J. Anat. (1995) 186, pp. 287–299, with 18 figures Printed in Great Britain

Light and electron microscope study of splenoportal milky spots in New Zealand Black mice: comparison between splenoportal milky spots and aberrant spleens

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(*Accepted 15 September 1994*)

ABSTRACT

The omentum contains peculiar lymphoid tissues termed omental milky spots. In mice, similar milky spots (splenoportal milky spots) are present in splenoportal fat bands developing along the splenic artery. We found that New Zealand Black (NZB) mice, which are known to develop spontaneous autoimmune diseases, have well developed splenoportal milky spots. However, little is known about these milky spots. Thus we investigated splenoportal fat bands in NZB mice by light and electron microscopy. Splenoportal fat bands contained sporadic aberrant spleens as well as abundant milky spots. In addition, transitional forms between splenoportal milky spots and aberrant spleens, although sporadic, were present in the fat bands. Splenoportal milky spots were supplied with offshoots from the splenic artery and were composed of abundant lymphocytes with macrophages, plasma cells, granulocytes, megakaryocytes and various stromal cells. In addition, they showed active neutrophilic myelopoiesis and probable megakaryopoiesis. Aberrant spleens were also supplied by branches from the splenic artery. They showed active granulopoiesis, megakaryopoiesis, and erythropoiesis. The transitional forms resembled splenoportal milky spots in structure, but the former showed extramedullary haematopoiesis of three cell lineages. The morphological transition from aberrant spleens, via transitional forms, to splenoportal milky spots seems to indicate that splenoportal milky spots represent splenoid lymphoid tissues.

INTRODUCTION

The omentum is known to contain peculiar lymphoid tissues designated as omental milky spots. Von Recklinghausen (1863) was the first to refer to the whitish spots in the omentum and in the serous layer of the thoracic cavity in young rabbits. Later, Ranvier (1874) named them 'taches laiteuses' (milky spots). In mice, the omentum is made up of an omental fat band and omental membranes. This fat band contains abundant vascularised milky spots (omental milky spots). An additional fat band (splenoportal fat band) is present along the splenic artery at the hilum of the spleen. It also contains abundant vascularised milky spots (splenoportal milky spots) (Takemori et al. 1994).

New Zealand Black (NZB) mice are known to develop spontaneous autoimmune diseases (Helyer & Howie, 1963; Izui et al. 1978; Reininger et al. 1990) and chronic lymphocytic leukaemia (Phillips et al. 1992). In these mice, it is suggested that thymic reticuloepithelial cells are functionally abnormal (Minoda & Horiuchi, 1983). We previously reported that omental milky spots are well developed in NZB mice (Takemori et al. 1994). Recently, we found that splenoportal fat bands contain three types of lymphoid tissue (splenoportal milky spots, aberrant spleens, and forms that are transitional between splenoportal milky spots and aberrant spleens). Until now, little attention has been directed towards these lymphoid tissues. We therefore investigated splenoportal fat bands by light and electron microscopy.

In this paper, morphological features of splenoportal milky spots, aberrant spleens, and transitional

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forms are described. In addition, the significance of these lymphoid tissues is discussed based on their morphological transition. With respect to milky spots, two types, vascularised and nonvascularised (Ranvier, 1874; Renaut, 1907; Tanaka, 1958; Takemori, 1979*a*, *b*, 1980; Takemori & Ito, 1980; Hirai et al. 1992; Takemori et al. 1994), are known to be present. To avoid confusion, we will use the term 'milky spots' as designating 'vascularised milky spots' in this paper.

MATERIALS AND METHODS

A total of 46 adult NZB/N mice (provided by Japan SLC Co. Ltd., Hamamatsu, Japan) of both sexes, aged 8 wk and weighing 23–32 g were used. They were maintained on a commercial pellet diet (MF, Oriental Yeast Co. Ltd, Tokyo, Japan) and water ad libitum under constant environmental conditions.

For dissection microscopy, a total of 10 mice, with equal numbers of both sexes, were used. After deep anaesthesia with diethyl ether, the mice were injected intraperitoneally with 12–15 ml of 2% glutaraldehyde in 0.05 M phosphate buffer (pH 7.4). One hour after the injection, the abdomen was opened, and the splenoportal fat bands and surrounding organs were observed using a binocular dissecting microscope.

For light microscopy, a total of 20 mice, equal numbers of both sexes, were used. Mice were killed by anaesthetic overdose, and splenoportal fat bands were removed as widely as possible. They were fixed in a solution containing 1% glutaraldehyde, 2% paraformaldehyde, and 1.5% sucrose in 0.05 M phosphate buffer (pH 7.4) for 2 h at 4 °C. After fixation, the specimens were embedded in paraffin according to conventional methods, and 10 transverse serial sections, 4 μm in thickness, were made. One of these sections was stained with haematoxylin-eosin and another with periodic acid-Schiff (PAS)-haematoxylin. All stained sections were observed by light microscopy. When no megakaryocytes were seen in the 2 sections stained with haematoxylin-eosin and PAS-haematoxylin, the remaining 8 sections were similarly stained. The presence of megakaryocytes was confirmed using these preparations.

For demonstrating erythroblasts in splenoportal milky spots, we employed immunohistochemical staining using deparaffinised sections of splenoportal fat bands according to the method of DeLellis et al. (1979). Briefly, rabbit antiserum specific for mouse haemoglobin (Cappel Laboratories, West Chester, PA) diluted to 1:200 was used as the primary antibody. To demonstrate reaction products, a peroxidase-antiperoxidase staining using MEB PAP-R test kit (Medical & Biological Laboratories Co. Ltd, Nagoya, Japan) was carried out. Sections were counterstained with haematoxylin, embedded in glycerin-gelatin (Muto Pure Chemicals Ltd, Tokyo, Japan) and observed by light microscopy. As a control, the primary antibody was substituted by normal rabbit serum. For demonstrating immunoglobulin-positive lymphocytes (B cells) in splenoportal milky spots, the same immunohistochemical method described above was carried out. Rabbit immunoglobulins specific for mouse immunoglobulin G (IgG), IgA or IgM (Serotec, Oxford, UK) diluted to 1:200 were used as primary antibodies.

For electron microscopy, a total of 16 mice, with equal numbers of both sexes, were used. Splenoportal fat bands, similarly obtained, were fixed in a solution containing 2% glutaraldehyde, 2% paraformaldehyde, and 1.5% sucrose in 0.05 M phosphate buffer (pH 7.4) for 2 h, then postfixed in a solution containing 2% osmium tetroxide and 1.5% sucrose in 0.05 M phosphate buffer for 2 h at 4 °C. The specimens were dehydrated in a graded series of ethanol. After substituting acetone for ethanol, they were embedded in Epok 812 (Oken Shoji Co. Ltd, Tokyo, Japan). Semithin and ultrathin sections were made with a diamond knife on an LKB Ultrotome. Semithin sections of aberrant spleens were stained with toluidine blue and were used for light microscopy. Ultrathin sections stained with uranyl acetate and lead citrate were observed with a Hitachi H-800 electron microscope at 75 kV.

RESULTS

Dissection microscopy of splenoportal fat bands

The splenoportal fat bands appeared to be well-developed adipose tissues which developed along the splenic artery at the hilum of the spleen (Fig. 1). They showed a band-like configuration (Fig. 2). Occasionally, 1–2 reddish rounded nodules ranging from 0.5 to 0.8 mm in diameter (aberrant spleens) were seen on the surface of some fat bands. The presence of these nodules was confirmed in 4 of 10 splenoportal fat bands examined.

