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S. Sasou
Iwate Medical University

R. Satodate
Iwate Medical University

T. Masuda
Iwate Medical University

K. Takayama
Iwate Medical University

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SCANNING ELECTRON MICROSCOPIC FEATURES OF SPLEEN IN THE RAT AND HUMAN: A COMPARATIVE STUDY

S. Sasou[*], R. Satodate, T. Masuda and K. Takayama

Division of Pathology of the Central Clinical Laboratory and Department of Pathology, Iwate
Medical University, School of Medicine, Uchimaru 19-1, 020 Morioka, JAPAN

Abstract

The marginal zone, white pulp and red pulp of rat and human spleen were studied by scanning electron microscopy and were compared. The marginal zone was observed in both species. The arterial termination in the marginal zone was quite different between both species. The follicular arteries terminated at the boundary of the white pulp and formed a vascular net regarded as the marginal sinus in rat. On the other hand, numerous arterial termini of the follicular and sheathed arteries were scatteringly found in the marginal zone in man. The central artery was surrounded with flat reticular cells in rat and human spleen. In the red pulp of rat, the arterial termini were funnel-shaped or tubular. The sheath of the sheathed arteries of man revealed a circumferential lamellar structure consisting of flat reticular cells, and most free cells of the sheath were washed away by perfusion.

*Address for correspondence:
S. Sasou
Division of Pathology, Central Clinical
Laboratory, Iwate Medical University School of
Medicine, Uchimaru 19-1, 020 Morioka, Iwate,
Japan. Phone No.: 0196-51-5111

Introduction

The spleen is an organ that consists of peripheral lymphoid tissue and the mononuclear phagocytic system, and plays a role in immunological response against blood-borne antigens as well as destruction of aged blood cells. The spleen has a red pulp in which macrophages clear out aged blood cells and foreign bodies, a marginal zone which acts as an immunological filter (Snook, 1964; Clarke and Weiss, 1971; van Rooijen, 1972; Abe and Ito, 1972), and a white pulp in which antibodies are produced and which has a circulating lymphocyte pool (Weiss, 1983).

We have studied the basic structure of the spleen and the relationship between its structure and functions in man and rats using scanning and transmission electron microscopies as well as light microscopy (Satodate et al., 1971, 1977, 1986; Sasou et al., 1976, 1980, 1982; Sasou and Satodate, 1979).

Materials and Methods

The spleens of adult male Wistar rats, each weighing from 200 to 300g, those of newborn Wistar rats and those of man were used.

Adult rats were intravenously given 2,500 I.U. of heparin and the newborn rats intraperitoneally one-third of this dose 15 minutes prior to anesthesia with ether and/or sodium pentobarbital. The spleens were perfused with a physiological saline solution via the abdominal aorta at a pressure of 150 cm H_2O or at a flow rate of 150-250 ml per 10 minutes in the adult rats, and via the left cardiac ventricle at a lower pressure in the newborn rats. The spleens were fixed by perfusion of a 2.8% glutaraldehyde solution adjusted to pH 7.4 with a phosphate buffer after they became pale in color. The spleens were removed and cut into several pieces. These pieces were further fixed in a glutaraldehyde solution for 24 h, and subsequently postfixed in a 1% osmium tetroxide solution for 16 h.

The human spleens were removed at gastrectomy from patients with gastric cancer. They were immediately perfused with a physiological saline solution containing 2,500 I.U. of heparin via the splenic artery until they

became pale in color. The spleens were perfused with 500 ml of a 2.5% glutaraldehyde solution adjusted to pH 7.4, and cut into pieces of about 30 x 5 x 5 mm. The pieces were further fixed in a 2.8% glutaraldehyde solution for 24 h, and then in an osmium tetroxide solution for 16 h.

The pieces of both rat and human spleens were postfixed in a 1% osmium tetroxide solution, dehydrated in a graded alcohol series, and cracked into small pieces in liquid nitrogen (Tokunaga et al., 1974). The cracked specimens were immersed in iso-amyl acetate for 20 minutes, dried at the critical point in CO_2, coated with carbon in a vacuum evaporator, and coated with platinum or gold by the ion-sputtering method. Finally, they were examined with scanning electron microscopes (Hitachi HSM-2B and S-430) at 20 keV.

