Mitogen-activated protein kinase phosphatase 2, MKP-2, regulates early inflammation in acute lung injury

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Cornell TT, Fleszar A, McHugh W, Blatt NB, LeVine AM, Shanley TP. Mitogen-activated protein kinase phosphatase 2, MKP-2, regulates early inflammation in acute lung injury. Am J Physiol Lung Cell Mol Physiol 303: L251–L258, 2012. First published June 8, 2012; doi:10.1152/ajplung.00063.2012.—Acute lung injury (ALI) is mediated by an early proinflammatory response resulting from either a direct or indirect insult to the lung mediating neutrophil infiltration and consequent disruption of the alveolar capillary membrane ultimately leading to refractory hypoxemia. The mitogen-activated protein kinase (MAPK) pathways are a key component of the molecular response activated by those insults triggering the proinflammatory response in ALI. The MAPK pathways are counterbalanced by a set of dual-specific phosphatases (DUSP) that deactivate the kinases by removing phosphate groups from tyrosine or threonine residues. We have previously shown that one DUSP, MKP-2, regulates the MAPK pathway in a model of sepsis-induced inflammation; however, the role of MKP-2 in modulating the inflammatory response in ALI has not been previously investigated. We utilized both MKP-2-null (MKP-2−/−) mice and MKP-2 knockdown in a murine macrophage cell line to elucidate the role of MKP-2 in regulating inflammation during ALI. Our data demonstrated attenuated proinflammatory cytokine production as well as decreased neutrophil infiltration in the lungs of MKP-2−/− mice following direct, intratracheal LPS. Importantly, when challenged with a viable pathogen, this decrease in neutrophil infiltration did not impact the ability of MKP-2−/− mice to clear either gram-positive or gram-negative bacteria. Furthermore, MKP-2 knockdown led to an attenuated proinflammatory response and was associated with an increase in phosphorylation of ERK and induction of a related DUSP, MKP-1. These data suggest that altering MKP-2 activity may have therapeutic potential to reduce lung inflammation in ALI without impacting pathogen clearance.

sample key words

Since it was first described in 1967 (2), acute lung injury (ALI), which results in the destruction of the alveolar capillary membrane leading to refractory hypoxemia (20), has continued to have a significant impact on public health with an estimated incidence of >200,000 adult cases per year in the United States (14), accounting for nearly 4 million hospital days. The mortality rate remains substantial at greater than 34% in adults (14) and ranging from 18 to 22% in children (7, 23). The mortality and morbidity associated with ALI necessitates a better understanding of the cellular processes involved in the pathophysiology of ALI to identify novel therapeutic targets with the potential of attenuating the pathological processes causing pulmonary dysfunction.

A key, initial cellular event in ALI is the recruitment of neutrophils into the lung parenchyma mediated by chemokines produced by inflammatory cells during the innate immune response (20), which occurs following both direct and indirect insults to the lung. Alveolar macrophages, the primary innate immune cell orchestrating the initial inflammatory response in direct lung injury, express Toll-like receptors (TLRs), which are capable of binding to highly conserved sequences expressed by “pathogen-associated molecular patterns” (PAMPS), also termed “microbial-associated molecular patterns” (MAMPs) (17, 18). Upon activation, the TLRs trigger a cascade of signaling events activating signaling pathways such as the NF-κB and mitogen-activated protein kinase (MAPK) pathways, which subsequently drive transcriptional expression of a variety of proinflammatory cytokines such as TNF-α, IL-1β, and CXCL8/IL-8 that contribute to the pathophysiology of ALI.

As a result, the regulation of the MAPK pathways is an important focus in the investigation of the pathologies associated with inflammation including ALI (12). The MAPKs are a series of kinases that mediate phosphorylation of threonine and tyrosine residues. The propagation and amplification of the signal from receptor to nucleus is dependent on a cascade of upstream kinases activating downstream kinases ending in phosphorylation and activation of the three terminal kinases: c-Jun NH2-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), and p38. The dual-specificity phosphatase (DUSP) family members, also referred to as MAPK phosphatases (MKPs), regulate the MAPK pathways by targeting both threonine and tyrosine residues of the MAPK pathways for dephosphorylation and, thus, deactivation, to “reset” this inflammatory signaling machinery (12).

