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Splenic haematopoiesis in patients with cirrhosis of the liver

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Summary. Hitherto it has generally been assumed that splenic haematopoiesis in adult humans occurs very infrequently and is predominantly associated with haematological disorders. In the present study of patients with cirrhosis of the liver, a marked splenic haematopoiesis was a constant finding. Moreover, low-level splenic erythro- and granulopoiesis was highly prevalent even in haematologically normal controls, while splenic thrombopoiesis was conspicuously absent in both groups. We suggest that splenic haematopoiesis results from entrapment and proliferation of circulating haematopoietic precursor cells in the splenic red pulp. This would account for the presence of splenic haematopoiesis in normal controls as well as in patients with cirrhosis of the liver. In the latter, stimulation of bone marrow haematopoiesis and increased splenic pooling of haematopoietic precursor cells may contribute to the marked increase of splenic haematopoiesis observed.

Key words: Cirrhosis of the liver – Extramedullary haematopoiesis – Spleen

Introduction

Extramedullary haematopoiesis may occur in a variety of diseases, namely in myeloproliferative disorders, e.g. osteomyelofibrosis (Wetzel et al. 1970), and in certain forms of anaemia (Lewis 1983). Haematopoietic foci in liver and/or spleen have also been described in association with metastatic tumours (Fischer et al. 1970; Berge 1974) and other conditions (Stutte 1985). Spurious observations of splenic haematopoiesis in patients with cirrhosis of the liver prompted us to conduct a systematic autopsy study. We were especially interested in possible interactions between bone marrow, liver and spleen with regard to haematopoiesis.

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Materials and methods

Spleen, liver and a vertebral body (L1) of 25 patients with cirrhosis of the liver (age: 31–81 years, median: 58,8 years) and of 25 controls matched for age and sex were removed at autopsy, weighed and processed according to standard histological techniques. At least three representative sections from different, randomly chosen areas of each organ were stained as follows: haematoxylin-eosin (bone marrow, liver and spleen), Giemsa (bone marrow and spleen), modified Masson trichrome (liver) and Naphthol-AS-D-Chloroacetatesterase (spleen). The total area of the spleen and liver tissue blocks cut and examined amounted to 1 cm^2 for each organ. Splenic haematopoiesis was identified in Giemsa-stained sections; in addition, the extent of splenic granulopoiesis was analysed in sections stained by the Naphthol-AS-D-Chloroacetatesterase method (ASDCL; Leder 1964). Liver and bone marrow were analysed morphometrically using a semiautomatic MOP-AM-01 system (Kontron, München, FRG) with regard to the degree of cirrhosis and the extent of haematopoiesis, respectively. These were determined by a point-counting method and subsequent analysis of the pertinent area fraction. At least 20 visual fields at a magnification of ×100 of each section were examined using a 1600-point grid. The different compartments of bone marrow and liver were projected onto the grid, and the number of points corresponding to each compartment was counted. After conversion into percentage values the mean area fraction of each compartment was obtained.

Results

Mean spleen and liver weight of the patients (414 g ± 307 g and 1642 g ± 476 g, respectively) differed significantly from the values obtained in controls (180.8 g ± 91.9 g and 1810 g ± 895.5 g, respectively) with splenomegaly being a common sequel of cirrhosis of the liver. The connective tissue contents in livers from patients greatly exceeded that in controls (40.7% ± 13.7% versus 2.47% ± 1.15%). Likewise, the patients' bone mar-

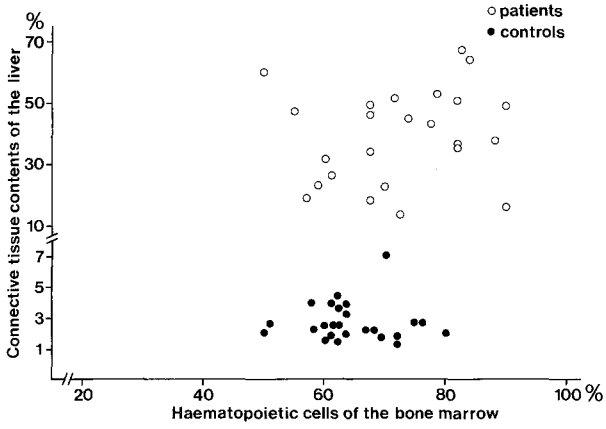

Fig. 1. Relationship between connective tissue contents of the liver and haematopoietic cells of the bone marrow

