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Different patterns of spleen involvement in systemic and malignant mastocytosis

A histological and immunohistochemical study of three cases

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Summary. Three cases of splenic involvement in three different types of generalized mastocytosis (systemic mast cell disease) are reported. The macroscopic, histological and ultrastructural modifications of the spleen are described. Each case exhibited a different morphological pattern. Giemsa staining, fluorescence after acridine orange staining and naphthol ASD chloracetate esterase reaction are shown to be valuable for diagnosis. By comparison, immunohistochemistry seemed not to be very useful, because no specific antigens are expressed. These findings are compared to previously published cases. Their value for the diagnosis and the prognosis are discussed.

Key words: Generalized mastocytosis – Systemic mast cell disease – Splenic mastocytosis – Mastocytosis with acute leukaemia

Introduction

In mast cell neoplasia, involvement of the spleen is frequent. Generalized mastocytosis (GM) (Lennert and Parwaresch 1979; Parwaresch et al. 1985), also called systemic mast cell disease (SMCD) (Travis and Li 1988; Travis et al. 1988a), includes different types of disease. Parwaresch et al. (1985) described a generally benign form called systemic mastocytosis (SM), with frequent skin involvement and an aggressive form, malignant mastocytosis (MM). Travis et al. (1988a) and Travis and Li (1988) described an indolent type of SMCD, an aggressive type of SMCD (non-leukaemic) and a type of SMCD associated with haematological disorders (Travis et al. 1988b). Both classifications also recognize mast cell leukaemia (Lennert and Parwaresch 1979; Travis et al. 1986) and mast cell sarcoma (Lennert and Parwaresch 1979; Parwaresch et al. 1985). Here we present three cases, showing the different patterns of splenic involvement in SM or indolent SMCD, in MM or aggressive SMCD and in SMCD with haematological disorder. The diagnostic and prognostic value of histopathology is discussed.

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Materials and methods

Tissue samples from spleen and lymph nodes were fixed in 4% buffered formalin solution and Bouin's solution. After paraffin embedding, 4-μm-thick tissue sections were stained with haematoxylin and eosin, periodic acid-Schiff (PAS), Giemsa, toluidine blue and alcian blue at various pHs, Gordon and Sweets' reticulin stain, and Perl's stain. Fluorescence of the granules after acridine orange staining was studied under ultraviolet illumination. Naphthol ASD chloracetate esterase activity was demonstrated according to Leder's method. Immunohistochemistry using peroxidase as a marker with an avidin-biotin complex amplification was performed on paraffin-embedded tissue with the following polyclonal and monoclonal antibodies: anti-IgG, -IgA, -IgM, kappa and lambda light chains, anti-lysozyme, anti-alpha-1-chymotrypsin and anti-alpha-1-antichymotrypsin, S100 protein, anti-macrophage KP1 (CD68) and anti-elastase NP 57 (kindly provided by Dr. Mason, Oxford, UK); B markers: LN1, LN2, MB2, L26 (CD 20); a series of B markers kindly provided by Dr. G. Delsol (Toulouse, France): BNF13, DBB42, DND53, DBA44 and also BNH9; T markers: MT1, UCHL1 (CD45RO); granulocytic markers: Leu M1 (CD15), markers of activation Ber H2 (CD30) and epithelial membrane antigen.

Unfortunatly no fresh tissue was available for immunohistochemistry or histoenzymology of frozen sections. Tissue samples were available for electron microscopy in cases 1 and 2.

Clinical data

Case 1. A splenomegaly was discovered in a 42-year-old woman in February 1987. There was no hepatomegaly and no peripheral lymphadenopathy. Abdominal echography and a thoraco-abdominal scan confirmed the presence of a homogeneous splenomegaly (165 mm in length) and disclosed paraaortic lymphadenopathy. Physical examination revealed multiple small, brownish skin lesions, often centred by telangiectasia, on the face, the neck and

the anterior part of the thorax. According to the patient, these skin lesions had been present since approximately the age of 15 years. The skin biopsy confirmed the clinical diagnosis of urticaria pigmentosa in the form of "telangiectasia macularis eruptiva perstans". Gastroduodenal endoscopy was normal, without symptoms of portal hypertension. No osteosclerosis was detected on skeletal radiography. The haemoglobin (Hb) was 12.3 g/dl and the haemogram was normal. Sternal puncture showed a slight dyserythropoiesis. The levels of blood folate and cyanocobalamin were normal, as were fibrinogen and Coombs' tests. Bone marrow biopsy showed global hyperplasia without osteosclerosis but with a dense reticulin framework. No mast cell infiltrate could be discovered.

The patient complained for several years of paroxysmal tachycardia, allergy to acetylsalicylic acid and non-steroidal anti-inflammatory drugs and intolerance to some foods and to alcohol.

A splenectomy was performed in April 1988. Splenic hilar, mesenteric and pre-aortic lymph nodes were biopsied.

In February 1989, the patient was in good health, but new clinical and biological symptoms appeared. Hepatomegaly was found in August 1988. Osteosclerosis was recognized on radiography of the femoral heads, of the hips and iliac bones, associated with band-like osteoporosis of the radial and cubital metaphysis. An MDP-technetium bone scintillogram showed an abnormal repartition of the marker with osteogenetic activity in the metaphysis of the bones of the superior and inferior members. A diffuse bone marrow infiltrate was demonstrated by nuclear magnetic resonance imaging in both iliac bones. The haemogram in February 1989 showed 9.8×10^9/l leucocytes with 980/ml eosinophils, 607000/ml platelets, but no myelaemia. Blood histamine was at that time higher than normal (146 ng/ml). This case can be interpreted as SM with skin involvement in Lennert's group classification and as indolent SMCD as defined by Travis et al.

