A Potential Mechanism for Immune Suppression by Beta-Adrenergic Receptor Stimulation following Traumatic Injury

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Keywords

Host defense · Immune response · Kinase · Phosphatase · Protein · Sepsis

Abstract

Background: β -Adrenergic agents suppress inflammation and may play an important role in posttraumatic infections. Mechanisms may include inhibition of MAP kinase signaling. We sought to determine whether MKP-1 contributed to catecholamine suppression of innate immunity and also wanted to know whether early catecholamine treatment after traumatic injury increases the risk of later nosocomial infection. **Methods:** We performed experiments using THP-1 cells and peripheral blood mononuclear cells from healthy individuals. We exposed cells to epinephrine and/or LPS and measured inflammatory gene transcription and MAP kinase activation. We inhibited MKP-1 activity to determine its role in catecholamine-induced immune suppression. Finally, we studied injured subjects to determine whether early catecholamine treatment was associated with nosocomial infection. **Results:** Epinephrine increases MKP-1 transcripts and protein and decreases LPS-induced p38 and JNK phosphorylation and TNF- α gene transcription. RNAi inhibition of

MKP-1 at least partially restores LPS-induced TNF- α gene expression ($p = 0.024$). In the clinical cohort, subjects treated with β -adrenergic agents had an increased risk of ventilator-associated pneumonia (aOR = 1.9; 95% CI = 1.3–2.6) and bacteremia (aOR = 1.5; 95% CI = 1.1–2.3). **Conclusions:** MKP-1 may have a role in catecholamine-induced suppression of innate immunity, and exogenous catecholamines might contribute to nosocomial infection risk.

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Introduction

Severe traumatic injury is often complicated by nosocomial infection and organ failure. Together, they contribute to prolonged ICU stays and, when shock is present, have high case-fatality rates [1, 2]. For those who survive, quality of life may be poor [3].

We have observed that at the level of gene expression, markers of both innate and adaptive immunity are suppressed within 96 h of injury in trauma victims who subsequently develop bacteremia [1]. Some investigators have observed discordant changes in innate and adaptive immunity, reflecting the complexity of the systems that

are activated and suppressed in response to traumatic injury and initial resuscitation practices. Others have also reported associations between immune suppression, subsequent infection, and mortality. Taken together, it is likely that early immune suppression is central to the subsequent development of infection, organ failure, and death.

A number of mechanisms contribute to early postinjury changes in the immune system [4]. Catecholamines are one class of mediators thought to influence inflammation and may contribute to posttraumatic immune suppression [5, 6]. Endogenous epinephrine and norepinephrine are elevated after injury, and both agents may also be administered to treat shock. Circulating or elevated local concentrations of catecholamines may contribute to the altered immunity seen in critically injured patients [5, 7]. There is evidence that vasopressor treatment during resuscitation is associated with mortality in severely injured patients, but less is known about its relationship with posttraumatic infection or sepsis [8].

We want to understand the mechanisms that contribute to catecholamine-induced immune suppression; specifically, those related to β -adrenergic agents. With this knowledge, it may be possible to intervene in ways that would mitigate immune suppression and the associated complications. The data presented here are in follow-up to our initial genome-wide interrogation of the effects of epinephrine on monocyte gene expression. In those experiments, we used a web-based entry tool (Ingenuity Systems Inc., Redwood City, CA, USA), to identify possible molecules and networks that might be important [9]. The results of this work implicated MKP-1 (DUSP-1) in the adrenergic influence on inflammation, and we followed this with a series of experiments to confirm the relationships between LPS and epinephrine exposure on monocyte MKP-1 and TNF- α gene expression [10]. The genome-wide expression results implicating MKP-1 (DUSP-1) are included in the online supplementary Tables S1 and S2 (for all online suppl. material, see www.karger.com/doi/10.1159/000486972).

In this report, we present the results of a series of experiments aimed to understand the role of MKP-1 and the related signaling pathways in catecholamine-induced immune suppression. We also examine the relationship between β -adrenergic vasopressor treatment (epinephrine or norepinephrine) in the first 96 h after severe injury and the subsequent development of nosocomial infections in order to better understand the clinical implications of immune suppression potentially related to adrenergic vasopressor treatment.

