An Electron Microscopic Study of Spleen in Myelofibrosis With Myeloid Metaplasia

By Mehdi Tavassoli and Leon Weiss

The ultrastructure of the spleen was studied in four patients with myelofibrosis with myeloid metaplasia (MMM) who underwent splenectomy. The basic structure of the spleen is not altered. Degenerative changes are seen in the white pulp, which appears atrophic. Hemopoietic tissue (erythropoietic and granulopoietic cells, as well as megakaryocytes) is seen in the sinuses, as well as in the cords of the red pulp, but is not seen in the white pulp. Nuclear and cytoplasmic abnormalities are seen in hemopoietic cells. Nuclear changes consist of the nuclear membrane projecting into the cytoplasm and forming nuclear blebs or loops. The nuclear blebs are sometimes connected to the main nuclear body by a stalk containing fibrillar structures. The nuclear loops may be so large as to engulf almost all the cytoplasm. Cytoplasmic changes are degenerative in nature and result in premature destruction followed by phagocytosis. Macrophages are ubiquitous in the red pulp, particularly in association with extracellular reticulum and the basement membranes. They often contain debris of developing hemopoietic cells. These findings support previous studies that indicate that a portion of splenic hemopoiesis in MMM is ineffective.

MYELOFIBROSIS with myeloid metaplasia (MMM) is a clinical and pathologic entity that has been classified in the spectrum of myeloproliferative disorders. It may occur de novo or during the course of other myeloproliferative diseases, such as polycythemia vera, chronic granulocytic leukemia, or essential thrombocytopenia. The disease is characterized by a leukoerythroblastic blood picture, progressive marrow fibrosis, splenomegaly (often massive and progressive), and usually hepatomegaly.1,8

The spleen plays an important part in the pathogenesis of this disease. As a rule, it is enormously enlarged and may cause mechanical problems because of its size (small stomach syndrome). Early in the course of the disease, it harbors extramedullary hemopoiesis. With progress of the disease, hypersplenism may emerge, leading to anemia or thrombocytopenia. The pathology of spleen in MMM has not been extensively studied, and all previous studies have been at the magnification of the light microscope.3,6,8,11 The present report is an electron microscopic study of spleen in four cases of myelofibrosis with myeloid metaplasia occurring de novo that were not secondary to polycythemia vera, chronic granulocytic leukemia, or other myeloproliferative diseases.

From the Department of Anatomy, Johns Hopkins University School of Medicine, Baltimore, Md., and the Division of Hematology, Scripps Clinic and Research Foundation, La Jolla, Calif.

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Mehdi Tavassoli, M.D.: Scripps Clinic and Research Foundation, La Jolla, Calif. 92037. Leon Weiss, M.D.: Professor of Anatomy, The Johns Hopkins University School of Medicine, Baltimore, Md. 21205.

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Table 1. Clinical Data Obtained During Hospitalization for Splenectomy

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yr)</th>
<th>Race</th>
<th>PCV (%)</th>
<th>WBC (cells/cu mm)</th>
<th>Platelets (X 10^4)</th>
<th>Marrow Fibrosis</th>
<th>Spleen Weight (g)</th>
<th>Liver</th>
<th>Indication for Splenectomy</th>
<th>Follow-up Period (yr)</th>
<th>Other Diagnoses</th>
<th>Drug Therapy Prior to Splenectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. W. C</td>
<td>53</td>
<td>M.</td>
<td>13</td>
<td>15,000</td>
<td>484</td>
<td>Extensive</td>
<td>3950</td>
<td></td>
<td>Hepatomegaly, Bx not done</td>
<td>2</td>
<td>None</td>
<td>Myleran, androgen</td>
</tr>
<tr>
<td>2. E. K</td>
<td>44</td>
<td>M.</td>
<td>25</td>
<td>11,300</td>
<td>63</td>
<td>Extensive</td>
<td>3170</td>
<td></td>
<td>Hepatomegaly, Bx Extramedullary hemopoiesis</td>
<td>6</td>
<td>None</td>
<td>Myleran</td>
</tr>
<tr>
<td>3. J. H</td>
<td>73</td>
<td>M.</td>
<td>35</td>
<td>18,000</td>
<td>49</td>
<td>Moderate</td>
<td>650</td>
<td></td>
<td>Hepatomegaly, Bx Extramedullary hemopoiesis</td>
<td>2.5</td>
<td>Gallstone</td>
<td>Prednisone</td>
</tr>
<tr>
<td>4. H. B</td>
<td>60</td>
<td>F.</td>
<td>40</td>
<td>47,900</td>
<td>4</td>
<td>Extensive</td>
<td>3000</td>
<td></td>
<td>Hepatomegaly, Bx not done</td>
<td>2</td>
<td>Diabetes</td>
<td>None</td>
</tr>
</tbody>
</table>

