Megakaryocytic Emperipolesis in the Rat Bone Marrow Induced by Lipopolysaccharide

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ABSTRACT. Megakaryocytic emperipolesis was observed in the rat bone marrow when lipopolysaccharide (LPS) was administrated intravenously at a daily dose of 0.5 mg/kg body weight for 4 weeks. Emperipolesis was significantly increased in the LPS-treated group (3.54%) as compared with the control group (1.35%). Histologically, several types of hematocytes were engulfed within megakaryocytes, but the host cells and entering hematocytes showed no degenerative alterations. Morphologically, most megakaryocytes showing emperipolesis were classified into the mature type indicating high platelet producing ability but platelet counts in the peripheral blood were markedly reduced in the LPS-treated group (63.7 × 10^9/mm³) as compared with the control group (128.4 × 10^9/mm³). These results suggest that the emperipolesis is a phenomenon closely related to the marked reduction in the number of platelets and accelerated platelet production of megakaryocytes.—KEY WORDS: LPS, megakaryocytic emperipolesis, rat.


Emperipolesis, a word of Greek derivation, was first introduced by Humble et al. to describe the round about wandering of lymphocytes within tumor cells or megakaryocytes in vitro [4]. Since then, the relationship between a host cell and entering cells in emperipolesis has been described [2, 7, 9, 13]. Host cells were megakaryocytes [13], lymphocytes [8] and tumor cells [10], and entering cells were neutrophils [2, 7], erythrocytes [13], erythroblasts [13] and lymphocytes [4]. These phenomena were distinguished from phagocytosis in macrophages and neutrophil, because entering cells retain their normal structure in the host cells [13]. In man, emperipolesis appeared in patients with idiopathic thrombocytopenic purpura, iron-deficiency anemia [9], malignant tumor [7, 10], uremia, diabetes [5] and severe blood loss [12]. In rodents, emperipolesis was also reported in some experimental conditions such as severe anemia and myelogenous leukemia [1, 13]. However, chemically-induced emperipolesis has not been investigated.

Endotoxin (LPS) causes the severe systemic toxicity and the local inflammation associated with gram-negative bacterial infections. LPS, however, produces endogenous mediators of inflammation and immunity known as cytokins [6].

The present paper describes the morphological features of megakaryocytic emperipolesis in the rat bone marrow induced by intravenous administration of LPS.

Twenty male rats (Sprague Dawley, 4 weeks old) were purchased from Clea Inc., Osaka, Japan. The animals were placed in a hanging stainless wire cage in a barrier sustained animal room at a temperature of 23±2°C and a humidity of 55±10% with 12 hr of light and darkness (from 8:00 to 20:00). They were fed a standard pellet diet (CE-2, CLEA, Osaka, Japan) and tap water ad libitum.

LPS from Salmonella abortus equi was obtained from SIGMA Chemical Co., U.S.A. (Lot. 14F4011). Ten rats were given LPS intravenously at a daily dose of 0.5 mg/kg body weight for 4 weeks. The other 10 rats in the control group were given saline.

At the end of the experimental periods, blood samples were taken from the jugular vein of the surviving animals under ether anesthesia and then they were euthanized.

The red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit value (Ht), total white blood cell count (WBC), and platelet count were determined.

At autopsy the left femoral bone with the bone marrow of each rat was removed and fixed in 10% neutral buffered formalin. Paraffin sections were made and stained with hematoyxin and eosin (H-E).

A bone marrow smear was also prepared from the right femoral bone and stained with May-Grünwald. Morphologically, megakaryocytes were classified into five types according to criteria proposed by Digs and Hewlett [3]. The classification of the megakaryocytes is as follows. The megakaryoblasts are classified as type 1, the immature megakaryocytes are classified as type 2, the intermediate megakaryocytes are classified as type 3, the mature megakaryocytes are classified as type 4 and the naked nucleus is classified as type 5.

Hematological analysis revealed anemic changes and a decrease in the platelet count (Table 1) in LPS-treated animals.

At necropsy, the spleen and adrenal glands of the LPS-treated rats were found to be hypertrophic. The lungs, testes, gastro-intestinal tracts, and mesenteric lymph nodes of these animals were also observed to have changed to a dark red color. It is presumed that most of these changes were caused by hemorrhage.

