Beta-Blockade Prevents Hematopoietic Progenitor Cell Suppression after Hemorrhagic Shock

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Abstract

Background: Severe injury is accompanied by sympathetic stimulation that induces bone marrow (BM) dysfunction by both suppression of hematopoietic progenitor cell (HPC) growth and loss of cells via HPC mobilization to the peripheral circulation and sites of injury. Previous work demonstrated that beta-blockade (BB) given prior to tissue injury both reduces HPC mobilization and restores HPC colony growth within the BM. This study examined the effect and timing of BB on BM function in a hemorrhagic shock (HS) model.

Methods: Male Sprague-Dawley rats underwent HS via blood withdrawal, maintaining the mean arterial blood pressure at 30–40 mm Hg for 45 min, after which the extracted blood was reinfused. Propranolol (10 mg/kg) was given either prior to or immediately after HS. Blood pressure, heart rate, BM cellularity, and death were recorded. Bone marrow HPC growth was assessed by counting colony-forming unit–granulocyte-, erythrocyte-, monocyte-, megakaryocyte (CFU-GEMM), burst-forming unit-erythroid (BFU-E), and colony-forming unit-erythroid (CFU-E) cells.

Results: Administration of BB prior to injury restored HPC growth to that of naïve animals (CFU-GEMM 59 ± 11 vs. 61 ± 4 , BFU-E 68 ± 9 vs. 73 ± 3 , and CFU-E 81 ± 35 vs. 78 ± 14 colonies/plate). Beta-blockade given after HS increased the growth of CFU-GEMM, BFU-E, and CFU-E significantly and improved BM cellularity compared with HS alone. The mortality rate was not increased in the groups receiving BB.

Conclusion: Administration of propranolol either prior to injury or immediately after resuscitation significantly reduced post-shock BM suppression. After HS, BB may improve BM cellularity by decreasing HPC mobilization. Therefore, the early use of BB post-injury may play an important role in attenuating the BM dysfunction accompanying HS.

SEVERE TRAUMA and hemorrhagic shock (HS) followed by resuscitation primes the immune system and leads to an inflammatory response [1]. One component of this response is an alteration bone marrow (BM) function, which manifests as persistent anemia and increased susceptibility to infection [2,3]. The persistent anemia is not related to ongoing blood loss or erythropoietin and can manifest for as long as two weeks after severe injury [3]. The etiology of the altered BM function and persistent anemia is multifactorial and is associated with impaired growth of hematopoietic progenitor cells (HPC) and stromal cells within the BM, along with mobilization of HPCs into the peripheral circulation [4]. Shah et al. [5] showed that HPCs mobilize to the peripheral blood (PB), sequester in injured tissue, and likely are involved in

tissue repair. The combined effect of BM progenitor growth suppression and increased mobilization of HPCs from the BM occurs in response to the degree and duration of physiologic stress, which is mediated by norepinephrine [6–8].

There is strong evidence of bidirectional communication between the sympathetic nervous system, the BM, and the immune system [9]. Both mature and immature immune cells, including HPCs and B and T lymphocytes, express alpha- and beta-adrenergic receptors, with a predominance of beta receptors [10,11]. The sympathetic nervous system has a direct effect on these cells, influencing their cytokine expression, function, and cytotoxic activity [10]. In addition, the sympathetic nervous system, through its BM innervations and release of norepinephrine, is a crucial regulator of HPC mobilization [8,12].

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Trauma results in substantial and sustained release of catecholamines that leads to a wide range of hemodynamic, metabolic, behavioral, and immune changes [1]. The regulation of the catecholamine response with beta-blockade (BB) is beneficial in humans after burns, trauma, non-cardiac surgery, and traumatic brain injury, as measured by improved outcomes [13–16]. Our previous work focused on the role of catecholamines in the context of tissue injury-induced BM dysfunction. We showed that norepinephrine impacts BM progenitor cell growth and mobilization in a dose-dependent manner and that non-selective BB with propranolol reduces HPC mobilization into the periphery and HPC growth in injured tissue [7,17]. The aim of this study was to examine both the impact and the effect of the timing of BB on BM progenitor cells in a HS model.