Results

The marginal zone

In rat spleen (Fig. 1a), the marginal zone was easily distinguished as a region between the white and the red pulps in conventional paraffin sections, and marginal metallophilic cells formed the boundary between the marginal zone and the white pulp (Satodate et al., 1971).

Scanning electron microscopy also easily revealed the marginal zone in rats. No venous sinuses were found within the marginal zone, although this zone was surrounded by venous sinuses of the red pulp. Stellate-shaped reticular cells in the marginal zone formed a meshwork that was continuous with the reticular meshwork of splenic cords. Free cells remained in the reticular meshwork even after perfusion. The marginal sinus (Figs. 1a and 2) was situated between the white pulp and the marginal zone. This was an anastomosis of the termini of the follicular arteries of the white pulp at the boundary between the white pulp and the marginal zone. Thus, it formed a vascular network that surrounded the white pulp. Cell-passing pores (Fig. 2), opening toward the marginal zone, were found in the walls of the marginal sinus. These pores were located at the junctions of the endothelial cells; i.e., cell-passing pores were widened gaps at the endothelial junction and were so large that blood cells may easily pass through them. The marginal sinus was also observed in newborn rats, but it was not well developed. In the areas where the marginal sinus was absent, flat demarcating reticular cells were found between the white pulp and the marginal zone. The processes of the reticular cells of the
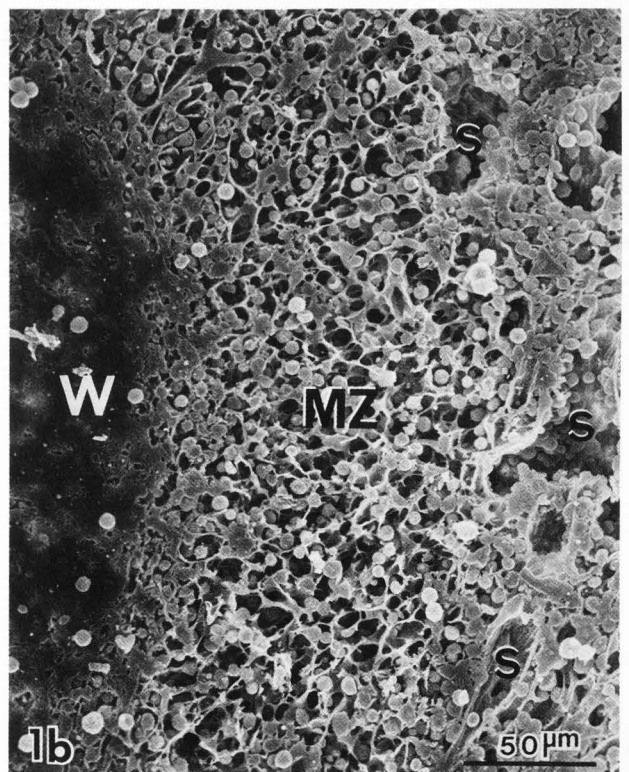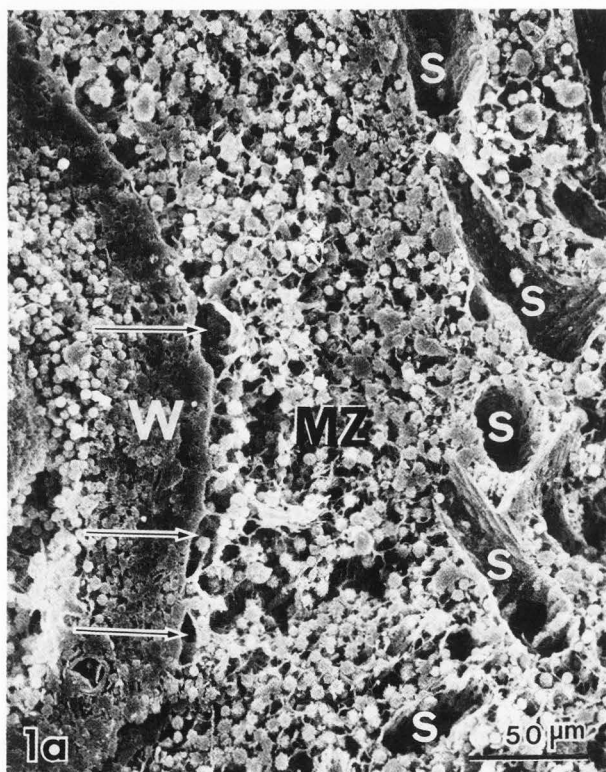

Fig. 1a. Rat spleen. The white pulp, marginal zone and red pulp are seen. The marginal sinus (arrows) is located between the white pulp and marginal zone. W, white pulp; MZ, marginal zone; S, venous sinus.