Table 1. Hematological examination of rats given LPS for 4 weeks

<table>
<thead>
<tr>
<th>Treatments</th>
<th>WBC (10^9/mm³)</th>
<th>RBC (10^12/mm³)</th>
<th>Hb (g/dl)</th>
<th>Ht (%)</th>
<th>Plt (10^9/mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>74.7±14.7</td>
<td>822.6±25.2</td>
<td>16.3±0.5</td>
<td>48.9±1.1</td>
<td>128.4±12.0</td>
</tr>
<tr>
<td>LPS</td>
<td>76.4±18.8</td>
<td>610.3±53.9***</td>
<td>12.4±0.4***</td>
<td>38.7±0.9***</td>
<td>63.7±9.9***</td>
</tr>
</tbody>
</table>

Significantly different from Saline group, Student's t-test, ***p<0.001.
Fig. 1. The bone marrow of an LPS-treated rat. A megakaryocyte (arrow) containing more than five hematoxylin cells. H-E, × 400.

Fig. 2. Megakaryocytic emperipolesis in an LPS-treated rat. A megakaryocyte contains mature neutrophils and erythrocyte (arrow). H-E, × 800.

Table 2. Differential counts of 100 consecutive megakaryocytes and the number of megakaryocytes in the bone marrow

<table>
<thead>
<tr>
<th>Treatments</th>
<th>EP(%)a)</th>
<th>Morphological classification (%)b)</th>
<th>No. of megakaryocytes in the bone marrowb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Type 1</td>
<td>Type 2</td>
</tr>
<tr>
<td>Saline</td>
<td>1.35</td>
<td>5.8</td>
<td>16.6</td>
</tr>
<tr>
<td>LPS</td>
<td>3.54***</td>
<td>3.8</td>
<td>13.2</td>
</tr>
</tbody>
</table>

EP, emperipolesis.
The number of megakaryocytes was counted at ×200 magnification in 10 randomly selected fields per animal.
a) Significantly different from Saline group, χ² test, *p<0.05, *** p<0.001.
b) Significantly different from Saline group, Student's t-test, * p<0.05.
MEGAKARYOCYTIC EMERIPOLYSIS INDUCED BY LPS

There was much more megakaryocytic emeripolesis in the LPS-treated group than in the control group. The number of entering cells per a megakaryocyte was also increased in the LPS-treated group (Fig. 1). Several types of hematocytes were engulfed within the megakaryocytic cytoplasm. Mature neutrophils were the most common cells and lymphocytes and erythrocytes were also found (Fig. 2). Both entering cells and megakaryocytes generally showed no sign of degenerative changes. Megakaryocytes with emeripolesis were enlarged in the LPS-treated rats. In addition, hyperplasia of megakaryocytes and granulocytes were observed in the bone marrow of LPS-treated rats.

On the megakaryocytes without distinction (Table 2), the incidence of the type 5 megakaryocyte was significantly increased, and that of the type 3 megakaryocyte decreased in the LPS-treated group, but most megakaryocytes with emeripolesis were classified into type 4, indicating high platelet producing ability.

The association of biological activity on endotoxins has been reported [6], but emeripolesis induced by endotoxin has not been reported. Moreover, chemically-induced emeripolesis has not been reported in rodents. The present study clearly demonstrated megakaryocytic emeripolesis in rat bone marrow produced by the 4-week intravenous treatment with LPS at a daily dose of 0.5 mg/kg.

The initial role of megakaryocytes is production of platelets. In general, megakaryocytes with emeripolesis always occurred when thrombocytogenetic activity increased [5, 9]. On the other hand, Sobolewski has observed that granular cytoplasm and platelet production was reduced in megakaryocytes with emeripolesis [11]. In the present study, megakaryocytic emeripolesis and the number of megakaryocytes was increased in the LPS-treated rats which showed a decrease in the number of platelets. In addition, most megakaryocytes with emeripolesis were classified as the mature type indicating high platelet producing ability, and naked nucleus type of megakaryocytes were increased in the LPS-treated group. These observations may indicate that the emeripolesis is associated with enhanced platelet production by megakaryocytes.

Although the cause of megakaryocytic emeripolesis is unknown, several hypotheses has been proposed [2, 7, 13]. The present results show the relations between emeripolesis and increased platelet production by megakaryocytes in relation to the reduced number of peripheral blood platelets. Further studies are needed to elucidate the exact mechanisms and biological significance of megakaryocytic emeripolesis.

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REFERENCES