Published in final edited form as:

Crit Care Med. 2012 February ; 40(2): 379–387. doi:10.1097/CCM.0b013e318232e404.

A novel role for matrix metalloproteinase-8 in sepsis

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Abstract

Objectives—Matrix metalloproteinase-8 (MMP-8) mRNA expression was previously found to be increased in whole blood of children with septic shock. The impact of this finding on the severity and inflammatory response to sepsis is unknown. Here, we investigate the relationship between MMP-8 and disease severity in a children with septic shock. We further corroborate the role of MMP-8 in sepsis in a murine model.

Design—Retrospective observational clinical study and randomized controlled laboratory experiments.

Setting—Pediatric intensive care units and an animal research facility at an academic children's hospital.

Patients/Subjects—Patients age ≤ 10 years admitted to the intensive care unit with a diagnosis of septic shock. For laboratory studies, we utilized male mice deficient for MMP-8 and male wild type C57/BL6 mice.

Interventions—Blood from children with septic shock was analyzed for MMP-8 mRNA expression and MMP-8 activity, and correlated with disease severity based on mortality and degree of organ failure. A murine model of sepsis was used to explore the effect of genetic and pharmacologic inhibition of MMP-8 on the inflammatory response to sepsis. Finally, activation of nuclear factor-κB (NF-κB) was assessed both *in vitro* and *in vivo*.

Measurements and Main Results—Increased MMP-8 mRNA expression and activity in septic shock correlates with decreased survival and increased organ failure in pediatric patients. Genetic and pharmacologic inhibition of MMP-8 leads to improved survival and a blunted inflammatory profile in a murine model of sepsis. We also identify MMP-8 as a direct *in vitro* activator of the pro-inflammatory transcription factor, NF-κB.

Conclusions—MMP-8 is a novel modulator of inflammation during sepsis and a potential therapeutic target.

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The authors have not disclosed any potential conflicts of interest.

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Keywords

Matrix Metalloproteinase-8; sepsis; septic shock; inflammation; nuclear factor- κ B; multi-organ failure

INTRODUCTION

Septic shock continues to be a major health concern, with high levels of morbidity, mortality, and associated costs (1–3). Multiple organ failure is major contributor to morbidity and mortality, making prevention of organ dysfunction a key goal in the clinical treatment of septic shock (4). Effective therapy for septic shock, beyond antibiotics and intensive care unit-based support, remains elusive. Thus, the search for new interventions continues.

One area of investigation focuses on regulating the robust inflammatory response that accompanies septic shock, as this inflammatory response is thought to contribute to multiple organ dysfunction. Matrix metalloproteinases (MMPs) may function as key regulators of this inflammatory response. Originally thought to function primarily in the degradation of the extracellular matrix (ECM), it is now evident that MMPs also modify a diverse group of non-ECM proteins, thus impacting a variety of biological processes, including the inflammatory cascade (5–10). Several studies have demonstrated a reduction of inflammatory markers and mortality in rodent models of sepsis with non-specific, global inhibition of MMPs (11–13).

Global inhibition of MMPs may not be clinically desirable because of their diverse functions, but targeting select MMPs may be a more viable therapeutic option. One such target is MMP-8, a neutrophil-derived collagenase involved in the degradation of type I collagen (14, 15). MMP-8 is also present in a multitude of cell types, including macrophages, fibroblasts, epithelial cells, and other immune cells. Additionally, MMP-8 has non-collagen proteolytic targets, including pro-inflammatory chemokines (16–18). Given this background and our previous clinical studies demonstrating high level expression of MMP-8 mRNA in children with septic shock (19, 20), we hypothesized that MMP-8 is a novel therapeutic target in septic shock.

MATERIALS AND METHODS

Patients and clinical samples

The clinical study protocol was approved by the Institutional Review Boards of participating institutions. Patient selection and data collection were conducted as previously described (20). Briefly, patients ≤ 10 years of age admitted to the pediatric intensive care unit were enrolled, and after meeting published criteria for sepsis or septic shock (21), had blood drawn within the first 24 hours of diagnosis (Day 1) and 48 hours after initial blood draw (Day 3). Organ failure occurring within 7 days of initial diagnosis was defined using published definitions (21, 22). Control patients had blood withdrawn at ambulatory centers using previously published inclusion and exclusion criteria (19, 23, 24). A total of 180 patients with septic shock and 32 patients with sepsis exist in the expression database.