Materials and Methods

Isolation of Peripheral Blood Monocytes

Human peripheral blood mononuclear cells (PBMCs) from healthy subjects were isolated from fresh peripheral venous blood following Ficoll-Paque (Ficoll-Paque Puls; GE Healthcare, USA) centrifugation separation. The PBMCs were then added to tissue culture plates for 2 h at 37°C and nonadherent cells were gently washed off of the plate, leaving adherent cells behind, and media were replenished. Cells were exposed to 1 μ M epinephrine and/or 1 ng/mL LPS or RPMI vehicle as control condition (for all experiments we used *Escherichia coli* LCD25; List Biologic Laboratories, Campbell, CA, USA). In these experiments and those that follow, cells were exposed to epinephrine (or vehicle) for <5 min prior to LPS exposure.

Quantitative PCR

Following RNA extraction, cDNA was reverse transcribed (Omniscript RT kit; Qiagen, USA) and MKP-1 and TNF- α mRNA levels were evaluated by qPCR via the SYBR green method (SYBR green master mix; Bio-Rad) with the following primers: MKP-1 primers: forward – GCCTTGCTTACCTTATGAGGAC, reverse – GGGAGAGATGATGACTTCGCC; TNF- α primers: forward – AGCCCATGTTGTAGCAAACC, reverse – TGAGGTACAGGCCTCTGAT.

All qPCR analyses were adjusted to GAPDH. GAPDH primers: forward – GGGGAGCCAAAAGGGTCATCATCT, reverse – GAGGGGCCATCCACAGTCTTCT.

Western Blotting

The THP-1 cell line (American Type Culture Collection [ATCC], Manassas, VA, USA) was used to test the effects of epinephrine on intracellular signaling pathways. THP-1 cells were differentiated with 100 nM vitamin D₃ (Sigma Aldrich, St. Louis, MO, USA) for 48 h to enhance their ability to respond to LPS [11]. The cells were then treated with 1 μ M epinephrine and/or 1 ng/mL LPS and total cellular protein was isolated at a series of time points following stimulation. Total MKP-1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), phosphorylated MKP-1 (p-MKP-1) (Cell Signaling, Danvers, MA, USA), p-JNK (Promega, Madison, WI, USA), p-p38 (Cell Signaling), and total ERK (Santa Cruz Biotechnology) protein levels were then evaluated via Western blotting as described in the following paragraph.

Total protein was electrophoresed in a 10% SDS-PAGE gel and transferred to a Hybond-ECL nitrocellulose membrane (Amersham Pharmacia Biotech, UK). The membrane was blocked for 1 h at room temperature with 5% bovine serum albumin (Sigma Aldrich) or 5% milk (Nestlé, Solon, OH, USA) and then incubated with rabbit-derived antibodies directed against the active phosphorylated forms of MKP-1 (Cell Signaling), p-JNK (Promega), p-p38 (Cell Signaling), and nonphosphorylated total ERK2 (Santa Cruz Biotechnology) and MKP-1 (Santa Cruz Biotechnology) overnight at 4°C. The membrane was then incubated with goat anti-rabbit, horseradish peroxidase-conjugated antibody. The membrane was developed using the SuperSignal chemiluminescent substrate (Thermo Fisher Scientific, Rockford, IL, USA) and exposed on Kodak KAR-5 film (Eastman Kodak, Rochester, NY, USA). All gels were blotted with total ERK2 to confirm equal protein loading and data were analyzed after adjusting to ERK2 densitometry. Films were scanned and densitometry measured using ImageJ (National Institutes of Health, USA).

Fig. 1. Time course of MKP-1 and TNF- α gene expression. THP-1 cells were exposed to LPS, epinephrine, both together, or control conditions for 0, 20, 40, 60, and 120 min. RNA was then extracted and cDNA quantified using rtPCR. All comparisons are made to time “0” which is not shown on the graph. Data were analyzed by two-way ANOVA using Bonferroni correction. **a** MKP-1 peaked at 40 min and to the greatest extent after combined exposure to

LPS and epinephrine. The overall p value was 0.020. * $p < 0.05$, comparing epinephrine and LPS with LPS alone. **b** TNF- α expression was lower at each of the time points (20, 40, 60, and 120 min) when cells were exposed to both LPS and epinephrine relative to LPS exposure alone. The overall p value was 0.021. * $p < 0.05$, comparing epinephrine and LPS with LPS alone. $n = 4$ for each experimental condition at each time point.

