The Liver Lymphatics as a Migratory Pathway of Macrophages from the Sinusoids to the Celiac Lymph Nodes in the Rat*

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Summary. A migratory pathway of macrophages as well as lymphatic communications from the liver to the celiac lymph nodes were studied both macroscopically and histologically. The injection of gelatinized carbon into the porta hepatis revealed a new pathway of the liver lymphatics running independently of the portal vein in addition to the ordinary periportal lymphatics. By obstruction of the efferent lymph flow of the celiac nodes and immunostaining with a monoclonal antibody to lymphatics, perilobular lymphatic vessels in the portal tract was readily demonstrated. It was suggested that heavily carbon-laden (HC) macrophages had migrated from the sinusoid into the interlobular connective tissue by 6h after an intravenous injection of carbon, and then entered the lymphatic vessels of the portal tract. By 9h to 12h after carbon injection, HC macrophages started to migrate into the celiac nodes via the two lymphatic pathways. From the marginal sinus in the celiac nodes, they moved into the interfollicular area of the superficial cortex, then accumulated in the paracortex by 12h to 24h. They finally ended up in the corticomedullary junction. Migrating HC macrophages showed morphological homogeneity. The liver lymphatic pathway in the rat and a significance for the translocation and function of migrating macrophages were discussed.

In rats, celiac lymph nodes are the regional lymph nodes of the upper abdominal organs. Usually they consist of 3 nodes and are horizontally located on the trifurcation of the celiac artery where the hepatic, gastric and splenic arteries originate. In this paper, for convenience, the nodes are named consecutively from right to left as the first, second and third celiac nodes (Figs. 1, 2). There are only a few reports concerning afferent lymphatic communications of the celiac nodes with abdominal organs in the rat (TILNEY, 1971; SEO, 1981). During a study of splenic carbon-laden macrophages (KOTANI et al., 1986), we found that in rats of several strains, the first and third celiac lymph nodes, but not other peripheral nodes including the second celiac node, turned black 1 day after intravenous injection of Pelikan ink (unpublished observation). When the lymph node was examined histologically, the first and third celiac nodes contained an enormous number of carbon-laden macrophages. It is known that the macrophage content of peripheral hepatic lymph is considerably higher than from any other source in sheep (SMITH et al., 1970). Furthermore, HARDONK et al. (1986) has reported that macrophages, possibly Kupffer cells, after loading with particulate material, have a capacity to migrate from the sinusoidal site to the portal tract and thence to hepatic lymph nodes in rats. They speculate an influx of these cells in the hepatic nodes is via the afferent lymph but not via the blood vessels. Hence, we considered that the blackening of the celiac nodes after carbon injection was due to accumulation of carbon-laden macrophages within the nodes which had migrated from the liver via the afferent lymphatics. However, the direct evidence to prove these lymph-borne migration of macrophages is lacking and the relevance of the hepatic lymphatics to the third celiac node is also unknown.

Intrahepatic lymphatic vessels are thought to be confined in the portal tract of the interlobular connective tissue, being called as perilobular lymphatic...
Fig. 1. Lymphatic communications between the liver and celiac lymph nodes. Gelatinized carbon injected into the porta hepatis. The spleen and other nonessential tissues removed. Periportal lymphatics (L1 arrow) entering the first celiac node (C1). A second new lymphatic (L2 arrow) from the liver running around the esophagus (E) either ventrally (---- in Fig. 2) or dorsally (---- in Fig. 2), entering the third celiac node (C3). The third and a part of the second celiac nodes blackened, while the first node only slightly blackened in this specimen. Arrowheads The efferent lymphatic (celiac duct) running along the celiac artery (CA) entering cysterna chyli (CC). x2.8

Fig. 2. A schematic summary of Figure 1. Two lymphatic pathways (L1 and L2) from the porta hepatis to the first (C1) and third (C3) celiac lymph nodes, respectively. G gastric blood and lymphatic vessels, the latter entering second (C2) celiac node. CA central artery, D pancreatico-duodenal blood and lymphatic vessels, S splenic node and vessels. a site for ligation of the celiac duct.