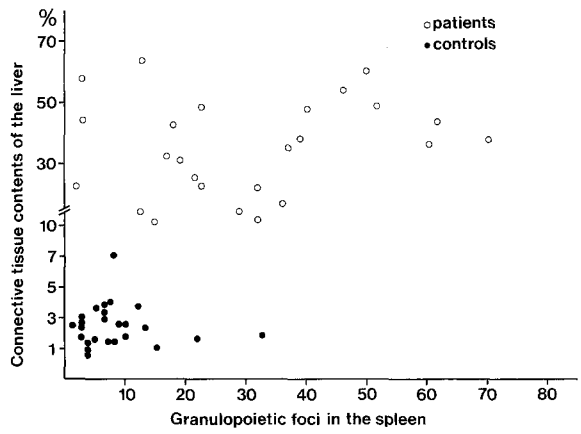

Fig. 2. Relationship between connective tissue contents of the liver and the number of granulopoietic foci in the spleen

row in the majority of cases microscopically exhibited a hyperplastic haematopoiesis. In six cases all haematopoietic cell lines were hyperplastic; in 11 cases an additional increase of immature erythropoietic cells was noted. This impression could be confirmed by morphometric determinations of the percentage of haematopoietic cells within the bone marrow. The values obtained ($73.81\% \pm 11.1\%$ in patients versus $63.29\% \pm 7.53\%$ in controls) demonstrated a statistically significant difference (t-test, $p < 0.05$). Further analysis confirmed that the expansion of haematopoiesis as well as the shift to the left in erythropoiesis correlated with the increase in connective tissue contents in progressive cirrhosis of the liver (t-test, $p < 0.05$, Fig. 1). The results of the systematic evaluation of spleen sections stained by the Giemsa- and the ASDCL-methods for haematopoiesis were as follows:

Despite an extensive search megakaryocytes could not be identified in either patients or controls.

Granulopoietic precursor cells were found within the splenic cords in every case studied. In controls a total of 170 granulopoietic foci was present, while in patients granulopoiesis occured 4.5 times more frequently: 778 foci were discernible. This difference did not appear to be related to age or sex of the subjects; statistically it proved to be highly significant (t-test; $p < 0.001$). However, a correlation between either connective tissue contents of the liver, the extent of haematopoiesis in the bone marrow and the degree of granulopoiesis in the spleen could not be established (Figs. 2 and 3).

In contrast to the diffuse distribution of granulopoietic cells within the splenic cords erythropoiesis in almost all cases occured as dense clusters

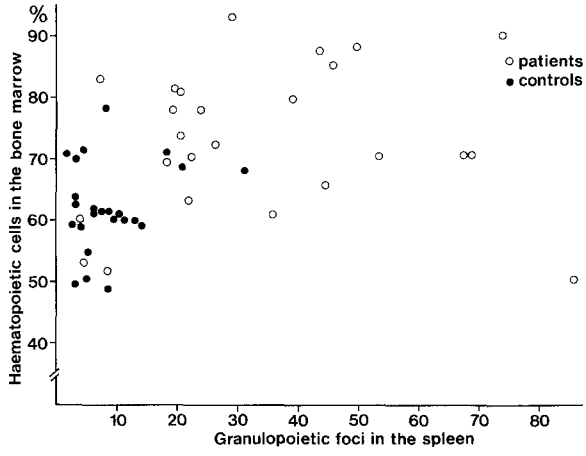

Fig. 3. Relationship between haematopoietic cells in the bone marrow and the number of granulopoietic foci in the spleen

("nests") of erythroblasts within the splenic sinuses. In controls a total of 89 erythropoietic nests could be identified; in each case between one and four clusters were present, and their occurrence was not related to either sex or age of the case. Compared with controls, however, the number of erythropoietic nests in the spleen of patients was significantly increased (t-test; $p < 0.001$), since splenic erythropoiesis was observed in 753 instances. Each patient exhibited between four and 94 erythropoietic nests in the spleen sections studied (1 cm^2). A correlation between splenic erythropoiesis and splenic weight, connective tissue contents of the liver or the extent of haematopoiesis in the bone marrow could not be established (Figs. 4 and 5).