Total RNA was isolated from whole blood samples using the PaxGene Blood RNA System (PreAnalytiX, Qiagen/Becton Dickinson, Franklin Lakes NJ) according to manufacturer's specifications. Microarray hybridization was performed by the Affymetrix Gene Chip Core facility at Cincinnati Children's Hospital Research Foundation as previously described using the Human Genome U133 Plus 2.0 GeneChip (Affymetrix, Santa Clara CA) and analyzed as

previously described (19, 24). All microarray data have been deposited in the NCBI Gene Expression Omnibus (accession numbers: GSE26440 and GSE26378).

Measurement of MMP-8 activity

MMP-8 activity in plasma samples was determined using a commercially available fluorimetric kit, according to the manufacturer's specifications (Anaspec, Fremont, CA). The kit utilizes a FRET peptide, which is cleaved by active MMP-8, thereby producing a measurable fluorescence at 490/520nm.

Murine model of sepsis

All aspects of this study complied with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23 revised 1996) and met approval of the Institutional Animal Care and Use Committee. Mmp-8 $-/-$ mice on a C57BL6-J background were provided by Dr. Steven Shapiro, University of Pittsburgh. Loss of the Mmp-8 gene was confirmed by PCR using Mmp-8 specific primers (data not shown). Wild type C57BL6-J mice were obtained from Harlan Laboratories (Indianapolis, IN). All mice were fed standard rodent chow and maintained on 12 hour light-dark cycles.

Mice aged 5–9 weeks underwent cecal ligation and puncture (CLP) as previously described (25). Briefly, mice were anesthetized and a midline laparotomy was performed. The cecum was isolated and ligated to 30% original diameter and two punctures were made using a 21-gauge needle with a small amount of fecal content expressed from each site, and the abdominal cavity was closed. Mice treated with vehicle received an intraperitoneal (i.p.) injection of 1% dimethyl sulfoxide (DMSO) in phosphate-buffered saline (PBS) at a dose of 10 mL/kg after abdominal closure. The MMP-8 inhibitor ((3R)-(+)-[2-(4-methoxybenzenesulfonyl)-1,2,3,4-tetrahydroisoquinoline-3-hydroxamate]) (EMD Chemicals, Gibbstown NJ) was dissolved in 1% DMSO in PBS to a final inhibitor concentration of 0.01mg/mL. Animals received a 0.1 mg/kg dose of inhibitor immediately following abdominal closure. All mice received 0.6 mL of normal saline subcutaneously at the conclusion of the operation. In experiments extending past 12 hours, animals were re-dosed with the MMP-8 inhibitor, or the vehicle control, at 12 hour intervals, up to 3 days after CLP. Following CLP, animals were monitored for survival (up to 10 days) or were sacrificed at 3, 6 or 24 hours for procurement of biological specimens.

Measurement of myeloperoxidase activity

Myeloperoxidase was measured as an indication of neutrophil infiltration in lung tissue, as previously described (26). Briefly, whole lung tissue was homogenized and myeloperoxidase activity was assessed using spectrophotometry and defined as the quantity of enzyme degrading 1 μ mol hydrogen peroxide/min at 37°C, expressed in units per 100 mg of tissue.

Measurement of plasma cytokines and chemokines

Plasma levels of interleukin-6 (IL-6), keratinocyte-derived chemokine (KC), IL-1 β , macrophage inflammatory protein-1 α (MIP-1 α), tumor necrosis factor α (TNF α), lipopolysaccharide induced CXC chemokine (LIX), and IL-10 were analyzed using a Luminex multiplex system (Luminex Corporation, Austin TX) according to instructions from the manufacturer.