The most well-studied DUSP in the regulation of the innate immune response is MKP-1, which negatively regulates cytokine production by deactivating JNK and p38 (5, 8, 10, 11, 15, 22). Another DUSP closely related to MKP-1 that is involved in regulating the inflammatory response is MKP-2 (1, 6). Our prior work investigating the role of MKP-2 in a model of sepsis triggered by cecal ligation and puncture indicated that MKP-2 regulates the inflammatory response by altering both ERK and MKP-1 activity. Since the role of DUSPs in ALI has not been previously examined, we sought to investigate the role of MKP-2 in ALI. We hypothesized that MKP-2 regulates the innate immune response such that in its absence MKP-2−/−
mice will have an attenuated inflammatory response in ALI models.

MATERIALS AND METHODS

Mice. All animal care and procedures were conducted under the guidelines and policies of the University of Michigan’s Unit for Laboratory and Animal Medicine. Review and approval of the study protocols were by the University Committee on the Use and Care of Animals. MKP-2−/− mice used in these experiments have been previously described (6). Experiments were performed on 6- to 8-wk-old MKP-2−/− mice as well as age- and sex-matched C57Bl/6 wild-type (WT) mice (Jackson Laboratories, Bar Harbor, ME).

Murine model of direct intratracheal induced lung injury. We use the direct visual instillation method described by Su et al. (16) to induce lung injury in our mice. Briefly, anesthesia was induced with ketamine (30 mg/kg) and xylazine (5 mg/kg). After visualization of the tracheal orifice, a 24-gauge angiocatheter (Becton Dickinson, Franklin Lakes, NJ) was placed through the orifice into the trachea. We confirmed correct catheter placement by attaching the catheter to a mouse volume controlled ventilator (model 687, Harvard Apparatus, Holliston, MA) and assessing chest expansion. Following catheter confirmation, 100 μl of lipopolysaccharide (LPS; 5 mg/kg) from Escherichia coli strain O55:B5 (Sigma-Aldridge, St. Louis, MO) or bacteria isolated from human patients [Staphylococcus aureus or Pseudomonas aeruginosa; 1 × 107 colony-forming units (cfu)] or phosphate-buffered saline (PBS) as control was instilled directly into the airways over several breaths.

Bronchial alveolar fluid differential cell counts. After induction of anesthesia with ketamine (30 mg/kg) and xylazine (5 mg/kg), bronchial alveolar lavage (BAL) fluid was collected by intratracheal instillation and aspiration of 1 ml of PBS three separate times for a total of 3 ml instilled. Average recovery for all three instillations was 2.7 ml and all three samples were pooled. BAL fluid results were normalized to the amount of fluid recovered from each mouse. There was no difference between the average amounts of fluid recovered from each group of mice. BAL fluid was centrifuged at 500 g for 5 min. The resulting supernatant was collected and stored at −80°C prior to cytokine determination. The cell pellet was resuspended in 800 μl of PBS. The cell suspension was then prepared on glass slides with use of a cytospin and fixed with CAMCO Differential Stain Pak (Fisher Scientific, Pittsburgh, PA).

Cultured cells. For all studies, MH-S (mouse transformed alveolar macrophage) cells were used (American Type Culture Collection; Manassas, VA). Cells were cultured at 37°C in 5% CO2 at a density of 1–2 × 105 cells/ml in RPMI medium containing 10% heat-inactivated fetal bovine serum (FBS) and penicillin-streptomycin. Cells were stimulated with either 100 ng/ml ultrapure LPS (E. coli, serotype O111:B4; Invivogen, San Diego, CA) or 10 μg/ml purified lipotechoic acid (LTA, S. aureus, Invivogen) for the times indicated.

