Electron Microscopic Studies on the Normal Human Spleen:
Especially on the Red Pulp and the Reticulo-endothelial Cells

YASUSHI HIRASAWA AND HIDEO TOKUIRO

The application of electron microscopy in the study of medical sciences during the last twenty years has provided not only an understanding of the ultrastructure of the cells and tissues, but also some of the functional significances of these structures.

Such merits of the electron microscope have also been bestowed upon hematology. However, the spleen still remains a “mysterious organ.”

The reasons why the spleen has remained a mysterious organ are: (1) It is hard to detect the specific function of the spleen; (2) The spleen is composed of the reticulo-endothelial system (R.E.S.) which is still subject to dispute; and (3) There are various limitations on doing electron microscopic examinations on the healthy, human spleen.

We have had the opportunity to study by electron microscopic observation the normal human spleen and have obtained some interesting information.

In this paper we treat the ultrastructure of the normal red pulp of the human spleen and discuss the various reticulo-endothelial cells, including the sinal endothelial cells, the reticulum cells, and the cordal phagocytes. Whether or not these cells are the same or different has often been argued.

Materials and Methods

Three cases each of benign stomach ulcer and traumatic injuries to the spleen, and one case each of pancreatic cyst and splanchnointosis, for a total of eight cases, were selected for the study. The patients ranged in age from 26 to 51 years. There were five males and three females.

General condition of the patients was good in all cases, with no complications arising before, during, or after the surgery. No preoperative transfusions were conducted.

Materials for the study were obtained during splenectomy. Immediately after laparotomy, the study tissue was taken with a Silvermann needle or with a knife.

No macroscopic or light microscopic abnormalities were found in these specimens.

The electron microscopic techniques were as follows. Fixation was carried out in veronal buffer OsO₄ solution (Caulfield’s method¹) and embedding with epoxy-resin (Luft’s method²), slicing with a Porter Blum microtome, staining with lead acetate, uranyl acetate, and phosphotungstic acid singly or in double strain, and observation and photography was carried out with a Hitachi HS7S electron microscope.

From the First Department of Internal Medicine, Chiba University, Chiba, Japan.
First submitted January 28, 1969; accepted for publication August 18, 1969.

YASUSHI HIRASAWA, M.D., PH.D.: Postdoctoral Fellow of the First Department of Internal Medicine, Chiba University, Chiba, Japan. HIDEO TOKUIRO, M.D., PH.D.: Instructor, First Department of Internal Medicine, Chiba University, Chiba, Japan.
Observations

The red pulp, which might be called the parenchyma of the spleen, consists of sinuses of the ductal system with irregular anastomoses, and pulp cord filling these irregular spaces with many cellular elements (Fig. 1).

The stereoscopic picture obtained upon combination of electron microscopic
Fig. 3.—Transverse section of sinus. Sinus wall consists of 23 endothelial cells. Leucocyte (L) passing through aperture (× 4800).

pictures at each cut surface of a sinus are shown in the schema in Fig. 2. In ultrathin slices, the outline of a sinus produced widely variable impressions depending upon the corresponding cut surface. (Figs. 2–7). However, imagining the sinus as an incomplete blood-vessel-like ductal structure is useful in understanding the structure of a sinus.

As seen from these photographs, the sinus is composed of a bundle of long spindle-shaped sinus endothelial cells covering the sinus wall along the long axis of the sinus, and the reticulum of the sinus wall incompletely surrounding the sinus at the basal part. Between the adjacent sinus endothelial cells only some mild interdigitation is noted in the basal part (Fig. 5), but no special junctional apparatus is noted. Sinus wall reticulum is entirely different from an ordinary basement membrane which has continuity and a membranous property. With 1–3-μ width and approximately 5-μ interval, these fibers give the appearance of rodlike fibers surrounding the long axis of the sinus in a ringlike fashion (the term ‘Reifenfaser’ excellently describes such a structure). A relatively free communication is thus present between the sinus and the pulp cord (Fig. 3).