Raw 264.7 Murine Macrophages

In vitro experiments were conducted as previously described (27). RAW 264.7 murine macrophages were purchased from American Type Culture Collection (Manassas, VA) and maintained at standard conditions. An NF- κ B-luciferase reporter plasmid was used to measure activation of NF- κ B. The PathDetect cis-reporting plasmid (Stratagene, Santa Clara, CA) contains the luciferase reporter gene under the control of five tandem NF- κ B binding motifs. The transfection control plasmid pGL4.74 [hRluc/TK] (Promega, Madison WI) contains the luciferase reporter gene hRluc (*Renilla reniformis*). RAW 264.7 cells were transfected with 1 μ g of the NF- κ B luciferase plasmid and 0.1 μ g of the pGL4.74 plasmid in triplicate, in 12 well plates at 75% confluency by incubation with FuGENE 6 (Roche Molecular Biochemicals, Indianapolis IN) and serum-free media overnight. The medium was changed to growth medium and cells were treated with 1 μ g/mL of human neutrophil-derived MMP-8 (Endo Life Sciences, Chadds Ford PA), LPS (80 pg/mL), denatured MMP-8 (incubated at 100° C for one hour), or MMP-8 + polymyxin B sulfate (300 ng/mL). Four hours after treatment, cellular proteins were extracted with Passive Lysis Buffer (Promega, Madison WI) and analyzed for luciferase activity according to the manufacturer's instructions for Dual Luciferase (Promega, Madison WI) with the use of a luminometer (AutoLumat LB953, Berthold, Oak Ridge TN). Luciferase activity was reported as relative induction over respective control cells.

Primary Murine Peritoneal Macrophages

6 week old male C57BL6-J mice were sacrificed with carbon dioxide. The peritoneum was exposed and 2 ml of sterile PBS was injected into the peritoneal space and withdrawn. The cells were centrifuged for 10 minutes at 1200 rpm. The cell pellet was re-suspended in 1 ml ACK lysing buffer (Invitrogen) and incubated for 3 minutes before the addition of 10 ml of media (RPMI supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin (Invitrogen)). The cells were pelleted again and re-suspended in 1 ml media. 250,000 cells were seeded in 1 ml media per well in a 12 well plate. After overnight incubation at 37° C/5% CO₂, media was removed and 500 μ l of fresh media was added to each well. Cells were treated with 1 μ g/ml MMP-8 (Enzo Life Sciences) or 1 μ g/ml LPS as a positive control. Conditioned media was collected at 24 hours.

Measurement of NF- κ B activation

Nuclear extracts from whole lung cells were isolated as previously described (26). The presence of the p-65 subunit of NF- κ B was measured using a commercially available, chemiluminescent kit according to the manufacturer's specifications (Active Motif, Carlsbad CA).

Statistical Analysis

Statistical analysis was performed using SigmaStat for Windows Version 3.10 (SysStat Software, San Jose CA). Data are represented as means $\pm$ SEM or as medians with interquartile range of n observations, where n represents the number of subjects in each group. For multiple group analysis at a single time point, one way analysis of variance (ANOVA) with Student-Newman-Keuls correction was used. For multiple group analysis at different time points, a two-way ANOVA with Student-Newman-Keuls correction was performed. If data failed to follow a normal distribution, a Mann-Whitney Rank Sum test or an ANOVA on ranks test was performed. For survival analysis, a Gehan-Breslow analysis was used. When comparing two groups at the same time point, a Student's t-test was performed. P values less than 0.05 were considered significant.

RESULTS

MMP-8 expression and activity are increased in human sepsis and septic shock

We mined our previously published mRNA expression database to determine the expression levels of MMP-8 mRNA in whole blood-derived RNA from children with sepsis and septic shock (20, 28). Blood samples were obtained within 24 hours of meeting criteria for either sepsis or septic shock (day 1) and 48 hours later (day 3). As shown in Figure 1A, children with sepsis and septic shock had significantly increased expression of MMP-8 mRNA as compared to healthy, control patients on day 1. Furthermore, patients with septic shock had increased MMP-8 mRNA expression as compared to patients with sepsis. This pattern of gene expression persisted into day 3. Figure 1B shows that MMP-8 mRNA expression was significantly higher in children with septic shock that did not survive the 28 day study period, as compared to survivors, suggesting that the degree of MMP-8 mRNA expression correlates with illness severity.