Experimental Methods

Animals

Male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 250–350 g were housed under barrier-sustained conditions and kept at 25°C with 12 h light/dark cycles. The rats had free access to water and chow (Teklad 22/5 Rodent Diet W-8640; Harlan Teklad, Madison, WI). All rats were maintained in accordance with the recommendations of the U.S. National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. All animal protocols were approved by the New Jersey Medical School Animal Care and Use Committee.

Experimental design

The animals were assigned randomly to one of the following experimental groups: Unmanipulated control (UC) (n = 8), BB alone (n = 6), sham shock (SS) (n = 8), sham shock + BB (SS + BB) (n = 6), HS alone (n = 6), and BB given before or after HS (HS + preBB; HS + postBB) (n = 5 and 4, respectively). The number represents the survivors in each group that underwent harvesting of BM. The pre-HS BB animals received intraperitoneal (IP) propranolol injections (10 mg/kg) for three days prior to HS. The animals that received BB post-HS received a single IP injection of propranolol (10 mg/kg) after adequate resuscitation following HS. Rats assigned to the BB alone group received a daily IP propranolol injection (10 mg/kg) for three days and were sacrificed on day four.

Hemorrhagic shock

Rats were weighed and anesthetized with IP sodium pentobarbital (50 mg/kg). The femoral artery and the right internal jugular vein were isolated using aseptic techniques and cannulated with polyethylene catheters (PE-50 and PE-10, respectively) containing 0.3 mL of heparinized saline (10 units/mL). Both catheters remained in situ for 3 h after resuscitation. The femoral artery catheter was used for continuous blood pressure (BP) monitoring (BP-2 Digital BP Monitor; Columbus Instruments, Columbus, OH). On cannulation of the vessels, the rats were allowed to equilibrate for 10–15 min. Rats assigned to the HS group then underwent blood withdrawal via the internal jugular vein catheter to a mean arterial blood pressure (MABP) of 30–45 mm Hg. This was maintained for 45 min, after which the animals were re-

suscitated by reinfusion of the removed blood (which was kept at 37°C) at a rate of 0.5–1 mL/min. Three hours after resuscitation, MABP and heart rate (HR) were recorded, the animals were sacrificed via cardiac puncture, and the BM was harvested for cell cultures. Throughout the experiment, the animals' temperatures were kept at approximately 37°C by using an electric heating pad under the surgical platform.

Animals assigned to the SS group underwent cannulation alone and were sacrificed 3 h 45 min later. The SS + BB animals received a single propranolol dose (10 mg/kg) 45 min after cannulation and were sacrificed at 3 h. The SS animals' HR and MABP were recorded on cannulation, as shock was not induced. Death of an animal during anesthesia, HS, or resuscitation prior to the 3-h sacrifice time was recorded to calculate the mortality rate.

Hematopoietic progenitor cell assays

The BM was harvested from both femurs by removing the epiphysis and aspirating using an 18-g needle on a 5-mL syringe containing 0.5 mL of Minimal Essential Medium (MEM)-alpha medium (Sigma Chemical, St Louis, MO). The aspirate was washed by adding 1 mL of cold medium and subjected to vigorous pipetting to ensure adequate cell separation. The cell suspension was then centrifuged at 300×g for 15 min, the supernatant liquid was discarded, and the pellet was resuspended in 1 mL of Dulbecco's MEM containing 10% fetal bovine serum (FBS). Bone marrow cellularity was assessed with a hemocytometer. Mononuclear cells (2×10^6) were plated in duplicate in Iscove's medium containing 30% FBS, 2% bovine serum albumin, 1% methylcellulose, rat growth factor, penicillin/streptomycin (GIBCO, Grand Island, NY), 2-mercaptoethanol 2×10^{-4} mol/L, and glutamine (Cellgro; Mediatech, Herndon, VA) supplemented with recombinant erythropoietin (rEpo) 1.3 U/mL and recombinant interleukin-3 (rhIL-3) (Genetics Institute, Cambridge, MA) 6 U/mL for burst-forming unit-erythroid (BFU-E)/colony-forming unit-erythroid (CFU-E) or recombinant granulocyte-macrophage colony stimulating factor (rGM-CSF) 3 U/mL for CFU-granulocyte, erythrocyte, monocyte, megakaryocyte (GEMM). Cultures were incubated at 37°C in 5% CO₂. Colonies of CFU-E were counted at day 7, CFU-GEMM at day 10, and BFU-E at day 15 by an observer blinded to the origin of the samples.