Case 2. In June 1988, an acute myeloblastic leukaemia was diagnosed in a 17-year-old woman. She suffered from fever and weight loss. Physical examination found no peripheral lymphadenopathy, no hepatomegaly, but splenomegaly was present. The haemogram showed anaemia (Hb 6.3 g/dl), thrombocytopenia (50×19^9 l), leucocytosis (31×10^9/l) with 87% myeloblasts, some with Auer rods. Sternal puncture confirmed the diagnosis of M2 (French, American, British classification) acute myeloblastic leukaemia and showed 0.5–1% normal mast cells, 1.5% normal basophilic granulocytes and 4–5% of cells measuring 15–30 μm in diameter, with bi- or multilobated nuclei and presenting azurophilic or basophilic granules and small clear vacuoles. These cells were probably abnormal mast cells. Cytogenetic study performed on peripheral blood blasts showed a t8–21 translocation.

The patient was treated with doxorubicin and cytarabine, the induction course being given in June 1988. In October 1988, she was still in complete remission but the splenomegaly had not disappeared. A splenectomy with liver biopsy was performed. In December 1988, the patient was treated by a bone marrow autograft. On day 50, she was in good health, without hepatomegaly or lymphadenopathy. The Hb level was 12.9 g/dl, and the haemogram showed a leucocytosis 11×10^9/l, with 5700/ml lymphocytes and 101000/ml platelets, without blasts. In June 1989, the patient relapsed and had 22.6×10^9/l leucocytes in the peripheral blood with 54% blasts and 56% blasts in the sternal marrow. Lumbar puncture was normal. The same cytogenetic pattern was again demonstrated (45xx, t8,21). A second complete remission was obtained after chemotherapy (mitozantrone, cytarabine) in August 1989. A second relapse occured in December 1989 and treated, with apparently complete remission.

During these relapses no abnormal mast cells could be found in the blood. Blood and urine histamine were not increased. Radiography of the skeleton showed only a slight densification of the bone structure of the iliac bone near the left sacro-iliac articulation.

This case falls into the SMCD with associated haematological disorder for Travis et al. and to SM with acute leukaemia of Lennert's group.

Case 3. In January 1988 a 21-year-old female complained of high fever with erythematous skin lesions. Physical examination disclosed hepatosplenomegaly but no peripheral lymphadenopathy. Biological data were as follows: Hb 8.5 g/dl; haemogram: 11.5×10^9/l leucocytes with eosinophilia (35%) and 128000/ml platelets. Sternal puncture showed a mast cell infiltrate but no myeloproliferative disease. The mast cells exhibited large, clear nuclei with medium-sized nucleoli. The cytoplasm contained a small number of granules and many clear vacuoles. Some mast cells showed erythrophagocytotic activity. No bone marrow biopsy was performed.

Coombs' tests were negative and protein electrophoresis was normal. Chest radiographs were also normal. A splenectomy was performed. The patient died a month after the intervention. This case can be classified as MM or an aggressive SMCD.

Results

In case 1, the spleen weighed 600 g, and measured $18 \times 12 \times 6$ cm. The parenchyma was deep purple and showed no discrete tumour. Small lymph nodes were found in the splenic hilum.

Microscopically the splenic architecture was well preserved. The follicles each exhibited a small germinal centre with normal mantle and marginal zones containing some immunoblasts. Mast cell infiltration was seen exclusively in the white pulp. The initial lesion seemed to be an accumulation of mast cells between the fibroblasts of the arteriolar adventitia. The earliest lesion therefore appeared to be in the periarteriolar lymphoid sheaths and around the centrofollicular arteries. When the number of mast cells increased, round nodules were formed. Mast cells progressively replaced the lymphocytes from the periarterial sheaths. The infiltrate penetrated the follicles, appearing as round pale nodules or crescents, (Fig. 1) extending from the periarteriolar sheets to the marginal zones (Fig. 2). In some sections, where the mast cells were numerous around a centrofollicular artery, they could appear to be similar to a germinal centre (Fig. 1). The large septa were infiltrated only at the periphery, where the arterioles were entering the splenic parenchyma. No infiltrate was seen around large vessels. The mast cells had a pale or faintly eosinophilic cytoplasm after haematoxylin and eosin staining (Fig. 2, inset) but a large number of metachromatic granules were easily recognized after Giemsa staining, although a few cells exhibited only a few granules. The nuclei were always oval, often elongated but never really fusiform; the membrane was thin and the chromatin finely punctate. One or two small nucleoli were present. The mast cells were associated with numerous dispersed eosinophilic granulocytes; iron-laden macrophages were also seen and some plasma cells were present at the periphery of the infiltrated periarterial sheaths. In and around the mast cell infiltrates, fine collagenous bands were present, with activated fibroblasts recognizable due to their large pale nuclei each with a medium-sized nucleolus. In the marginal zone, activated follicular dendritic cells exhibited large pale nuclei. The red pulp was normal without any mast cell infiltrate. Some macrophages containing haemosiderin and eosinophils and plasma cells were seen in the cords. The hilar lymph nodes were normal.

