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Selective inhibition of histone deacetylase 6 alters the composition of circulating blood cells in a lethal septic model

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ABSTRACT

Background: Phagocytes, especially monocytes, macrophages, and dendritic cells, play a pivotal role in the innate and adaptive immune responses during sepsis. We have shown that inhibition of histone deacetylase 6 improves survival and increases bacterial clearance in a mouse model of cecal ligation and puncture (CLP). The aim of this study was to determine whether this effect was associated with changes in the number and composition of different blood cell types in the circulation.

Methods: C57BL/6J mice were subjected to CLP, and 1 h later given an intraperitoneal injection of either Tubastatin A dissolved in dimethyl sulfoxide, or dimethyl sulfoxide only. Sham-operated animals were treated in an identical fashion but not subjected to CLP. Forty-eight hours later, peripheral blood was obtained *via* cardiac puncture and analyzed using a HemaTrue veterinary hematology analyzer.

Results: Tubastatin A administration increased the number of circulating monocytes in the sham-operated and the CLP animals. In comparison with the sham, CLP animals displayed an increase in the granulocyte percentage in white blood cells and decrease in the lymphocyte number and percentage, with a resultant increase in the granulocyte-to-lymphocyte ratio. Treatment of CLP animals with Tubastatin A decreased the granulocyte percentage and restored the lymphocyte number and percentage, which decreased the granulocyte-to-lymphocyte ratio. In the sham animals, Tubastatin A increased red blood cell number, hematocrit, and hemoglobin. This effect was not seen in CLP animals.

Conclusions: Tubastatin A treatment has significant impact on the composition of circulating blood cells. It increases the number of circulating monocytes and the red blood cell mass in sham-operated animals. In the CLP animals, it increases the monocyte count,

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decreases the percentage of granulocytes, restores the lymphocyte population, and decreases the granulocyte-to-lymphocyte ratio. These results may explain why Tubastatin A treatment improves survival in the septic models.

1. Introduction

Severe sepsis causes tremendous burden for health care systems with 750,000 new cases and more than 225,000 deaths annually in the United States [1–3]. Severe sepsis and septic shock are among the most elusive syndromes in medicine for which all clinical trials have so far failed to show efficacy [4,5].

Histone acetylation is an essential epigenetic mechanism that determines the amplitude of cellular and subcelluar signaling by controlling the chromatin structure, accessibility of transcription factors to the DNA, and the subsequent gene transcription. This process regulated by the opposing actions of two families of enzymes: histone acetyltransferases and histone deacetylases (HDACs). Histone acetylation relaxes the chromatin structure and promotes gene transcription, whereas histone deacetylation compacts the chromatin structure favoring gene silencing.

The 18 HDAC enzymes are grouped into four classes in humans and mice. Classical HDACs (classes I, II, and IV) are Zn^{2+} dependent, whereas the class III sirtuins act through a NAD^{+}-dependent mechanism [6]. HDAC6 belongs to class IIb HDAC based on domain organization and is unique among the classical HDAC family, in that it is a cytoplasmic microtubule-associated enzyme. HDAC6 deacetylates tubulin, heat shock protein 90 (HSP90), and cortactin form complexes with other partner proteins and involve in a variety of biological processes [7].

Our laboratory was the first to demonstrate that administration of suberoylanilide hydroxamic acid, a histone deacetylase inhibitor (HDACI), improved survival in lethal rodent models of lipopolysaccharide-induced endotoxemia and cecal ligation and puncture (CLP)-induced severe sepsis [8–10]. Selective inhibition of HDAC6 with Tubastatin A displays even better survival outcomes in the lethal CLP sepsis model and increases bacterial clearance in circulation (unpublished data). However, the mechanisms underlying the increased survival outcomes and bacteria clearance after Tubastatin A treatment remain unclear. The present study was therefore designed to determine whether these effects are associated with changes in the number and composition of different blood cell types in the circulation.

2. Materials and methods

2.1. Sepsis model: CLP

Male C57BL/6J mice (18–26 g) were purchased from The Jackson Laboratory and housed for 3 d before manipulations. The CLP murine model [11], modified by our laboratory, was used to induce fecal peritonitis. In brief, the peritoneal cavity was opened under inhaled isoflurane anesthesia. Cecum was eviscerated, ligated below the ileocecal valve using a 5-0 silk suture, and punctured through (two holes) with a 20-ga needle. The punctured cecum was squeezed to expel a small amount of fecal material and returned to the peritoneal cavity. The abdominal incision was closed in two layers with 4-0 silk suture. Animals were resuscitated by subcutaneous injection of 1 mL of saline. Sham-operated animals were handled in the same manner, except that the cecum was not ligated or punctured. This protocol was approved by the Animal Review Committee at the Massachusetts General Hospital. All surgery was performed under anesthesia, and all efforts were made to minimize suffering.

2.2. Administration of Tubastatin A and experimental design

Animals were randomly assigned to the following four groups ($n = 5$ for sham control and $n = 12$ for CLP): (a) sham-operated animals (sham control); (b) sham-operated animals injected with Tubastatin A (sham + Tub.A); (c) dimethyl sulfoxide (DMSO) vehicle–treated animals after CLP (CLP control), and (d) Tubastatin A treated animals after CLP (CLP + Tub.A). Mice received intraperitoneal Tubastatin A dissolved in DMSO (70 mg/kg) or vehicle DMSO 1 h after the procedure. Sham-operated animals were subjected to laparotomy and intestinal manipulation, but the cecum was neither ligated nor punctured.

2.3. Peripheral blood analysis

Peripheral blood was obtained *via* cardiac puncture 48 h after procedure using a 1 mL heparinized syringe. Three hundred microliter of aliquots were then sampled and analyzed within 10 min of collection using a HemaTrue veterinary hematology bench top analyzer (Heska Corporation, Loveland, CO) [12]. The number and percentage of monocytes, granulocytes, and lymphocytes in white blood cell, the number of red blood cells (RBCs) and platelets, hematocrit, and hemoglobin were measured in all animals.

2.4. Statistical analysis

Results were represented as mean ± standard error of mean. Differences between the four groups were assessed using one way analysis of variance followed by Bonferroni *post hoc* testing for multiple comparisons. Student *t*-test was used to compare the differences between two groups. All analyses were performed using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA). *P* values ≤ 0.05 were considered statistically significant.

3. Results

3.1. Tubastatin A increases circulating monocyte number in sham-operated and CLP animals 48 h after the procedure

HDAC6 inhibitor Tubastatin A increased circulating monocyte number in sham-operated (0.3 ± 0.1 *versus* $0.6 \pm 0.1 \times 10^9$/L,