Short Communication

SERUM TNF ACTIVITY HAD NO INFLUENCE ON MEGAKARYOCYTIC EMPERIPOLESIS IN THE RAT BONE MARROW

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Abstract: The relationship between megakaryocytic emperipolesis in the bone marrow and lipopolysaccharide (LPS)-induced tumor necrosis factor (TNF) was investigated in adrenalectomized or dexamethasone (Dex)-treated rats. Adrenalectomized rats were injected LPS intravenously at a single dose of 0.5 $\mu\text{g}/\text{kg}$ body weight 48 h after the operation, and Dex-treated rats were given LPS at a dose of 0.5 mg/kg body weight 1 h after the Dex-treatment. Serum TNF activity markedly increased in the adrenalectomized rats (3279.3 units/ml), but reduced in the Dex-treated rats (227.3 units/ml) 1 h after LPS-treatment. Megakaryocytic emperipolesis was not influenced by these treatments 24 h after LPS-administration. Thus, LPS-induced serum TNF activity could not increase megakaryocytic emperipolesis. (J Toxicol Pathol 8: 151~154, 1995)

Key words: Emperipolesis Lipopolysaccharide, Tumor necrosis factor

Megakaryocytic emperipolesis, *i.e.* entrance of hematopoietic cells into the megakaryocyte cytoplasm, was observed in lipopolysaccharide (LPS)-treated rat bone marrow^{1,2}. This phenomenon should be distinguished from phagocytosis, because entering cells retain normal structures in megakaryocytes³⁻⁵. Tavassoli⁶ suggested that the high demand for cell delivery from the bone marrow into peripheral blood might make blood cells take a transmegakaryocyte path to enter the blood circulation and that megakaryocytic emperipolesis was related to cellular traffic across the marrow-blood barrier. Parmley *et al.*⁷ proposed that an undefined insult to the marrow, increased megakaryocyte turnover or death, may contribute to the neutrophil emperipolesis by megakaryocytes. De Pasquale *et al.*⁸ also reported that the megakaryocyte cytoplasm might provide a sanctuary for normal granulocytes under an unfavorable bone marrow environment. Some

investigators have been proposing several hypotheses of the cause of megakaryocytic emperipolesis, but the exact mechanism is still unknown.

On the other hand, LPS brings about broad biological activities through the effects of macrophage-derived mediators such as TNF⁹⁻¹². Therefore, it is reasonable to consider that LPS-induced TNF may increase the incidence of megakaryocytic emperipolesis. However, the effect of LPS-induced TNF on megakaryocytic emperipolesis has not been studied. This study was carried out to clarify this point.

Forty-eight 6-week-old male rat of Sprague Dawley strain were purchased from Japan Clea Inc., Osaka. Animals were placed in a hanging stainless wire cage in a barrier sustained animal room at a temperature of $23 \pm 2^\circ\text{C}$ and a humidity of $55 \pm 10\%$ with 12 h of light and darkness (from 8:00 to 20:00). They were fed a standard pellet diet (CE-2, CLEA Osaka) and tap water *ad libitum*. Rats were divided into 8 groups. In Groups 1 and 2, animals were adrenalectomized bilaterally and were given saline (Group 1) or 0.5 $\mu\text{g}/\text{kg}$ body weight of LPS (from *Escherichia coli*, Dibco Laboratories, U.S.A.) (Group 2) 48 h after

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Accepted for publication: February 28, 1995

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Table 1. Effect of Adrenalectomy or Dexamethasone Treatment on the Serum TNF Activity and Megakaryocytic Emperipolesis in LPS-treated Rats

Groups	Treatments	Experimental periods (hr)	TNF activity (units/ml)	EP-incidence ^{a)} (%)	Average No. of cells per megakaryocyte ^{b)} (Mean $\pm$ S.D.)	EP-index (a $\times$ b)	No. of megakaryocytes (Mean $\pm$ S.D.)	No. of neutrophils (Mean $\pm$ S.D.)
1	Ope+Saline	1 24	<10 <10	1.80 1.90	1.57 $\pm$ 0.26 1.63 $\pm$ 0.13	2.83 3.10	183.7 $\pm$ 13.1 183.7 $\pm$ 22.1	760.3 $\pm$ 32.3 774.0 $\pm$ 63.6
2	Ope+LPS ¹⁾	1 24	3279.3 $\pm$ 2156.2 <10	2.07 2.57	1.92 $\pm$ 0.56 1.53 $\pm$ 0.05	3.97 3.93	194.3 $\pm$ 25.1 173.0 $\pm$ 22.6	785.7 $\pm$ 78.8 814.7 $\pm$ 133.6
3	Sham ope+Saline	1 24	<10 <10	1.63 1.63	1.76 $\pm$ 0.30 1.69 $\pm$ 0.35	2.87 2.75	172.3 $\pm$ 16.2 178.0 $\pm$ 15.0	710.3 $\pm$ 19.3 676.3 $\pm$ 144.3
4	Sham ope+LPS ¹⁾	1 24	862.0 $\pm$ 339.0 <10	1.53 1.37	1.70 $\pm$ 0.08 1.59 $\pm$ 0.10	2.60 2.18	202.0 $\pm$ 7.8 191.0 $\pm$ 28.9	753.3 $\pm$ 30.3 785.7 $\pm$ 90.7
5	Dex+Saline	1 24	<10 <10	1.56 1.57	1.51 $\pm$ 0.20 1.55 $\pm$ 0.23	2.36 2.43	178.7 $\pm$ 18.8 161.7 $\pm$ 20.1	779.7 $\pm$ 43.3 760.7 $\pm$ 134.1
6	Dex+LPS ²⁾	1 24	227.3 $\pm$ 82.5 <10	2.03 10.87***	1.62 $\pm$ 0.16 1.78 $\pm$ 0.10	3.29 19.35	177.7 $\pm$ 14.8 238.3 $\pm$ 9.7*	767.7 $\pm$ 23.6 673.0 $\pm$ 73.9
7	Vehicle+Saline	1 24	<10 <10	1.73 1.20	1.50 $\pm$ 0.21 1.78 $\pm$ 0.50	2.60 2.14	174.0 $\pm$ 20.3 170.3 $\pm$ 33.1	755.7 $\pm$ 65.2 708.7 $\pm$ 95.9
8	Vehicle+LPS ²⁾	1 24	3156.3 $\pm$ 349.4 <10	2.23 11.00***	1.55 $\pm$ 0.71 1.81 $\pm$ 0.09	3.46 19.91	168.7 $\pm$ 5.3 256.0 $\pm$ 26.2*	756.0 $\pm$ 47.9 602.7 $\pm$ 100.1