Occasionally in the sinusoidal wall, trapping of erythrocytes is recognized as shown in Fig. 8. The shape and alignment of the erythrocytes in such
instances were always the same, indicating the direction of the blood stream from the pulp cord to the sinus lumen.

Part of the sinus reticulum revealed a transition into the reticulum in the pulp cord. Both the sinus wall and pulp cord reticulum had a homogeneous and structureless appearance, and no clear difference was found between these two. In the former, needlelike fine fibers were rarely noted, while collagen fibers were frequently found in the latter.

The inferior part of the sinusoidal wall reticulum was covered by fixed reticulum cells of the pulp cord and cordal phagocytes. The fundamental unit of the sinus wall was composed of an overlapping between the sinus endothelial cells—sinus wall reticulum—and fixed reticulum cells and/or cordal phagocytes (Fig. 6).

The basic structure of the pulp cord is the spongy network of the reticulum which compartmentalizes the lumen irregularly, and the cytoplasmic processes of reticulum cells and phagocytes which extend holding the reticulum. The
structure of pulp cord was definitely different from that of the sinus which always had characteristics of a ductal structure. Differentiation between these two was easy.

Thus, the principal structures of the red pulp are composed of the reticulum as a strut and the reticulo-endothelial cells (in a wide sense) such as sinus endothelial cells, reticulum cells, and cordal phagocytes which are closely related with the reticulum.

The detailed structures of these 3 cells are shown in Table 1. The characteristic findings of these cells are described below.

Sinus endothelial cells are characterized by small processes extending from the cell surface to the lumen of the sinus, abundant micropinocytotic vesicles, and dense bodies around the nucleus. Occasionally, phagocytosed material may be seen in the cytoplasm (Fig. 9).

Some of the micropinocytotic vesicles are sometimes of the coated type.3

Fixed reticulum cells (Figs. 6, 10) have the highest electron density of any cell in the spleen. Besides its large nucleus, it has a rough-suraced endoplasmic reticulum, and a free ribosome represent a whole organelle.

Other important features of this cell are the cytoplasmic processes which extend irregularly into the pulp cord and the cytoplasm which is fixed to the reticulum in several places as if it were holding the reticulum in its arms.
Fig. 7.—Horizontal section of bundles of sinal endothelium. The long axis of endothelium at right angles to reticulum (r) (× 9600).

Fig. 8.—Erythrocyte trapped in sinal wall. Figure suggests direction of blood flow between sinus and pulp cord (× 12,000).

The cordal phagocytes (Figs. 6, 11) are characterized by abundant and variable-sized phagocytosed particles, and have a light protoplasm with poor ribosomes. Like the fixed reticulum cells, the phagocytes have localized parallel arrays of rough-surfaced endoplasmic reticulum (Fig. 12), and they also stick to the reticulum. These features may suggest that both cells have the same origin.

Another unsettled question which is usually discussed is whether the vascular system is open or closed. In our electrom microscopic studies on the normal human spleen, blood vessels of the arterial system that directly connect with the sinus have not been demonstrated. The details of these findings will be discussed in a subsequent paper.

DISCUSSION

As stated above, the sinus on the basis of its ductal structures and the pulp cord, mainly consisting of irregular interspaces, are clearly distinguishable. At first glance, the red pulp (Fig. 13) appears to be a meshwork of reticulum with no organized distribution of various cells. By painting out the free, wandering component from the red pulp (Fig. 14), sinus and the pulp cord can be seen arranged with the various features mentioned above.
Table 1.—Electron Microscopic Findings of Structural Cells in Human Spleen