Bacterial colony counts from whole lungs. Mice were euthanized; the lungs were removed, weighed, and placed in sterile PBS. Equal amounts of lung tissue were homogenized by use of Fisher Scientific TissueMiser (Fisher Scientific) for 30 s. Between samples, the homogenizer was washed with sterile water then with 70% ethanol before being blended. The lung homogenates were serial diluted at 1:10 dilutions (1:10, 1:1,000; 1:10,000, and 1:100,000) and each dilution was plated on tryptic soy plates (S. aureus) or 2YT plates (P. aeruginosa). Plates with less than 150 colonies were counted and averaged to determine the final colony-forming units.

Quantitative RT-PCR. RNA was isolated from the MH-S cells by using the RNeasy Mini Kit (Qiagen, Valencia, CA) following LPS or LTA stimulation for the times indicated. Quantitative RT-PCR was performed on the samples by use of MDP-2 TaqMan Gene Expression Assay (cat. no. 4331182; Applied Biosystems, Foster City, CA) following cDNA production with the High Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems). Fold increases in mRNA were calculated by the delta-delta Ct method with GAPDA as the reference housekeeping gene used for normalization.

siRNA transfection. MH-S cells were plated in RPMI media supplemented with glutamine and 10% fetal bovine serum without antibiotics to a concentration of ~0.3 × 106 cells/well in a six-well plate. Following overnight incubation (16 h) the cells were transfected using DharmaFECT 4 (Dharmacon, Lafayette, CO) per the manufacturer’s protocol with On-Targetplus SmartPool MKP-2 small interfering RNA (siRNA) (siMKP-2; cat. no. L-003963-00) or On-Targetplus SmartPool control siRNA (cat. no. D-001810-10) from Dharmacon. Transfected cells were cultured for an additional 48 h prior to overnight incubation in low serum medium (0.5% FBS) and stimulation with LPS.

In vitro phosphatase assay. Cells were exposed to the experimental conditions indicated in RESULTS. After two washes with 0.9% NaCl, total cellular proteins were extracted in lysis buffer containing 50 mM Tris·HCl, pH 7.5, 250 mM NaCl, 3 mM EDTA, 3 mM EGTA, 1% Triton X-100, and 0.5% Nonidet P-40 (NP-40) without phosphatase inhibitors. Specific MKP-2 activity was measured by using a nonradioactive malachite green phosphatase assay kit (Millipore, Billerica, MA) following immunoprecipitation of MKP-2 by use of the Catch and Release v.2.0 immunoprecipitation kit (Millipore). All procedures were performed according to the manufacturer’s protocol, and changes in absorbance were measured at 650 nm in a Spectra-MAX 250 (Molecular Devices, Sunnyvale, CA) plate reader.

Immunoblotting. Following experimental conditions, MH-S cells were harvested and lysed in RIPA buffer containing 25 mM Tris·HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, and 0.1% SDS with 10 μl Halt Protease Inhibitor Cocktail (Pierce, Rockford, IL). Samples were separated by SDS-PAGE and transferred to nitrocellulose. Nitrocellulose blots were probed with antibodies...
against MKP-2 (sc-1200; Santa Cruz Biotechnology, Santa Cruz, CA), MKP-1 (sc-1199; Santa Cruz Biotechnology), phospho-p42/p44 ERK (9101; Cell Signaling, Danvers, MA), p42/p44 ERK (9102; Cell Signaling), p38 (9212; Cell Signaling), JNK (9252; Cell Signaling), phosphor-p38 (V1211; Promega, Madison, WI), phosphor-JNK (V7931; Promega), or GAPDH (Ab9483; Abcam, Cambridge, MA) as indicated in RESULTS.

ELISA. Immunoreactive IL-1β concentrations from cell culture supernatants and TNF-α concentrations from BAL fluid were determined by using a commercially available mouse IL-β, TNF-α, and MIP-1α enzyme-linked immunoabsorbant assay (ELISA) kit (Invitrogen, Carlsbad, CA). All procedures were performed in triplicate and according to the manufacturer’s protocol.