Statistical analysis

All data are represented as means ± standard deviations. The data were subjected to repeated measures one-way analysis of variance (ANOVA) followed by Tukey-Kramer's multiple comparison post test using the GraphPad Prism statistical package (version 4.0; Graph Pad, San Diego, CA). A p value <0.05 was considered significant.

Results

Death and bone marrow cellularity

The overall mortality rate for all groups ranged from 11–40% (Table 1). There was one death in the UC group related to anesthesia. In the remaining experimental groups, animals that died during shock or resuscitation prior to the 3-h time of sacrifice were included in the mortality calculation, but none of these animals underwent harvesting of BM. The use of BB

TABLE 1. BONE MARROW CELLULARITY AND MORTALITY RATE FOR ALL EXPERIMENTAL GROUPS

	Mortality rate (%)	Femur marrow cellularity (cells $\times 10^6$ /mL) $\pm$ standard deviation
Unmanipulated control	11	198 $\pm$ 15
Beta-blockade alone	12	218 $\pm$ 10
Sham shock	20	190 $\pm$ 62
Sham shock + beta-blockade	15	199 $\pm$ 47
Hemorrhagic shock	40	107 $\pm$ 24 ^a
Hemorrhagic shock + prior beta-blockade	33	207 $\pm$ 20
Hemorrhagic shock + followed by beta-blockade	38	195 $\pm$ 56

^aP < 0.05 compared with control group.

did not increase the mortality rate in any of the treatment groups (Table 1). The SS and the SS + BB groups had mortality rates of 20% and 14%, respectively. The HS group had the highest mortality rate at 40%. There was no difference in the mortality rate if the BB was given pre-HS compared with HS alone (38% vs. 40%). There was a slightly better mortality rate if BB was given post-HS, but this was not statistically significant (33% vs. 40%).

The BM of HS animals had significantly fewer cells than the controls (107 $\times 10^6$ /mL vs. 198 $\times 10^6$ /mL; $p < 0.05$). The BM cellularity of SS, BB, SS + BB, HS + preBB, and HS + postBB animals was similar to that of the UC and at least double that of the HS animals (Table 1).

Physiologic monitoring

The HR of all animals was similar before induction of shock (343 $\pm$ 26 beats/min [bpm]), except in the rats that received BB pre-HS (302 $\pm$ 15 bpm). Hemorrhagic shock resulted in sustained tachycardia at 3 h after resuscitation (Fig. 1). Administration of BB after HS reduced the HR at 3 h by more than 30% compared with HS alone. Similarly, a 20% reduction in

FIG. 1. Heart rate (beats/min) according to treatment. UC = unmanipulated control; HS = hemorrhagic shock; preBB = beta-blockade before hemorrhagic shock; postBB = beta-blockade after hemorrhagic shock.

HR at 3 h post-shock was seen when BB was given prior to HS compared with animals subjected to HS alone. Three hours after shock, there was no significant difference in MABP among the groups (data not shown).

Bone marrow hematopoietic progenitor cell cultures

Similar to our previously published data, both HS and SS caused a significant reduction in BM HPC growth compared with UC (Fig. 2). The degree of HPC growth suppression was most pronounced in the HS group, with a more than 50–60% reduction in CFU-GEMM, BFU-E, and CFU-E cells compared

FIG. 2. Colonies/plate of colony-forming unit-granulocyte-erythrocyte, monocyte, megakaryocyte (A), burst-forming unit-erythroid (B), and colony-forming unit-erythroid (C) cells. SS = sham shock; HS = hemorrhagic shock; BB = beta-blockade; HS + preBB = hemorrhagic shock with prior beta-blockade.

with UC. In the SS group, HPC growth was suppressed by about 25–30% of control values. Beta-blockade given for three days prior to HS protected the BM, with return of BM HPC growth to UC values in all erythroid progenitor cells (Fig. 2)(CFU-GEMM 59 ± 11 vs. 61 ± 4 colonies/plate, BFU-E 68 ± 9 vs. 73 ± 3 and CFU-E 81 ± 35 vs. 78 ± 14 colonies/plate). Administration of BB alone did not have any effect on BM HPC growth.