Fig. 1b. Human spleen. The structure is very similar to that of rat spleen. However, the marginal sinus is not found between the white pulp and the marginal zone. W, white pulp; MZ, marginal zone; S, venous sinus. (From Sasou and Satodate, 1979)
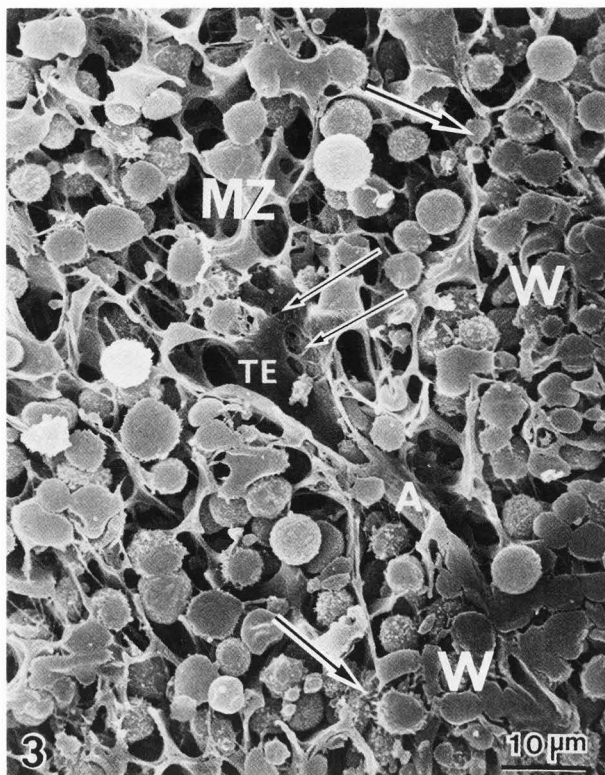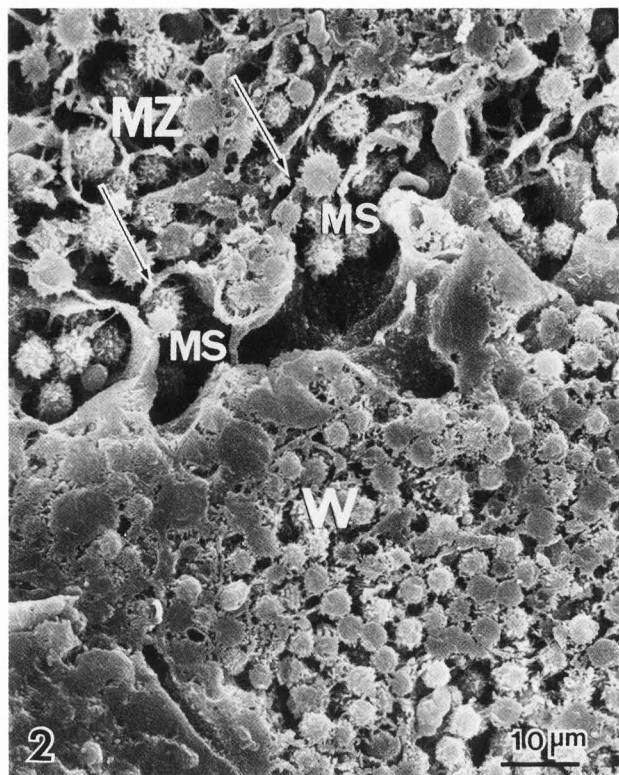
Fig. 2. Rat spleen. The marginal sinus (MS) is found between the white pulp (W) and the marginal zone (MZ), and contains the cell-passing pores (arrows).

Fig. 3. Human spleen. The terminal end (TE) of the follicular artery (A) is found in the marginal zone (MZ), and has a few fenestrations (thin arrows) in the enothelial cells. Thick arrows show the pores of the flat reticular cells demarcating the marginal zone (MZ) from the white pulp (W). (From Sasou and Satodate, 1979)

marginal zone were attached to these flat reticular cells. The demarcating reticular cells did not form a complete layer and gaps were observed among them. Cells passing through the gaps were found.