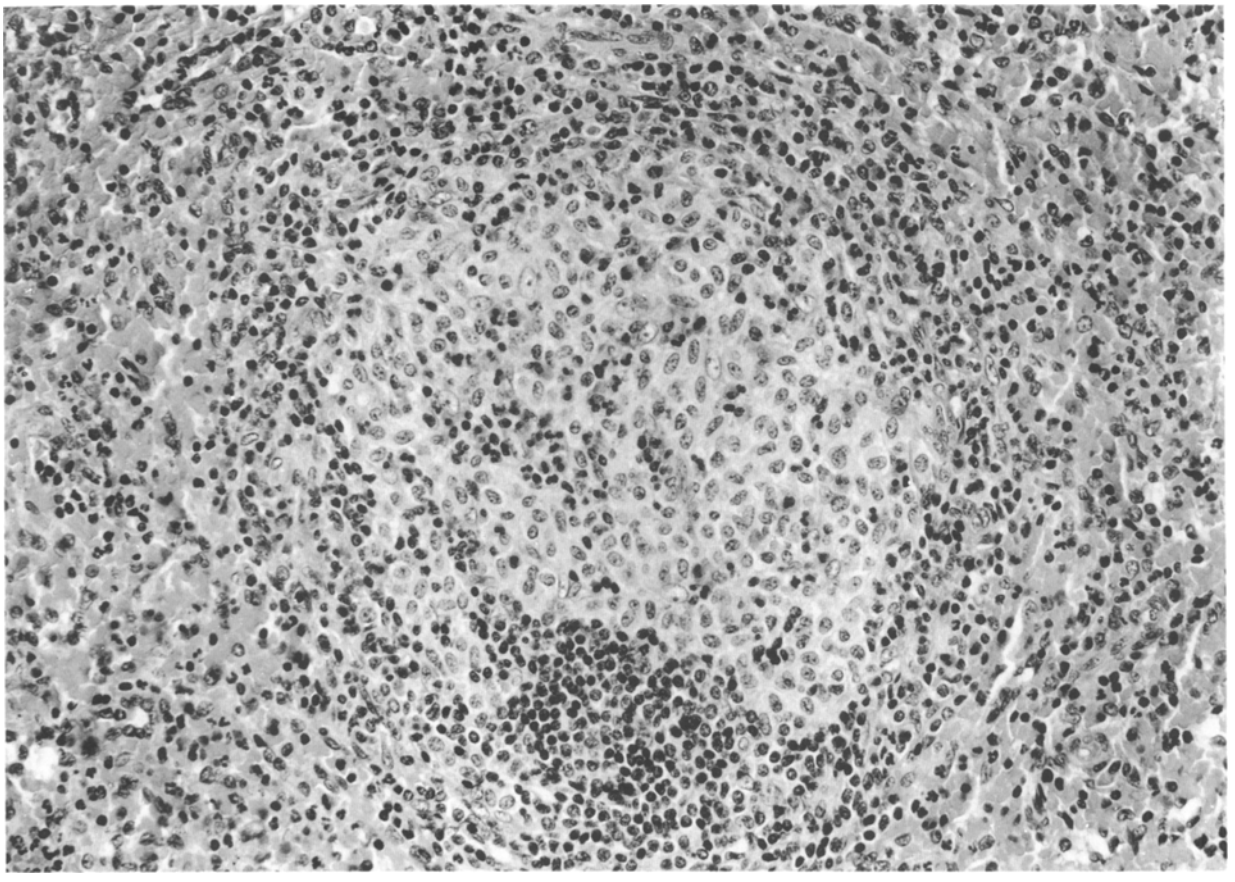

Fig. 1. Case 1. Splenic nodule constituted by mast cells in a follicle. Haematoxylin and eosin, ×310