Light microscopy of splenoportal milky spots, aberrant spleens, and transitional forms

In the superficial area of splenoportal fat bands, splenoportal milky spots of various sizes were seen.

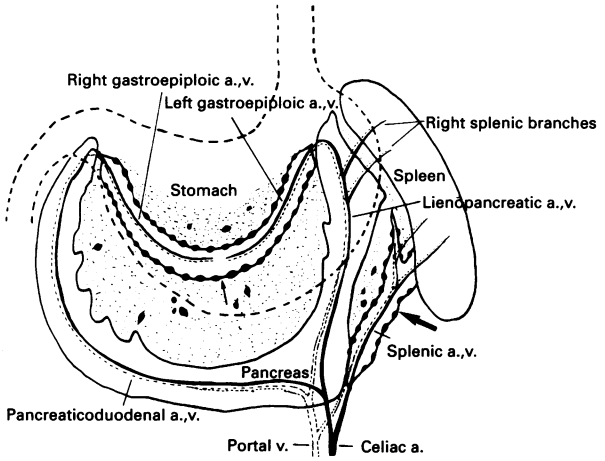

Fig. 1. Schematic drawing showing the relationship between the omentum, spleen and pancreas. The large arrow indicates a splenoportal fat band, and the small arrow indicates an omental fat band.

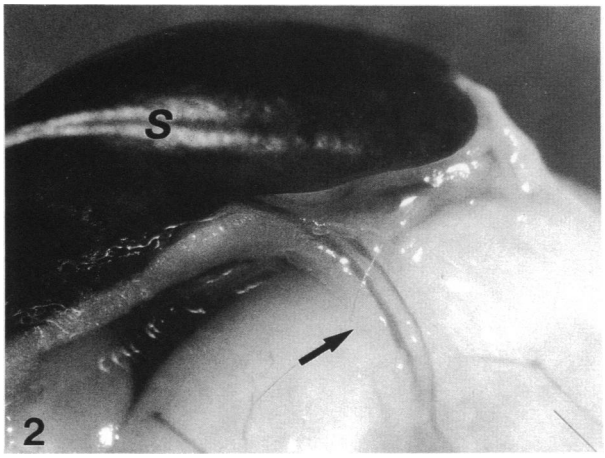

Fig. 2. Photograph of a splenoportal fat band (arrow). The splenic artery and vein are seen in the splenoportal fat band. S, spleen.

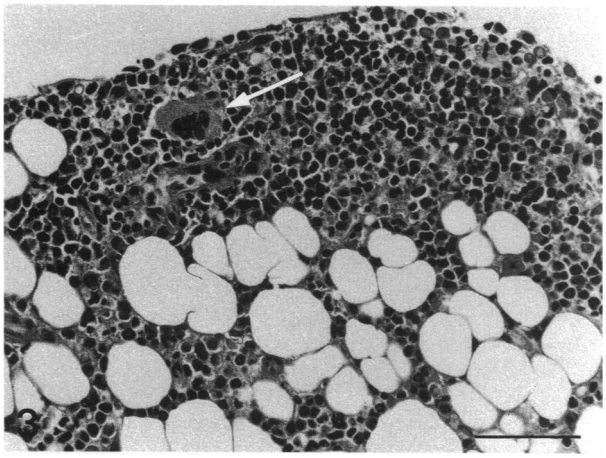

Fig. 3. A splenoportal milky spot. The surface of the milky spot is covered by a layer of mesothelial cells. Beneath the mesothelial layer, abundant lymphocytes and various mononuclear cells are seen. The arrow indicates a megakaryocyte. The milky spot is supplied with blood vessels. No apparent lymphatic vessels are seen. PAS-haematoxylin. Bar, 50 μm.

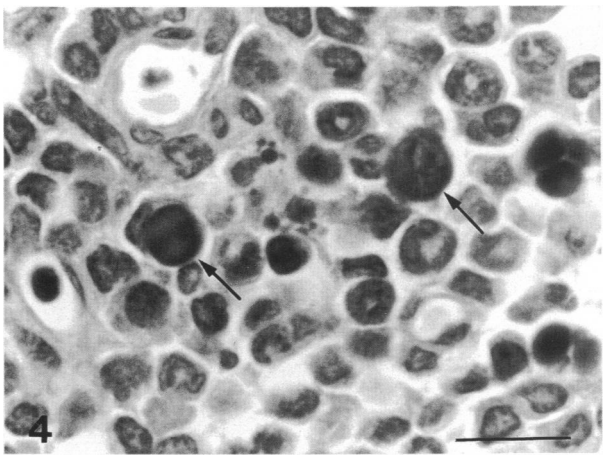

Fig. 4. Clustered neutrophils in various stages of maturation in a splenoportal milky spot. The cells with ring-shaped nuclei represent immature neutrophils (promyelocytes or myelocytes). Arrows indicate PAS-positive plasma cells (Mott cells). PAS-haematoxylin. Bar, 10 μm.

They were supplied by branches from the splenic artery, and were composed of abundant lymphocytes together with macrophages (including monocytes), plasma cells, neutrophils, eosinophils, megakaryocytes, reticulum cells, and various stromal cells (Fig. 3). The surface was covered by a layer of mesothelial cells and occasional macrophages. In the deeper areas, clusters of neutrophils in various stages of maturation with occasional mitotic figures were frequently seen (Fig. 4). The identification of immature neutrophils was based on their ring-shaped nuclei with relatively fine chromatin networks. Megakaryocytes were seen sporadically, although not in all the splenoportal milky spots. The identification of megakaryocytes was easily made, based on their large size (20–40 μm in diameter), multilobulated nuclei, and faintly PAS-positive cytoplasm. The presence of megakaryocytes was confirmed in 95% (19/20) of the splenoportal fat bands by light microscopy. No erythroblasts were seen in splenoportal milky spots by immunohistochemistry for detecting mouse haemoglobin (not shown). Most of the lymphocytes in splenoportal milky spots were positive for immunoglobulins. In particular, IgM-positive lymphocytes were predominant (Fig. 5). Lymphocytes which were faintly or moderately positive for IgG and IgA were also present (not shown). Well developed plasma cells in splenoportal milky spots had strongly PAS-positive cytoplasm (Fig. 4). They were distinctly positive for IgM and faintly positive for IgG and IgA (Fig. 5). Lymphatic vessels, although rare, were seen (not shown).

Reddish rounded nodules observed by dissection

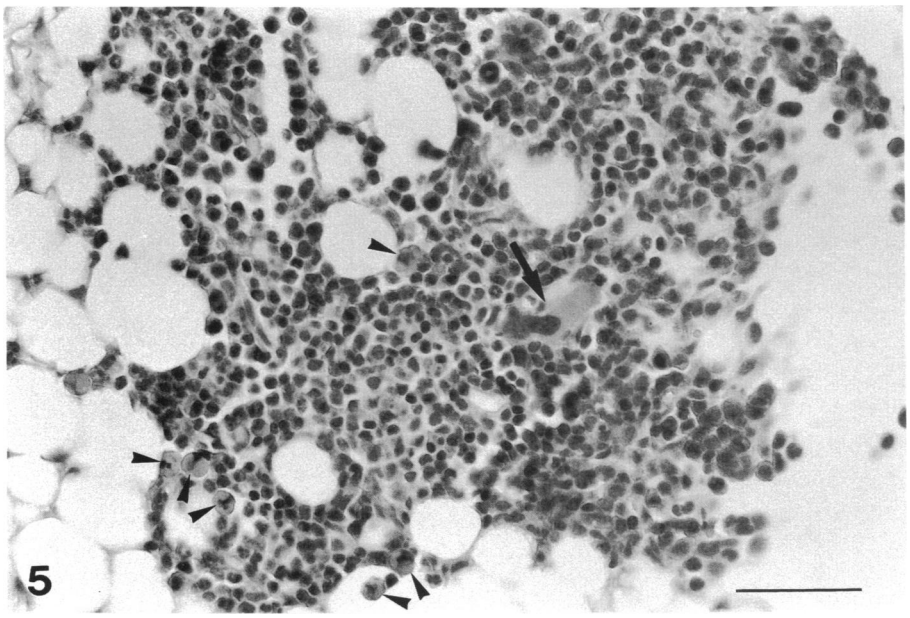

Fig. 5. Splenoportal milky spot stained for IgM. Most of the lymphocytes and plasma cells are positive for IgM. Well developed plasma cells are indicated by arrowheads. A megakaryocyte (arrow) is negative for IgM. Peroxidase-antiperoxidase stain (counterstained with haematoxylin). Bar, 50 μm.

