was strongly induced by CLP but also was not substantially affected by deficiency in both MSK1 and MSK2 (**Fig. 7d**). IL-10 was also induced by CLP in wild-type mice and, consistent with results obtained with the intraperitoneal LPS model, double-knockout mice expressed less IL-10 (**Fig. 7d**).

We also examined the consequence of deficiency in both MSK1 and MSK2 in a model of acute toxic contact eczema induced by phorbol 12-myristate 13-acetate (PMA). In this model, local inflammation is induced in the ear by topical administration of PMA. In wild-type mice, this localized inflammation resulted in an increase in ear thickness that normally peaked at 8 h

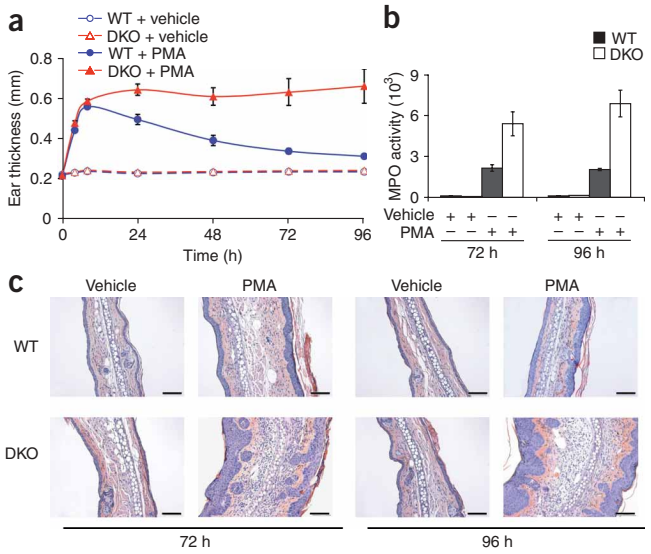

Figure 8 Involvement of MSK1 and MSK2 in the sensitivity of mice to PMA-induced eczema. (**a**) Ear thickness of wild-type and double-knockout mice treated on the ears with vehicle (acetone) or PMA (2.5 μg/ear). Vehicle, $n = 8$ mice per genotype; PMA, $n = 12$ mice per genotype. Data are pooled from two experiments (mean ± s.e.m.). (**b**) MPO activity in punch biopsies (4 mm in diameter) of ears from wild-type and double-knockout mice treated on the ears with vehicle (acetone) or PMA (2.5 μg/ear), presented as the number of polymorphonuclear cells ($\times 10^3$) per punch biopsy. Vehicle, $n = 8$ mice per genotype; PMA, $n = 12$ mice per genotype. Data are pooled from two experiments (mean ± s.e.m.). (**c**) Hematoxylin and eosin–stained transverse ear sections from wild-type and double-knockout mice treated on the ears for 72 h or 96 h with vehicle or PMA (2.5 μg/ear). Scale bars, 100 μm. Vehicle, $n = 4$ mice per genotype; PMA, $n = 8$ mice per genotype. Data are representative of two experiments.

and then slowly resolved (**Fig. 8a**). In double-knockout mice, the inflammation failed to resolve, and we did not find any substantial decrease in ear thickness even by 96 h after PMA treatment (**Fig. 8a**). We estimated the extent of the recruitment of polymorphonuclear cells by MPO (myeloperoxidase) assay of extracts of ear biopsies. Consistent with the lack of resolution of ear thickness, MPO activity was much higher in the double-knockout mice than in wild-type mice at both 72 h and 96 h after PMA treatment (**Fig. 8b**). Histological analysis of the inflamed tissue confirmed there was more cellular infiltration in the dermis, as well as more total ear and epidermal thickness, in double-knockout mice than in wild-type mice (**Fig. 8c**). These results suggest that MSKs have a protective function in acute endotoxic shock and toxic contact eczema but may have a deleterious function in sepsis.