Statistical analysis. Results are reported as means ± SE. Statistical significance for parametric data was determined by using an unpaired t-test for experiments comprising two groups. A one-way analysis of variance (ANOVA) was used to determine significance of overall interactions for experiments comprising three or more time points. A two-way ANOVA with Bonferroni posttest was used to determine significance.

Fig. 2. Attenuated proinflammatory response to intratracheal LPS. Mice received intratracheal instillation of 5 mg/kg of LPS or PBS (100 μl total volume), and BALs were performed at the times indicated. A: ELISA for immunoreactive MIP-1α or TNF-α on BAL fluid indicates attenuated cytokine production in MKP-2−/− mice compared with wild-type mice at 4 h after LPS. Baseline cytokine levels were undetectable in both phenotypes, and although the 24-h concentrations remained elevated there was no statistical difference between these values and baseline values. Total number of inflammatory cells (B), macrophages (C), and neutrophils (D) identified in BAL samples from both MKP-2−/− and wild-type mice at 4 and 24 h after intratracheal LPS show a decrease in neutrophil infiltration at 4 h but increased infiltration at 24 h after LPS in MKP-2−/− mice compared with wild-type mice. *P < 0.05; **P < 0.01, ***P < 0.001; NS, not significant; n = 7 wild-type mice and 8 MKP-2 mice; 2-way ANOVA with Bonferroni posttest was used to determine significance.
analysis of variance was used to determine significance of overall interactions for experiments comprising of two groups over time. A Bonferroni posttest was used to determine the significance of the different time points when a one- or two-way ANOVA was performed. Statistical tests were conducted by use of Sigma Stat 3.0 (Systat, San Jose, CA).

RESULTS

Decreased inflammation in the lungs of MKP-2−/− mice. We first investigated the baseline immune state of the lungs in MKP-2−/− mice by examining BAL fluid in untreated mice and observed no difference between MKP-2−/− and WT mice in either the total number (Fig. 1A) or the subpopulations of inflammatory cells (Fig. 1B). Baseline cytokine/chemokine concentrations of MIP-1α and TNF-α were below the level of detection by ELISA in both groups.

We next investigated the impact of a direct stimulus on the inflammatory response in the lung. We initially used intratracheal injection of LPS as an established model of ALI because we were interested in determining the regulation of an inflammatory response that was independent of bacterial clearance. Four hours after intratracheal instillation of LPS, significant reductions of a key chemokine (MIP-1α, 78% decrease) and cytokine (TNF-α, 54% decrease) secretion into the BAL fluid was observed in MKP-2−/− mice compared with WT mice (Fig. 2A). The BAL fluid cytokine concentrations for both the MKP-2−/− and WT mice decreased substantially by 24 h after LPS. Although the MIP-1α concentrations remained higher in the BAL fluids from the MKP-2−/− mice compared with WT mice (67.9 ± 21.3 pg/ml vs. 22.2 ± pg/ml) these values were not significantly different. There was no significant difference in TNF-α concentrations in BAL fluid 24 h after LPS between the two groups (Fig. 2A). In fact, even though the 24-h cytokine concentrations were detectable they were not significantly different from baseline levels, which were undetectable.

The total number of inflammatory cells retrieved from the BALs also differed between the WT and MKP-2−/− mice over time. Four hours after LPS, the total number of inflammatory cells in the BAL from WT mice was 42% greater than in MKP-2−/− mice (Fig. 2B). Interestingly, this difference did not persist out to 24 h after LPS (Fig. 2B). In accounting for the difference in total inflammatory cell infiltration, there was no detectable difference in macrophage numbers in BAL at either 4 or 24 h after LPS between the two groups (Fig. 2C). However, WT mice had nearly a threefold increase in neutrophil infiltration at 4 h compared with MKP-2−/− mice (Fig. 2D); however, although less than the peak numbers at 4 h, this difference in neutrophil infiltration was reversed by 24 h after LPS in that the MKP-2−/− mice showed a twofold increase in neutrophils compared with the WT mice (Fig. 2D).