To further demonstrate a link between MMP-8 mRNA expression and illness severity, we measured MMP-8 mRNA expression in 180 children with septic shock and grouped these patients into quartiles of MMP-8 mRNA expression. We then queried the clinical database to determine the maximal number of organ failures, over the first 7 days of admission, for patients in each quartile. As shown in Figure 1C, patients in the 3rd and 4th quartiles of MMP-8 mRNA expression had a significantly higher number of organ failures, as compared to patients in the 1st quartile, further demonstrating a link between MMP-8 mRNA expression and illness severity.

To confirm that increased MMP-8 mRNA expression led to increased MMP-8 activity, MMP-8 activity was measured in plasma samples from separate cohorts of healthy control patients and children with septic shock. As shown in Figure 1D, MMP-8 activity was significantly increased in the plasma of children septic shock, as compared to healthy control patients.

Collectively, these data demonstrate that substantially increased MMP-8 mRNA expression and activity are early and persistent features of pediatric sepsis and septic shock, and that the degree of MMP-8 expression correlates with illness severity. Thus, it is possible that MMP-8 plays an integral role in the biological response to sepsis and septic shock.

Deletion of *Mmp-8* improves survival and blunts the inflammatory response in a murine model of sepsis

To further explore the role of MMP-8 in sepsis, *Mmp-8* deficient mice (*Mmp8* ^{-/-}) and C57BL6-J wild type (WT) mice were subjected to CLP. As shown in Figure 2A, *Mmp-8* ^{-/-} mice displayed a significant survival advantage over their WT counterparts over the 10 day observation period after CLP. As MMP-8 is an important component of neutrophil function, we next determined if absence of MMP-8 compromised bacterial clearance. We found no difference in plasma, pulmonary, peritoneal, or splenic bacterial colony counts 24 hours after CLP in *Mmp8* ^{-/-} mice as compared to WT mice, thus indicating that absence of MMP-8 does not compromise bacterial clearance in mice (data not shown).

Morbidity and mortality from sepsis is thought to occur, in part, from an excessive host inflammatory response to bacterial challenge, and the lung is often a primary target of this robust inflammatory response. We assessed whole lung inflammation by measuring myeloperoxidase (MPO) activity as a surrogate of neutrophil infiltration. MPO levels were assessed in whole lung specimens from WT and *Mmp8* ^{-/-} mice at 3, 6, and 24 hours after CLP. As shown in Figure 2B, *Mmp8* ^{-/-} mice showed a reduction in lung MPO activity after CLP at 3 and 6 hours after CLP as compared to WT mice. These data demonstrate that

the survival advantage seen in *Mmp8*^{-/-} mice correlates with decreased early lung neutrophil infiltration.

Following the observations that genetic ablation of MMP-8 improves survival and reduces early lung neutrophil infiltration, we hypothesized that MMP-8 possesses a regulatory function in the sepsis inflammatory cascade. To test this hypothesis, we analyzed the circulating concentrations of several cytokines and chemokines in the plasma of WT and *Mmp8*^{-/-} mice after CLP, at baseline and at several time points after CLP. As shown in Figure 3, significant reductions in the circulating levels of the pro-inflammatory cytokines IL-6 and IL-1 β were observed in *Mmp8*^{-/-} mice, compared to WT mice, at 6 hours after CLP. The reduction of IL-6 persisted at 24 hours. Additionally, plasma concentration of the anti-inflammatory cytokine, IL-10, was significantly increased in *Mmp8*^{-/-} mice, as compared to their WT counterparts, at 24 hours after CLP. Cumulatively, these results suggest that MMP-8 plays a role in regulating the inflammatory response to sepsis, with genetic ablation of MMP-8 leading to a survival advantage and correlative reduction in early lung neutrophil infiltration and selected systemic cytokines.