RNA Inhibition

Vitamin D₃-differentiated (48 h, 100 nM) THP-1 cells were administered 50 nM of noncoding or MKP-1 siRNA (Santa Cruz Biotechnology) in Ribojuice transfection reagent (EMD Millipore, Germany) for 24 h. These cells were then exposed to 1 μ M epinephrine and/or 1 ng/mL LPS for 1 h. MKP-1 and TNF- α expression levels were evaluated via qPCR.

Clinical Cohort to Study the Relationship between β -Adrenergic Therapy and Infectious Complications after Trauma

For this analysis, we studied subjects who had been enrolled into our single-center study of genetic factors associated with posttraumatic sepsis. This cohort has been presented in detail elsewhere and is summarized here [12, 13]. Subjects were enlisted from a prospectively enrolled cohort of severely injured trauma patients admitted to the intensive care units at Harborview Medical Center in Seattle, WA, USA. Clinical data were obtained from two sources: (1) detailed information about injuries, including the Injury Severity Scale (ISS) score and the Abbreviated Injury Scale (AIS) score, were obtained from the institutional trauma registry, and (2) additional data were obtained from the electronic medical record. The University of Washington Human Subjects Division approved all study procedures. Sepsis and in-

fectious complications were based upon data obtained from the electronic medical records. Bacteremia is defined as any positive culture from blood sampled from a peripheral vein or from a central venous catheter excluding *Staphylococcus epidermidis*, which we considered a contaminant. The diagnosis of ventilator-associated pneumonia at our institution generally requires quantitative cultures of samples obtained from the lower respiratory tract either by lavage or brushings. We followed the criteria established by Fagon et al. [14] in defining ventilator-associated pneumonia in our study.

Data Presentation and Statistical Analyses

Data were managed and analyzed using the following statistical programs: GraphPad Prism V.6 (USA), Stata/IC for Windows V21.1 (StataCorp, College Station, TX, USA), and SPSS for Windows V14.0 (USA). For analysis of the qPCR and cell signaling data, ANOVA with Bonferroni correction was used to adjust for multiple comparisons where appropriate. The Dunnett post hoc test was used when all treatment groups were compared to the control group. The clinical data were first analyzed using unadjusted comparisons between the subjects treated with β -adrenergic vasopressors and those who were not. Logistic regression analysis (forward stepwise) was used to adjust for multiple risk factors and confounding variables in order to determine the risk of infectious

Fig. 2. MKP-1 and TNF- α gene expression in human peripheral blood monocytes. In order to confirm that THP-1 cells reflected what could be occurring in vivo, we isolated PBMCs from healthy adults and exposed these cells to the same experimental conditions for 40 min. Data were analyzed by ANOVA. **a** Combined exposure induced the highest MKP-1 response, followed by LPS alone and then epinephrine alone. The overall p value was <0.0001 . * $p < 0.05$, using Dunnett's post hoc adjustment. **b** TNF- α gene expression was lower in the cells exposed to both agents compared to LPS exposure (9.8 ± 3.1 and 17 ± 6.3 , respectively). * $p < 0.05$, ANOVA with Bonferroni correction. The data displayed are based upon PBMCs isolated from 6 healthy subjects tested under each experimental condition.

complications associated with vasopressor medication use in the first 3 days after injury. These data are presented as adjusted odds ratios (aOR) with 95% confidence intervals (95% CI) and the associated p values.

Results

Epinephrine Decreases TNF- α Gene Transcription and Increases MKP-1 Gene Transcription

We wanted to confirm our previous genome-wide observation that MKP-1 gene transcription is increased by epinephrine. We first performed experiments using THP-1 cells in order to clarify the time course of both MKP-1 and TNF- α gene transcription. Elevations in both MKP-1 (Fig. 1a) and TNF- α (Fig. 1b) were greatest at 40 min. We then conducted experiments to determine whether human PBMCs demonstrated similar responses. At 40 min, MKP-1 mRNA increased in response to LPS, epinephrine, or both (Fig. 2a). TNF- α gene transcription was lower in the presence of epinephrine pretreatment when compared with LPS treatment alone (Fig. 2b). In summary, the observations in human PBMCs were consistent with our observations in the THP-1 cells.