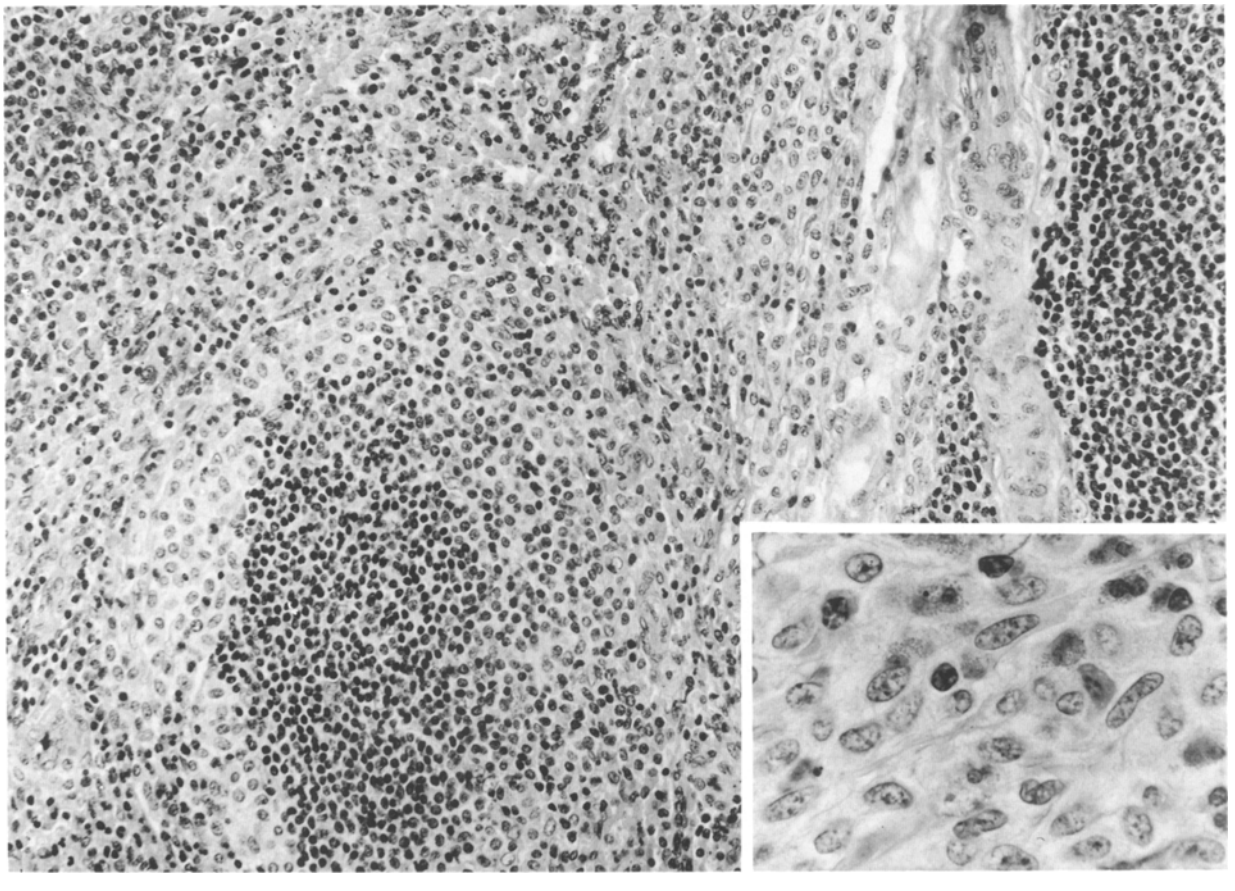

Fig. 2. Case 1. Another follicle is partly surrounded by a crescent of mast cells infiltrating the mantle zone. Inset (×700): mast cells have regular, oval or elongated nucleus with small nucleoli. The cytoplasm is large, clear, and faintly granular. Haematoxylin and eosin, ×260

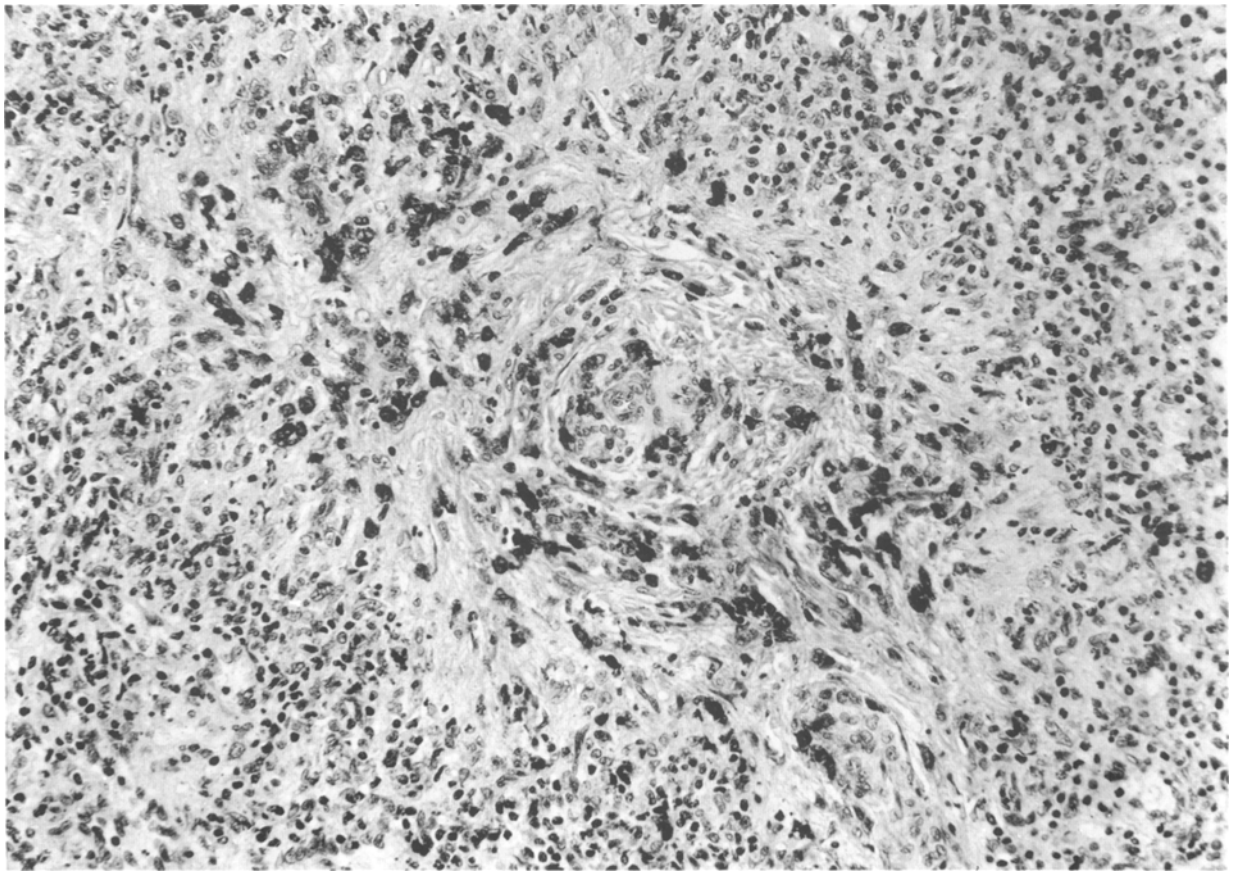

Fig. 3. Case 2. The follicle is partly destroyed by a large fibrous scar, heavily infiltrated by mast cells. Giemsa, ×260