DISCUSSION

Although Erk1/2 and p38 have been shown before to be important in proinflammatory cytokine production and inflammation, it is now becoming apparent that these MAPKs also activate negative feedback pathways that are critical for preventing uncontrolled inflammation. This negative feedback occurs through intracellular mechanisms and through the release of anti-inflammatory cytokines. For example, p38 is able to inhibit the MAPK kinase kinase TAK1 through phosphorylation of the TAB1 regulatory subunit[15], whereas both Erk1/2 and p38 can induce DUSP1 expression that promotes the inactivation of p38 and Jnk[17–20]. In addition, both Erk and p38 promote expression of tristetraprolin, which induces degradation of TNF mRNA[31]. TLR signaling also induces the production of IL-10, an anti-inflammatory cytokine known to inhibit proinflammatory cytokine production by macrophages[27].

Here we have shown that the Erk1/2- and p38-activated kinases MSK1 and MSK2 use many mechanisms to limit the proinflammatory effects of TLR4 signaling. Knockout of MSK1 and MSK2 resulted in less DUSP1 expression. The *Dusp1* promoter contains a conserved CREB-binding site[32], *Dusp1* is a CREB-dependent immediate-early gene in neurons[24], and knockout of MSK1 blocks CREB phosphorylation and *Dusp1* transcription in response to brain-derived neurotrophic factor in cortical neurons. By chromatin immunoprecipitation analysis, we found that phosphorylated CREB and/or ATF1 bound to the *Dusp1* promoter after LPS stimulation in BMDMs, by a mechanism dependent on MSK activity. Notably, phosphorylation of CREB at Ser133 was not essential for *Dusp1* transcription in response to LPS; this observation suggests that MSK1 and MSK2 regulate the *Dusp1* promoter through ATF1 phosphorylation or by an undescribed mechanism.

Similar to MSK1 and MSK2 double-knockout mice, DUSP1-deficient mice do not have an obviously altered phenotype in the absence of LPS challenge, but like the double-knockout mice, DUSP1-knockout mice do show greater susceptibility to LPS-induced endotoxic shock[17–20]. Although the phenotypes of DUSP1-deficient mice and mice deficient in both MSK1 and MSK2 are similar, the lower DUSP1 expression alone is unlikely to explain all the effects seen in the double-knockout mice. A chief difference between the two animal models is IL-10 expression, which is lower in double-knockout mice but higher in DUSP1-deficient mice. Because LPS-induced IL-10 production was decreased by inhibition of p38, the higher IL-10 concentrations in DUSP1-deficient mice are consistent with the prolonged p38 activation in these mice. In contrast, the lower LPS-induced IL-10 transcription in the double-knockout mice, together with the lack of decrease in Erk1/2 and p38 activation, suggests that MSKs may directly regulate IL-10 transcription. Consistent with that, two MSK substrates, CREB and histone H3, have been proposed to be involved in *Il10* transcription[26,33–35]. In BMDMs, MSKs were required for binding of phosphorylated CREB and/or ATF1 to the *Il10* promoter after LPS stimulation, and substitution of the Ser133 phosphorylation site in endogenous CREB resulted in less *Il10* transcription in response to LPS.

Deficiency in both MSK1 and MSK2 also resulted in more production of TNF, IL-6 and IL-12 at later time points after LPS stimulation. As double-knockout and wild-type cells produced similar amounts of IL-6 and IL-12 in the presence of an IL-10-neutralizing antibody, and as deficiency in both MSK1 and MSK2 had little effect on IL-6 and IL-12 production on an IL-10-deficient background, MSK1 and MSK2 seem to regulate IL-6 and IL-12 by an autocrine inhibition loop involving IL-10. In contrast, augmented TNF production in the double-knockout mice seemed to be the result of IL-10-dependent and IL-10-independent mechanisms. More p38 activation, due to the lower DUSP1 expression, may be involved. In addition, MSK-mediated regulation of the transcription of tristetraprolin, an mRNA-binding protein that destabilizes TNF mRNA, might be involved[31].

