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EFFECTS OF ENDOTOXIN ON THE SPLENIC MICROCIRCULATION AND ITS CELLULARITY

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Abstract

This report describes the effects of endotoxin treatment on the intrasplenic microcirculation and cellularity in rats. Four and 16 h after a single intravenous injection of endotoxin (2 mg/100g body weight), altered intrasplenic microcirculation was observed. The open circulation was reduced from 97% in the control rats to 79% in the endotoxin treated rats, while the closed circulation increased from 3% in the controls to 21% in the endotoxin treated rats. Such changes in the splenic microcirculation may be partly due to the presence of fibrin and the pooling of polymorphonuclear leukocytes and red blood cells in the red pulp. The most apparent cellular changes seen in the white pulp of endotoxin treated rats 16 h after endotoxin injection are the disappearance of lymphocytes from the periarterial lymphatic sheath and the appearance of many giant macrophages within the white pulp. The giant macrophages contain lymphocytes undergoing various stages of degradation. This suggests that the lymphocytes may be injured by endotoxin treatment and are subsequently phagocytosed by macrophages.

Introduction

A large dose of endotoxin administered intravenously into the dogs and rats causes pronounced hemodynamic alteration and subsequent death. The hemodynamic changes associated with endotoxin shock are severe hypotension, a decrease in the cardiac output and venous return (4, 7, 9) and alteration in the regional blood flow (6). Several studies suggest that the spleen may play an important role in endotoxin-induced lethality (1, 8). It is known that splenectomy will protect mice against endotoxin-induced lethality suggesting that spleen cells are important in producing these lethal effects (1). A mouse resistant to the lethal effects of endotoxin (C3H/HeJ) could be killed by endotoxin after the adoptive transfer of spleen cells derived from an endotoxin-sensitive but histocompatible mouse (C3H/HeN) (8). Although the spleen may be involved in the lethal effect of endotoxin administration, little is known about the effects of endotoxin on the splenic microcirculation and the cellularity (11). The present paper describes the changes in splenic microcirculation and cellularity associated with endotoxin shock.

Materials and Methods

Animals: Male Sprague-Dawley rats (200-250g) were obtained from Charles River Breeding Laboratories, Wilmington, MA. Prior to the experimental treatment, rats were maintained on Purina chow and water ad libitum for one week following receipt from the supplier.

Endotoxin treatment: A 0.6% solution of endotoxin (Escherichia coli 055:B5, Difco Laboratories, Detroit, MI) was prepared in saline. Rats under light ether anesthesia were injected through the penis vein with 0.7 ml of endotoxin (2 mg/100g body weight) (LD 90). Rats injected with saline served as controls.

Intrasplenic microcirculation: Intrasplenic microcirculation in rats was studied by means of the microsphere method (3) 4 h and 16 h following endotoxin administration. Rats were anesthetized with pentobarbital (4 mg/100 g body weight), secured to the guillotine platform, and injected in the dorsal penis vein with 2 x 10^8 microspheres.
carbonized plastic microspheres of 3 to 4 µm (3M Company, St. Paul, Minn.) in 0.4 ml of saline. The splenic blood flow was stopped 5-6 seconds after the initiation of microsphere injection by dropping the guillotine through the thorax at the level between the diaphragm and the heart. The spleen was removed and cut transversely into 1.5 mm thick slices 1h, 4h, and 16h following endotoxin injection and fixed in Karnovsky's paraformaldehyde and glutaraldehyde (10). The slices were washed in cacodylate buffer, dehydrated through graded ethanol, and embedded in a mixture of butoxyethanol and glycomethacrylate (Polysciences, Warrington, PA). Sections of 3 µm in thickness were stained with hematoxylin and eosin. For each spleen, the percentage of microspheres in the cords and sinuses was determined by counting 1,000 microspheres in several random tissue sections.

Electron microscopy: For electron microscopy, tissue slices fixed in Karnovsky's fixative were post-fixed in osmium tetroxide, dehydrated in ethanol, and embedded in Araldite. Thin sections were stained with uranyl acetate and lead citrate.