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MATERIALS AND METHODS

Clinical Materials

Four spleens were selected from patients who underwent splenectomy between 1964 and 1970. All four cases had a diagnosis of myelofibrosis with myeloid metaplasia that had been established both clinically and histologically. The diagnosis was established on the basis of clinical features of the disease, hematologic findings including a leukoerythroblastic blood picture, pronounced morphologic abnormalities of the red cells, and fibrosis of the marrow. A follow-up period ranging from 2 to 6 yr and subsequent pathologic studies of the spleen and liver (in two cases where liver biopsies were done) confirmed the diagnosis. Clinical data obtained during the course of hospitalization for splenectomy are summarized in Table I.

Tissue Preparation and Microscopy

The tissue was fixed in Karnovsky’s glutaraldehyde-formaldehyde mixture for 24 hr at pH 7.4, was rinsed in cacodylate buffer, and was postfixed in OsO₄ that was similarly buffered for 1 hr. After dehydration in graded alcohol, it was embedded in Araldite. The blocks were sectioned in a Reichert microtome with a Dupont diamond knife. Sections in the range of silver were stained with uranyl acetate and lead citrate and were studied in a Siemens Elmiscop I. Thicker sections of plastic-embedded materials were stained with 1% toluidine blue in borate buffer (pH 8) and were studied by light microscopy. Larger portions of spleen were embedded in paraffin, were sectioned at 5 μ, and were stained with PAS-hematoxylin and reticulum stain.

RESULTS

The observations relate to all four spleens, except when otherwise specified.

Gross Appearance

The spleens were massively enlarged (Table 1). They were firm in consistency and were reddish-brown in color. The capsule was smooth but showed patchy areas of thickening related to underlying infarctions. In case 4, several infarctions were seen, with the greatest measuring 7 cm in diameter. On section, the parenchyma bulged slightly and appeared dark brown and firm with relatively little blood lost from the cut surface. There were few areas of infarction, except in case 4 where some 22 infarcted areas were encountered. The red pulp was markedly expanded and widely separated the lymphatic nodules, which appeared sparse and small.

Light Microscopy

The basic anatomy of spleen was preserved. The capsule and trabeculae showed focal thickening adjacent to areas of infarction. The lymphatic nodules were small and sparsely distributed. The red pulp comprised most of the splenic tissue and was highly congested and cellular. There was no noticeable increase in silver staining extracellular reticulum of fibrous tissue. Foci of hemopoietic cells were discernible in both splenic sinuses and intersinal cords (Fig. 1). No hemopoietic cells were seen within the white pulp. There was a conspicuous number of macrophages that were best seen in plastic-embedded sections stained with toluidine blue. They contained fragments of erythrocytes and other cellular debris.
Fig. 1. Light micrograph shows splenic red pulp in MMM. Pulp is congested by presence of red cells, hemopoietic cells, and macrophages. Several sinuses are clearly seen (S): one of them contains many red cell precursors (arrow). X 640.

Fig. 2. Splenic white pulp in MMM. Note myelin figures in cytoplasm of lymphocytes (arrows). X 6000.
Electron Microscopy

The white pulp was normal in organization. Myelin figures were frequently encountered in the cytoplasm of the lymphocytes and reticular cells covering the extracellular reticulum of the white pulp (Fig. 2). No foci of erythropoiesis, granulopoiesis, or megakaryopoiesis were seen in the white pulp.

The red pulp was highly cellular. There was also a considerable increase in vascularity. Small arterioles and vascular sinuses were frequently encountered. Some of these arterioles terminated in the red pulp with a slitlike opening. Occasionally, these openings were in close vicinity of a sinus, but we did not encounter direct communication between arterioles and sinuses. The extracellular reticulum of the cordal tissue appeared somewhat thick and usually contained collagen. The cytoplasm of macrophages was usually spread along the surface of extracellular reticulum (Figs. 4, 5, 8, 9). This portion of the cytoplasm often contained bands of microfilaments that, in appropriate sections, were extending into the adjacent extracellular reticulum or the sinus basement membranes.