In summary, these data indicate an attenuated early (4 h) inflammatory response from direct endotoxin challenge in the lungs of MKP-2−/− mice compared with WT mice.

Lack of MKP-2 does not affect bacterial clearance in the lung. We next assessed whether the attenuated response to endotoxin in the MKP-2−/− mice would alter clearance of live pathogens by instilling viable, clinical bacterial isolates directly into the trachea of the mice. Following intratracheal inoculation of 1 × 10^7 cfu of Pseudomonas aeruginosa bacteria, we did not detect a difference between the numbers of colonies isolated from the lungs of WT vs. MKP-2−/− mice at either 4 h (Fig. 3A) or 24 h (Fig. 3B). Nor did we detect a difference in the number of colonies isolated from the lungs of WT vs. MKP-2−/− mice following intratracheal instillation of 1 × 10^2 cfu of the gram-positive bacteria Staphylococcus aureus at either 4 h (Fig. 3C) or 24 h (Fig. 3D). These data indicated that, although the MKP-2−/− mice demonstrated an attenuated early inflammatory response, they retain similar capacity to clear pathogenic organisms.

Fig. 3. No difference in bacterial clearance between MKP-2−/− and wild-type mice following intratracheal bacteria. Lung homogenates were plated to determine the colony-forming units (cfu) of Pseudomonas aeruginosa at 4 h (n = 6 wild-type, 5 MKP-2−/− mice; A) and 24 h (n = 8 in each group; B) or Staphylococcus aureus at 4 h (n = 8 wild-type, 7 MKP-2−/− mice; C) and 24 h (n = 6 wild-type, 7 MKP-2 mice; D) after the mice received 1 × 10^5 cfu of either bacteria (2-way ANOVA with Bonferroni posttest was used to determine significance).
TLR stimulation induction of MKP-2. To further delineate the mechanism of the attenuated inflammatory response in the MKP-2<sup>−/−</sup> mice and the impact of different TLR ligands, we examined the effect of TLR engagement on the kinetic expression of MKP-2 in alveolar macrophages (using the MH-S cell line). Both LPS and LTA induced MKP-2 in MH-S cells, although with slightly different kinetics of expression (Fig. 4). Upon LPS stimulation, MKP-2 RNA was expressed within 0.5 h, peaked at 1 h, and declined by at 4 h (Fig. 4A). LTA stimulation resulted in MKP-2 RNA being expressed within 0.5 h, peaking at 1 h, then remaining constant for 4 h (Fig. 4B). These data correlated with the time course of protein expression following stimulation with LPS that showed peak protein production at 4 h (Fig. 4C) and LTA resulting in protein peak production at 6 h (Fig. 4D). Thus, although the kinetics of expression varied modestly, both TLR agonists, LPS and LTA, associated with gram-negative (TLR4) or gram-positive (TLR2) pathogen-triggered inflammation, respectively, induced expression of MKP-2.

MKP-2 activity induced by TLR activation. Despite being able to identify induction of MKP-2 in response to stimuli such as growth factors, oncogenes, and oxidative stress (9, 13), investigators have not previously quantified endogenous, MKP-2-specific phosphatase activity. To that end, we employed an immunoprecipitation strategy utilizing an anti-MKP-2 monoclonal antibody to optimize an in vitro phosphatase assay. Using a phospho-threonine substrate, we specifi-
cally measured induction of MKP-2 activity following TLR4 stimulation. This approach was based on the detection of free phosphate in the lysates of unstimulated (control) MH-S cells, compared with cells stimulated with LPS. With use of this assay, the peak time point of MKP-2 production (2 h after LPS stimulation) was associated with significant induction of MKP-2-specific phosphatase (Fig. 5A).

Since the phosphatase activity of MKP-2 has been shown to target the terminal kinases of the MAPK pathway (4), we investigated whether any of these MAP kinases coimmunoprecipitated with MKP-2 following LPS stimulation. Consistent with the MKP-2 expression and activity observed at 2 h post-LPS, we detected an association between ERK and endogenous MKP-2 at this time point (Fig. 5B). We were unable to detect an association between p38 and JNK with MKP-2, suggesting that the principal target of MKP-2 regulation in our model was p-ERK.