Electron microscopy showed that the white pulp mast cells were associated in close contact with many eosinophils (5 eosinophils per mast cell). Mast cells were activated with numerous interdigitating cytoplasmic extensions, many vacuoles and a variable number of normal granules. Eosinophil granules were often heterogeneous, with alteration of the crystalloid and sometimes of the matrix. Some eosinophils exhibited degranulation lysis. Charcot-Leyden crystals were seen in the extracellular spaces.

The diagnosis was SM according to Parwaresch et al. (1985) or indolent SMCD (Travis et al. 1988a; Travis and Li 1988).

In case 2, the spleen weighed 330 g and measured 17 × 6 × 5 cm. Small white macroscopic nodules were visible. The most important finding in this case was the extent of fibrosis. All the lymphoid tissue appeared to have been destroyed by collagenous fibrosis (Fig. 3). This fibrosis caused either collagenous scars replacing the follicles or perifollicular systematized concentric bands. Collagenous bands were also seen around the centrofollicular arterioles replacing the lymphoid periarteriolar sheets. The same banding pattern was observed around the septa. The preserved red pulp therefore appeared as irregularly shaped areas surrounded by fibrotic areas. In the fibrosis, remnants of lymphoid tissue could be seen, but the architecture was completely destroyed. No germinal centres, no mantle zones, no marginal zones, and no periarteriolar sheaths were recognized. A large number of iron-laden macrophages were present, dispersed or in small clusters. Some plasma cells were also seen, mainly at the periphery of the fibrosis, replacing the periarteriolar sheaths. Only a careful search disclosed small groups of cells with faintly eosinophilic cytoplasm dispersed between the collagenous bands, around the vessels or dispersed in between the lymphocytes and macrophages. Giemsa staining was very useful, showing an unexpectedly large number of mast cells, the majority containing a large number of metachromatic granules (Fig. 4A, B). Nevertheless, many were degranulated to some extent, with pale cytoplasm. Hence the number of granules varied greatly among the cells. A few eosinophils were dispersed in the mast cell infiltrate. Giemsa staining clearly revealed the destruction of the lymphoid tissue by fibrosis and mast cell infiltrates. It showed also the predominance of mast cell infiltrates around the centrofollicular and terminal arterioles and around capillaries. In the red pulp, a few mast cells were seen around the terminal arteries in the cords.

At the ultrastructural level, mast cells could be recognized in the white pulp mainly clustered around arterioles and capillaries. Some eosinophils were associated with the mast cells (1 per 5 mast cells). The mast cells