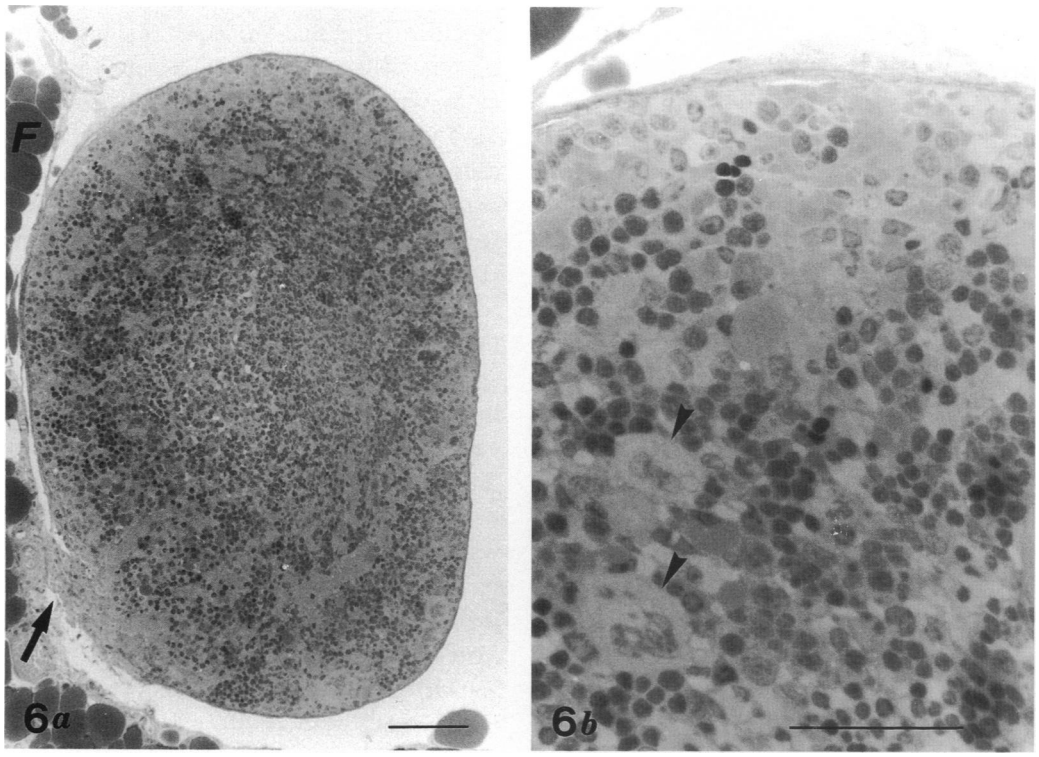

Fig. 6. An aberrant spleen on a splenoportal fat band. A semithin epoxy section stained with toluidine blue. (*a*) The aberrant spleen appears to be an oval nodule encased in a capsule. The central area is occupied by abundant lymphocytes showing a follicle-like structure. A short connective tissue stalk (arrow) links the aberrant spleen to the fat band (F). Bar, 0.1 mm. (*b*) Higher magnification of the subcapsular area. Megakaryocytes (arrowheads), erythroblasts, erythrocytes, and various mononuclear cells including immature myeloid cells (chiefly, immature neutrophils) are seen in this area. Erythroblasts are seen as small mononuclear cells having markedly condensed nuclear chromatin networks, and immature myeloid cells are seen as mononuclear cells having prominent nucleoli and fine chromatin networks. The ultrastructure of this aberrant spleen is shown in Figures 17 and 18. Bar, 50 μm.

light microscopy were found to correspond to aberrant spleens. They were present on the surface of the fat bands. Each aberrant spleen was linked to the fat band by a short connective tissue stalk (Fig. 6). They were completely encased in a capsule, and supplied by branches from the splenic artery. Beneath the

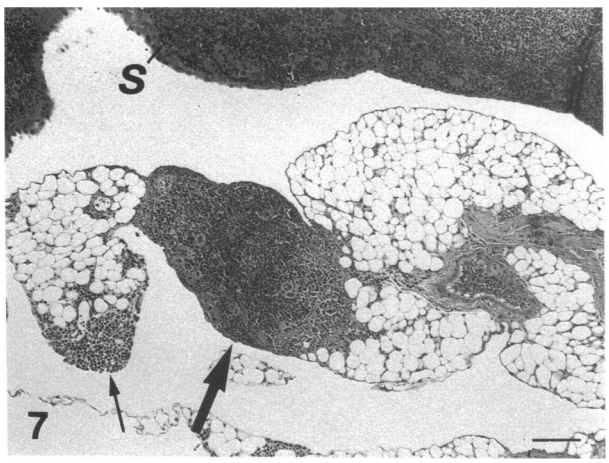

Fig. 7. A splenoportal fat band containing a transitional form and a splenoportal milky spot. The large arrow indicates a transitional form in a splenoportal fat band. The small arrow indicates a splenoportal milky spot. The transitional form contains many megakaryocytes. The central area is occupied by abundant lymphocytes. The transitional form resembles the spleen. S, spleen. Haematoxylin–eosin. Bar, 0.1 mm.

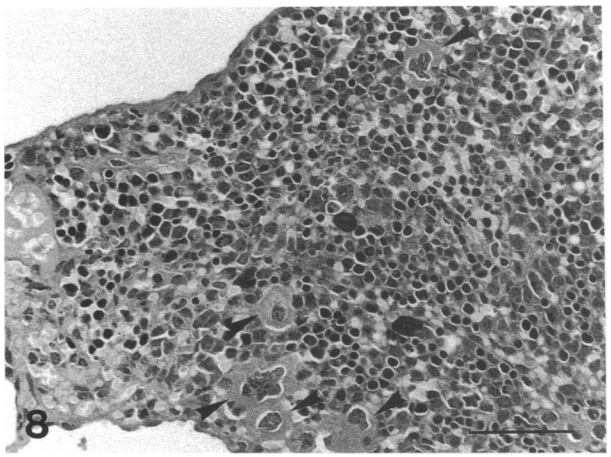

Fig. 8. A transitional form. The surface is covered by a capsule-like structure, beneath which megakaryocytes (arrowheads) are seen. In this micrograph, it is difficult to distinguish erythroblasts from lymphocytes. PAS-haematoxylin. Bar, 50 μm.

capsule, megakaryocytes of various sizes, erythroblasts, erythrocytes and immature and mature granulocytes (chiefly neutrophils) were frequently seen. The central area was densely packed with abundant lymphocytes, forming a follicle-like structure. No fat cells were present in aberrant spleens.