The role of MKP-2 in regulating signaling pathways. We next aimed to definitively establish MKP-2’s role in regulating inflammatory signaling pathways in alveolar macrophages by investigating the effect of knockdown of MKP-2 on MAPK pathway activation in response to TLR stimulation. The ability to alter the endogenous induction of MKP-2 by using siRNA strategy was demonstrated as MKP-2 siRNA (but not scrambled siRNA) significantly reduced LPS-induced MKP-2 expression (Fig. 6A). MKP-2 knockdown resulted in an attenuated inflammatory response as measured by a 68% reduction of LPS induced IL-1β production by MH-S cells (Fig. 6B).

The effect of MKP-2 knockdown on the MAP kinases resulted in an increase in p-ERK after LPS stimulation (Fig. 6C). In contrast to the increased phosphorylation of ERK, knockdown of MKP-2 resulted in decreased phosphorylation of both JNK and p38 (Fig. 6C). Similar phosphorylation patterns of JNK and p38 following LPS stimulation of bone marrow-derived macrophages isolated from MKP-2−/− mice resulted from an increase the DUSP MKP-1 (6). We therefore investigated the impact of MKP-2 knockdown on the levels of MKP-1 and detected an increase in MKP-1 protein 2 h after LPS stimulation in MH-S cells that were subjected to MKP-2 knockdown (Fig. 6C). The increased amounts of MKP-1 were also noted to have a change in mobility. Quantitative RT-PCR for MKP-1 mRNA demonstrated no differences in MKP-1 expression between scrambled siRNA and siMKP-2 (data not shown). Taken together these data expand our previous data that in the absence of MKP-2 ERK phosphorylation and the amount of MKP-1 are both increased.

DISCUSSION

We have shown that the DUSP MKP-2 regulates the early inflammatory response in a mouse model of ALI as well as in alveolar macrophages. The absence of MKP-2 via either knockout (mouse model) or knockdown (in vitro MH-S model) attenuated the early production of proinflammatory cytokines (Figs. 2A and 6B). The early, attenuated proinflammatory response in the lungs resulted in decreased infiltration of neutrophils at 4 h after LPS but increased neutrophil infiltration in the BAL fluid at 24 h. Despite the early, reduced neutrophil recruitment, bacterial clearance remained adequate to inhibit the proliferation of both gram-negative and gram-positive bacteria (Fig. 3).

In elucidating the potential mechanism of this regulation, it was first determined that TLR2 and 4 stimulation significantly induced MKP-2 mRNA production (Fig. 4, A and B). Similar trends in the kinetics of expression of MKP-2 protein after TLR stimulation with an early peak of expression observed at 2–4 h after TLR4 activation and 4–6 h after TLR2 stimulation was observed (Fig. 4, C and D). Importantly, in addition to demonstrating expression of MKP-2 protein, induction of endogenous, MKP-2-specific phosphatase activity was shown by establishing an in vitro phosphatase assay performed with immunoprecipitation of endogenously expressed MKP-2 (Fig. 5). The only other prior reports of MKP-2 phosphatase activity involved transfection-based overexpression of MKP-2, but no quantification of endogenous activity (3). Thus these novel data demonstrated that the increased endogenous expression of MKP-2 correlated to increased phosphatase activity attributed specifically to MKP-2 that was induced by TLR2 and TLR4 activation.