Results

The relative blood flow to the open and closed circulation in the rat spleen was estimated by the distribution of microspheres in the cords and sinuses. In the control rats, 97% of the blood entering the spleen traveled the open circulation and the other 3% traveled the closed circulation (Table 1). In the endotoxin treated rats, the open circulation was reduced to 79%, while the closed circulation increased to 21% four hours after endotoxin injection. Similar alteration in the intrasplenic microcirculation was also observed in rats 16 h after endotoxin injection (Table 1).

Several distinct morphological features were readily recognizable in the spleen of endotoxin treated rats: (a) presence of fibrin in the red pulp, (b) accumulation of granulocytes and aggregation of platelets in the red pulp, (c) appearance of giant macrophages in the white pulp and (d) disappearance of lymphocytes from the periarterial lymphatic sheath. The fibrin seen in the red pulp of the endotoxin treated rats was located in the intercellular space (Figure 1). It appeared as patches scattered throughout the cordal space. The fibrin was observed in the spleen 4 h after endotoxin treatment. More fibrin was seen in the spleen of 16 h endotoxin treatment than in the spleen of 4 h endotoxin treatment (Figures 2 and 3).

Accumulation of a large number of polymorphonuclear leukocytes in the splenic red pulp became evident at 4 h after endotoxin injection. Similar observations were also found at 16 h, but not at 1 h following endotoxin treatment. Platelets appearing in aggregates were noted at 4 h and some of them were phagocytosed by the macrophages in the cords (Figure 4). Pooling of red blood cells in the spleen was not yet evident at 1 h. It became apparent at 4 and 16 h.

The most conspicuous cellular changes in the white pulp of endotoxin treated rats were the disappearance of lymphocytes from the periarterial lymphatic sheath and the appearance of numerous giant macrophages containing lymphocytes undergoing various degrees of degradation (Figures 5-7). Those changes were apparent at 16 h but not at 1 and 4 h following endotoxin treatment. Giant macrophages were rarely seen in the red pulp adjacent to the white pulp.

<table>
<thead>
<tr>
<th>Time After Endotoxin Injection (hour)</th>
<th>Number of Animals</th>
<th>Percentage* of Microspheres in Cords</th>
<th>Percentage* of Microspheres in Sinuses</th>
<th>Microspheres Counted</th>
</tr>
</thead>
<tbody>
<tr>
<td>0†</td>
<td>4</td>
<td>97.7±0.6</td>
<td>2.3±0.6</td>
<td>4,200</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>79.3±3.1</td>
<td>20.7±3.1</td>
<td>5,015</td>
</tr>
<tr>
<td>16</td>
<td>5</td>
<td>77.2±5.5</td>
<td>22.8±5.5</td>
<td>4,900</td>
</tr>
</tbody>
</table>

* Mean ±1 standard deviation.
† Rats injected with saline served as controls.
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Fig. 1. Red pulp of the spleen of an endotoxin treated rat (4 h after endotoxin injection). Fibrin (arrow) is localized in the intercellular space in the cords. Bar = 0.5 µm.

Fig. 2. Red pulp of the spleen of an endotoxin treated rat (4 h after endotoxin injection). Fibrin is indicated by arrows. Bar = 1 µm.

Fig. 3. Red pulp of the spleen of an endotoxin treated rat (16 h after endotoxin injection). Fibrin is indicated by arrows. Bar = 1 µm.

Fig. 4. Red pulp of the spleen of an endotoxin treated rat (4 h after endotoxin injection). Several platelets (P) are phagocytosed by a macrophage (M). Bar = 1 µm.

Fig. 5. White pulp of the spleen of an endotoxin treated rat (16 h after endotoxin injection). A giant macrophage contains several digested vacuoles (V) and pyknotic nuclei (N). Bar = 1 µm.
Fig. 6. White pulp of the spleen of an endotoxin treated rat (16 h after endotoxin injection). The number of lymphocytes in the periarterial lymphatic sheath (P) diminished. The macrophages are indicated by arrow heads. The white pulp is outlined by arrows. C, central artery; N, lymphatic nodule. (a) Bar = 50 µm; (b) Bar = 25 µm.