Pharmacologic inhibition of MMP-8 activity replicates the *Mmp8*^{-/-} phenotype

While the experiments involving *Mmp8*^{-/-} mice implicate a role for MMP-8 in sepsis, there remains the possibility that chronic deletion of MMP-8 can lead to unexpected developmental adaptations that confer a survival advantage in the context of sepsis. Therefore, we attempted to replicate the phenotype observed in *Mmp8*^{-/-} mice via pharmacologic inhibition of MMP-8 in WT mice subjected to CLP. To directly measure the efficacy of our MMP-8 inhibitor, we measured MMP-8 activity in the plasma of WT mice treated with the MMP-8 inhibitor and subjected to CLP. Figure 4A shows that intraperitoneal (i.p.) administration of the MMP-8 inhibitor, after CLP, effectively decreased plasma MMP-8 activity in WT mice 6 hours after CLP. This trend continued at 24 hours, but did not reach statistical significance (Figure 4B).

To show that this reduced activity correlated to improved outcomes, we observed mice for survival during a 10 day observation period after CLP. Pharmacologic inhibition of MMP-8 activity correlated with a significant survival advantage as compared to vehicle-treated WT mice (Figure 4C). Thus, pharmacologic inhibition of MMP-8 reduces mortality in a rodent model of sepsis, successfully replicating the phenotype observed in *Mmp8*^{-/-} mice.

To examine if this replicated phenotype was also due to regulation of the inflammatory cascade by MMP-8, we again measured whole lung MPO as a marker of neutrophil infiltration. While whole lung MPO tended to be lower in mice treated with the MMP-8 inhibitor, compared to vehicle-treated animals, this trend did not reach statistical significance (Fig 4D).

To further assess the role of pharmacologic inhibition on the inflammatory cascade, we measured the plasma concentrations of several cytokines and chemokines 6 hours after CLP in WT mice treated with vehicle or the MMP-8 inhibitor. As shown in Figure 5, pharmacologic inhibition of MMP-8 activity in WT mice significantly reduced plasma concentrations of IL-6, IL-1 β , MIP-1 α and TNF α at 6 hours after CLP, as compared to vehicle-treated WT mice.

To test the specificity of the MMP-8 inhibitor, we treated *Mmp8*^{-/-} mice with the MMP-8 inhibitor or vehicle after CLP. We found no significant difference in lung MPO activity or plasma cytokine/chemokine concentrations between *Mmp8*^{-/-} mice treated with vehicle or inhibitor (data not shown).

Cumulatively, these data demonstrate that we can reduce mortality and systemic inflammation with pharmacologic inhibition of MMP-8, thus generally reproducing the phenotype observed in *Mmp8*^{-/-} mice.

MMP-8 activates the pro-inflammatory transcription factor NF-κB

To begin assessing a potential mechanism by which genetic ablation or inhibition of MMP-8 blunts inflammation following sepsis, we evaluated the effect of MMP-8 on activation of the pro-inflammatory transcription factor, NF-κB. RAW 264.7 murine macrophages were transfected with an NF-κB-dependent luciferase reporter and subsequently treated with active MMP-8 (1 μg/ml) isolated from human neutrophils. Macrophages treated with MMP-8 showed a significant increase in luciferase activity (Figure 6A). LPS (1 μg/ml) was used as a positive control, and showed a vigorous activation of NF-κB.

To assess the potential role of LPS contamination of the MMP-8 preparation, we carried out a series of control experiments. We did not observe increased luciferase activity when macrophages were exposed to the same concentration of LPS (80 pg/mL) as that found in MMP-8 preparation, nor when we denatured the MMP-8 protein by boiling (Figure 6A). However, the increase in luciferase activity was maintained when active MMP-8 was delivered in conjunction with the endotoxin binder, polymyxin-B. Thus, MMP-8 can directly activate NF-κB *in vitro*, and this effect does not appear to be an artifact of LPS contamination of the MMP-8 preparation.

To confirm that MMP-8 led to a pro-inflammatory phenotype in macrophages, we measured the production of several pro-inflammatory cytokines by primary murine peritoneal macrophages treated with MMP-8 *ex vivo*. Peritoneal macrophages from WT mice were exposed to media alone, media with LPS (1 μg/ml) as a positive control, or media with MMP-8 (1 μg/ml) for 24 hours. Figure 6B shows that treatment with MMP-8 increased supernatant concentrations of IL-6, KC, TNFα, and MIP-1α, as compared to cells treated with media alone. This confirms that MMP-8 induces a pro-inflammatory phenotype in cultured macrophages.