Given that prior investigators had identified the ERK MAPK as a putative target (4, 9, 13, 19), we focused our studies on the potential regulation of ERK activation. Coimmunoprecipitation showed an interaction between MKP-2 and ERK 2 following LPS stimulation at the time of peak MKP-2 expression (Fig. 5B), suggesting that MKP-2 was targeting ERK for deactivation. To confirm our hypothesis, we employed siRNA to silence/knock down MKP-2 in cells prior to LPS stimulation and examined the

Fig. 5. LPS stimulation of MH-S cells results in endogenous MKP-2 associating with ERK and increased MKP-2 phosphatase activity. Cells were stimulated with LPS (100 ng/ml) for 2 h prior to immunoprecipitation (ip) with either anti-MKP-2 or IgG isotype-matched irrelevant antibody. A: an in vitro phosphatase assay using malachite green to detect free phosphate release was used to determine the phosphatase activity following MKP-2 immunoprecipitation. **P < 0.01; t-test used to analyze 4 separate experiments. B: Western blot detected coimmunoprecipitation of ERK, JNK, and p38 with endogenous MKP-2. The figure represents 3 separate experiments.
Consistent with our hypothesis, p-ERK was significantly augmented in cells in which MKP-2 was knocked down compared with those cells transfected with scrambled siRNA, whereas p38 or JNK was decreased (Fig. 6C). These data provided evidence for a role of MKP-2 as an endogenous downregulator of ERK activation following LPS stimulation. Our data are in agreement with prior studies indicating that MKP-2 interacts with ERK and involves cross talk with MKP-1 to modulate the early innate immune response (6). This work expands our previous findings by showing that MKP-2’s regulatory role is present in the lung in response to both direct and indirect inflammatory stimuli and that this regulation does not hallucinate.
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not impact processes involving bacterial clearance. As with our previous work, these data show an attenuation of cytokine/chemokine production and are in agreement with our hypothesis that this attenuation is regulated by an increase in MKP-1 levels. The shift in MKP-1 mobility (Fig. 6C) suggests the possibility that the increased levels of MKP-1 may be due to increased phosphorylation of MKP-1. The mechanisms resulting in increased levels of MKP-1 are beyond the scope of this manuscript and experiments are underway to further investigate this mechanism. These data expand the impact of the attenuation of cytokine/chemokine production by showing an alteration in neutrophil migration. The simple explanation for the decrease in early neutrophil migration is the decreased production of MIP-1α. However, it is possible that MKP-2 may directly impact neutrophil migration and function; we are currently investigating the role of MKP-2 in neutrophils.

These data also indicate for the first time an interaction between ERK and endogenous MKP-2. Interestingly, these data are not consistent with the work of Al-Mutairi et al. (1), who reported an increased inflammatory response and an increase in intracellular pathogens in MKP-2−/− mice. These differences are likely explained by the variance in the experimental design in that our investigations looked at early cytokine production whereas these investigators examined late (>24 h) cytokine production. In fact, when Al-Mutairi et al. investigated the induction of inducible nitric oxide synthase following an early inflammatory stimulus (<24 h), they similarly reported a decreased inflammatory response in the MKP-2−/− mice. The differences in results in the clearance of organisms between our data and the report from Al-Mutairi et al. may be due to the fact that they utilized the intracellular pathogen Leishmania mexicana whereas we used Staphylococcus and P. aeruginosa, neither of which is an intracellular pathogen. Because the immune response necessary to clear these pathogens differs greatly, it is not unexpected that the impact on pathogen eradication was different. Thus the role of MKP-2 appears to be dependent on targeting early vs. late immune responses, as well as the type of inflammatory insult.

In summary, we have shown that MKP-2 regulates the early proinflammatory response in mouse models of ALI and that in the absence of MKP-2 there is less neutrophil migration into the lung while preserving the clearance of gram-positive and gram-negative bacteria. Thus further investigations MKP-2 as a therapeutic target to attenuate the inflammatory response after the onset of ALI may be warranted.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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AUTHOR CONTRIBUTIONS

TTC: conception and design of research, performed experiments, analyzed data, interpreted results of experiments, prepared figures, drafted manuscript, edited and revised manuscript, approved final version of manuscript; AF: performed experiments, analyzed data, interpreted results of experiments; WM: performed experiments, analyzed data, interpreted results of experiments; NBB: conception and design of research, interpreted results of experiments; AML: conception and design of research, performed experiments, analyzed data, interpreted results of experiments, edited and revised manuscript, approved final version of manuscript.

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