Fig. 3. Distension of the celiac duct (arrowheads) by ligation. C3 third celiac node, CA celiac artery. x7.3
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vessels (RUSZNYÁK et al., 1967; YOFFEY and COURTICE, 1970). Identification of lymphatics solely by light microscopy is difficult at best (RUSZNYÁK et al., 1967; NIIRO and O'MORCHOE, 1986). And the lymphatic vessels in the portal tract often collapsed after ordinary tissue preparations. Hence, a route of migrating cells from the sinusoidal site into these lymphatic vessels has been difficult to study and few report being available by now.

The aim of the present study was to demonstrate lymphatic communications between the liver and the celiac nodes and the migratory pathway of macrophages from the original site to the final destination both macroscopically and histologically. To examine the migratory route of carbon-laden macrophages within the interlobular connective tissues, liver lymphatics were passively diluted by a surgical obstruction of the hepatic lymph flow. And a monoclonal antibody to rat lymphatic vessels (B27, EZAKI et al., in this supplement) was used for the immunostaining of the dilated lymphatic vessels. By these procedures, the lymphatic vessels and migrating macrophages within them became readily demonstrable.

MATERIALS AND METHODS

Animal

Inbred DA rats of 2.5 to 7 months of age, reared under a specific pathogen-free condition, were supplied from the Laboratory Animal Center for Experimental Research in Kumamoto University Medical School.

Lymphatic communications between the liver and the celiac lymph nodes

To visualize draining lymphatics of the liver, gelatinized carbon was injected into the porta hepatitis of 14 female rats (Figs. 1, 2). Animals were killed by exsanguination under ether anesthesia and the thoracic duct was ligated at a level just below the diaphragm. A prewarmed tuberculin syringe containing carbon with 5% gelatin was connected to a fine dental needle (internal bore of 1/10 mm) by polyethylene tubing (MIYAKAWA et al., 1990). The needle was inserted into the connective tissue space of the porta hepatitis between the bile duct and portal vein and about 0.5 ml of the ink solution was slowly injected. The liver lymphatics were soon filled with carbon and became visible. Portions of the celiac nodes draining the liver, the efferent lymphatics of the celiac nodes (termed as the celiac duct in this study), and the cisterna chyli were also filled with carbon. In comparison, the lymphatic connections of the upper abdominal organs other than the liver were also studied by carbon injection. Carcasses were dipped in buffered formalin for 1 day to solidify gelatinized carbon within lymphatic vessels. Photographs of the specimens were taken by macrophotographic apparatus (PMT35, Olympus, Tokyo).

Time kinetics of carbon-laden macrophages in the liver and celiac lymph nodes

Male rats were injected intravenously with Pelikan ink (Günther Wagner, C11-1431a, Hannover, FRG) at a dose of 25 mg/100 g body weight. Two rats for each time point were killed by cervical severing under ether anesthesia at 1 h, 6 h, 9 h, 12 h, 24 h, 3 day and 7 day after carbon injection. The liver and celiac nodes were excised after examining the gross appearance of the celiac nodes. Tissues were fixed in Carnoy's fluid and embedded in paraffin. Serial 6 μm sections were stained with methyl green and pyronin (MGR). The carbon-laden macrophages that localized in the paracortex of the celiac lymph nodes 12 h after carbon injection (100 cells/rat, 2 rats) were examined for the cell size according to the previous report (MIYAKAWA et al., 1990).