We next evaluated the effect of MMP-8 on *in vivo* NF-κB activation. The concentration of the p65 active subunit of NF-κB was measured in murine whole lung nuclear protein extracts at 6 hours after CLP. We found that *Mmp8*^{-/-} mice showed a significant reduction of NF-κB activation in lung nuclear extracts as compared to WT mice (Figure 6C). WT mice treated with the MMP-8 inhibitor had a trend toward decreased NF-κB activation in lung nuclear extracts as compared to WT mice treated with vehicle, but this did not reach statistical significance (Figure 6D). Collectively, these data demonstrate that MMP-8 has the potential to directly activate NF-κB, thus suggesting a potential mechanism by which MMP-8 regulates the inflammatory state in experimental sepsis.

Discussion

Our study implicates a novel role for MMP-8 in sepsis biology. Increased MMP-8 expression and activity correlate with increased mortality and illness severity in children with septic shock. The biological significance of this observation is corroborated by animal-based studies, which demonstrate that genetic ablation or pharmacologic inhibition of MMP-8 activity confers a survival advantage after a sepsis challenge. In addition, the data suggest that the survival advantage correlates with a tempered inflammatory response to sepsis, including blunting of NF-κB activation.

A role for MMP-8 in sepsis has been previously suggested (29, 30), but our study is the first to show a significant increase of MMP-8 mRNA and MMP-8 activity in clinical septic

shock. This increase correlated with a poor prognosis, with non-survivors showing a significant increase of MMP-8 mRNA as compared to survivors. Furthermore, increased MMP-8 mRNA expression correlated with the degree of organ failure encountered after septic shock. This suggests that MMP-8 is playing a role in the response to sepsis in pediatric patients, though the exact nature of that role remains unclear.

Earlier studies suggest that MMP-8 regulates the inflammatory cascade in varying disease models, though these studies have provided divergent conclusions (31–37). For example, several studies have suggested a *pro-inflammatory* role for MMP-8. Two studies showed that genetic ablation of MMP-8 led to reduced neutrophil influx at the target organ and subsequent tempering of inflammation, with MMP-8 mediating inflammation via cleavage and activation of LPS-induced CXC chemokine (LIX) (31, 32). A third study also showed similar results, with genetic and pharmacologic inhibition of MMP-8 reducing inflammation and decreasing disease severity in a rodent model of encephalomyelitis (34). In contrast to these studies, there have been several reports of an *anti-inflammatory* role for Mmp-8. In three separate studies, MMP-8 has been shown to reduce the murine inflammatory response to intra-tracheal injection of LPS, bleomycin, or allergens (35–37).

Collectively, these studies broadly confirm a role for MMP-8 in regulating the inflammatory response, but offer different conclusions as to the nature of this role. It appears that MMP-8 plays a complex role in regulating inflammation that may vary depending on the nature of the inflammatory stimulus as well as the compartment being studied. However, no study has addressed the role of MMP-8 in regulating the inflammatory response to poly-microbial sepsis. We designed a study using a poly-microbial, murine model of sepsis to complement our clinical studies. Our study showed that in a murine model of poly-microbial sepsis, MMP-8 is acting in a pro-inflammatory manner. With elimination of MMP-8, key markers of inflammation were reduced, both systemically and in the lung. Mmp8 deficient mice also showed reduced mortality, mirroring the clinical results in our pediatric population.

Using a specific MMP-8 inhibitor, we were able to generally replicate the phenotype observed in the Mmp8 deficient mice, observing a reduction in mortality and inflammatory markers with pharmacologic inhibition of MMP-8. To confirm the specificity of our pharmacologic inhibition, we treated Mmp8 deficient mice with the MMP-8 inhibitor and found no significant attenuation of inflammation compared to Mmp8 deficient mice treated with vehicle.