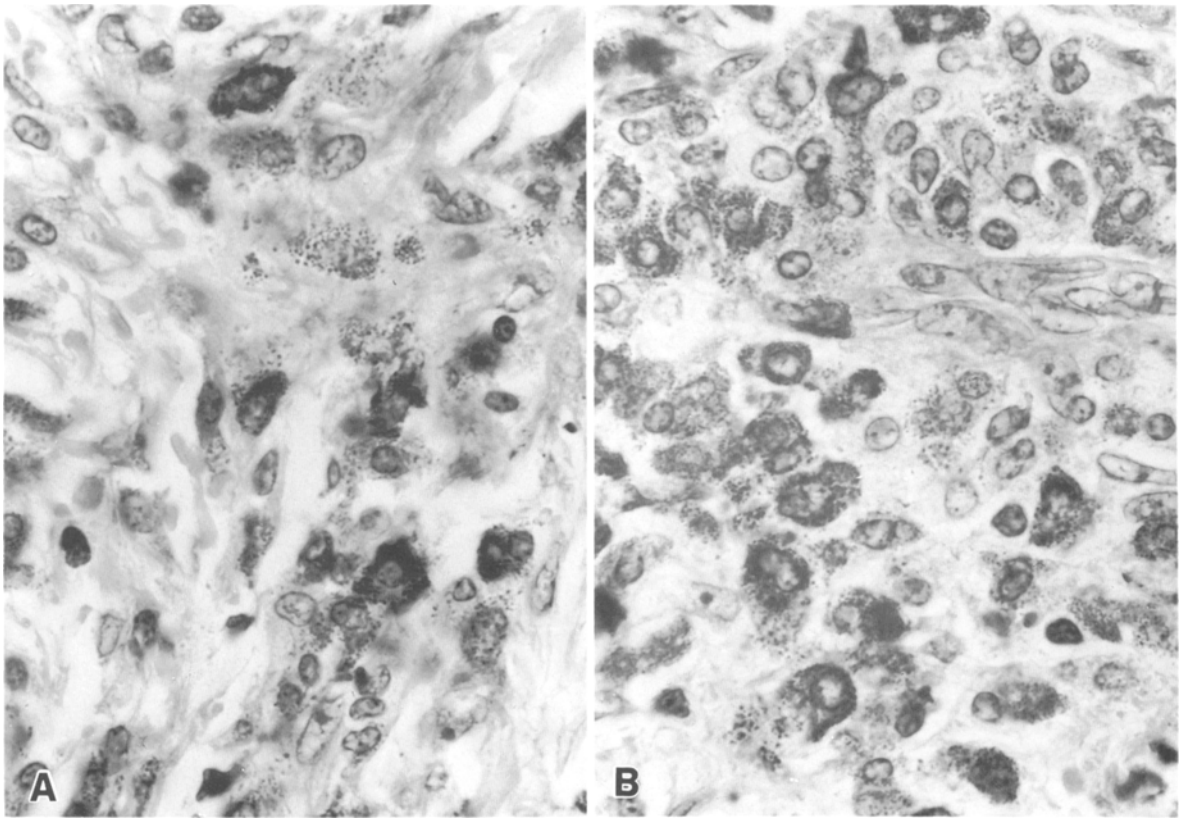

Fig. 4A, B. Case 2. These different fields show: the morphology of mast cells containing a variable quantity of metachromatic granules; the variable amount of collagen; and the perivascular localization of the mast cells. Giemsa, × 100

contained a variable number of granules and many vacuoles. One-third exhibited intact granules. One-third were completely degranulated. The remaining third displayed cytoplasmic vacuolization with degenerative changes of the organelles and granules. Between the mast cells, numerous fibroblasts with thick bundles of collagen were detected.

The liver biopsy showed normal hepatic architecture with many mast cells associated with eosinophils, lymphocytes and plasma cells infiltrating the portal spaces, and clusters of mast cells were seen in the intralobular sinusoides. Large strands of collagen fibres enlarged the portal spaces without cirrhosis.

This case was diagnosed as SM (Parwaresch et al. 1985) or SMCD associated with myeloblastic acute leukaemia (Travis et al. 1988 b) in remission.

In case 3, the spleen weighed 1300 g and exhibited a dark red parenchyma without tumour or nodules. The follicles were atrophic, represented by small round clusters of lymphoid cells without germinal centres. The architecture of the red pulp was preserved. The cords and, to a lesser degree, the sinuses were infiltrated by mast cells, associated with nests of erythroblasts, myelocytes and megakaryocytes (Fig. 5). Histiocytes with erythrophagocytosis and haemosiderin were also present. Extensive fibrosis had developed in some cords. Mast cells had also infiltrated the atrophic lymphoid tissue. Most of the mast cells contained numerous metachromatic granules, but many were partially degranulated. The nuclei in most cells were irregular, resembling nuclei of interdigitating or Langerhans cells; in others they were large, pale, and with thick membranes. A few were multilobated, giving the appearance of bi- or trinucleated cells. Most of these irregular nuclei showed medium-sized or large nucleoli. Numerous eosinophils, either myelocytes or granulocytes, were associated with the mast cells in the white and red pulp (Fig. 6A). Megacaryocytes and erythroblasts could also be recognized (Fig. 6B). One point should be stressed: there was no tumour nodule and no clusters of mast cells. These cells infiltrated the splenic parenchyma diffusely and were dispersed in between the cells of the myeloid metaplasia and the reactive cells. The liver biopsy showed portal infiltration by mast cells. In the sinusoides there were many partially degranulated mast cells associated with neutrophil and eosinophil myelocytes, clusters of erythroblasts and megakaryocytes. The Kupffer cells were hypertrophied, exhibiting erythrophagocytosis. A variable number of mast cells were also present in the portal tracts associated with lymphocytes and plasma cells. Some mast cells were very large. Many had irregular, multi-indented or multilobated nuclei with medium-sized nucleoli. No fibrosis could be seen either around the portal tract or in the parenchyma. A paraaortic