Obstruction of the hepatic lymph flow

The efferent lymphatics of the celiac nodes (celiac duct) was ligated (Figs. 2, 3) by which the whole afferent lymph flow entering the celiac nodes as well as the efferent flow were dammed up. A midsagittal incision of the abdominal wall was made using 3 male rats under ether anesthesia. The intestines, stomach and spleen were wrapped in small wet gauzes and pulled out of the abdomen. The celiac lymph nodes and a triangular area surrounded by the celiac artery, mesenteric artery and the portal vein were located. The celiac duct ran parallel with the celiac artery in this area and entering the cisterna chyli (Fig. 2). Under a stereomicroscope, serosa covering the celiac artery was gently peeled at a point about 5 mm apart from the aorta. Then a small hole was carefully made on the serosa at the inner edge of the celiac artery. An another perforation was made at a point inside the portal vein. Silk thread was inserted between the two perforations and the celiac duct was ligated. The celiac duct became distended soon after the ligation (Fig. 3). The abdomen was sutured after monitoring the distension of the celiac duct. Six hours after the operation, 25 mg/100 g body weight of Pelikan ink was injected intravenously and rats were sacrificed 18 h later. In order to prevent a leakage of lymph
from distended lymphatic vessels during tissue preparations, a vascular radix of each liver lobe was ligated at a level of the porta hepatitis. Each lobe en bloc was then either fresh frozen or fixed in buffered formalin. For the light microscopy, 3 μm-thick serial paraffin sections were made and stained with periodic acid-Schiff (PAS).

**Immunostaining of lymphatic vessels with a monoclonal antibody**

For immunostaining of intrahepatic lymphatic vessels, an indirect immuno-alkaline phosphatase method (MATSUNO, EZAKI and KOTANI, 1989) was performed. Briefly, 6 μm-thick cryosections were first incubated with a monoclonal antibody directed against rat lymphatic vessels (B27, EZAKI et al., 1990, in this supplement). After washing, the sections were reacted with alkaline phosphatase-labeled second antibody. The phosphatase activity was developed with a substrate of naphthol AS-MX phosphoric acid and the Fast Red TR salt. The reaction product of this substrate emitted stable orange fluorescence (MATSUNO, EZAKI and KOTANI, 1989). The specific staining was observed under an epifluorescence microscope (Microphot FX, Nikon, Tokyo) at a wavelength of 490 nm. To visualize carbon particles and negatively stained cells in the dark field, trans-illumination with an attenuated light was simultaneously made.

**RESULTS**

**Lymphatic communications between the liver and the celiac lymph nodes**

The injection experiment of gelatinized carbon revealed both afferent and efferent lymphatics of the celiac lymph nodes (Figs. 1, 2). The first celiac node drained the liver, duodenum and pancreas. From the porta hepatitis, one pathway of the liver lymphatics, usually two in number, ran down along the portal vein and reached the first celiac node. They correspond to the ordinary liver lymphatics, so-called perportal lymphatics (TILNEY, 1971). The second node drained the stomach where afferent lymphatics ran parallel with the gastric artery. The third node drained the spleen (splenic node) but not the stomach. In addition, we found that the third node also drained the liver via another pathway of the liver lymphatics (Figs. 1, 2). In all rats examined, this second lymphatic was usually single in number and the size was larger than the perportal ones. From the porta hepatitis the second pathway went around the distal part of the esophagus, either ventrally (9 of 14 rats) or dorsally (5 of 14 rats). Then it ran downwards along the left crus of the diaphragm beneath the gastric vessels and entered the dorsum of the third celiac node. The celiac duct, the efferent lymphatic of the celiac nodes, usually arose from all three groups of the nodes and soon came together to form one common duct. It ran parallel to the celiac artery and entered the cisterna chyli just behind the beginning of the celiac artery from the aorta (Figs. 1, 2).