The chords were striking in their content of large numbers of macrophages, which contained the breakdown products of erythropoietic or granulocytic precursors (Figs. 3–9). Little phagocytosis of erythrocytes was recognized. The macrophages were large with many extensions and foldings. A portion of their cytoplasm appeared to be fixed to extracellular reticulum or the basement membrane of sinuses, while the bulk of the cell extended into a cord and contained phagocytic material. A common picture was a macrophage extending a small portion of cytoplasm into the lumen of a sinus with the bulk of the cell in the cord and filled with phagocitized cells or cell debris (Fig. 7). Often macrophages appeared to be engulfing hemopoietic precursors that were normal in appearance.

Aside from macrophages, the cells of the red pulp were largely hemopoietic. The three cell lines (erythropoietic and granulopoietic cells, and megakaryocytes) were seen (Figs. 3–6). They were in various stages of maturation. Although the ratio of different cell types varied from one spleen to the other and from one area to the next, there appeared to be an equal distribution of erythropoiesis and granulopoiesis with a tendency for increased proportion of early stages of maturation. The following figures were obtained by counting 500 hemopoietic cells in a random sample of low resolution micrographs from all four cases: erythroid precursors 44% (early forms 15%, intermediate 13%, and late stage 16%); granulocytic precursors 56% (early stages 22%, intermediate 22%, neutrophilic metamyelocytes 7%, recognizable eosinophilic precursors 3%, and recognizable basophilic precursors 2%).

Erythropoiesis tended to occur in distinct foci. Granulopoietic foci were less distinct. Hemopoietic cells were widely distributed within the red pulp. They were present in the cords, within the lumen of sinuses, and even in arterioles. There were no differences in the stages of maturation of hemopoietic cells in the cords in relation to those in sinuses. Mitosis occurred in both the cord and sinuses. The cytoplasm of hemopoietic cells frequently contained myelin figures and autophagosomes. Unusual nuclear formations were present. They included a projection of nuclear membrane into the cytoplasm (Figs. 6 and 9). This projection contained a small amount of nucleoplasm and enclosed a part of
Fig. 3. Cord that is congested by presence of many hemopoietic cells in various stages of maturation; several of them have large nuclei with an open chromatin pattern and nucleoli. Other cells are in later stages of maturation and are clearly recognizable as white cell or red cell precursors. Portions of the cytoplasm of two macrophages are also seen (arrows). They contain debris of degenerated cells. X 4500.
Fig. 4. Granulopoiesis is evident in this figure obtained from a cord. Granulocytic precursors show profiles of rough endoplasmic reticulum and variable numbers of granules. Extracellular reticulum (ER) contains bands of collagen (C). Several macrophages are also seen and contain a heavy load of degenerated cells and cell debris. Note association of macrophages with extracellular reticulum. X 5000.
Fig. 5. Several hemopoietic cells in splenic cord. Portion of a megakaryocyte (MEGA) is also included in the upper part of the figure. Cytoplasm of a macrophage is spreading along extra-cellular reticulum (ER) and contains fragments of a degenerated cell (arrow). X 4500.

Fig. 6. Structure of a sinus is seen in lower left part of this figure. Note interrupted basement membrane (BM). Lumen contains hemopoietic cells particularly granulocytic precursors. Nucleus of one cell shows a nuclear bleb (arrow). Cord also contains hemopoietic cells. Two nucleated red cells (R) are also seen. Their nuclei are eccentric and are probably in the process of extrusion. One cell lies within the lumen of the sinus with its nucleus directing toward the cord. The other cell lies within the cord with its hemoglobinized portion directing toward the sinuses. This arrangement is suggestive of transmural passage of these nucleated cells. X 3600.
Fig. 7. Portion of a splenic sinus is seen on left. Lumen is not included in the figure. Two endothelial lining cells are seen (END). Their nuclei show characteristic foldings, and hemoglobin-containing micropinocytic vesicles (small arrows) are evident in their cytoplasm, particularly on the luminal side. Note again the interrupted basement membrane (M) and their association with dark bands of microfilaments (large arrows) located within the cytoplasm of lining cells. Portion of red cell (H) containing two Heinz bodies is seen in passage between the two lining cells. A macrophage (M) has extended a portion of its cytoplasm into the sinus wall (between two arrowheads). Bulk of the cell, however, remains in the cord. X 10,000.