Transitional forms between aberrant spleens and splenoportal milky spots, though sporadic, were admixed with splenoportal milky spots in the fat bands (Fig. 7). The transitional forms were also supplied by branches from the splenic artery. There was a remarkable similarity between transitional forms and splenoportal milky spots in their localisation pattern in fat bands. However, unlike splenoportal milky spots, the surface of transitional forms was covered by a capsule-like structure. Furthermore, megakaryocytes of various sizes, erythroblasts, erythrocytes, and granulocytes (chiefly neutrophils) in various stages of maturation were frequently seen in the transitional forms (Fig. 8). The presence of erythroblasts was confirmed by immunohistochemistry for detecting mouse haemoglobin (Fig. 9). The central area of transitional forms was densely packed with lymphocytes.

Electron microscopy of splenoportal milky spots and aberrant spleens

Electron microscopy revealed the detailed cell composition of the milky spots. The surface of splenoportal milky spots was loosely covered by a layer of mesothelial cells, beneath which abundant lymphocytes were present. In some milky spots, the covering of mesothelial cells was interrupted by intruding macrophages (Fig. 10). The identification of macrophages was based on their indented nuclei, prominent cell membrane invaginations, cytoplasmic dense bodies, cytoplasmic vacuoles, and coated vesicles. The submesothelial and deep areas were occupied by abundant lymphocytes with plasma cells, macrophages (including monocytes), neutrophils, eosinophils, megakaryocytes, reticulum cells and various stromal cells. Plasma cells showed clustering in the milky spots. Some had distended cisterns of rough endoplasmic reticulum that contained amorphous electron-opaque material (Fig. 11), thus exhibiting the features of Mott cells (Alanen et al. 1985). These cells appeared to correspond to PAS-positive plasma cells. Monocytes were sporadically admixed with lymphocytes. The identification of monocytes was based on indented nuclei, moderately condensed nuclear chromatin networks, rounded or elongated granules of various sizes, and occasional cell membrane invaginations (Fig. 12). Bundles of microfilaments were sometimes seen in the cytoplasm. Cells transitional between macrophages and monocytes were occasionally observed. In addition, immature haematopoietic cells of an undetermined nature (Fig. 13) and myeloblast-like cells (Fig. 14) were sporadically admixed with lymphocytes in the deep area of the milky spots. Immature haematopoietic cells showed prominent nucleoli, fine nuclear chromatin networks, and abundant ribosomes, and were poor in organelles.

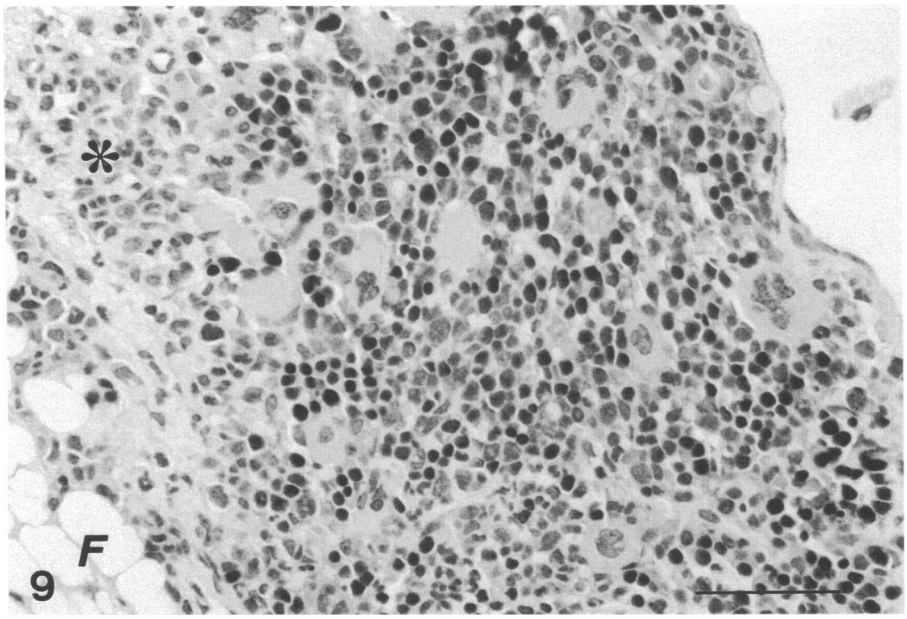

Fig. 9. A transitional form is immunostained for mouse haemoglobin. Erythroblasts and erythrocytes, which are stained brown, are clearly identified by this staining. Many megakaryocytes are present. Clustered neutrophils (asterisk) are seen near the fatty tissue (F). Peroxidase-antiperoxidase stain (counterstained with haematoxylin). Bar, 50 μm.

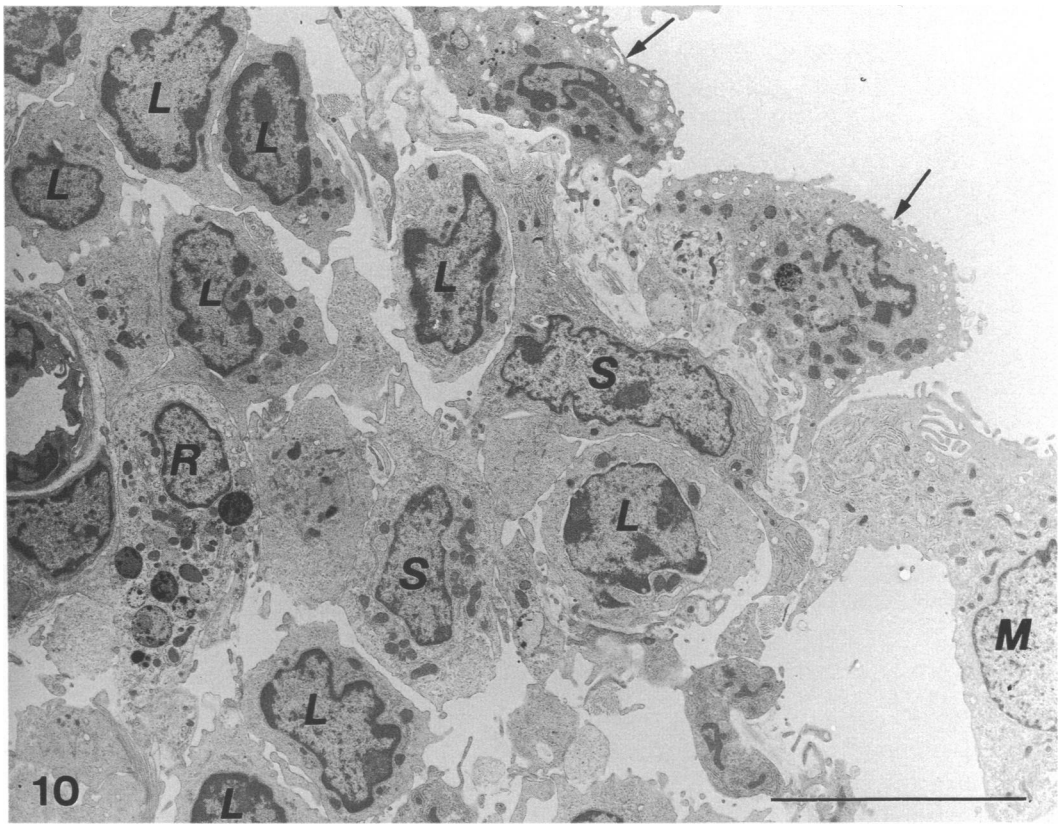

Fig. 10. Electron micrograph of a splenoportal milky spot. The surface is covered by 2 macrophages (arrows) and a mesothelial cell (M), beneath which lymphocytes (L), a reticulum cell with well developed dense bodies (R), and some fibroblast-like stromal cells (S) are seen. Bar, 10 μm.