In the human spleen, it was usually difficult to identify the marginal zone by light microscopy in conventional paraffin sections. However, perfusion rendered it more visible (Fig. 1b). Basic structure of the marginal zone in human spleen was similar to rat spleen, although no vessels corresponding to the marginal sinus of rat spleen were found in human spleen. Instead, follicular and sheathed arteries entered the marginal zone and their arterial termini were frequently found in the marginal zone (Fig. 3). The terminal ends of these arteries dilated, and opened into the reticular meshwork of the marginal zone. The endothelial surface of the arterial termini in the marginal zone were smooth and a few fenestrations were found in them. In the region between the marginal zone and the white pulp, flat reticular cells surrounding the white pulp were found. Various-sized pores were found in the flat reticular cell (Fig. 3). A number of cells passed through these pores. Small venous sinuses were found in the marginal zone close to

the red pulp and were continuous with the large venous sinuses of the red pulp (Fig. 4). The wall of the small venous sinus in the marginal zone consisted of broader endothelial cells than that of the large venous sinus of the red pulp.

The white pulp

The white pulp consisted of the periarterial lymphatic sheath and lymphatic follicles. In the lymphatic follicles of rat spleens, it was difficult to wash away the free cells by perfusion. On the contrary, the cells of the periarterial lymphatic sheath were easily washed away by perfusion, resulting in a convenient condition for scanning electron microscopy. A few flat reticular cells with smooth surface surrounded the central artery in a circumferential arrangement in the periarterial lymphatic sheath (Fig. 5). In human spleen, at times, a few flat reticular cells surrounding the central artery were found in the periarterial region (Fig. 6), although free cells were difficult to be removed from the white pulp by perfusion.

The red pulp

We have examined the arterial termini of rat spleen and the sheathed arteries of human spleen.

In rat spleen, the central artery ran into the red pulp and terminated in the splenic cords.
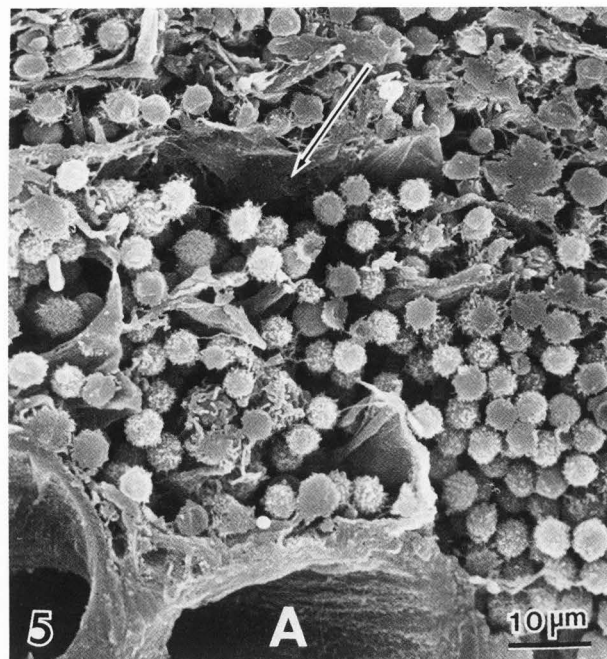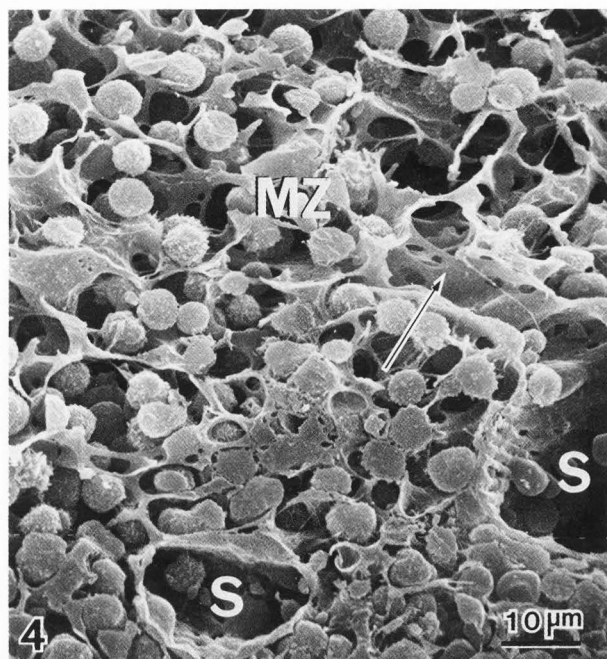
Fig. 4. Human spleen. A small venous sinus (arrow) is found in the marginal zone (MZ) and is
continuous with a large one (S) in the red pulp.