Discussion

On the basis of the microsphere method (2, 3), the present study shows that the intrasplenic microcirculation is altered in rats with endotoxin shock. In the normal anesthetized rats, about 97% of the blood takes the open route of circulation and 3% travels the closed route. In contrast to the normal rats, about 77-79% of the blood takes the open route and the rest travels the closed route in the endotoxin treated rats. The reduction in the proportion of the blood traveling the open circulation in the spleen of endotoxin treated rats could be due, in part, to the presence of fibrin and the pooling of polymorphonuclear leukocytes, red blood cells and platelets in the red pulp. It has been suggested that endotoxin induced inflammatory reactions of the splenic red pulp initiate platelet aggregation and fibrin formation by activation of coagulation mechanisms following exposure of blood to reticular fibers and subendothelial basement membranes (11). It has been shown in an earlier paper that the pooling of red blood cells in the red pulp could result in a reduction in the open circulation (3). Since rat peritoneal macrophages synthesize potent vasoactive arachidonic acid metabolites such as thromboxane A₂ and prostacyclin in response to endotoxin (5), production of vasoactive substances by the endotoxin-stimulated macrophages in the spleen may affect the intrasplenic microcirculation. The disappearance of lymphocytes from the periarterial lymphatic sheath (PALS) at 16 h could be due to active migration of lymphocytes out of the white pulp, degeneration of lymphocytes within the white pulp, or both. The fact that many giant macrophages containing pyknotic lymphocytes appear in the white pulp, while the number of lymphocytes diminishes in the PALS indicates that some lymphocytes in the PALS may die there and are subsequently phagocytosed by macrophages. How the lymphocytes are injured by the endotoxin treatment is not known and remains to be studied. A decreased number of lymphocytes in the paracortical area of the lymph nodes was also noted in mice treated with sublethal doses of endotoxin (13). The paracortical region of the lymph node
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and the PALS of the spleen are predominantly occupied by T lymphocytes (12). Disappearance of lymphocytes from these two areas suggests that endotoxin treatment may have a more deleterious effect on T lymphocytes than on B lymphocytes.

Acknowledgements

The author gratefully acknowledges the technical assistance of Bill Beach and Margie Bryant.

References


Discussion with Reviewers

M. Tavassoli: Is there any evidence from the literature that endotoxin can induce lymphocyte migration?

Author: It has been shown that the treatment of a large dose of endotoxin in mice results in a reduction of lymphocytes in the lymph nodes (reference No. 13). However, it is not known whether the reduction of lymphocytes in the lymph nodes is due to active lymphocyte migration.

M. Tavassoli: Is there any evidence that endotoxin can differentially affect B and T cells?

Author: A decreased number of lymphocytes observed in the paracortical area but not in the nodule of the lymph nodes following an injection of sublethal dose of endotoxin in mice (reference No. 13) indicates that endotoxin treatment affects T cells more than B cells with regard to cell distribution.

M. Tavassoli: The presence of fibrin deposits suggests activation of coagulation system. Was there any evidence of systemic activation of this system? This could be easily demonstrated by RIA for fibrin split products or by deposition of fibrin in other tissues. Alternatively examination of blood smear should demonstrate fragmented red cells produced by fibrin strands with the circulation.

Author: Endotoxin treatment induces systemic intravascular activation of the coagulation system and the deposition of fibrin occurs not only in the spleen but also in the hepatic sinusoids and other vascular beds (Ref. No. 11).

M. Tavassoli: The presence of fibrin deposits in splenic cords has an interesting connotation. The rate of circulation in splenic cords is near-stasis and other metabolic conditions also favor activation of the coagulation system. That this activation normally does not take place may be related to local presence of lysosomal enzymes or local activation of plasminogen system (Weiss L, Tavassoli M: Anatomical hazards to the passage of erythrocytes through the spleen. Seminars in Hematol 7:372, 1970). Thus, an equilibrium, similar to but distinct from that in general circulation, is reached preventing fibrin deposition in the cord. It would be suitable if the author could comment on those factors, induced by endotoxin, that alter this state of equilibrium and leads to fibrin deposition. This may have far-reaching implication in control of hemostasis and thrombosis.

Author: Endotoxin-induced inflammatory reactions of the splenic red pulp initiate platelet aggregation and fibrin formation by activation of the coagulation system following disruption of reticular cells and exposure of blood to reticular fibers and subendothelial basement membranes (Ref. No. 11).

* All four questions jointly asked by M. Tavassoli and C. Hardy.