The function of MSK was not limited to BMDMs, as we obtained similar results with *in vivo* models of endotoxic shock and eczema in which deficiency in both MSK1 and MSK2 resulted in more inflammation. The double-knockout mice, however, did not show greater sensitivity in the CLP model. This may have been because CLP is a more acute model than is intraperitoneal injection of a low dose of LPS, which makes it more difficult to observe an increase in sensitivity. Unexpectedly, although there was similar mortality in the wild-type

and double-knockout mice before excision of the cecum, after excision, the double-knockout mice experienced no mortality, whereas all but one of the remaining wild type mice died. This suggests that knockout of MSK could be protective in sepsis but deleterious in acute endotoxic shock. Many differences between these two models have been described, which makes it difficult to directly extrapolate findings from one model to the other. In contrast to intraperitoneal injection of LPS, CLP induces a bacterial peritonitis that activates a wider range of immune pathways and involves both inflammatory and immunosuppressive stages[30]. Relevant to that, it has been shown that CLP is not affected by loss of TLR4 (the receptor for LPS), which indicates that several pathogen-associated molecular patterns are important in this model[36,37]. The function of cytokines in the two models is also different; for example, TNF blockade is protective after LPS injection but not in CLP[38]. In fact, endogenous TNF has been suggested to have protective effects in some circumstances in CLP; TNF-neutralizing antibodies have a harmful effect if administered immediately after CLP[39], and TNF receptor 1–deficient mice show greater mortality in a CLP model[40].

The function of IL-10 is also complex. Neutralization of IL-10 with antibodies before induction of CLP[41] or IL-10 knockout[42] increases mortality in CLP. In contrast, blocking IL-10 after CLP induction (a situation more similar to what would occur in the double deletion of MSK1 and MSK2) either by neutralizing antibodies[43] or inhibition of IL-10 transcription[44] has a protective effect. In addition, larger amounts of IL-10 have been correlated with poor outcome in clinical sepsis[45]. IL-12 is another cytokine that was higher in the double-knockout mice after LPS stimulation. IL-12 deletion[46] and IL-12-neutralizing antibodies[47] both sensitize mice to mortality in CLP. One possible explanation for the CLP results obtained with the double-knockout mice is that MSK has an immunosuppressive effect that may decrease bacterial clearance, resulting in more peritonitis.

These results collectively establish the critical function of MSK1 and MSK2 in limiting proinflammatory signaling 'downstream' of TLRs. It is possible that some of the toxicity associated with p38 inhibitors could be due to the inhibition of negative feedback mechanisms mediated by MSK1 and MSK2. Thus, agents that selectively activate MSK1 and MSK2 may have therapeutic benefits in situations in which more production of IL-10 and less production of proinflammatory cytokines is desired.

METHODS

Animals. Mice lacking MSK1 and MSK2 have been described[22]. IL-10-deficient mice[48] on a C57BL/6 background were from The Jackson Laboratory. An inducible mutation in endogenous *Creb1* resulting in a S133A substitution was generated by the insertion of a *lox*P-flanked 'minigene' (**Supplementary Fig. 7**). For generation of the *Creb1* 'knock-in' mutation in BMDMs, these mice were crossed onto a strain with tamoxifen-inducible Cre. The tamoxifen-inducible transgene encoding Cre had no effect on LPS-induced CREB phosphorylation or cytokine transcription (**Supplementary Fig. 9** online). All strains were backcrossed onto the C57BL/6 strain for a minimum of six generations. Mice were housed in specific pathogen–free condition in accordance with European Union regulations. Work was approved by local ethical review and either was done with a UK Home Office project license (*in vivo* LPS injection) or was approved by the Danish Laboratory Animal Research committee (journal number 2005/561-963; CLP and acute toxic contact eczema).