Fig. 5. Rat spleen. Flat reticular cells with a smooth surface are found in the periarterial lymphatic
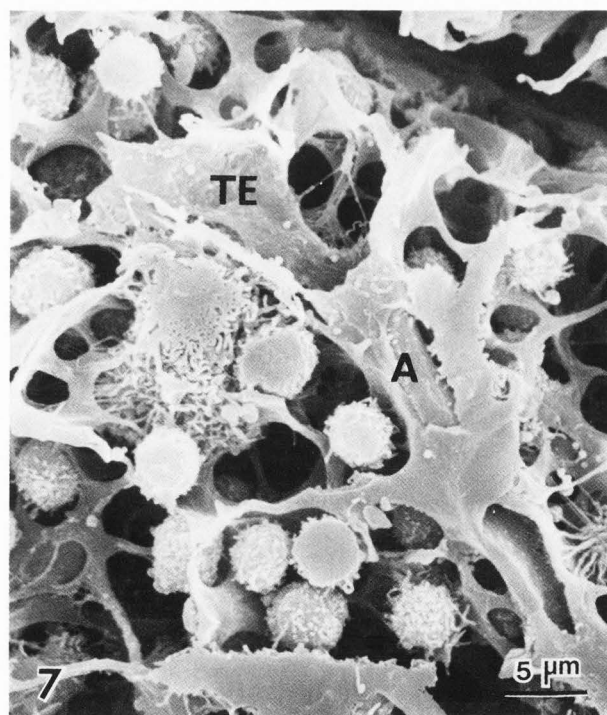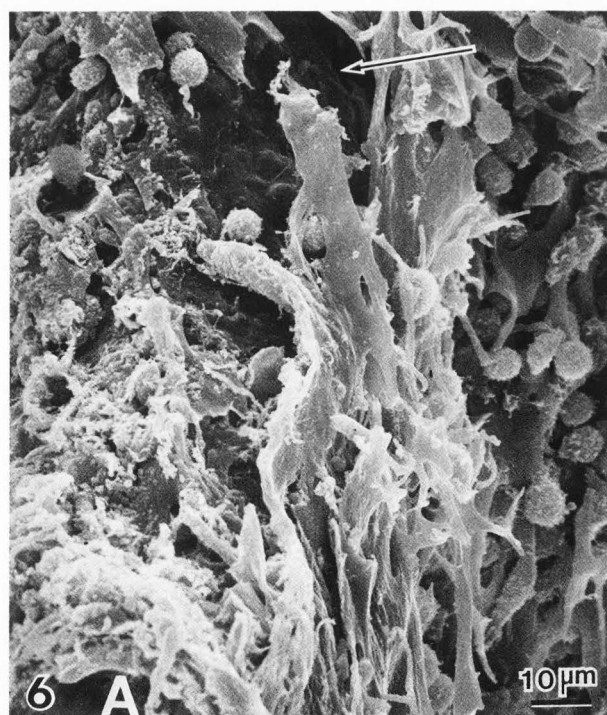sheath (arrow). A, central artery.

Fig. 6. Human spleen. Flat reticular cells surround the central artery (A) and form the intercellular
space (arrow) between them.