Myeloblast-like cells showed relatively fine nuclear chromatin networks, prominent nucleoli, somewhat electron-opaque cytoplasm, abundant ribosomes, and occasional rounded cytoplasmic granules ranging from 0.1–0.3 μm in diameter (probable primary granules). Neutrophils in various stages of maturation

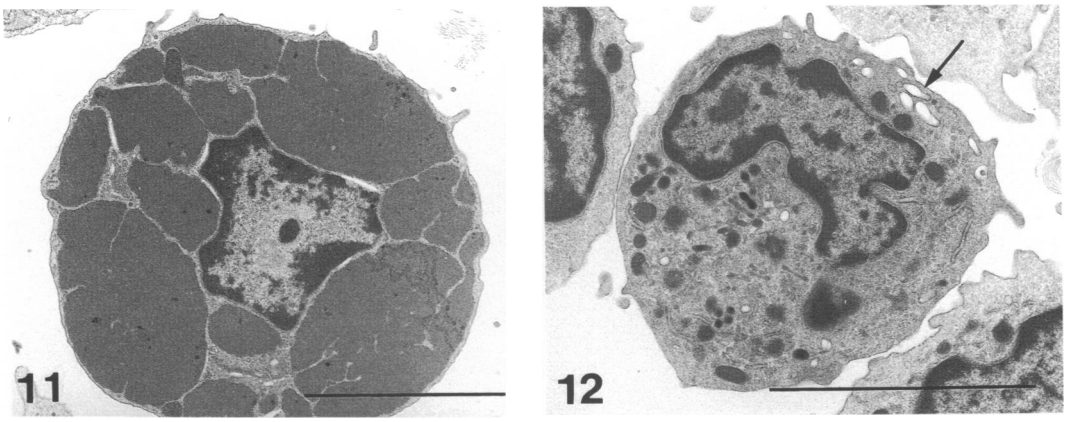

Fig. 11. Electron micrograph of a Mott cell in the deep area of a splenoportal milky spot. The cell shows distended cisterns of rough endoplasmic reticulum containing homogeneous electron-opaque material. Bar, 5 μm.

Fig. 12. Electron micrograph of a monocyte in the deep area of a splenoportal milky spot. The cell shows an indented nucleus, membrane invaginations (arrow), and rounded and elongated granules. Bar, 5 μm.

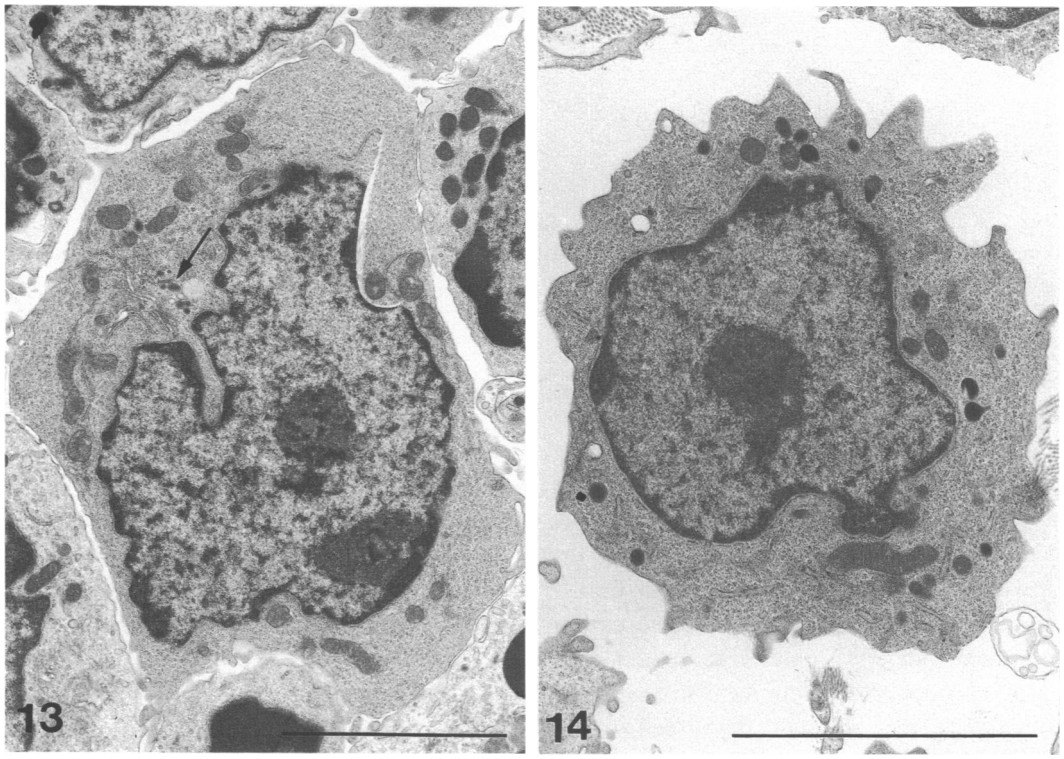

Fig. 13. Electron micrograph of an immature haematopoietic cell of undetermined nature in the deep area of a splenoportal milky spot. The cell shows a prominent nucleolus, a relatively fine nuclear chromatic network, an indented nucleus, abundant ribosomes, and a Golgi apparatus. Near the Golgi apparatus are a number of small granules (arrow). Bar, 5 μm.

Fig. 14. Electron micrograph of a myeloblast-like cell in the deep area of a splenoportal milky spot. The cell shows a prominent nucleolus, a relatively fine nuclear chromatic network, abundant ribosomes, somewhat electron-opaque cytoplasm, and several rounded cytoplasmic granules (probably neutrophilic primary granules). Bar, 5 μm.

showed frequent clustering. In addition, occasional mitotic figures were seen in immature neutrophils (Fig. 15). These findings were considered to represent neutrophilic myelopoiesis. The identification of immature neutrophils was made based on their ring-shaped or slightly indented nuclei, prominent nucleoli, fine nuclear chromatin networks, somewhat electron-opaque cytoplasm, slightly dilated rough endoplasmic reticulum, and rounded granules ranging from 0.1 to 0.3 μm in diameter (primary granules). Mature