<table>
<thead>
<tr>
<th>Shape and Size</th>
<th>Sinal Endothelium</th>
<th>Fixed Reticulum Cell</th>
<th>Cordal Phagocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Elongated more than 30 μ in length with a few slender projections into the sinus lumen</td>
<td>Stellate or irregular in shape and large in size; Irregularly elongated extensions interspersing in the cordal lumen</td>
<td></td>
</tr>
<tr>
<td>Nucleus</td>
<td>Oval; a few indentations on the basal surface</td>
<td>Round</td>
<td>Irregular in shape</td>
</tr>
<tr>
<td>Nucleolus</td>
<td>One in number and small in size</td>
<td>Serrated margin</td>
<td></td>
</tr>
<tr>
<td>Mitochondria</td>
<td>Rod in shape and in large numbers</td>
<td>One and large</td>
<td>One or more and large</td>
</tr>
<tr>
<td>Golgi-complex</td>
<td>Moderately developed ones in the basal area</td>
<td>Large and several in number</td>
<td>Small and rich</td>
</tr>
<tr>
<td>Rough</td>
<td>Poorly developed</td>
<td>Developed, usually in flat sack form</td>
<td>Moderately developed</td>
</tr>
<tr>
<td>Smooth</td>
<td>Slightly developed</td>
<td>Poorly developed</td>
<td>Sometimes found in close parallel array</td>
</tr>
<tr>
<td>Smooth</td>
<td>Slightly developed</td>
<td>Poorly developed</td>
<td>Rich and variable</td>
</tr>
<tr>
<td>Free Ribosomes</td>
<td>Scarce</td>
<td>Rich</td>
<td>Scarce</td>
</tr>
<tr>
<td>Phagocytosed Materials</td>
<td>May be seen occasionally</td>
<td>Very seldom observed</td>
<td>Rich and variable in size</td>
</tr>
<tr>
<td>Micro-pinosytotic Vesicles</td>
<td>Numerous; smooth and fuzzy surface</td>
<td>Scarce, mostly located on the cell surface where the reticulum is attached</td>
<td></td>
</tr>
<tr>
<td>Cytoplasmic Filaments</td>
<td>Rich</td>
<td>Usually found</td>
<td>Seldom found</td>
</tr>
<tr>
<td>Basal Dense Condensation</td>
<td>(−) Attached to the reticulum on one end</td>
<td>Interdigititation (−)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>Dense bodies (−)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The cells which cover the sinusoidal wall should be regarded as a group of independent cells under the name of sinus endothelial cells, because they are arranged in a regular way along the long axis of the sinus, and their ultrastructural features are quite different from those of other cell groups in the spleen.

As shown in Table 1, many characteristics of the sinus endothelium are similar to those of the vascular endothelium. In the former, however, the basement membrane is not continuous and the cell-to-cell attachment is poorly developed. Moreover, sinus endothelial cells have more coated vesicles, dense bodies, and phagocytosed material that are hardly seen in the vascular endothelium. These findings not only make it easy to distinguish the former from the latter, but also suggest that the former has more active function in uptake and transport of materials than the latter.

The cytoplasmic filaments seen in the sinus endothelial cells are responsible for the contractile ability of the cells. Basal dense material might appear to act as "half desmosome" and an adjoining apparatus to the reticulum.

The cell typical of the fixed reticulum cell is attached to the reticulum of the sinus wall and the pulp cord in several places with irregular processes of...
Fig. 11.—Cordal phagocyte. Attachment to reticulum (r) and interdigitation with phagocyte below are seen (× 10,500).

Fig. 12.—Rough-surfaced endoplasmic reticulum in close parallel array seen in cordal phagocyte (× 27,300).

The cytoplasm. The central part of the cytoplasm is occupied by a large nucleus which contains the loose chromatin network. There are generally scarce perinuclear organella, and only a rough-surfaced endoplasmic reticulum and free ribosomes are apparent. These characteristics were more pronounced in the reticulum cells within the pulp cord than in those directly beneath the sinus wall. These cellular characteristics are definitely different from those in the sinus endothelial cells. The difference in their location and arrangement is also distinct. These two groups of cells thus appear to represent distinctly different cell groups.