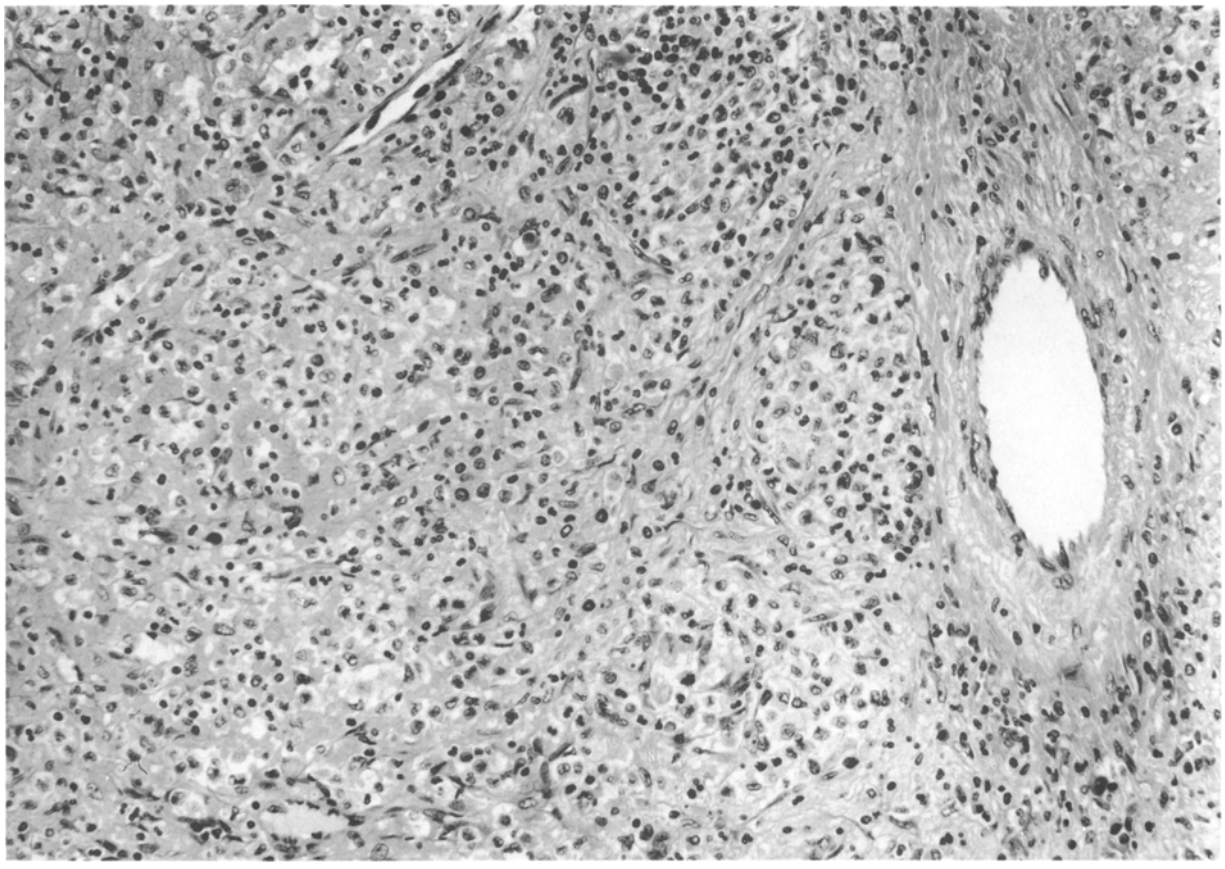

Fig. 5. Case 3. Diffuse infiltration by mast cells. Haematoxylin and eosin, × 260

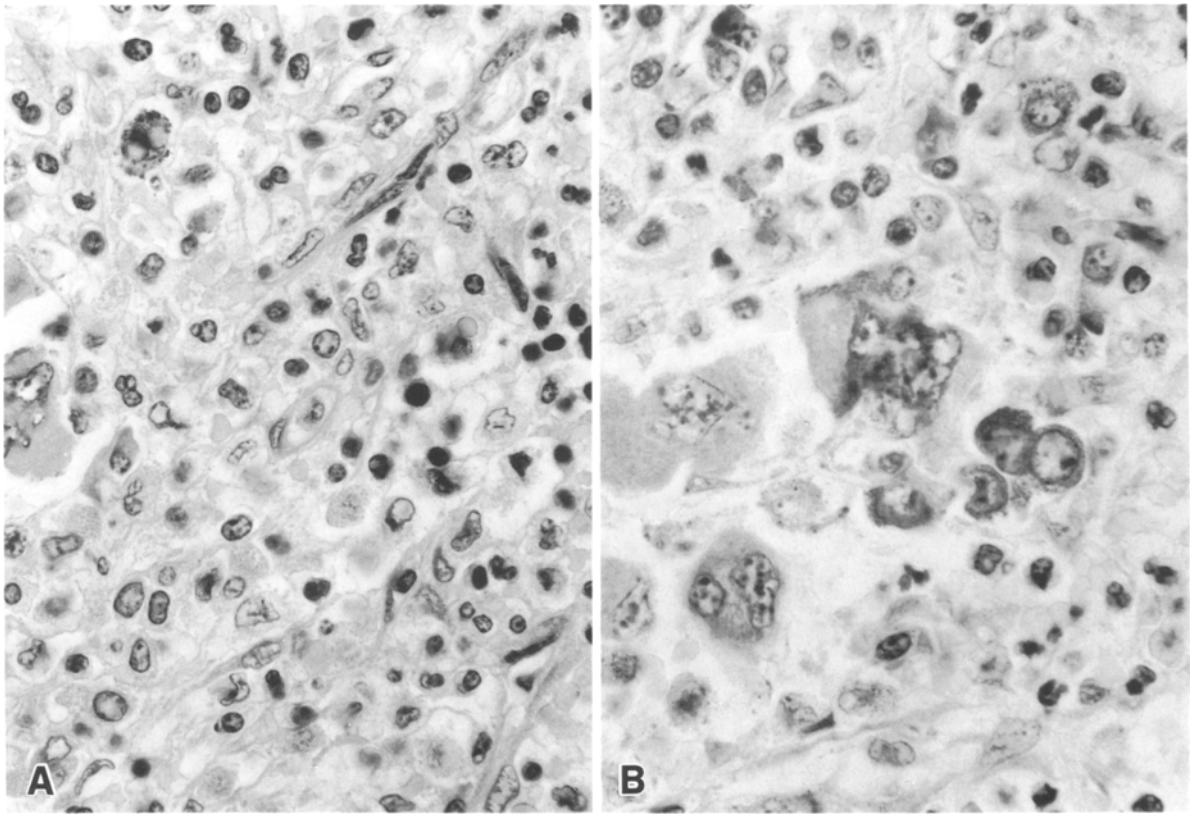

Fig. 6A. Case 3. The red pulp is infiltrated here by a mixture of mast cells, myeloid cells and granulocytes. Haematoxylin-eosin, × 750. **B** Many megakaryocytes and clusters of large erythroblasts are associated. Giemsa, × 900

lymph node was normal with normal mast cells present in the sinuses and the parenchyma.

This case was diagnosed as MM (Parwaresch et al. 1985) or aggressive SCMD (Travis et al. 1988a; Travis and Li 1988). The histopathological pattern of spleen and liver infiltration resembled those described for mast cell leukaemia.