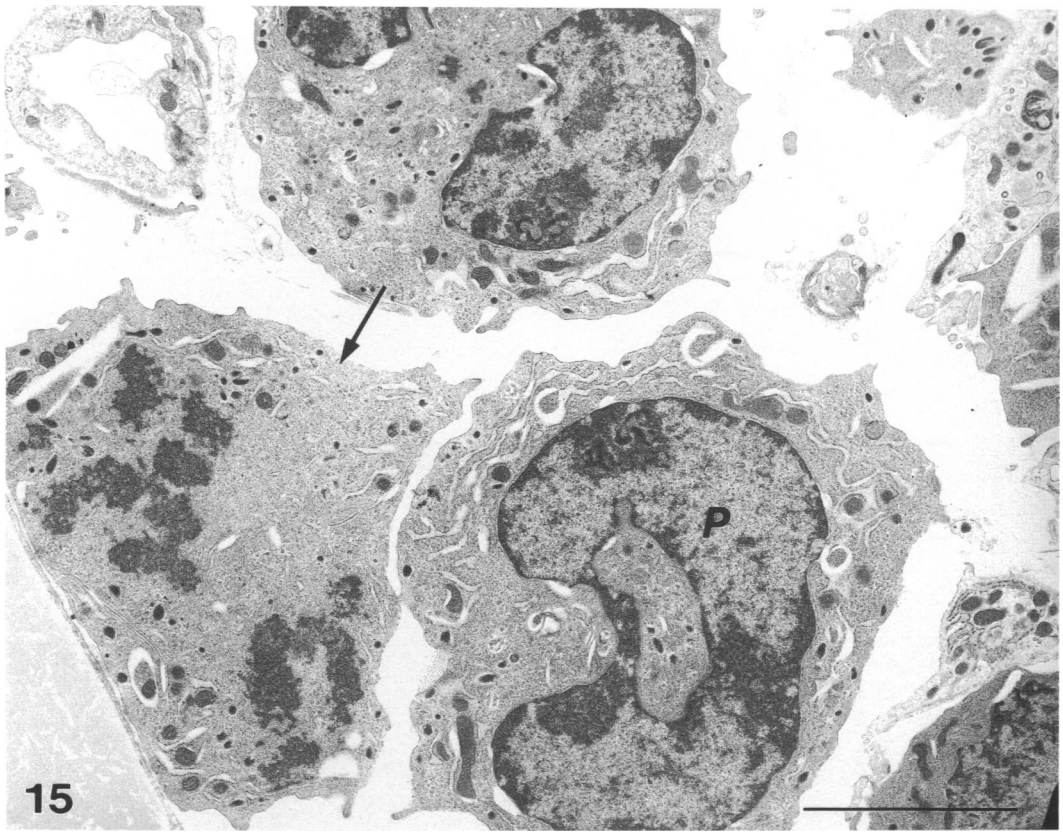

Fig. 15. Electron micrograph of neutrophils in various stages of maturation in the deep area of a splenoportal milky spot. One promyelocyte (P) has a ring-shaped nucleus with a fine chromatin network, slightly dilated cisterns of rough endoplasmic reticulum, and small rounded granules ranging 0.1–0.3 μm in diameter (primary granules). An arrow indicates an immature neutrophil with a mitotic figure. Bar, 5 μm.

neutrophils were easily identified as they showed segmented nuclei with markedly condensed nuclear chromatin networks, electron-opaque cytoplasm, and primary and rod-shape secondary granules. There were sporadic megakaryocytes (Fig. 16). The identification of megakaryocytes was easily made based on the giant size of the cells, multilobulated nuclei, bull's-eye granules (alpha granules), and a demarcation membrane system. No erythroblasts were seen even by electron microscopy. Reticulum cells in the milky spots frequently possessed well developed dense bodies in the cytoplasm (Fig. 10). The cytoplasm of capillary endothelial cells showed multiple fenestrations, about 50 nm in diameter, with a thin diaphragm (not shown).

Unlike splenoportal milky spots, the surface of aberrant spleens was tightly covered by a thick capsule consisting of a superficial layer of mesothelial cells, and underlying layers of fibroblast-like cells with abundant intercellular collagen fibres (Fig. 17). Beneath the capsule, erythroblasts (Figs 17, 18), megakaryocytes (Fig. 18) and granulocytes in various stages of maturation (chiefly, neutrophils) were present. Clustered erythroblasts in various stages of maturation were seen to be admixed with reticulocytes and mature erythrocytes (Fig. 17). Mitotic figures in erythroblasts and denucleating erythroblasts were occasionally observed. These findings clearly indicated true erythropoiesis. The identification of erythroblasts was based on the electron-opaque cytoplasm containing abundant polyribosomes and condensed nuclear chromatin networks. The more haemoglobin the cytoplasm of erythroblasts contained, the more electron-opaque it appeared. Megakaryocytes did not differ from those seen in splenoportal milky spots. Immature or small megakaryocytes were occasionally seen near mature megakaryocytes (Fig. 18). Frequent neutrophilic myelopoiesis and sporadic eosinophilic myelopoiesis were also observed (not shown). The central area of aberrant spleens was occupied by abundant lymphocytes with occasional plasma cells and reticulum cells.

The presence of transitional forms was so sporadic that no transitional forms were found in the fat bands processed for electron microscopy.

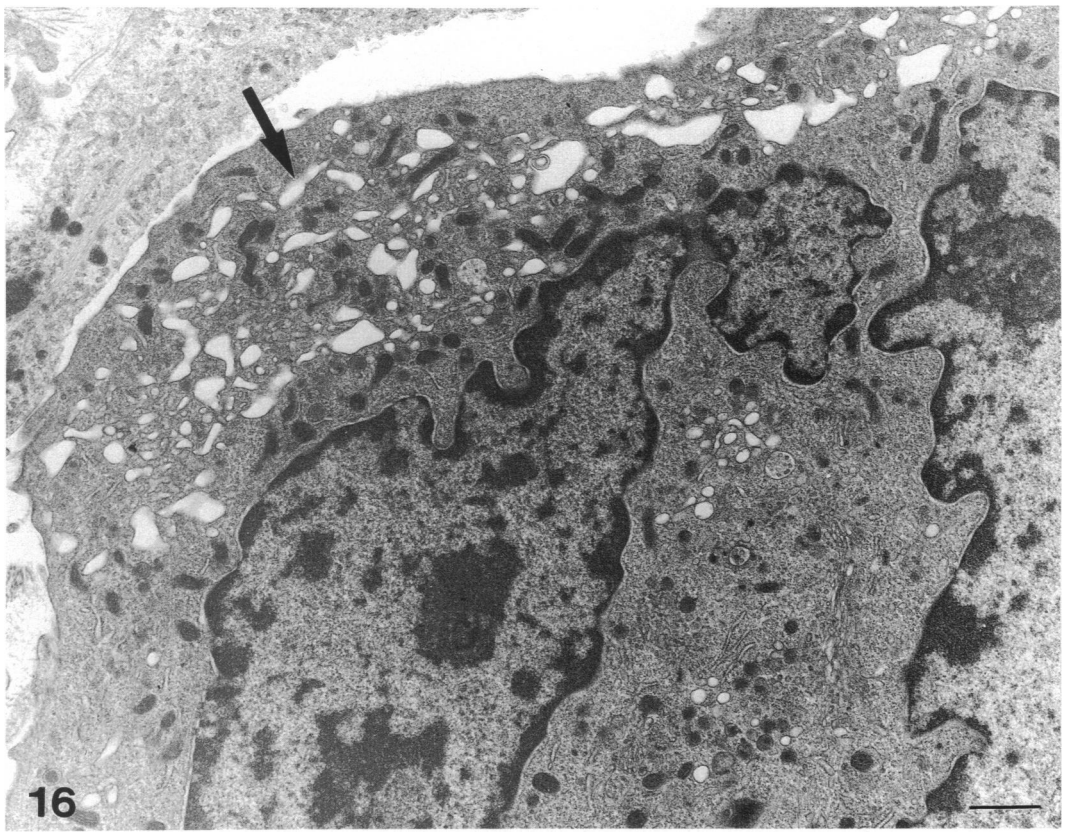

Fig. 16. Electron micrograph of a megakaryocyte in a splenoportal milky spot. The megakaryocyte has multilobulated nuclei with a relatively fine chromatin network. The cytoplasm has a well developed demarcation membrane system (arrow), small rounded granules with a bull's-eye configuration and abundant ribosomes. Bar, 1 μm.