cytoplasm. These projections were sometimes small (nuclear blebs), but they could be large and extend over a large proportion of cytoplasm (nuclear loop). The nuclear loops may be so large as to encompass almost all the cytoplasm. The nuclear bleb was sometimes connected to the main nuclear body by a stalk-containing fibrillar structure (Fig. 9, inset). Megakaryocytes were unusually large and rich in granules. They were not present in large numbers. In case 3, many plasma cells were encountered in the cords.
Fig. 8. This figure shows extensive phagocytic activity of splenic cord in MMM. Cytoplasmic portions of several macrophages have dominated this figure; one of them shows several foldings (arrows). Note the association of macrophages with extracellular reticulum, which contains bands of collagen (C). One macrophage has extended its cytoplasm along the extracellular reticulum, engulfing what is probably a granulocytic precursor (G). X 10,000.
Fig. 9. The field from splenic cord is dominated by macrophages; many of them contain cell debris and fragments of degenerated cells. Note again the association of macrophages with collagen-containing extracellular reticulum (C). Cytoplasm of macrophage on the left shows extensive foldings (arrows). Structure in lower part of this figure (A) may be an arteriolar ending. Note intracellular microfilaments. Cell in the center is a granulocyte; its nucleus shows two large blebs (B). X 10,000. Figure in upper left corner shows nucleus of a cell extending a fibrillar structure into the cytoplasm. X 10,000.

Sinuses appeared normal in structure (Figs. 6 and 7), and their lumen was packed with hemopoietic cells (Fig. 6).

DISCUSSION

In myeloid metaplasia, the basic anatomy of the spleen is not altered. Infiltration by hemopoietic cells is limited to the red pulp; the white pulp does not harbor the hemopoietic tissue. The malpighian nodules, however, are widely
separated because of the red pulp expansion, and they appear smaller than usual. These findings are in agreement with those of most previous investigators.\textsuperscript{1,3,6,8,11} We have further observed degenerative changes in lymphocytes of the white pulp. This is probably because hemopoietic proliferation in the red pulp encroaches on the white pulp and causes its gradual atrophy. A similar picture has been reported in the spleen from cases of chronic granulocytic leukemia.\textsuperscript{12}

Although hemopoiesis within the lumen of sinuses has been stressed in this disorder,\textsuperscript{6} our observations indicate that the hemopoietic tissue is equally distributed in the cords and sinuses. However, the intravascular hemopoiesis, is significant because in adult mammals normal hemopoiesis occurs almost exclusively extravascularly.\textsuperscript{8} Only mature cells enter the circulation. Furthermore, the spleen functions as a filter in the circulation, trapping immature or abnormal cells.\textsuperscript{13} This function depends on the structure of the wall of the splenic sinuses: Bands of microfilaments, basally located in the sinal endothelium, are inserted into the basement membrane (Fig. 7), thus limiting the pore size through which the cells must pass to enter the circulation.\textsuperscript{14} When a cell, such as an immature hemopoietic cell, is larger than this pore size and is not pliable enough, it is trapped in the cord where it is subject to phagocytic action of cordal macrophages.

Abnormalities of hemopoietic cells were frequently encountered. Nuclear abnormalities consist of nuclear blebs and loops. These abnormalities have been occasionally encountered in normal leukocytes,\textsuperscript{15} but their frequent occurrence in neoplastic diseases of hemopoietic tissue has been reported.\textsuperscript{16-20} They may be an index of the neoplastic nature of hemopoiesis in MMM. Cytoplasmic abnormalities (myelin figures, autophagosomes) are probably degenerative in nature and may result in premature destruction and phagocytosis of hemopoietic cells before they can be discharged into the circulation. Thus, a variable portion of splenic hemopoiesis in MMM appears to be ineffective hemopoiesis. This conclusion is in agreement with the results obtained by radioactive isotope utilization studies,\textsuperscript{7,21,22} which have also indicated various degrees of ineffective hemopoiesis in this disease. Phagocytosis of immature hemopoietic cells was a frequent finding in our material. Over a long period of time, exaggerated phagocytic function of the spleen may result in hypertrophy of the spleen's reticuloendothelial elements that, in turn, results in hypersplenism—a frequent finding in long-standing disease.

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REFERENCES

5. Bouroncle BA, Doan CA: Myelofibrosis,
8. Ward HP, Block MH: The natural history of agnogenic myeloid metaplasia (AMM) and a critical evaluation of its relationship with the myeloproliferative syndrome. Medicine (Baltimore) 50:357, 1971
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