In a more clinically relevant model, BB was administered after HS. There was a statistically significant increase in BM growth of CFU-GEMM, BFU-E, and CFU-E when BB was given post-HS compared with HS alone (Fig. 3). Beta-blockade given after HS produced BM protection, as quantified by HPC colony growth, which was similar to that of UC

animals. Similarly, when BB was given to SS animals, Bone marrow HPC colony growth was improved.

Discussion

Hemorrhagic shock and resuscitation represent an ischemia/reperfusion stimulus that promotes the development of multiple organ dysfunction syndrome by priming the immune system for an exaggerated inflammatory response [1]. A persistent inflammatory response leads to sustained elevation of catecholamine concentrations. This immediate and sustained release of catecholamines has been confirmed in human beings by elevations in urinary epinephrine and norepinephrine for as long as one week after injury [6]. During this time, injury-induced BM dysfunction manifests as sustained HPC growth suppression and increased HPC mobilization and sequestration in injured tissues lasting one week [3–5]. In addition to their cardiovascular, hemodynamic, and metabolic effects, catecholamines are the primary mediators of neural regulation of the immune system. They achieve this modulation via their direct action on adrenergic receptors on immature BM HPCs as well as on mature circulating immune cells [9,10]. At the BM level, both the degree and the duration of catecholamine stimulation seem to impact BM HPC growth [6,7,12]. In our study, SS, an isolated soft tissue injury, produced a 25–30% suppression of BM HPC growth. This is consistent with our previous finding that lung contusion alone leads to suppression of BM HPC growth [4,5,17]. Yet in this study and previous studies, HS resulted in more than 50–60% BM HPC growth reduction [2,3].

Physiologic concentrations of circulating catecholamines over six days produce a trophic effect on BM cells, manifested as an increase in the number of multipotential $CD34^+$ progenitor cells in the BM [12]. Our previous work showed that with severe stress (10^{-3} M), both epinephrine and norepinephrine completely inhibited BM erythroid progenitor growth in vitro [18]. In vivo, norepinephrine causes significant dose-dependent suppression of BM HPC growth [7]. These observations suggest that the relation between BM HPC growth and the sympathetic response is both dose- and duration-dependent. Therefore, because the most severe injuries elicit the strongest catecholamine response, this leads to the highest degree of BM HPC growth suppression. These findings are supported by Schraml et al. [19], who found that the degree of BM suppression correlates directly with the severity of adrenergic stress. They utilized adrenergic agonists in increasing doses to affect HPC cell division and thereby decrease HPC colony growth in a dose-dependent manner [19]. In our previous in vitro work with norepinephrine, we found that norepinephrine's effect was mediated through the BM stroma [6]. In the absence of stroma, norepinephrine's dose-dependent effects were not observed [6]. Although the BM stroma has a role, the direct effect of norepinephrine on erythroid progenitor cells has not been defined.

Beiermeister et al. [17] showed that treatment with beta-adrenergic receptor blockers protected BM HPC growth after lung injury. In a subsequent study, a team from the same laboratory [20] found that the protection is mediated by beta-2 and beta-3 receptors; the beta-1 receptor had no impact on BM HPC growth [20]. In this study, using an HS model, we demonstrated similarly that the BM is protected via non-selective BB administration either before or immediately after HS.

FIG. 3. Colonies/plate of colony-forming unit-granulocyte-, erythrocyte-, monocyte-, megakaryocyte (A), burst-forming unit-erythroid (B), and colony-forming unit-erythroid (C). SS = sham shock; SS/BB = sham shock with beta-blockade; HS = hemorrhagic shock; HS + postBB = hemorrhagic shock followed by beta-blockade.

Propranolol influences cellular immune suppression by preventing natural killer (NK) cell mobilization after hemorrhagic shock [21]. The protection of BM HPC progenitor cell growth with BB given before or after HS may be indirect, attributable to the preserved BM cellularity resulting from decreased HPC mobilization. This idea is supported by the study of Spiegel et al. [12] demonstrating the influence of adrenergic stimuli on HPC motility, proliferation, and repopulation. In addition, antioxidative treatment or inhibition of p38/mitogen-activated protein kinase (MAPK) signaling restored HPC growth in the presence of adrenergic stimuli [12,22]. Also, Liu et al. [23] showed that the hypercatecholaminergic state that follows HS and resuscitation enhances neutrophil mobilization and that its effects can be blocked by prior treatment with propranolol.