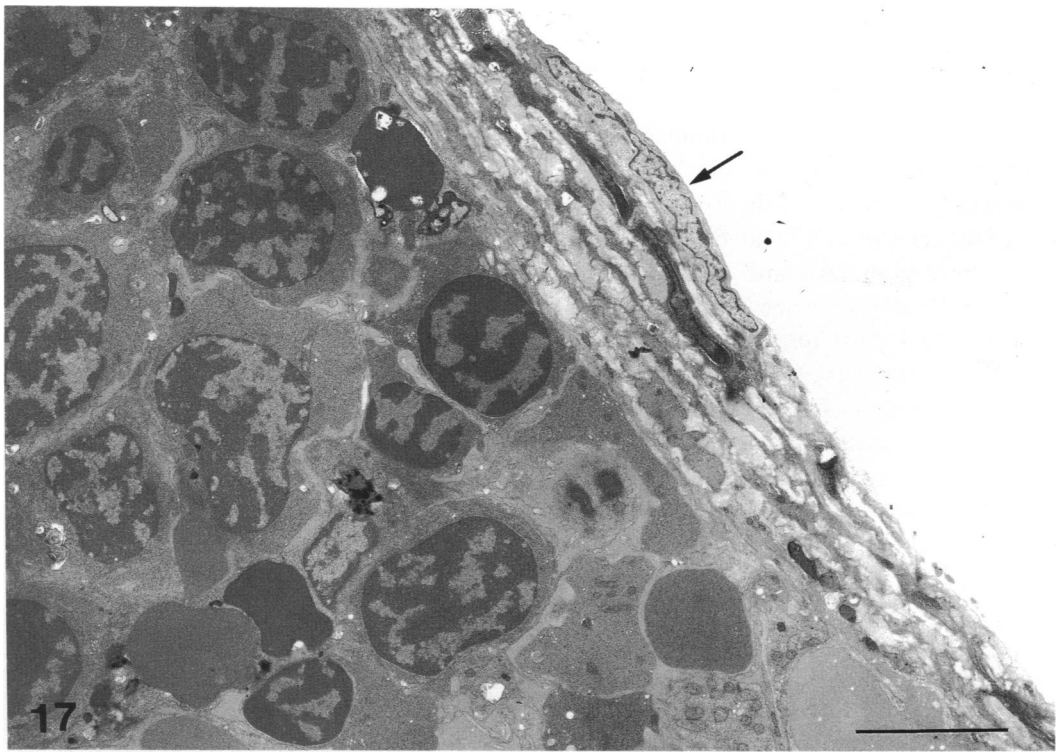

Fig. 17. Electron micrograph of an aberrant spleen. The surface is tightly covered by a capsule. The capsule consists of a superficial layer (arrow) of mesothelial cells, and underlying layers of fibroblast-like cells with abundant intercellular collagen fibrils. Beneath the capsule are many erythroblasts in various stages of maturation, and erythrocytes including reticulocytes. Bar, 5 μm.

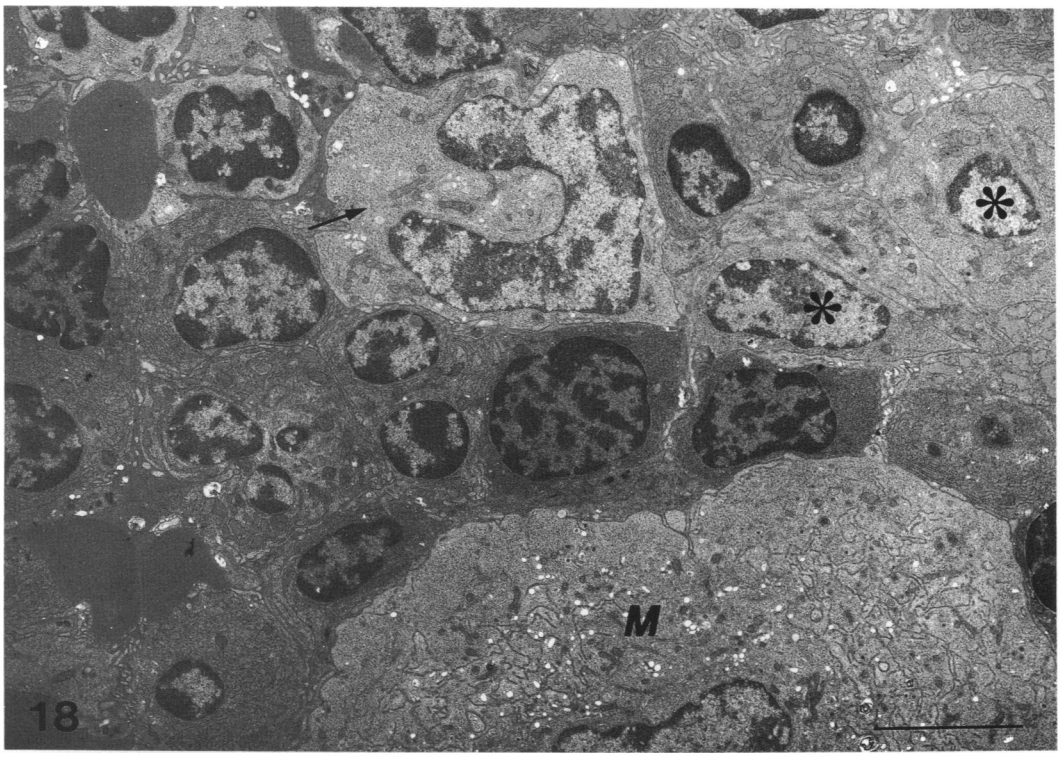

Fig. 18. Electron micrograph of the subcapsular area of an aberrant spleen. A megakaryocyte (M) and an immature megakaryocyte (arrow) are admixed with erythroblasts, plasma cells, lymphocytes and immature haematopoietic cells of undetermined nature (asterisks). Bar, 5 μm.

DISCUSSION

Light and electron microscopy revealed that splenoportal milky spots in NZB mice were composed of abundant lymphocytes with macrophages (including monocytes), plasma cells, neutrophils, eosinophils, megakaryocytes, reticulum cells, and various stromal cells. The basic structure and cell composition of splenoportal milky spots in NZB mice did not differ from those of omental milky spots in normal mice (Hamazaki, 1925; Murata, 1955; Tanaka, 1958; Bartoszewicz & Dux, 1968; Dux et al. 1977, 1986; Takemori, 1979*a*, *b*, 1980; Beelen et al. 1980*a*, *b*; Takemori & Ito, 1980, 1981; Cranshaw & Leak, 1990; Hirai et al. 1992), except that the former contained occasional megakaryocytes. We had already investigated omental milky spots in NZB mice, and separately reported their structural features (Takemori et al. 1994). A comparison between omental and splenoportal milky spots revealed that both possess the same features with respect to their structure and cell composition.

Omental milky spots are known to have the following roles and functions. (1) They represent a major immune system in the peritoneal cavity (Dux et al. 1977, 1986; Beelen, 1991; Shimotsuma et al. 1991). (2) They produce free peritoneal cells, such as macrophages and lymphocytes (Murata, 1955; Tanaka, 1958; Takemori, 1979*a*, 1980; Beelen et al. 1980*a*, *b*; Takemori & Ito, 1981; Cranshaw & Leak, 1990). (3) They are a major gate through which circulating inflammatory cells (granulocytes, monocytes, and lymphocytes) migrate from omental vessels into the peritoneal cavity (Beelen et al. 1980*a*, *b*; Takemori & Ito, 1981; Cranshaw & Leak, 1990). (4) They play an important role in scavenging foreign substances in the peritoneal cavity (Webb, 1931; Murata, 1955; Takemori & Ito, 1980, 1981). (5) They produce ascites by filtering fluid from the capillaries to the peritoneal cavity (Bartoszewicz & Dux, 1968; Cranshaw & Leak, 1990). (6) They show extramedullary neutrophilic myelopoiesis (Takemori, 1979*a*, 1980; Hirai et al. 1992; Takemori et al. 1994) and probable megakaryopoiesis (Takemori et al. 1994).