Various theories have been advanced in the literature concerning the difference and identity between these two kinds of cells in splenic red pulp. As the cells constituting the sinus and pulp, some investigators have recognized the presence of special cell groups, while others have not. From the points discussed above, the two types of cells were considered definitely different. In the normal spleen, the sinuses are composed of a regular ductal arrangement of sinus endothelial cells. On the other hand, the irregular network of the pulp cord consists of the reticulum and the cytoplasmic processes of the reticulum cells and the cordal phagocytes.
Consequently, we cannot agree with the opinion that the sinus endothelial cell is the reticulum cell which is located on the sinusoidal wall.

Recognition of the structure of the sinus and pulp cord in the normal spleen is extremely important for an understanding of the structure of the spleen in pathological conditions (for instance, in the interpretation of the origin of a proliferating sinus in the spleen of patients with portal hypertension), and also for the understanding of R.E.S.

Many discussions have been made on the R.E.S. since Aschoff.10 Akazaki’s theory11,12 is popular in Japan at the present time. According to his theory, the R.E.S. is divided into reticulum cells (identical with histiocytes) and reticulo-endothelial cells. These two cells are embryologically different according to his conclusion. The endothelial cells of the splenic sinus are different from the reticulo-endothelial cells such as the endothelium of the bone marrow and Kupffer’s stellate cells of the liver, and more closely resemble the general endothelium of blood vessels.

Concerning the difference and identity between the actual reticulo-endothelial cells and splenic sinus endothelial cells, a thorough discussion will be conducted in another paper. In the present study, electron microscopic studies on splenic sinus endothelium revealed characteristics that are more common with capillary endothelium than with those in any of the cells indigenous to the spleen. This would suggest a close embryological relationship between these two.
Fig. 14.—Same field as Fig. 13. Free cellular components painted out. Sinal endothelial cells outlined; reticulum painted with Indian ink. Structural difference between sinal lumen and cordal lumen distinct.

In the pulp cord, phagocytes are present as an essential component. According to the literature, opinions are divided as to whether these should be treated as reticulum cells,\textsuperscript{7-8,13,14} or not.\textsuperscript{4,6}

According to our observations, this cell attaches to the reticulum at various points of the irregularly extended cytoplasmic processes and has the rough-surfaced endoplasmic reticulum with locally lamellar arrangement. These findings are generally recognized as one of the characteristics of reticulum cells. Fixed reticulum cells and phagocytes in the pulp cord of the spleen contrast very well in electron microscopy. However, characteristics are common between these two kinds of cells, indicating a possible common origin of the two cells. Based on these points it seems reasonable to call the cordal phagocyte “a cordal phagocytic reticulum cell.”

However, the organella of the two cells was quite different. In the fixed reticulum cells, no phagocytosed material was usually found. This might indicate that completion of the intracellular structural changes appears to be necessary in response to the proper stimuli before the reticulum cells can perform phagocytosis.

**Conclusion**

Electron microscopy and stereoscopic reconstruction was carried out on normal human splenic red pulp. The splenic sinus is built on the basis of a regular arrangement of sinus endothelial cells forming duct-shaped struc-
tures. The pulp cord has a basic structure of a spongy network consisting of protoplasmic processes of the fixed reticulum cells and cordal phagocytes. Differentiation between these two structures is readily made.

Between the sinus endothelial cells and fixed reticulum cells, a distinct difference was noted in their location, arrangement pattern, manner in which they joined the reticulum, and the characteristics of the intercellular organelle. These two groups of cells thus definitely represent different types of cells. From this aspect, the theory that the sinus endothelial cells are simply reticulum cells facing the sinus lumen cannot be supported.

In view of the characteristics of the fine structures of the sinus endothelial cells, generally, they resemble the endothelium of blood vessels. However, in the sinus endothelial cells, there appears to be a more positive, greater cell function than in the endothelium of blood vessels.

REFERENCES