In this study, the use of BB before or after HS was associated with no worsening of the mortality rate. The HS group had the highest mortality rate at 40%. The addition of BB pre-HS or post-HS resulted in similar mortality rates (38% and 33%). The reason for slightly lower rate in the group given BB after HS is unknown, but it may be explainable by BB-mediated inhibition of the Bezold-Jarisch reflex [24]. Wisbach et al. [24] showed that propranolol given to rats after hemorrhage decreased cardiac contractility and increased stroke volume, resulting in more effective resuscitation. Previous *in vivo* studies used propranolol dosing regimens from a low of 0.5 mg/kg to a high of 20 mg/kg [25]. Our study utilized a nonselective BB, propranolol, that was given at 10 mg/kg. This dose was chosen because our goal was to administer a dose that exhibited a physiologic effect without risking higher mortality. This dose did in fact result in a lower HR. Our BP data showed no significant differences between the groups. Our findings are supported by Wisbach et al. [24], who showed a 35% reduction in HR with minimum variation in cardiac output, MAP, or systemic vascular resistance in rats given propranolol during HS. Those authors proposed that the reduction in HR resulted in a significant decrease in myocardial oxygen demand, which increases diastolic filling time, leading to a greater stroke volume that maintains BP [24]. It is possible that a lower dose of propranolol would provide the same degree of BM HPC protection, but more work is needed to determine the optimal dose and duration of action of BB at the BM level.

The desired goal of the stress response is the mobilization of energy stores and modulation of metabolic and immune function to cope with injury. After severe injury and shock, there are surges in catecholamines and cytokines that disrupt the BM milieu [26,27]. The exact mechanisms involved in BM growth and HPC trafficking in response to trauma have yet to be elucidated fully. Katayama et al. [8] showed a direct link between the sympathetic nervous system and HPC egress from the BM under normal conditions. Current evidence suggests the BM milieu functions differently depending on the concentration of its mediators [26,27]. In addition, high catecholamine concentrations up-regulate metalloproteinases and down-regulate SDF-1 expression, leading to enhanced HPC mobilization [12]. Also, suppression of HPC growth was observed in response to a number of cytokines whose concentrations usually are elevated after trauma, such as interleukin (IL)-6, transforming growth factor (TGF)- β , and tumor necrosis factor (TNF)- α [18,28,29]. The interaction between cytokines and the sympathetic nervous system also was demonstrated by Katayama et al. [8] who showed that

adrenergic signaling controlled granulocyte colony-stimulating factor (G-CSF)-induced HPC mobilization. During times of severe and prolonged stress, catecholamines may act as synergists with other inflammatory mediators [1]. Therefore, the use of BB abrogates the detrimental effects of the hypercatecholamine state.

In summary, this study showed that propranolol given before or after HS preserves BM cellularity and prevents BM HPC growth suppression. Therefore, nonselective BB may minimize BM dysfunction and prevent the persistent anemia that follows severe injury. Further study is needed to determine the timing, duration, and optimal dosing of BB after trauma.

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References

1. Molina PE. Neurobiology of the stress response: Contribution of the sympathetic nervous system to the neuroimmune axis in traumatic injury. *Shock* 2005;24:3–10.
2. Livingston DH, Gentile PS, Malangoni MA. Bone marrow failure after hemorrhagic shock. *Circ Shock* 1990;30:255–263.
3. Livingston DH, Anjaria D, Wu J, et al. Bone marrow failure following severe injury in humans. *Ann Surg* 2003;238:748–753.
4. Badami CD, Livingston DH, Sifri ZC, et al. Hematopoietic progenitor cells mobilize to the site of injury after trauma and hemorrhagic shock in rats. *J Trauma* 2007;63:596–602.
5. Shah S, Ulm J, Sifri ZC, et al. Mobilization of bone marrow cells to the site of injury is necessary for wound healing. *J Trauma* 2009;67:315–322.
6. Fonseca RB, Mohr AM, Wang L, et al. Adrenergic modulation of erythropoiesis following severe injury is mediated through bone marrow stroma. *Surg Infect* 2004;5:385–393.
7. Penn A, Mohr AM, Shah SG, et al. Dose response relationship between NE and erythropoiesis: Evidence for a critical threshold. *J Trauma* 2007;63:479.
8. Katayama Y, Battista M, Kao W, et al. Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. *Cell* 2006;124:407–421.
9. Dwight MN, Virginia MS. Autonomic innervations and regulation of the immune system. *Brain Behav Immun* 2007;21:736–745.
10. Muthu K, Iyer S, L-K He, et al. Murine hematopoietic stem cells and progenitors express adrenergic receptors. *J Neuroimmunol* 2007;186:27–36.
11. Kohm AP, Sanders VM. Norepinephrine and beta-2-adrenergic receptor stimulation regulate CD4⁺ T and B lymphocyte function *in vitro* and *in vivo*. *Pharmacol Rev* 2001;53:487–525.
12. Spiegel A, Shvitiel S, Kalinkovich A, et al. Catecholaminergic neurotransmitters regulate migration and repopulation of immature human CD34⁺ cells through Wnt signaling. *Nature Immunol* 2007;8:1123–1131.