Epinephrine Pretreatment Increases MKP-1 and p-MKP-1, and Decreases MAPK Phosphorylation following Monocyte LPS Stimulation

MKP-1 regulates signaling by dephosphorylating mitogen-activated protein kinases (MAPKs). We wanted to know whether increased MKP-1 gene transcription leads to increased MKP-1 protein with a resultant decrease in MAPK phosphorylation. We examined the effect of epinephrine and LPS exposure on total MKP-1, p-JNK, and p-p38 kinase protein levels by Western blot analysis (Fig. 3). LPS alone had little effect on MKP-1 protein levels, whereas epinephrine increased MKP-1 protein, beginning at 30 min and increasing through 120 min. The highest MKP-1 protein levels were seen at 60 min in cells exposed to both LPS and epinephrine (Fig. 3a). The levels of phosphorylated MKP-1 were more variable under all conditions than the other proteins, but this active form of the molecule was most elevated in response to epinephrine and LPS together, at the 60- and 120-min time points (Fig. 3b).

The changes in the phosphorylated forms of p38 and JNK corresponded to the aforementioned increased in MKP-1. In response to LPS, both p-p38 and p-JNK followed a pattern where levels peaked at 30 min and de-

Fig. 3. MKP-1 protein and phosphorylated MAPKs levels. THP-1 cells were exposed to LPS and/or epinephrine and total cellular protein harvested after 0, 30, 60, and 120 min. Measurement of protein expression was determined by Western blot and adjusted for by ERK concentrations. **a** MKP-1 increased in response to epinephrine at 30 min and remained elevated throughout. **a, b** Both MKP-1 and p-MKP-1 increased greatest in response to a combina-

tion of LPS and epinephrine, peaking at 60 min. **c, d** Suppressed phosphorylation of p-p38 and p-JNK occurred in response to epinephrine stimulation. Exposure to epinephrine decreased levels of p-JNK and p38 at 30 and 60 min induced by LPS alone. * $p < 0.05$, ANOVA adjusted with Bonferroni correction. **e** A representative Western blot. $n = 5$ for each experimental condition at each time point with 3 technical replicates for each blot.

(Figure continued on next page.)

Fig. 4. MKP-1 and p-p38 protein expression in the presence of triptolide. **a** In the presence of triptolide, MKP-1 protein was suppressed to similar levels under all experimental conditions. **b** p-p38, in the presence of LPS, epinephrine, and triptolide was restored to levels similar to those of LPS without epinephrine (100 ± 35 and 84 ± 32 , respectively). * $p = 0.42$, indicating inhibition of MKP-1 effects on MAPK signaling. $n = 5$ for each experimental condition.

creased toward baseline levels by 120 min. The effect of epinephrine on MAPK phosphorylation induced by LPS was evident at 30, 60, and 120 min for both p38 and JNK but most marked at 30 and 60 min (Fig. 3c, d). In summary, epinephrine suppressed MAPK phosphorylation that was seen in response to LPS.

MKP-1 Inhibition Restores LPS-Induced p38 Phosphorylation and Partially Restores TNF- α Gene Transcription

In order to determine whether dephosphorylation of MAPKs is a mechanism by which epinephrine might influence inflammatory signaling, MKP-1 activity was inhibited using triptolide. Triptolide, an extract from the herb *Tripterygium wilfordii* Hook F. used in traditional

Fig. 5. Epinephrine-induced MKP-1 contributes to decreased TNF- α expression. We tested whether epinephrine-induced MKP-1 expression directly contributes to the diminished TNF- α response using siRNA inhibition of MKP-1. Control and MKP-1 siRNA were administered to vitamin D₃-differentiated THP-1 cells for 24 h. Subsequently, 1 μ M epinephrine and/or 1 ng/mL LPS were administered to the siRNA-treated THP-1 monocytes and RNA was harvested 1 h after stimulation. **a** We observed that MKP-1 siRNA modestly inhibited MKP-1 mRNA expression in all

3 stimulation conditions. The overall p value by ANOVA was <0.0001 and the Bonferroni-corrected p value of 0.025 is shown. **b** MKP-1 silencing partly rescued the epinephrine-induced suppression of mRNA when compared with control (noncoding) siRNA. The overall p value by ANOVA was <0.0001 . The two bars to the right indicate that gene silencing partially restored TNF- α gene expression (8 ± 0.6 and 11 ± 1.4 ; corrected p value of 0.024). For the gene silencing experiments, $n = 3$ for each experimental condition.