Fig. 7. Rat spleen. In the red pulp, the terminus of an artery (A) reveals a funnel-shaped ending (TE)
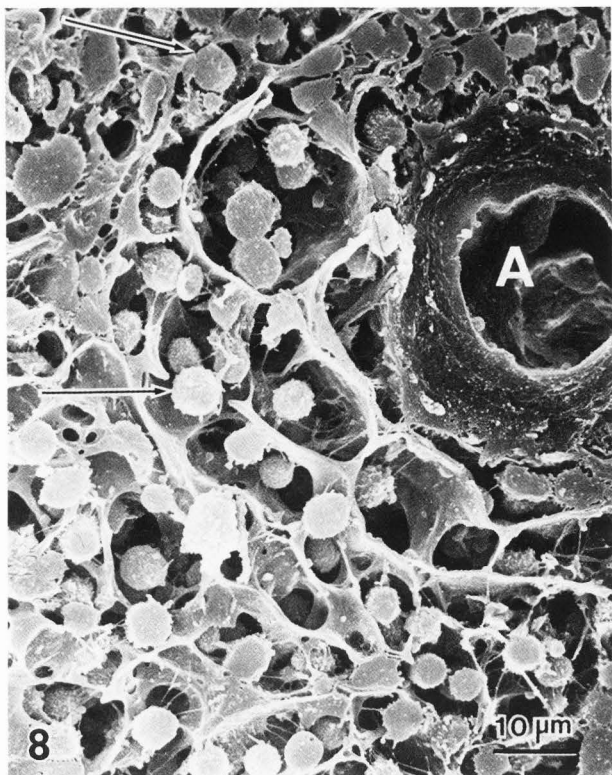and opens into the reticular meshwork of the splenic cord.

Fig. 8. Human spleen. Around the sheathed
artery (A) in the splenic cord, spaces are
seen between each layer of the reticular
cells. Arrows show free cells passing
through the pores of the flat reticular
cells.

The processes of reticular cells adhered to the
arterial wall of the splenic cord and supported
them. The arterial termini in the splenic cord
were located at various distances from the
venous sinuses. Some of these termini were
found in the center of the splenic cord. All
termini opened into the reticular meshwork of
the splenic cord (Fig. 7). No evidence of
direct communication between arterial terminus
and the venous sinus was found. The arterial
termini were funnel-shaped or tubular. Pores
were found in the wall of the arterial termini.
Endothelial processes of the terminal arteries
were continuous with the processes of the
reticular cells of the splenic cord at the
terminal ends.

In human spleen, numerous sheathed
arteries, commonly regarded as ellipsoid, were
found in the splenic cord (Fig. 8). The sheath
was an array of a few layers of flat reticular
cells which circularly surrounded the artery.
The space between each layer of the
circumferentially arranged reticular cells was
wide. Pores were found in these flat reticular
cells and free cells passed through them (Fig.
8).

Discussion

The marginal zone

The marginal zone has an intense blood flow
and is the site where blood-borne antigens appear
and where contact with lymphocytes and
macrophages occurs (Brown et al., 1973; Mitchell
and Abbot, 1971; Nossal et al., 1966; van
Rooijen, 1973, 1977; Veerman and van Rooijen,
1975). Our findings further emphasize that the
marginal zone has convenient structures for
immunological response to the blood-borne
antigens (Sasou et al., 1976, 1980, 1982) and
has a different function from the white and red
pulps (Satodate et al., 1977). In rats, the
marginal sinus is found as a vascular network
covering the white pulp and demarcating it from
the marginal zone. It contains a number of
cell-passing pores for blood cell passage. These
findings suggest that the marginal sinus is a
suitable structure for distributing blood evenly
into the marginal zone. In man, numerous
arterial termini are found in the marginal zone.
Ink particles have been reported to accumulate in
the marginal zone or the perifollicular space of
the spleen in both man and animals (Altshul and
Hummason, 1947; Snook, 1964; van Rooijen, 1972;
Sasou et al., 1976). Therefore, the arterial
termini in the marginal zone of human spleen may
function as a distributor or an equalizer similar
to the function of the marginal sinus in rat
spleen.

The marginal zone has been suggested as the
main site of lymphocyte migration from the blood
stream (Brelinska and Pilgrim, 1982).
Lymphocytes can move from the marginal zone to
white pulp through the boundary between the two
areas (Mitchell, 1973; Nieuwenhuis and Ford,
1976; Brelinska et al., 1984). The anatomical
sites of this cell movement are the gaps in rat
spleen and the pores in human spleen, that we
found between or in the flat reticular cells of
the boundary between the white pulp and the
marginal zone. This has also been confirmed in
transmission electron microscopic studies (Sasou
et al., 1976).