**Time kinetics of carbon-laden macrophages in the liver and celiac lymph nodes**

When Pelikan ink was injected intravenously, not only the first but also the third celiac nodes turned black by 12 h. This blackening (Fig. 4) persisted till the end of the experiment, 7 day while the second node remained unchanged. Results of the time kinetic study were essentially similar to the report of HARDONK et al. (1986). In the liver, many carbon-laden macrophages appeared in the sinusoid immediately after injection (Figs. 5, 6).
Figs. 4-8. Legends on the opposite page.
Figs. 9-12. Legends on the opposite page.
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It was not until 6 h that carbon-laden macrophages first appeared in the connective tissue of the portal tract. In the celiac lymph nodes 6 h after carbon injection, carbon-laden macrophages were hardly found and only free carbon particles were seen. The latter had been ingested by macrophages in the medullary sinus. At 9 h to 12 h, carbon-laden macrophages first appeared in the marginal sinus of the first and third celiac nodes (Fig. 5). At 12 h to 24 h, they were successively seen in the interfollicular area of the superficial cortex and in the paracortex of both nodes (Fig. 6). Most of the macrophages were heavily carbon-laden, and showed common morphological features (Fig. 7). They were spherical or ovoid cells and homogenous in size (mean diameter, 7.5 μm; range, 6.7–9.2 μm). They had an eccentric round or oval nucleus and a cap-like accumulation of ingested carbon particles in the cytoplasm. They were termed as heavily carbon-laden (HC) macrophages. Within the afferent lymphatics of the celiac nodes, HC macrophages were often found (Fig. 8a, b), while they were scarcely seen within and around the high endothelial venules (Fig. 6). From 1 day to 2 day, aggregation of HC macrophages was observed and some of them fused to form giant cells in the paracortex (Figure not shown). Seven days after carbon injection, HC macrophages accumulated at a boundary between the paracortex and medulla. HC macrophages were seldom seen in the second celiac node throughout the experiment.

**Demonstration of intrahepatic lymphatics by obstruction of efferent lymph flow and immunostaining with a monoclonal antibody**

One day after ligation of the celiac duct, the celiac lymph node showed marked dilation of the sinus and reduction in the cellularity of the parenchyma (Figure not shown). The liver showed a lymphedema partially, i.e., not all of the lobules were edematous. This may be because the hepatic lymph bypasses into the ascending vessels that run along the hepatic veins and drain into the intrathoracic nodes (Rusznyák et al., 1967; Yoffey and Courtice, 1970). Thus, we searched the liver lobules where the space of Disse dilated conspicuously (Fig. 9). In the portal tract surrounding such lobules, the interstitial space was edematous and clefts being continuously lined by thin endothelia were often found. Most of them were neither the portal veins nor the hepatic arteries but seemingly the lymphatic vessels. Sometimes many HC macrophages were seen in and near to the clefts (Fig. 10). When such a place was immunostained with B27 monoclonal antibody, these clefts were positively stained with B27 (Figs. 11, 12) and thus, proven to be lymphatic vessels.

**DISCUSSION**

The present study has demonstrated a new pathway of the liver lymphatics in addition to the ordinary periportal pathway. Draining lymphatics generally follow adjacent veins toward the regional node (Tilney, 1971), such as the periportal pathway which were descending along the portal vein. In contrast, this second pathway ran independently of the portal vein and entered the third celiac node via the distal part of the esophagus. A similar pathway has been reported but its entrance into the third node is not shown (Seo, 1981). Since the size of the vessel was larger than the periportal lymphatics, we consider that the third celiac node is the important lymph node draining the liver as well as the first node. In this respect, Smith et al. (1980) reported that the inhibition of lymph-borne migration of cells from the liver to the celiac nodes was only partially successful by stripping the loose connective tissue from the portal vein and bile duct in the rat. A possible reason for the incomplete inhibition in their study would be that

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**Figs. 9 and 10.** Paraffin section of the liver 1 day after the celiac duct ligation and 18 h after carbon injection. PAS stain. ×380. **Fig. 9.** Edema of Disse’s space. Arrows indicate sinusoidal endothelia detached from the liver cell cord. Erythrocytes are packed in the sinusoidal space. **Fig. 10.** Many HC macrophages within and near to a cleft (arrows) in the connective tissue of the portal tract.

**Figs. 11 and 12.** Cryosection of the liver 1 day after celiac duct ligation and 18 h after carbon injection. Immunostained with B27 monoclonal antibody. Counterstained with hematoxylin. **Figs. 11a and 12a.** Transillumination with visible light. The portal tract where the hepatic artery (A), portal vein (V), bile duct (B) and lymphatic vessel (L) are seen. Fig. 11a: ×380, Fig. 12a: ×240. **Figs. 11b and 12b.** High power view of the indicated part (arrows) of Fig. 11a, 12a, respectively. Double illuminations of ultraviolet light (epifluorescence) and attenuated visible light (transillumination). HC macrophages in (double arrows) or near to (single arrow) brightly fluorescent lymphatic vessels (L). Fig. 11b: ×1,550, Fig. 12b: ×1,040
only the ordinary pathway were interrupted and the second pathway left intact by this procedure.