(herbal) treatments of rheumatoid arthritis, is known to be a potent MKP-1 inhibitor [15–19]. Triptolide pretreatment suppressed MKP-1 protein expression under all conditions (Fig. 4a). Exposure to triptolide, in the presence of epinephrine, restored phosphorylated p38 levels to those seen with LPS alone (Fig. 4b). Because triptolide blocks other inflammatory signaling molecules and pathways, including TNF- α , it was not possible to determine whether its effects on MKP-1 inhibition restore TNF- α protein levels [20–23]. Therefore, these experiments indicate a role for β -adrenergic stimulation in MAPK dephosphorylation; however, they do not prove that this restores TNF- α production.

In order to better clarify the role of MKP-1 in β -adrenergic suppression of TNF- α , we used anti-MKP-1

siRNA to knock down monocyte MKP-1. Following MKP-1 siRNA administration in THP-1 monocytes, we were able to partially inhibit MKP-1 gene expression induced by epinephrine, LPS, and epinephrine plus LPS compared with noncoding (control) siRNA administration (Fig. 5a). We also observed that MKP-1 siRNA knockdown resulted in rescued TNF- α mRNA expression when epinephrine was combined with LPS exposure (Fig. 5b).

β -Adrenergic Vasopressor Treatment in Critically Injured Patients Was Associated with Ventilator-Associated Pneumonia and Bacteremia

In a multicenter cohort of severely injured trauma victims, the use of vasopressor agents as part of their initial

Table 1. Demographics, injury characteristics, and outcomes for the entire cohort ($n = 1,645$)

<i>Demographics</i>	
Age, years	40 (1–52)
Sex	
Male	1,189 (72)
Female	456 (28)
Race/Ethnicity	
Caucasian	1,227 (75)
African-American	101 (6)
Hispanic	124 (7)
Asian	71 (4)
American Indian	36 (2)
Unknown	86 (5)
Chronic comorbidities	
Diabetes	117 (7)
Cardiovascular	419 (26)
Respiratory	179 (11)
Hepatic	102 (6)
Cancer	59 (4)
<i>Injury characteristics</i>	
Injury severity score	33 (25–75)
Severe brain injury	984 (60)
Severe thoracic injury	977 (60)
Severe abdominal injury	469 (29)
Severe lower extremity injury	257 (15)
Severe upper extremity injury	651 (39)
Severe spine injury	533 (32)
Injury mechanism	
Blunt	1,507 (91)
Penetrating	138 (8)
Initial base deficit >6	642 (39)
PRBC transfused, units	4 (1–8)
Vasopressor treatment	192 (11)
<i>Operative procedures performed</i>	
Laparotomy	311 (19)
Thoracotomy	151 (9)
Fracture fixation	888 (54)
<i>Outcomes</i>	
Mortality	215 (13)
ICU length of stay, days	10 (5–17)
Hospital length of stay, days	20 (12–31)
Duration of mechanical ventilation, days	8 (4–14)
Ventilator-free days	19 (8–23)
Ventilator-associated pneumonia	557 (34)
Bacteremia	261 (16)
Gram positive	178 (11)
Gram negative	98 (6)

Continuous data are presented as medians (25–75th percentiles) and categorical data are indicated as n (%). Severe traumatic brain, thoracic, abdominal, and extremity injuries refer to the number of patients with abbreviated injury scores ≥ 3 in the indicated body region. The entire cohort included 2,953 subjects, of whom 1,213 with <3 days of mechanical ventilation and 95 with burn injuries were excluded. PRBC, packed red blood cells; ICU, intensive care unit.