Giemsa staining on paraffin was the most useful technique for identifying mast cells. Even in degranulated cells, the rare metachromatic granules could be detected. Naphthol ASD chloracetate esterase activity was detected in cases 1 and 2, but not in case 3 due to Bouin's fixation. Alcian blue at variable pHs was positive in all three cases, as was toluidine blue on paraffin-embedded tissue sections. Acridine orange fluorescence under ultraviolet light unambiguously demonstrated mast cells and their granules.

In all three cases, the mast cells expressed the common leucocyte antigen (CD45). Both the mast cells and the granulocytic series were CD15-positive and their cytoplasm was elastase-positive (NP57). The mast cells were also positive for the histiocytic marker CD68 (KP1), and scored strongly positive for lysozyme and weakly positive for α1-anti-trypsin and α1-anti-chymotrypsin. In contrast to previous reports, some mast cells in all three cases were positive for S100 protein. Neuron-specific enolase was also detected in the mast cells of the three cases. No T-cell markers were found to be expressed in the three cases. The only monoclonal antibody recognizing B-related antigens to score positive was DBB42, which also labelled the granulocytic series.

Discussion

The spleens in these three reported cases of generalized (or systemic) mastocytosis each exhibited a different macroscopic and histopathological pattern. These should be known by pathologists so that they can diagnose the disease and give a prognosis. The first case, SM or indolent SMCD was characterized by the splenic architecture being well preserved, a perivascular predominance of the mast cells which infiltrated the white pulp, and the absence of atypical and/or degranulated mast cells. This patient is still active and in apparently good health. The disease probably began 10–15 years before the discovery of the splenomegaly. The second case was characterized by severe fibrosis with destruction of the lymphoid tissue. Mast cell infiltration was only detected after Giemsa staining. In this case of SM or SMCD with myeloblastic acute leukaemia no leukaemic involvement of the spleen could be found. The third case, MM or an aggressive SMCD without leukaemia, was characterized by a diffuse infiltration of the white and red pulp by mast cells, many being degranulated and many having irregular nuclei.

Mast cell infiltration can easily be overlooked in conventional haematoxylin and eosin staining. The most discriminating stain is Giemsa, which clearly demonstrated the metachromatic granules on paraffin-embedded sections (Lennert and Parwaresch 1979; Parwaresch et al. 1985). On paraffin sections, PAS, alcian blue, toluidin blue, naphthol ASD chloraceate esterase activity assays or fluorescence of the granules after orange acridine impregnation can be useful in identifying the mast cells. All these techniques can be used to recognized partially degranulated mast cells. On frozen sections, toluidine blue or aminocaproate esterase activity assays are good methods for mast cell recognition.

Mast cells are positive for some B-cell (KiB3, DBB 42), T-cell (CD4) or histiocyte (lysozyme, CD68) markers. They are also positive for elastase. However all these markers are non-specific. Immunohistochemistry, is therefore not a technique that is well adapted for the identification of mast cells (Forni et al. 1983; Horny et al. 1988). It is clear that the method of choice is Giemsa staining of paraffin-embedded sections and imprints. This stain allowed the discrimination of mast cell infiltrates from malignant lymphoma (lymphoplasmacytoid lymphoma, T-cell lymphoma) from acute granulocytic leukaemic involvement, from histiocytosis X or from inflammatory granulomatous lesions with fibrosis.

There are few reports of splenic changes in generalized or diffuse mastocytosis (Brunning et al. 1983; Ende and Chernisse 1958; Lennert and Parwaresch 1979; Parwaresch et al. 1985; Travis and Li 1988; Travis et al. 1988a, b; Webb et al. 1982; Wolf and Neiman 1989). The spleen is involved in 11–86% of the GM or SMCD with a median of 47% (Travis and Li 1988; Travis et al. 1988a). In most of the published cases, the weight of the spleen was increased. For example, in a series of 14 spleens studied by Travis and Li (1988), their weights ranged from 102 to 1140 g, with an average of 623 g and a median of 453 g. Some spleens are difficult to cut due to fibrosis as in the above case 2 and sometimes due to calcification. Mast cells may be rare in the intensely fibrotic lesions (Webb et al. 1982). In most of the cases, the capsule is thickened. Multiple 1–2 mm nodular areas (our case 1) can be seen in the spleen parenchyma (Travis and Li 1988; Travis et al. 1988a, b). All the descriptions have stressed the predominance of the infiltrate around the vessels (Brunning et al. 1983; Webb et al. 1982; Wolf and Neiman 1989) in the white pulp and particularly in the marginal zone (Webb et al. 1982; Wolf and Neiman 1989). The presence of sometimes severe fibrosis has also been reported in many of the cases (Webb et al. 1982; Wolf and Neiman 1989). Diffuse infiltration of the red and white pulp has only been described in patients with mast cell leukaemia. Eosinophilia is a consistent finding. Extramedullary haematopoiesis is frequent.

Various authors have stressed that degranulated and/or atypical mast cells indicate a poor prognosis (Horny et al. 1988; Lennert and Parwaresch 1979; Parwaresch et al. 1985; Travis and Li 1988; Travis et al. 1988a). These modifications were present in our cases 2 and 3.

In conclusion, the topography and the organization of the splenic infiltrate, and the presence or absence of degranulated and/or atypical mast cells give valuable in-

formation for diagnosis and prognosis. The prognostic value of the intensity of fibrosis is not known.

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