The first appearance of HC macrophages in the marginal sinus and within the afferent lymphatics obviously show a lymph-borne migration of HC macrophages into the celiac nodes. Since HC macrophages were found in the paracortex later and there was no clear relationship of these cells to the high endothelial venule, a blood-borne migration of HC macrophages would be minor. HC macrophages appeared simultaneously in the marginal sinus of the first and third celiac nodes and both nodes turned black by 12 h after carbon injection. This clearly indicates that both nodes receive the afferent lymph containing HC macrophages from the draining organs.

The finding of HC macrophages in the sinusoid preceded the appearance of these cells in the interlobular connective tissues. The study of lymphatic obstruction and immunostaining revealed HC macrophages in and near to the lymphatic vessels of the portal tract. Both results suggest that HC macrophages have migrated from the sinusoid into the interlobular connective tissues and then entered the lymphatic vessels in the portal tract. It has been shown that B27 monoclonal antibody is a valuable tool for detecting small lymphatic vessels within the organ such as intrahepatic lymphatic vessels.

HC macrophages showed common morphological features. We have found a new macrophage subpopulation that actively migrate into the splenic white pulp (MIYAKAWA et al., 1990). They bear a distinct phenotype as revealed by monoclonal antibodies to rat macrophage subpopulations and have a similar morphology to HC macrophages in this study. We are not sure whether HC macrophages represent Kupffer cells as suggested by others (SMITH, MCINTOSH and MORRIS, 1970; HARDONK et al., 1986). Characterization of HC macrophages including phenotype, origin, and relevance to Kupffer cells or other macrophages is now under study.

Taken together, it is shown that the blackening of not only the first but also the third celiac node is caused by lymph-borne migration of HC macrophages direct from the liver via the ordinary and the second lymphatic pathways, respectively. The meaning of this extensive migration of HC macrophages is not clear. It is reported that there are significantly more cells in peripheral liver lymph than in lymph from other non-lymphoid tissues and a large traffic of cells passed from the blood to the lymph through this organ (SMITH, MCINTOSH and MORRIS, 1970). Not only macrophages (SMITH, MCINTOSH and MORRIS, 1970; HARDONK et al., 1986) but also lymphocytes (FICHTELIUS and GROTH, 1963) and lymphoblasts (SMITH, MARTIN and FORD, 1980) are reported to show this lymph-borne migration. The liver therefore might function like the lymph node (GOWANS and KNIGHT, 1964) translocating cells in the blood to the lymph in a large scale through the high endothelial venule. In addition, migrating macrophages may have some immunological roles, such as transport of antigenic informations to the regional lymph nodes (MAYRHOFER, HOLT and PAPADIMITRIOU, 1986). Alternatively the liver lymphatics might act as a specific clearance system removing excess materials from the blood like the biliary system conveying hemoglobin breakdown products. Loading of carbon particles by intravenous injection may be an example of this situation. In this regard, lymphatic translocation of hematogenous particles such as titanium dioxide (HUGGINS and FROEHLICH, 1966) and tantalum dust (DUMONT and MARTELLI, 1969) has been reported.

In conclusion, the present study revealed a migratory route of carbon-laden macrophages from the liver to the celiac lymph nodes of rats after intravenous injection of carbon particles. In the beginning HC macrophages appeared at the liver sinusoid and then they migrated into the connective tissue of the portal tract where they entered the lymphatic vessels. Via two lymphatic pathways, HC macrophages in the hepatic lymph entered the marginal sinus of either the first or third celiac lymph nodes. Then they migrated into the interfollicular area of the superficial cortex and accumulated in the paracortex and finally ended up in the corticomedullary junction. In addition, a morphological homogeneity of HC macrophages was noted.

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REFERENCES