resuscitation was associated with subsequent mortality [8]. If β -adrenergic stimulation suppresses immunity, it is reasonable to expect an increase in infectious complications in patients treated with β -adrenergic agents. Therefore, we examined whether infusion of either epinephrine or norepinephrine during the initial few days after injury (days 0–3) was associated with subsequent nosocomial infection. We focused on ventilator-associated pneumonia (the most common infection) and bacteremia (representing failure of innate immune responses to localize an infection). Both of these are important complications of traumatic injury [1, 24]. There were 1,645 subjects who received ≥ 3 days of mechanical ventilation. A summary of their demographics, injury characteristics, and outcomes are shown in Table 1. β -Adrenergic agent use was uncommon during the first 4 days after injury. A total of 192 subjects (12%) received β -adrenergic agents at any point during days 0–3. The 1st postinjury day had the highest β -adrenergic use (132 subjects) and postinjury day 0 had the lowest use (56 subjects). Of those receiving β -adrenergic infusions, 125 received the infusion for less than 48 h and 67 subjects received more than 48 h of infusion. Demographic information, injury characteristics, and outcomes are shown in Table 2 for subjects according to whether they received β -adrenergic agents. Those who did were slightly older, had more severe shock, had higher injury severity scores and received more blood products than those who did not receive these agents. First, by unadjusted analyses, β -adrenergic treatment was associated with an increase in the incidence of both ventilator-associated pneumonia (RR = 1.6; 95% CI = 1.3–1.9) and bacteremia (RR = 1.7; 95% CI = 1.4–2.1). Using logistic regression to adjust for other risk and potentially confounding factors, we observed that β -adrenergic agents were associated with an increased risk of both ventilator-associated pneumonia (aOR = 1.9; 95% CI = 1.3–2.6) and bacteremia (aOR = 1.5; 95% CI = 1.1–2.5). The results of these analyses are shown in Table 3.

Although logistic regression is meant to help adjust for the potential confounding effects of other factors on the outcomes of interest, differences between our two groups that led to the use of vasopressors might not be adequately captured in the available data that may also contribute to infection risk. Propensity score analysis has been used to help address this potential shortcoming [25, 26]. We performed a propensity-matched analysis by first calculating the propensity for receiving early adrenergic vasopressors. We then matched subjects who received early vasopressors in a ratio of 1:2 to subjects who did not, who

Table 2. Demographics, injury characteristics, and outcomes according to whether subjects received β -adrenergic agents

	β -Adrenergic treatment (<i>n</i> = 192)	No β -adrenergic treatment (<i>n</i> = 1,453)	<i>p</i> value
<i>Demographic and injury characteristics</i>			
Age, years	40 (24–58)	37 (23–52)	0.04
Male	149 (78)	1,040 (72)	0.08
APACHE II	26 (22–30)	29 (25–33)	<0.0001
Initial base deficit ≥ 6 , mEq/L	91 (47)	551 (37)	0.01
≥ 6 units PRBCs in first 24 h	60 (31)	314 (21)	0.003
Injury severity score	35 (26–44)	30 (24–41)	<0.0001
Severe abdominal injury	63 (32)	406 (28)	0.16
Severe thoracic injury	117 (61)	860 (59)	0.64
Severe brain injury	122 (64)	862 (59)	0.26
Severe spinal injury	78 (41)	455 (31)	0.01
Severe extremity injury	95 (49)	653 (45)	0.24
<i>Outcomes</i>			
Ventilator-associated pneumonia	99 (52)	458 (32)	<0.0001
Bacteremia	48 (25)	213 (15)	<0.0001
Duration of mechanical ventilation, days	14 (7–23)	7 (4–13)	<0.0001
Ventilator-free days	1 (0–14)	20 (11–24)	<0.0001
Duration of ICU stay, days	15 (8–24)	9 (5–16)	<0.0001
Duration of hospital stay, days	22 (11–38)	20 (13–30)	0.63
Mortality	70 (37)	145 (10)	<0.0001

Continuous data are presented as medians (25–75th percentiles) and compared with the Mann-Whitney U test. Categorical data are presented as *n* (%) and compared using the Pearson χ^2 test. APACHE II, acute physiology and chronic health evaluation II; severe head/chest/abdominal injury, body region abbreviated injury score ≥ 3 ; PRBC, packed red blood cells; ICU, intensive care unit.

were within 0.05 probability. This resulted in 192 subjects being matched to 384 who were not treated with adrenergic agents. The OR was 2.2 (95% CI: 1.5–3.2) for ventilator-associated pneumonia and 1.7 (95% CI: 1.1–2.6) for bacteremia in the propensity-matched analyses. A summary of the propensity-matched analyses for bacteremia and ventilator-associated pneumonia is included in on-line supplementary Table S3.

Based upon the logistic regression analyses, the risk of nosocomial infection associated with β -adrenergic agents was comparable to the risk associated with large volume transfusion of red blood cells. This relationship was supported by the findings of the propensity-matched analysis. In summary, our data demonstrate a relationship between exogenous β -adrenergic drug use during the first 3 days after injury and subsequent bacteremia and ventilator-associated pneumonia, suggesting a potential immune-suppressive effect of these agents.