Cell culture. BMDMs were isolated from the femurs of adult mice as described[10]. Bone marrow cells were differentiated for 7 d on bacteria-grade plastic dishes in DMEM supplemented with 10% (vol/vol) FBS, penicillin (100 units/ml), streptomycin (100 μg/ml), amphothericin B (0.25 μg/ml), L-glutamine (5 mg/ml) and mouse recombinant macrophage colony-stimulating factor (5 ng/ml; R&D Systems). Where appropriate, the mutation resulting in the CREB-S133A substitution was induced by the addition of 0.1 μM tamoxifen on day 5 of culture. After 7 d, adherent cells were removed with Versene solution (Gibco), then were counted and replated at a constant density (2×10^5 cells/ml). At 24–48 h after being replated, cells were stimulated for various times with LPS (100 ng/ml; Sigma), Pam_3CSK_4 (1 μg/ml), LTA from *Staphylococcus aureus* (5 μg/ml) or CpG DNA (ODN1826; 2.5 μM; all from InvivoGen). For experiments with kinase inhibitors, PD184352 (2 μM) or SB203580 (5 μM) was added to the culture media 1 h before stimulation. For immunoblot analysis, cells were lysed in 1% (wt/vol) SDS sample buffer. For RNA analysis, cells were lysed in RNA lysis buffer (Qiagen) and RNA was isolated with an RNeasy Microkit (Qiagen).

***In vivo* LPS injection.** Mice 6–8 weeks of age were injected intraperitoneally with LPS (1.8 mg/kg; Sigma). Mice were killed 1 h or 4 h after LPS injection and plasma samples were collected for cytokine analysis.

CLP. Mice 7–8 weeks of age were starved for 16 h and the cecum was ligated just below the ileocecal valve, a procedure that maintained bowel continuity[49]. The cecum was punctured at both ends with a 21-gauge needle and a single droplet of fecal material was extruded from each puncture site. The abdomen was closed in two layers, then mice were resuscitated by subcutaneous injection of 3 ml saline per 100 g body weight, a microchip was inserted subcutaneously into the back of each mouse and the mouse was returned to its cage; this was considered 'time 0' for subsequent measurements. After 10 h, the cecum was excised and the abdominal cavity was irrigated with warm saline. The abdominal cavity was closed and mice were resuscitated by subcutaneous injection of 3 ml saline per 100 g body weight. For 'sham' treatment, mice underwent an identical procedure except the cecum was not ligated or punctured. A blood sample was obtained after 6 h and mice were resuscitated every 10 h by subcutaneous injection of 3 ml saline per 100 g body weight. The temperature of the mice was measured at various times. If body temperature dropped below 30 °C in a moribund mouse or if there was a prolonged drop below 30 °C for several hours, the mice were humanely killed. In a similar experiment, 100 μl of blood was obtained at 1 h, then the mouse was killed at 4 h, which allowed another blood sample to be obtained. Plasma cytokines in the blood samples were then measured by Luminex assay. Pipeline Biotech did the CLP model.

LPS-induced endotoxic shock. Mice 10–12 weeks of age were anesthetized and temperature sensors were surgically implanted in the peritoneal cavity. Then, 14 d later, mice were challenged with LPS (2.5 mg/kg; Sigma) and their intraperitoneal temperatures were then monitored constantly with the Remo 400 detection system. A drop in temperature of more than 1.5–2 °C relative to the average mouse diurnal temperature was used as an indicator of LPS-induced death and mice were killed.

Acute toxic contact eczema model. Male mice 6–8 weeks of age were treated with PMA (Sigma) on the ears (2.5 μg/ear). Ear thickness was measured with a Digimatic Indicator (Mitutoyo) before the first challenge of PMA and again at various times after challenge; mice were anesthetized during ear measurement.