To further explore the role of MMP-8 in regulation of the inflammatory cascade, we studied the effect of MMP-8 on a key regulator of the inflammatory response, NF- κ B. Using *in vitro* experiments, we discovered that MMP-8 directly activates the pro-inflammatory transcription factor NF- κ B in RAW 264.7 macrophages, while also increasing the expression of several pro-inflammatory cytokines in primary murine peritoneal macrophages. Rapidly activated after sepsis, NF- κ B is known to up regulate the transcription of several genes involved in the inflammatory response (38), and increased NF- κ B activity has been correlated with disease severity in patients with septic shock (39). We next confirmed our *in vitro* findings in our murine model of sepsis, finding a significant reduction of the p65 NF- κ B subunit in nuclear extracts from whole lung. Thus, MMP-8 may be a direct activator of NF- κ B, and this observation may partially account for the reduction of inflammation observed with genetic ablation or pharmacologic inhibition of MMP-8.

In conclusion, our clinical data indicate an important role for MMP-8 in human sepsis and septic shock, and this assertion has been corroborated in a murine model of sepsis. Therefore, we have identified inhibition of MMP-8 as a potential therapeutic strategy for sepsis.

Acknowledgments

Support

Supported by grants from the National Institutes of Health: T32 GM008478 (PDS), R01 GM067202 (BZ), R01 GM064619 (HRW), 1RC1HL100474 (HRW), and R01 GM096994 (HRW).

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Figure 1.

MMP-8 mRNA expression and activity are increased in children with sepsis or septic shock, and the degree of expression correlates with illness severity. **(a)** Relative expression of MMP-8 mRNA in whole blood of children with either sepsis ($N = 32$) or septic shock ($N = 98$), as compared to healthy control subjects ($N = 32$). For patients with sepsis and septic shock, data are provided for day 1 (within 24 hours of meeting criteria) and for day 3 (48 hours after the initial sample). # $p < 0.05$ vs. controls, * $p < 0.05$ vs. sepsis (ANOVA on Ranks). **(b)** Relative expression of MMP-8 mRNA in whole blood-derived RNA 24 hours after meeting criteria for septic shock in 28 day survivors ($n = 81$) and non-survivors ($n = 17$). * $p < 0.05$ vs. survivors (Mann-Whitney Rank Sum test). **(c)** Stratification of 180 children with septic shock based on quartiles of whole blood MMP-8 mRNA expression. Patients were grouped into quartiles of MMP-8 mRNA expression, and the maximal number of organ failures over the first 7 days of admission for patients in each quartile are plotted on the y-axis. # $p < 0.05$ vs. patients in the 1st quartile (ANOVA on Ranks). **(d)** MMP-8 activity (pM) in plasma of healthy control subjects ($N=10$) as compared to pediatric patients within 24 hours of meeting clinical criteria for sepsis ($N= 20$) or septic shock ($N= 20$). # $p < 0.05$ vs. healthy controls (Mann-Whitney rank sum test). All data are expressed as medians with interquartile ranges, 5th/95th percentiles, and outliers.

Figure 2.

Mmp8 $-/-$ mice show a survival advantage and decreased lung neutrophil activity after CLP as compared to WT mice. **(a)** Cumulative survival rates of WT (N=18) and Mmp8 $-/-$ (N=20) mice up to 10 days after CLP (time depicted in hours). Observations occurred every 12 hours for the first 48 hours after CLP, and every 24 hours thereafter. The y-axis depicts the proportion of mice alive at each time point. * $p < 0.05$ as compared to WT (Gehan-Breslow). **(b)** Whole lung MPO activity (U/100 mg of tissue) in WT and Mmp8 $-/-$ mice at baseline (N= 3 and 3) and at 3 (N=6 and 6), 6 (N=9 and 11, respectively), and 24 hours (N=9 and 9) after CLP. Data are represented as means $\pm$ SEM. * $p < 0.05$ vs. WT. $\ddagger$ $p < 0.05$ vs. Mmp8 $-/-$ control. # $p < 0.05$ vs. WT control (Two Way ANOVA).

Figure 3.