Discussion

Activation of the innate immune response occurs in response to traumatic injury and is part of what has been termed a “genomic storm” [27]. Sympathetic activation, leading to local and systemic release of adrenergic mediators, is an important component of this response and contributes in significant, yet incompletely understood, ways to the response to injury. Our observations help us understand the mechanisms underlying the influence of sympathetic activation and the clinical use of β -adrenergic medications on inflammatory signaling and on important clinical outcomes. We have shown that β -adrenergic stimulation suppresses intracellular inflammatory signaling pathways and that β -adrenergic agent infusion after trauma is associated with an increased incidence of ventilator-associated pneumonia and bacteremia.

Sympathetic activation is essential for the “fight or flight” response; however, it is also known to regulate the

Table 3. Logistic regression analyses of factors associated with ventilator-associated pneumonia and bacteremia

Risk factor	Adjusted odds ratio	95% CI	p value
<i>Ventilator-associated pneumonia</i>			
Vasopressor treatment	1.9	1.3–2.6	<0.0001
Age	1.007	1.001–1.012	0.01
APACHE II	1.02	1.01–1.04	<0.0001
Sex	0.78	0.61–0.99	0.05
≥6 units PRBC in 24 h	1.9	1.5–2.5	<0.0001
<i>Bacteremia</i>			
Vasopressor treatment	1.5	1.1–2.3	0.02
Injury severity score	1.01	1.001–1.02	0.038
APACHE II	1.02	1.001–1.043	0.04
≥6 units PRBC in 24 h	2.0	1.5–2.7	<0.0001
Severe brain injury	0.7	0.5–0.88	0.005

Results of forward stepwise logistic regression models for each of ventilator-associated pneumonia and bacteremia. Vasopressor treatment is defined as any β -adrenergic agent administered during the first 72 h after injury. Severe brain injury is defined as a head abbreviated injury score ≥ 3 . Injury severity score and APACHE II were included as continuous variables. APACHE II, acute physiology and chronic health evaluation II; PRBC, packed red blood cells.

function of many immune cell types, including various lymphocyte, monocyte, and granulocyte populations. van der Poll et al. [5] demonstrated that β -adrenergic stimulation alters monocyte cytokine responses to endotoxin. They observed that in vivo β -adrenergic stimulation altered the mononuclear cell cytokine and inflammatory profiles and posited that these changes may occur in critically ill patients with sepsis [6, 7]. However, they did not explore the potential mechanisms involved.

The mechanisms of the effect of adrenergic suppression of innate immune responses are likely multiple. We chose to focus on MKP-1 based upon our genome-wide gene expression data and observations by others supporting a role for β -adrenergic modulation of MKP-1 gene transcription [28–30].

There are a number of limitations of our data and observations related to the experimental and clinical observations. These are discussed here and will need to be addressed in future studies. First, our RNA inhibition experiments were only partially successful in knocking down MKP-1 and therefore were only partially able to restore TNF- α gene expression. However, it is likely that even full MKP-1 inhibition will not completely restore TNF- α or other gene transcription levels given that other

mechanisms and pathways are doubtless involved. Nevertheless, it is possible that we simply were not able to achieve full gene inhibition with our methods. Determining the role of these competing hypotheses requires additional studies. It is also important to re-emphasize that our experimental observations relied primarily on data generated using the THP-1 cell line which may differ in important ways from in vivo responses of human circulating and tissue immune cells.

Our observation of an association between early norepinephrine or epinephrine infusion and subsequent nosocomial infection supports the notion that these agents have clinically relevant immune-suppressive actions, but does not prove this definitively. Subjects who received these agents were more ill than those who did not and it is not possible to address and control for all the unmeasured factors that were associated with their use and with subsequent nosocomial infections. Alternatively, the effect of vasopressors on infection risk may be completely unrelated to immune suppression. For example, it is possible that vasopressors contribute to splanchnic vasoconstriction, increasing intestinal bacterial translocation, and in this way place the patient at greater risk of subsequent infection. Neither of our multivariate statistical methods can definitively rule out the possibility of such alternate mechanisms.

This limitation is important to acknowledge but should not invalidate our interpretation, which is not different to that in other observational studies of critically ill patients. For example, initial studies observing a relationship between a low plasma to red blood cell transfusion ratio in patients receiving massive transfusion was clearly confounded by the sicker patients receiving relatively more units of red blood cells [31, 32]. Nevertheless, the importance of a balanced approach to transfusion eventually emerged and the initial observations were central to the development of prospective observational studies and clinical trials.