MPO assay. Punch biopsies 4 mm in diameter were obtained from mouse ears 72 h and 96 h after PMA challenge and were immediately placed in liquid nitrogen. MPO assays were done as described[50]. Biopsies were homogenized in 200 μl of 0.5% (wt/vol) hexadecyltrimethylammonium bromide. Samples were incubated for 1 h at 37 °C and then were centrifuged for 5 min. MPO activity was assayed in the supernatant as follows. First, 20 μl supernatant was added to 96-well plates containing 100 μl tetramethylbenzidine ('ready-to-use' TMB ONE Substrate; Kem-En-Tec Diagnostics). After 10 min of incubation in the dark at 25 °C, the reaction was stopped by the addition of 100 μl of 0.2 M H_2SO_4. Results were determined at 450 nm with an ELISA 'reader' (iEMS Reader MF; Laboratory Systems). A standard curve was constructed with polymorphonuclear cells obtained from the blood from healthy human volunteers. Polymorphonuclear cells were prepared with Polymorphprep according to the manufacturer's instructions (Axis-Shield). These experiments were approved by the local ethical committee of Aarhus and signed informed consent was obtained from the volunteers.

Additional methods. Information on antibodies, chromatin immunoprecipitation, quantitative PCR, cytokine assays, flow cytometry and histology is available in the **Supplementary Methods** and **Supplementary Table 1** online.

Accession codes. UCSD-Nature Signaling Gateway (http://www.signaling-gateway.org): A001717, A001718, A000025, A001562 and A001563.

Note: Supplementary information is available on the Nature Immunology website.

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COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/natureimmunology/.