13. Herndon DN, Hart DW, Wolf SE, et al. Reversal of catabolism by beta-blockade after severe burns. *N Engl J Med* 2001;345:1223–1229.
14. Arbabi S, Ahrens KS, Wahl WL, et al. Beta-blockade use is associated with improved outcomes in adult burn patients. *J Trauma* 2004;56:265–271.
15. Auerback AD, Goldman L. Beta-blockers and reduction in cardiac events in non-cardiac surgery: Clinical applications. *JAMA* 2002;287:1445–1447.
16. Inaba K, Teixeira P, David JS, et al. Beta-blockers in isolated blunt head injury. *J Am Coll Surg* 2008;206:432–438.
17. Beiermeister K, Brett K, Patel P, et al. The effect of beta blockade on progenitor cell growth and mobilization. *Shock* 2009;31S, 7.
18. Fonseca RB, Mohr AM, Wang L, et al. The impact of a hypercatecholamine state on erythropoiesis following severe injury and the role of IL-6. *J Trauma* 2005;59:884–890.
19. Schraml E, Fuchs R, Kotzbeck P, et al. Acute adrenergic stress inhibits proliferation of murine hematopoietic progenitor cells via p38/MAPK signaling. *Stem Cells Develop* 2009;18:215–227.
20. Beiermeister KA, Keck BM, Sifri ZC, et al. Hematopoietic progenitor cell mobilization is mediated through beta-2 or beta-3 receptors after injury. *J Trauma* 2010;69:338–343.
21. Oberbeck R, van Griensven M, Nickel E, et al. Influence of beta-adrenoreceptor antagonists on hemorrhage-induced cellular immune suppression. *Shock* 2002;18:331–335.
22. Piccoli C, D'Aprile A, Ripoli M, et al. Bone marrow derived hematopoietic stem/progenitor cells express multiple isoforms of NADPH oxidase and produce constitutively reactive oxygen species. *Biochem Biophys Res Commun* 2007;353:965–972.
23. Liu Y, Yuan Y, Li Y, et al. Interacting neuro-endocrine, innate, and acquired immune pathways regulate neutrophils mobilization from bone marrow following hemorrhagic shock. *J Immunol* 2009;182:572–580.
24. Wisbach G, Tobias S, Woodman R, Spalding A. Preserving cardiac output with beta-adrenergic receptor blockade and inhibiting the Bezold-Jarisch reflex during resuscitation from hemorrhage. *J Trauma* 2007;63:26–32.
25. Bernard AC, Fitzpatrick EA, Maley ME, et al. Beta adrenoceptor regulation of macrophage arginase activity. *Surgery* 2000;127:412–418.
26. Lapidot T, Petit I. Current understanding of stem cell mobilization: The role of chemokines, proteolytic enzymes, adhesion molecules, cytokines and stromal cells. *Exp Hematol* 2002;30:973–981.
27. Nervi B, Link DC, DiPersio JF. Cytokines and hematopoietic stem/progenitor cell mobilization. *J Cell Biochem* 2006;99:690–705.
28. Mohr AM, Upperman JS, Taneja R, et al. Differential effects of acute hypoxia and endotoxin on the secretion and expression of bone marrow interleukin-1 and interleukin-6. *Shock* 1997; 7:324–331.
29. Wu J, Livingston D, Hauser C, et al. Trauma inhibits erythroid burst-forming unit and granulocyte-monocyte colony-forming unit growth through the production of TGF-beta1 by bone marrow stroma. *Ann Surg* 2001;234:224–232.

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