We included both norepinephrine and epinephrine as the adrenergic agents of potential importance as they both have β -adrenergic actions. It is likely that both of these agents have similar effects in immune cells that are mediated via the β_2 receptor and cyclic AMP intracellular signaling pathways. We reported on the effects of epinephrine as the β -agonist in our experiments; however, others have demonstrated similar effects on immune cell function with norepinephrine and the pure β -adrenergic agonist, isoproterenol.

Our experimental model does not capture all the complexity of the posttraumatic state. However, it is reason-

able to consider that circulating and resident immune cells exposed to β -adrenergic agents at relatively high levels, as seen with infusion, develop immune suppression, establishing the groundwork for subsequent infection. There are data to support this notion. First, we know that a subgroup of patients who develop bacteremia after trauma have evidence of early suppression of innate immunity [1]. We also know that patients who develop persistent immune suppression after trauma have defects in innate and adaptive immunity beginning early after injury [33]. Our data here raise the possibility that β -adrenergic stimulation and activation of the cyclic AMP pathway play a role in this clinical trajectory.

MKP-1 activation is likely not the sole mechanism whereby adrenergic stimulation might alter immunity, nor is it definite that the changes we induced and observed in PBMCs are directly related to the nosocomial infections we observed in our cohort of trauma patients. Drawing this connection is premature. However, now that we have at least one cellular mechanism to study, we can look in more detail at the immune cells in critically ill patients and how they respond to adrenergic agents and inhibitors.

There are alternative vasopressor agents that could be used in the resuscitation of trauma and other critically ill patients. Vasopressin, for example, is in wide clinical use. However, whether it or other nonadrenergic agents improve outcomes over adrenergic drugs is not certain [34]. Vasopressin also signals through a G-protein-coupled receptor and activates cyclic AMP. Therefore, its effects on immune signaling, including induction of MKP-1 gene transcription, may not differ from β -adrenergic agents. Nevertheless, studies aimed to determine its effect on inflammatory signaling are warranted. α -Adrenergic agonists are also potentially useful, but generally have been limited to patients with neurogenic shock.

Alternatively, β -adrenergic inhibitors could be used once shock has resolved and the patient has stabilized. The use of β -blockers has been examined in a number of studies of critically ill patients. Burn injury patients seem to derive the greatest benefit from β -adrenergic inhibition, primarily through a more rapid reversal of the effects of the hypermetabolism associated with burn injury [35, 36]. There is also some evidence indicating that β -adrenergic inhibition can restore monocyte immune function and may reduce susceptibility to infection after burn injury [37], but we can only speculate on this possibility. It is possible that the immune-suppressive effects of adrenergic stimulation are established and will not be completely reversible by subsequent β -adrenergic inhibi-

tor treatment. A prospective study aimed at testing the effects of β -adrenergic inhibition on innate immune function is warranted and is perhaps the best way to determine the importance of β -adrenergic stimulation on immune function.

Finally, our clinical observations only address the effects of exogenous β -adrenergic agent infusion and not the potential effects of the endogenous release of both epinephrine and norepinephrine as part of the stress response. It is possible that endogenous epinephrine release might influence subsequent immunity and patients who do not receive exogenous treatment will also benefit from β -adrenergic inhibition.

In summary, our data suggest a role for MKP-1 in the catecholamine-induced suppression of innate immunity. Given the use of catecholamines in critically ill patients, and the association with infectious complications that we observed in our traumatized subjects, it will be important to determine ways to reduce their use and inhibit their deleterious effects.

Disclosure Statement

The authors have no conflicts of interest relevant to this paper.

Funding Sources

This work was supported by NIGMS grants R01 GM066946 and T32 GM007037 and by the American Association for the Surgery of Trauma research and education foundation scholarship award.

Author Contributions

Nicholas J. Shubin: study design, conduct of experiments, data collection and analysis, and drafting of the manuscript. Tam N. Pham: study design, data analysis, and critical review of the manuscript. Kristan Lea Staudenmayer: literature reviews, study design, conduct of experiments, data collection and analysis, and critical review of the manuscript. Brodie A. Parent: data analysis and critical review of the manuscript. Qian Qiu: data analysis and critical review of the manuscript. Grant E. O'Keefe: literature review, study design, data collection and analysis, and drafting and review of the manuscript.

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