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1. Kawai, T. & Akira, S. TLR signaling. *Semin. Immunol.* **19**, 24–32 (2007).
2. Miggin, S.M. & O'Neill, L.A. New insights into the regulation of TLR signaling. *J. Leukoc. Biol.* **80**, 220–226 (2006).
3. Lee, M.S. & Kim, Y.J. Signaling pathways downstream of pattern-recognition receptors and their cross talk. *Annu. Rev. Biochem.* **76**, 447–480 (2007).
4. Han, J., Lee, J.D., Bibbs, L. & Ulevitch, R.J.A. MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. *Science* **265**, 808–811 (1994).
5. Freshney, N.W. *et al.* Interleukin-1 activates a novel protein kinase cascade that results in the phosphorylation of Hsp27. *Cell* **78**, 1039–1049 (1994).
6. Rouse, J. *et al.* A novel kinase cascade triggered by stress and heat shock that stimulates MAPKAP kinase-2 and phosphorylation of the small heat shock proteins. *Cell* **78**, 1027–1037 (1994).
7. Cuenda, A. *et al.* SB 203580 is a specific inhibitor of a MAP kinase homologue which is stimulated by cellular stresses and interleukin-1. *FEBS Lett.* **364**, 229–233 (1995).
8. Bain, J. *et al.* The selectivity of protein kinase inhibitors: a further update. *Biochem. J.* **408**, 297–315 (2007).
9. O'Keefe, S.J. *et al.* Chemical genetics define the roles of p38α and p38 in acute and chronic inflammation. *J. Biol. Chem.* **282**, 34663–34671 (2007).
10. Beardmore, V.A. *et al.* Generation and characterization of p38β (MAPK11) gene-targeted mice. *Mol. Cell. Biol.* **25**, 10454–10464 (2005).
11. Kang, Y.J. *et al.* Macrophage deletion of p38α partially impairs lipopolysaccharide-induced cellular activation. *J. Immunol.* **180**, 5075–5082 (2008).
12. Lang, T. & Mansell, A. The negative regulation of Toll-like receptor and associated pathways. *Immunol. Cell Biol.* **85**, 425–434 (2007).
13. Dambach, D.M. Potential adverse effects associated with inhibition of p38α/β MAP kinases. *Curr. Top. Med. Chem.* **5**, 929–939 (2005).
14. Liew, F.Y., Xu, D., Brint, E.K. & O'Neill, L.A. Negative regulation of toll-like receptor-mediated immune responses. *Nat. Rev. Immunol.* **5**, 446–458 (2005).
15. Cheung, P.C., Campbell, D.G., Nebreda, A.R. & Cohen, P. Feedback control of the protein kinase TAK1 by SAPK2a/p38α. *EMBO J.* **22**, 5793–5805 (2003).
16. Chen, P. *et al.* Restraint of proinflammatory cytokine biosynthesis by mitogen-activated protein kinase phosphatase-1 in lipopolysaccharide-stimulated macrophages. *J. Immunol.* **169**, 6408–6416 (2002).
17. Salojin, K.V. *et al.* Essential role of MAPK phosphatase-1 in the negative control of innate immune responses. *J. Immunol.* **176**, 1899–1907 (2006).
18. Chi, H. *et al.* Dynamic regulation of pro- and anti-inflammatory cytokines by MAPK phosphatase 1 (MKP-1) in innate immune responses. *Proc. Natl. Acad. Sci. USA* **103**, 2274–2279 (2006).
19. Zhao, Q. *et al.* MAP kinase phosphatase 1 controls innate immune responses and suppresses endotoxic shock. *J. Exp. Med.* **203**, 131–140 (2006).
20. Hammer, M. *et al.* Dual specificity phosphatase 1 (DUSP1) regulates a subset of LPS-induced genes and protects mice from lethal endotoxin shock. *J. Exp. Med.* **203**, 15–20 (2006).
21. Arthur, J.S. MSK activation and physiological roles. *Front. Biosci.* **13**, 5866–5879 (2008).
22. Wiggin, G.R. *et al.* MSK1 and MSK2 are required for the mitogen- and stress-induced phosphorylation of CREB and ATF1 in fibroblasts. *Mol. Cell. Biol.* **22**, 2871–2881 (2002).
23. Soloaga, A. *et al.* MSK2 and MSK1 mediate the mitogen- and stress-induced phosphorylation of histone H3 and HMG-14. *EMBO J.* **22**, 2788–2797 (2003).
24. Arthur, J.S. *et al.* Mitogen- and stress-activated protein kinase 1 mediates cAMP response element-binding protein phosphorylation and activation by neurotrophins. *J. Neurosci.* **24**, 4324–4332 (2004).
25. Darragh, J. *et al.* MSKs are required for the transcription of the nuclear orphan receptors Nur77, Nurr1 and Nor1 downstream of MAPK signalling. *Biochem. J.* **390**, 749–759 (2005).
26. Zhang, X., Edwards, J.P. & Mosser, D.M. Dynamic and transient remodeling of the macrophage IL-10 promoter during transcription. *J. Immunol.* **177**, 1282–1288 (2006).
27. Murray, P.J. Understanding and exploiting the endogenous interleukin-10/STAT3-mediated anti-inflammatory response. *Curr. Opin. Pharmacol.* **6**, 379–386 (2006).