3 animals in each group were used.

1) LPS : 0.5 μ g/kg, 2) LPS : 0.5 mg/kg.

Ope : adrenalectomy, Sham ope : sham operation, Dex : dexamethasone 3 mg/kg, Vehicle : 50% DMSO, EP : megakaryocytic emperipoleses.

Statistical significance was analyzed using chi-square test (EP-incidence), Student's *t*-test (Other data).

* : $p < 0.05$, *** : $p < 0.001$ (Significantly different compared to Group 5), * : $p < 0.05$, *** : $p < 0.001$ (Significantly different compared to Group 7).

the operation, respectively. Animals in Groups 3 and 4 (Sham-operated groups) were given LPS or saline in same way. Then adrenalectomized rats were maintained with 0.9% saline solution as drinking water.

Animals in Groups 5 and 6 were intraperitoneally administered 3 mg/kg body weight of Dex, glucocorticoid (SIGMA Chemical Co., U.S.A.) dissolved in 50% dimethylsulfoxide (DMSO) solution (vehicle). These rats were given saline or 0.5 mg/kg body weight of LPS 1 h after Dex-treatment, respectively. It is well known that adrenalectomy enhances the susceptibility of rats to endotoxin¹³, and serum TNF activity markedly increased in the adrenalectomized rats as Zuckerman *et al.* reported in mice¹². On the other hand, it was demonstrated that Dex-treatment inhibited the induction of TNF synthesis of macrophages by LPS *in vitro*⁹.

Animals in Groups 7 and 8 were treated with vehicle and received LPS or saline in the same way, respectively. Three rats in each group were sacrificed at 1 and 24 h after LPS injection, respectively. Blood samples for TNF assay were collected from the abdominal aorta of each animal under ether anesthesia. Serum TNF assay was done according to the procedure of Flick and Gifford¹⁴. The femoral bone including the bone marrow was removed and fixed in 10% neutral buffered formalin. Following decalcification, the tissues were prepared routinely for paraffin sections and stained with hematoxylin and eosin (HE). Emperipolesis index and the number of hematopoietic cells were counted according to the previous methods².

The results are shown in **Table 1**. Serum TNF level markedly increased in Group 2 (3279.3 units/ml) as compared with Group 4 (862.0 units/ml) on 1 h, but it could not be detected (<10 units/ml) at 24 h. On the other hand, serum TNF activity was not detected (<10 units/ml) in Groups 1 and 3 during the experimental periods. At 24 h, although not significant, megakaryocytic emperipolesis showed a trend to increase in Group 2 (2.57%) as compared with Group 4 (1.37%). In addition, emperipolesis index and the number of hematopoietic cells showed no significant difference between the adrenalectomized and

sham-operated rats with LPS-treatment during the experimental periods. On the other hand, serum TNF level markedly decreased in Group 6 (227.3 units/ml) as compared with Group 8 (3156.3 units/ml) at 1 h, but it could not be detected (<10 units/ml) at 1 h in both groups. In Groups 5 and 7 serum TNF activity was not detected (<10 units/ml) during the experimental periods.

Megakaryocytic emperipolesis significantly increased in the LPS-treated groups (Group 6, 10.87%; Group 8, 11.00%: $p < 0.001$) as compared with the saline control groups (Group 5, 1.57%; Group 7, 1.20%) at 24 h. The number of megakaryocytes also increased in the LPS-treated groups (Group 6, 238.3 cells; Group 8, 256.0 cells; $p < 0.05$) as compared with the saline control groups (Group 5, 161.7 cells; Group 7, 170.3 cells) at 24 h. But there was no significant difference between the Dex and non-Dex group in LPS-treated rats. The number of neutrophils in the bone marrow showed no significant difference in both groups during the experimental periods.

As mentioned above, serum TNF activity was markedly enhanced in the adrenalectomized rats with LPS-treatment, but emperipolesis incidence, number of entering cells per megakaryocyte and emperipolesis index showed no increase in these animals. On the other hand, serum TNF activity was considerably reduced in the Dex-treated rats with LPS-treatment, but these stated parameters also showed no changes. Thus, it seems that serum TNF activity has no direct effect on megakaryocytic emperipolesis. However, in our previous study, megakaryocytic emperipolesis showed a positive correlation with the numbers of megakaryocytes (host cells) and mature neutrophils (entering cells) in the bone marrow of rats treated with LPS². Therefore, there is still a possibility that some cytokines such as megakaryocyte or neutrophil proliferating factors will produce emperipolesis. Further studies on granulocyte-colony stimulating factor and thrombopoietin will be needed to elucidate this point.

Acknowledgement : The authors would like to thank Dr. Kunio Doi (Department of Biomedical Science, Faculty of Agriculture, The University of Tokyo) for reviewing the manuscript and his valuable suggestions.

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