The white pulp

The presence of deep lymphatic current or
vessel has been reported in the periarterial
region of the white pulp of the spleens (Snook,
1946; Fukuda, 1963; Janout and Weiss, 1972). A
strong lymph current was found in the
periarterial lymphatic sheath in rats by
Koshikawa et al. (1984). We also found that the
cells in the region around the artery of the
white pulp were easily washed away by perfusion
in rats. The region around the arteries of the
white pulp, i.e., deep lymphatic pathway of rat
spleen, consists of a few layers of flat
reticular cells. The deep lymphatic pathway of
the spleen differs from the ordinary lymphatic
vessels. Although no vascular structure for
lymphatic current is observed there, lymph
streams the intercellular space of reticular
cells around the artery.

The red pulp

Arterial termini in the splenic cord have a
tubular or funnel-shaped ending. Various shapes

of arterial termini such as tubular, ampullar and saccular shapes have been reported (Suzuki et al., 1977; Irino et al., 1978; Fujita et al., 1985). The difference in shape is found even in the same species. The use of different preparatory procedures may explain the difference in the results. In human spleen, numerous sheathed arteries are found in the splenic cord. Most cells of the sheath are washed away by perfusion. This suggests the presence of a blood flow from the artery to the splenic cord in the sheath. Furthermore, we have found that the sheath is a circumferential structure with a wide space between each layer of flat reticular cells. The sheath is filled with macrophages (Solnitzky, 1937). Blue and Weiss (1981) reported that macrophages within the sheath take up intravenously injected particles, and that the ellipsoid is the major site for clearance of blood-borne particles. It seems that the structure of the sheath may play an important role in the contact of macrophages with blood-borne particles.

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Discussion with Reviewers

F. Campbell: It appears to me that the slits between the endothelial cells of the marginal sinus shown in Fig. 1 are rather wide. Is this due to shrinkage of the cells or to perfusion of buffer and fixative through the spleen?
Authors: Influence of perfusion and fixation must be seriously considered in morphological examination. We have very carefully prepared the specimens. This feature was also obvious in the rat spleens which were not perfused.

F. Campbell: Weiss (Blood, 43: 665, 1974) has described numerous reticular fibers in the marginal zone of rat spleen, but no mention of fibers is made in the present study. I see what seems to me to be fibers in many of the micrographs. Can reticular fibers be identified in the spleen preparations described here?
Authors: Yes, we have also found reticular fibers in the marginal zone of rat and human spleens.

M. Tavassoli: It is my impression that spleen is the single lymphoid organ that does not have lymphatic vessels and that is why it sees blood-borne antigens and not lymphatic-borne antigens. Am I in error? If I am, would the authors explain where these lymphatics are? Are they afferent or efferent and in the latter case, where do they drain?
Authors: Deep lymphatic pathway of the spleen completely differs from ordinary lymphatic vessels as we have mentioned here. Lymph efferently flows between flat reticular cells which arrange around the artery in the white pulp. No real lymphatic vessel is found in parenchyma of the spleen. And no afferent of lymph current exists.

M. Tavassoli: How do the authors reach the conclusion that cell-passing pores are at the junction between two cells? It is not easy to derive such conclusions from SEM. TEM, or even better, freeze-fracture studies are more reliable in this regard. This is particularly important because cell migration in other lymphohemopoietic tissues (bone marrow, post capillary venules of lymph node) is a transcellular (and not intracellular) phenomenon and it is possible and even probable that it may be the same in spleen.
Authors: We think that the cell-passing pores are formed at the endothelial junction of the marginal sinus. But, it seems to us that your question is concerned with cell movement at other places in the spleen. Free cells commonly move through gaps and pores of the flat reticular cells in the spleen, although, at times, transcellular movement is found at the boundary of the white pulp and the wall of the white pulp side of the marginal sinus.

Reviewer III: The authors state that all the arterial terminals open into the reticular meshwork of the splenic cord. It is possible that a small percentage of arterial termini may not open into the reticular meshwork. Let us assume it is three percent. In order to be able to detect such a low percentage of arterioles not opening into the meshwork, it is necessary to examine a large number of arterial termini. Therefore, I would like to ask the following questions: (a) How many arterial terminals are observed in each spleen section? and (b) what is the total number of arterial terminals examined?
Authors: We examined numerous arterial termini, although we did not count them.