28. Carl, V.S., Gautam, J.K., Comeau, L.D. & Smith, M.F. Jr. Role of endogenous IL-10 in LPS-induced STAT3 activation and IL-1 receptor antagonist gene expression. *J. Leukoc. Biol.* **76**, 735–742 (2004).
29. Stiles, B.G., Campbell, Y.G., Castle, R.M. & Grove, S.A. Correlation of temperature and toxicity in murine studies of staphylococcal enterotoxins and toxic shock syndrome toxin 1. *Infect. Immun.* **67**, 1521–1525 (1999).
30. Rittirsch, D., Hoesel, L.M. & Ward, P.A. The disconnect between animal models of sepsis and human sepsis. *J. Leukoc. Biol.* **81**, 137–143 (2007).
31. Brook, M. *et al.* Posttranslational regulation of tristetraprolin subcellular localization and protein stability by p38 mitogen-activated protein kinase and extracellular signal-regulated kinase pathways. *Mol. Cell. Biol.* **26**, 2408–2418 (2006).
32. Sommer, A., Burkhart, H., Keyse, S.M. & Luscher, B. Synergistic activation of the mkp-1 gene by protein kinase A signaling and USF, but not c-Myc. *FEBS Lett.* **474**, 146–150 (2000).
33. Platzer, C. *et al.* Cyclic adenosine monophosphate-responsive elements are involved in the transcriptional activation of the human IL-10 gene in monocytic cells. *Eur. J. Immunol.* **29**, 3098–3104 (1999).
34. Saraiva, M. *et al.* Identification of a macrophage-specific chromatin signature in the IL-10 locus. *J. Immunol.* **175**, 1041–1046 (2005).
35. Park, J.M. *et al.* Signaling pathways and genes that inhibit pathogen-induced macrophage apoptosis—CREB and NF-κB as key regulators. *Immunity* **23**, 319–329 (2005).
36. Echtenacher, B., Freudenberg, M.A., Jack, R.S. & Mannel, D.N. Differences in innate defense mechanisms in endotoxemia and polymicrobial septic peritonitis. *Infect. Immun.* **69**, 7271–7276 (2001).
37. Peck-Palmer, O.M. *et al.* Deletion of MyD88 markedly attenuates sepsis-induced T and B lymphocyte apoptosis but worsens survival. *J. Leukoc. Biol.* **83**, 1009–1018 (2008).
38. Riedamann, N.C., Guo, R. & Ward, P.A. The enigma of sepsis. *J. Clin. Invest.* **112**, 460–467 (2003).
39. Echtenacher, B., Falk, W., Mannel, D.N. & Krammer, P.H. Requirement of endogenous tumor necrosis factor/cachectin for recovery from experimental peritonitis. *J. Immunol.* **145**, 3762–3766 (1990).
40. Hildebrand, F., Pape, H.C., Hoevel, P., Krettek, C. & van Griensven, M. The importance of systemic cytokines in the pathogenesis of polymicrobial sepsis and dehydroepiandrosterone treatment in a rodent model. *Shock* **20**, 338–346 (2003).
41. van der Poll, T. *et al.* Endogenous IL-10 protects mice from death during septic peritonitis. *J. Immunol.* **155**, 5397–5401 (1995).
42. Latifi, S.Q., O'Riordan, M.A. & Levine, A.D. Interleukin-10 controls the onset of irreversible septic shock. *Infect. Immun.* **70**, 4441–4446 (2002).
43. Song, G.Y., Chung, C.S., Chaudry, I.H. & Ayala, A. What is the role of interleukin 10 in polymicrobial sepsis: anti-inflammatory agent or immunosuppressant? *Surgery* **126**, 378–383 (1999).
44. Kalechman, Y. *et al.* Anti-IL-10 therapeutic strategy using the immunomodulator AS101 in protecting mice from sepsis-induced death: dependence on timing of immunomodulating intervention. *J. Immunol.* **169**, 384–392 (2002).
45. Gogos, C.A., Drosou, E., Bassaris, H.P. & Skoutelis, A. Pro- versus anti-inflammatory cytokine profile in patients with severe sepsis: a marker for prognosis and future therapeutic options. *J. Infect. Dis.* **181**, 176–180 (2000).
46. Moreno, S.E. *et al.* IL-12, but not IL-18, is critical to neutrophil activation and resistance to polymicrobial sepsis induced by cecal ligation and puncture. *J. Immunol.* **177**, 3218–3224 (2006).
47. Steinhauser, M.L., Hogaboam, C.M., Lukacs, N.W., Strieter, R.M. & Kunkel, S.L. Multiple roles for IL-12 in a model of acute septic peritonitis. *J. Immunol.* **162**, 5437–5443 (1999).
48. Kuhn, R., Lohler, J., Rennick, D., Rajewsky, K. & Muller, W. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* **75**, 263–274 (1993).
49. Hubbard, W.J. *et al.* Cecal ligation and puncture. *Shock* **24**, 52–57 (2005).
50. Ottosen, E.R. *et al.* Synthesis and structure-activity relationship of aminobenzophenones. A novel class of p38 MAP kinase inhibitors with high antiinflammatory activity. *J. Med. Chem.* **46**, 5651–5662 (2003).