Little is known about the haematopoietic ability of milky spots. In splenoportal milky spots in NZB mice, clustered neutrophils in various stages of maturation with occasional mitotic figures were seen by light and electron microscopy. These findings seem to represent true neutrophilic myelopoiesis. Similar neutrophilic myelopoiesis was also confirmed in aberrant spleens, and in transitional forms. The presence of megakaryocytes in splenoportal milky spots was so sporadic that it was difficult to find immature megakaryocytes and their mitotic figures. It was not easy to

identify megakaryoblasts or their precursor cells in the milky spots solely by conventional light and electron microscopy. Nevertheless, considering the giant size of megakaryocytes, it is apparent that they did not migrate directly into the milky spots from other haematopoietic organs. A possible explanation for the presence of megakaryocytes in splenoportal milky spots is that megakaryocytic precursor cells or stem cells, which might have migrated into the milky spots from the bone marrow or spleen, differentiated and developed into mature megakaryocytes. If this deduction is correct, the presence of megakaryocytes, although sporadic, may be interpreted as probable megakaryopoiesis. Mouse spleens are known to have megakaryopoietic ability and to contain abundant megakaryocytes (Seifert & Marks, 1985). Aberrant spleens and transitional forms also showed frequent megakaryocytes, as in mouse spleens. In addition, megakaryocytes in various stages of maturation were also seen in aberrant spleens by electron microscopy. Thus the frequent presence of megakaryocytes in these tissues can be interpreted as megakaryopoiesis. No erythroblasts were seen in splenoportal milky spots. On the other hand, clustered erythroblasts of various maturation stages, dividing erythroblasts, and reticulocytes were observed in aberrant spleens by light and electron microscopy. Transitional forms also contained many erythroblasts and erythrocytes. These findings seemed to indicate that erythropoiesis was present both in aberrant spleens and in transitional forms. Here we should emphasise that neither megakaryocytes nor erythroblasts were present in omental milky spots or splenoportal milky spots in normal mice (unpublished data). What is the difference between NZB and normal mice? Raveche et al. (1983) reported that NZB mice had increased stem cell activity. In fact, haematopoietic cells of an undetermined nature, and myeloblast-like cells are occasionally seen in splenoportal milky spots as well as in omental milky spots (Takemori et al. 1994) in NZB mice. To demonstrate the presence of pluripotent or committed stem cells in milky spots, sophisticated immunocytochemistry using specific antibodies for committed stem cells is needed. However, splenoportal fat bands are unsuitable for immunocytochemistry because the abundance of fat cells makes cryosectioning very difficult. Thus a less sensitive immunocytochemical method using deparaffinised sections was available in this study.

With respect to the nature of omental milky spots, Koten & Otter (1991) reported that omental milky spots might represent an intestinal thymus. As shown in the present study, most of the lymphocytes in splenoportal milky spots were composed of immunoglobulin-positive lymphocytes (B cells). Similar findings were seen in omental milky spots in NZB mice (data will be reported separately). In other words, non-B cells, probable T cells, were far less frequent than B cells in omental and splenoportal milky spots. Concerning T cells, Dux et al. (1986) and Shimotsuma et al. (1991) reported that they are present in omental milky spots. We also attempted to demonstrate the presence of T cells in splenoportal milky spots; however, their presence could not be confirmed because of marked nonspecific reactions. Generally, there is no relation between the thymus and omental milky spots from anatomical or histological points of view. Thus the claim of Koten & Otter (1991) is improbable. On the other hand, we have surmised that omental milky spots may represent splenoid lymphoid tissues (Takemori et al. 1994). This speculation was derived from the following features of omental milky spots in normal mice. (1) They show neutrophilic myelopoiesis (Takemori, 1979*a*, 1980; Hirai et al. 1992). (2) Erythropoiesis can be induced in omental milky spots by the intraperitoneal administration of erythropoietin (Hirai et al. 1992). (3) Sporadic megakaryopoiesis is seen in omental milky spots in NZB mice (Takemori et al. 1994). (4) Unlike lymph nodes, they are mainly supplied by blood vessels and poorly by lymphatic vessels. (5) Omental milky spots located in the left half of the omental fat band are supplied by the left gastroepiploic artery which is a branch of the lienopancreatic artery (Fig. 1). In other words, omental milky spots share the same blood vessel with spleens. As already stated, spleens in mice are known to show active haematopoiesis, including granulopoiesis, erythropoiesis, and megakaryopoiesis (Seifert & Marks, 1985). On the other hand, lymph nodes in adult mice never show extramedullary haematopoiesis. The neutrophilic myelopoiesis, inducible erythropoiesis, and sporadic megakaryopoiesis in omental milky spots may indicate that they resemble spleens rather than lymph nodes. In terms of their vascularisation, there is a close relationship between omental milky spots and spleens. Nevertheless, the above-described features appear to give indirect evidence for our hypothesis. To establish this, we investigated splenoportal fat bands in NZB mice because the fat bands contain aberrant spleens and transitional forms, as well as splenoportal milky spots. Aberrant spleens were rounded, reddish nodules with a follicle-like structure. They were present on the surface of the fat bands, and were completely encased by a capsule. They showed active extramedullary haematopoiesis, including megakaryopoiesis, erythro-

poiesis, and granulopoiesis. Aberrant spleens thus seemed to resemble spleens both in structure and cell composition. Transitional forms also showed megakaryopoiesis, erythropoiesis and neutrophilic myelopoiesis. Their surface was covered by a capsule-like structure. These findings show that transitional forms resemble aberrant spleens to some extent. Nevertheless, the localisation pattern of transitional forms was different from that of aberrant spleens; they were located with splenoportal milky spots in the fat bands. In other words, transitional forms possess intermediate features between aberrant spleens and splenoportal milky spots. The morphological transition from aberrant spleens, via transitional forms, to splenoportal milky spots seems to indicate that splenoportal milky spots represent splenoid lymphoid tissue. Taking into account the similarity between omental and splenoportal milky spots, the deduction that omental milky spots also represent splenoid lymphoid tissues may be made.

It is well known that NZB mice represent a pathological strain. The use of this strain may bias interpretation of the data. Nevertheless, findings obtained from NZB mice may be helpful in understanding the nature of milky spots. Extreme examples may sometimes provide suggestive information.

Many investigators have used peritoneal macrophages and lymphocytes for various immunological experiments because these cells are present in abundant numbers in the peritoneal cavity. However, there are few investigators who appreciate the origin of these cells in connection with milky spots. Furthermore, no one has recognised the splenoid nature of milky spots. Here, we emphasise that recognition of the splenoid nature of milky spots is a prerequisite for a correct evaluation of the data derived from the experiments using a peritoneal immunological system.

In conclusion, splenoportal milky spots, aberrant spleens, and transitional forms coexisted in splenoportal fat bands in NZB mice. They were supplied by branches from the splenic artery. Furthermore, the morphological transition from aberrant spleens, via transitional forms, to splenoportal milky spots was confirmed. These findings appear to indicate that splenoportal milky spots represent splenoid lymphoid tissues.

ACKNOWLEDGEMENTS

The authors thank Mr D. Hamilton and Miss J. Cosgriff for preparing the manuscript, and Mr K. Miyakawa and Miss M. Yamamoto for their technical assistance.

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