Mmp8^{-/-} mice show an overall decreased systemic inflammatory profile of cytokines and chemokines after CLP. The circulating plasma concentrations of (a) IL-6 (ng/mL), (b) KC (ng/mL), (c) IL-1β (pg/mL), (d) MIP-1α (pg/mL) (e) TNFα (pg/mL) (f) LIX (ng/mL) and (g) IL-10 in mice at 0, 3, 6, and 24 hours after CLP in WT (N=3, 6, 9, and 10, respectively) and Mmp8^{-/-} mice (N= 3, 6, 11, 10, respectively). Data are represented as means ± SEM. * p < 0.05 vs. WT at same time point, # p < 0.05 vs. WT time 0, ‡ p < 0.05 vs. Mmp8^{-/-} time 0 (Two-Way ANOVA).

Figure 4.

WT mice treated with the MMP-8 inhibitor have a survival advantage after CLP. **(a, b)** Plasma MMP-8 activity (pM) at 6 hours (a) and 24 hours (b) after CLP in WT mice treated with vehicle (N=9) as compared to WT mice treated with MMP-8 inhibitor (N=9). Data are represented as medians with interquartile ranges, 5th/95th percentiles, and outliers. * $p < 0.05$ vs. WT + vehicle (Mann-Whitney Rank Sum Test). **(c)** Cumulative survival rates of WT mice treated with vehicle (N=17) or MMP-8 inhibitor (N=17) up to 10 days after CLP (time depicted in hours). Observations occurred every 12 hours for the first 48 hours after CLP, and every 24 hours thereafter. The y-axis depicts the proportion of mice alive at each time point. * $p < 0.05$ as compared to vehicle-treated WT mice (Gehan-Breslow). **(d)** Whole lung MPO activity (U/100mg of tissue) in control WT mice (N=3), vehicle-treated WT mice (N=15) and inhibitor-treated WT mice (N=14) at 6 hours after CLP. Data are represented as means $\pm$ SEM. # $p < 0.05$ vs. control (One Way ANOVA).

Figure 5.

WT mice treated with the MMP-8 inhibitor show an overall decreased systemic inflammatory profile of cytokines and chemokines 6 hours after CLP. The figure depicts the circulating plasma concentrations of (a) IL-6 (ng/mL), (b) KC (ng/mL), (c) IL-1 β (pg/mL), (d) MIP-1 α (pg/mL), (e) TNF α (pg/mL), (f) LIX (ng/mL) and (g) IL-10 for control WT mice (N=3), vehicle-treated WT mice (N=10), and inhibitor-treated WT mice (N=10) at 6 hours after CLP. Data are represented as means $\pm$ SEM. * $p < 0.05$ vs. vehicle-treated WT, # $p < 0.05$ vs. WT control (One Way ANOVA).

Figure 6.

MMP-8 activates the pro-inflammatory nuclear transcription factor NF- κ B. **(a)** Luciferase activity from RAW 264.7 murine macrophages transfected with an NF- κ B driven reporter plasmid. RAW 264.7 murine macrophages were treated for 4 hours with high-dose LPS (1 μ g/mL) as a positive control, human MMP-8, human MMP-8 with polymyxin B, low dose LPS (80pg/mL), or denatured human MMP-8, with each condition in duplicate in 4 separate experiments. Data are expressed as means $\pm$ SEM fold induction over control cells (transfected and treated with media alone). * $p \leq 0.05$ vs. control (ANOVA) **(b)** Supernatant cytokine concentration from cultures of primary murine peritoneal macrophages treated with MMP-8 *ex vivo*. Macrophages were treated with MMP-8 or LPS (positive control) for 24 hours and supernatant was collected and analyzed for cytokine concentration. Data are expressed as means $\pm$ SEM fold induction over control cells (treated with media alone). * $p < 0.001$ vs. control (ANOVA) **(c)** Fold induction, as compared to sham mice, of the NF- κ B p-65 subunit in whole lung nuclear extracts from WT (N=8) and Mmp8 $-/-$ (N=7) mice 6 hours after CLP. * $p < 0.05$ vs. WT mice (t-test) **(d)** Fold induction, as compared to sham mice, of the NF- κ B p-65 subunit in whole lung nuclear extracts from vehicle-treated (N=8) and inhibitor-treated (N=7) WT mice 6 hours after CLP. All data are expressed as means $\pm$ SEM.