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Persistent injury-associated anemia: the role of the bone marrow microenvironment

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ABSTRACT

Background: The regulation of erythropoiesis involves hematopoietic progenitor cells, bone marrow stroma, and the microenvironment. Following severe injury, a hypercatecholamine state develops that is associated with increased mobilization of hematopoietic progenitor cells to peripheral blood and decreased growth of bone marrow erythroid progenitor cells that manifests clinically as a persistent injury-associated anemia. Changes within the bone marrow microenvironment influence the development of erythroid progenitor cells. Therefore, we sought to determine the effects of lung contusion, hemorrhagic shock, and chronic stress on the hematopoietic cytokine response.

Materials and methods: Bone marrow was obtained from male Sprague–Dawley rats ($n = 6$/group) killed 7 d after lung contusion followed by hemorrhagic shock (LCHS) or LCHS followed by daily chronic restraint stress (LCHS/CS). End point polymerase chain reaction was performed for interleukin-1β, interleukin-10, stem cell factor, transforming growth factor-β, high-mobility group box-1 (HMGB-1), and B-cell lymphoma-extra large.

Results: Seven days following LCHS and LCHS/CS, bone marrow expression of prohematopoietic cytokines (interleukin-1β, interleukin-10, stem cell factor, and transforming growth factor-β) was significantly decreased, and bone marrow expression of HMGB-1 was significantly increased. B-cell lymphoma-extra large bone marrow expression was not affected by LCHS or LCHS/CS (naïve: 44 ± 12, LCHS: 44 ± 12, LCHS/CS: 37 ± 1, all $P > 0.05$).

Conclusions: The bone marrow microenvironment was significantly altered following severe trauma in a rodent model. Prohematopoietic cytokines were downregulated, and the proinflammatory cytokine HMGB-1 had increased bone marrow expression. Modulation of the bone marrow microenvironment may represent a therapeutic strategy following severe trauma to alleviate persistent injury-associated anemia.

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Introduction

Anemia after trauma has been associated with acute hemorrhage, decreased red blood cell survival, iron dysregulation, inhibition of proliferation and differentiation of erythroid progenitor cells, and loss of hematopoietic progenitor cells (HPCs) to peripheral blood and sites of injury.[1-4] Severe trauma also leads to the development of an inflammatory response as well as catecholamine release.[1] Marked and sustained increases in catecholamines initiate a prolonged hypermetabolic response that is seen in critically ill trauma patients that remain in the intensive care unit. Persistent injury-associated anemia is the phenomenon where chronically elevated levels of catecholamines following injury have been shown to worsen bone marrow dysfunction by inhibiting differentiation of HPCs, decreasing bone marrow HPC growth, and potentiating prolonged HPC mobilization to peripheral blood.[2] Many trauma patients require repeated red cell transfusions which places them at increased risk for infection, organ failure, and death.[3-5] Erythropoietin levels are preserved or elevated following trauma, and exogenous use of erythropoietin has not been shown to be resolve anemia.[4,5] Trauma patients do not exhibit appropriate compensatory reticulocytosis, implying that persistent injury-associated anemia is related to bone marrow dysfunction.[2,6]

The inflammatory response associated with hypercatecholaminemia may also alter the profile of several signaling cytokine molecules. An alteration in these soluble mediators likely plays a role in bone marrow dysfunction following traumatic injury.[1,7] Persistent injury-associated anemia may be secondary to the antagonistic actions of proinflammatory cytokines.[2,6] Hematopoietic failure has also been associated with an overproduction of proinflammatory cytokines, a feature which may also be present following a hypercatecholamine state.[6,7] Cytokines released under stress conditions can actively instruct HPC into particular cell lineages on demand.[7] Cytokines have been shown to bind directly to receptors on hematopoietic stem cells and regulate many functions such as survival, proliferation, differentiation, maturation, and functional activation.[8,9] The various cytokines affecting HPC and hematopoiesis exhibit pleiotropy and redundancy.[8] Cytokines can be lineage specific or regulate cells in multiple lineages.[9] For some cell types, the simultaneous action of multiple cytokines is required.[9]

Cytokines control both basal and emergency hematopoietic cell proliferation. Cytokines that influence hematopoiesis include interleukin-1α (IL-1α), interleukin-1β (IL-1β), interleukin-10 (IL-10), transforming growth factor-β1 (TGF-β1), stem cell factor (SCF), high-mobility group box-1 (HMGB-1), and B-cell lymphoma-extra large (Bcl-xL). Although the interaction of these molecules is highly complex and poorly understood, it is thought that bone marrow dysfunction following trauma may be associated with changes in the profile of these cytokines, which ultimately cause an increased release of erythroid and myeloid progenitor cells into peripheral blood, decreased progenitor cell growth, and impaired growth of bone marrow stroma.[10] IL-1α, IL-1β, IL-10, SCF, and Bcl-xL promote differentiation of HPC toward myeloid lineage. TGF-β1 and HMGB-1 are proinflammatory and downregulate hematopoiesis. Therefore, during a posttraumatic hypercatecholamine state, it would be expected that prohematopoietic cytokines would be downregulated and proinflammatory cytokines would be upregulated.

An acute injury and hypercatecholaminemic state can be reproduced in a rodent model utilizing lung contusion followed by hemorrhagic shock (LCHS) and LCHS with daily chronic restraint chronic stress (LCHS/CS).[2,4] These models have been shown to lead to bone marrow dysfunction manifest as increased mobilization of HPC to peripheral blood, decreased bone marrow cellularity and HPC colony growth, and decreased hemoglobin levels consistent with persistent injury-associated anemia.[2] The purpose of this study was to examine the profile of prohematopoietic and proinflammatory cytokines in the bone marrow of these models following trauma and stress to provide greater understanding of the pathophysiology of persistent injury-associated anemia.

Materials and methods

Animals

Male Sprague–Dawley rats (Charles River, Raleigh, NC) ($n = 6$/group) weighing 300-400g were used for all rodent models. Rodents were housed in pairs at the animal facility and were provided free access to water and standard rat chow (Envigo Teklad 7912, Harlan Laboratories Inc, Tampa, FL). Male rats were chosen to avoid the potentially confounding effects of estrogens on the physiologic response to hemorrhagic shock (HS). Approval was obtained from the Institutional Animal Care and Use Committee.

Rodent group design

Rodents were randomly assigned to one of the following groups: naïve controls, lung contusion followed by hemorrhagic shock (LCHS), and LCHS followed by daily chronic restraint stress (LCHS/CS). Naïve and LCHS rodents underwent daily handling, whereas LCHS/CS rodents were exposed to daily chronic restraint stress. A sham procedure was not performed for naïve animals. On day 7, all rodents were killed via cardiac puncture and bone marrow was harvested. Day 7 was chosen as the day to harvest the animals based on the previous work demonstrating that a 7-d interval allows for complete healing of lung tissue following lung contusion (LC) alone but that animals that are also subjected to HS and/or chronic stress (CS) do not have complete healing after 7 d.[8-10]

LC with HS

In order to create a clinically relevant model of severe trauma, rodent models underwent LCHS. Following anesthesia with IP pentobarbital (50 mg/kg), a unilateral LC was made using a blast wave from a nail gun (PowerShot Model 5700M, Saddle