Discussion

It has generally been assumed that in the normal human spleen haematopoiesis ceases postnatally

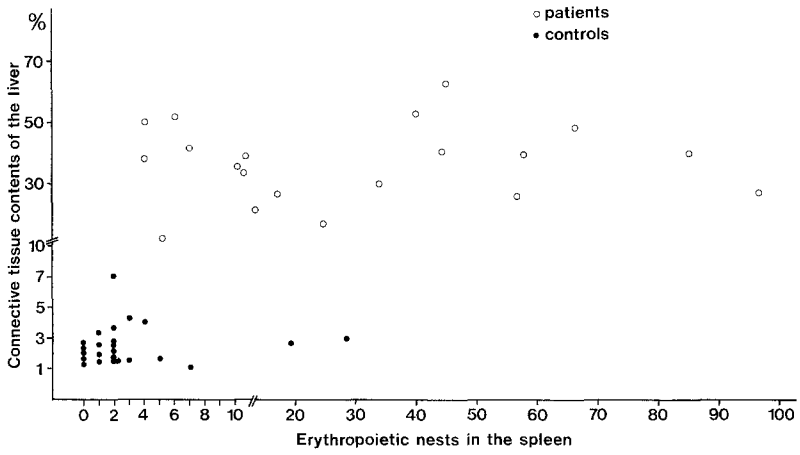

Fig. 4. Relationship between connective tissue contents of the liver and the number of erythropoietic nests in the spleen

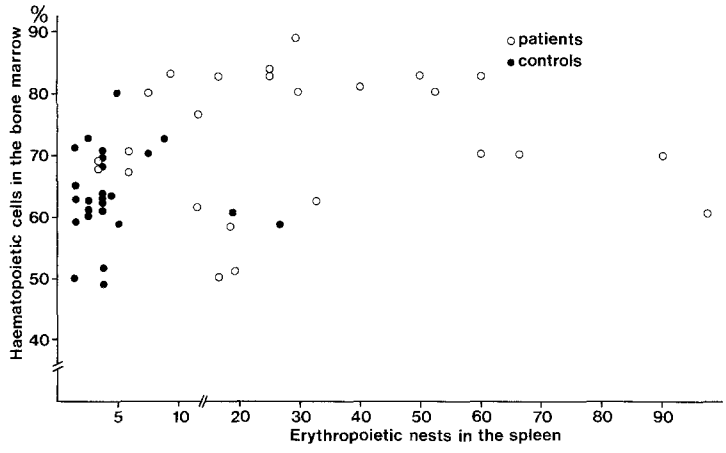

Fig. 5. Relationship between haematopoietic cells in the bone marrow and the number of erythropoietic nests in the spleen

(Freedman and Saunders 1981; Seifert and Marks 1985; Wintrobe et al. 1981). Even the participation of the human spleen in haematopoiesis during fetal life has been questioned repeatedly (Kelemen et al. 1979; Wolf et al. 1983). The spleen's haematopoietic potential appears to be realized only in certain pathological conditions, namely in myeloproliferative disorders and chronic haemolytic anaemias (Lewis 1983; Stutte 1985).

In the present study, however, splenic erythropoiesis was observed in 20 of 25 healthy controls and in all 25 patients with cirrhosis of the liver. Likewise, splenic granulopoiesis was found in 24 controls and in all patients studied, while splenic thrombopoiesis, i.e. megakaryocytes could not be demonstrated. In controls 89 erythropoietic nests could be identified; each spleen contained a mean of 3.5 nests/cm^2. In patients 753 erythropoietic nests, i.e. a mean of 30.12 nests were visible. The majority of these nests consisted of 20 or more erythroblasts each. Assuming the differentiation and maturation of each erythroblast into erythrocytes this number indicates a final yield of 240 to 280 erythrocytes or of 5600 erythrocytes per cm^2 of spleen tissue studied. If an effective erythropoiesis (Dacie 1960) is taken for granted and if nests with four to eight erythroblasts are not taken into account these findings show that in patients with cirrhosis of the liver a marked splenic erythropoiesis is present (Fig. 6).

Granulopoietic precursor cells were observed in all patients and controls (31.12 versus 6.8 foci; t-test, $p < 0.001$). They were easily identifiable in Giemsa-stained sections and readily distinguishable from mature granulocytes or mast cells by their characteristic morphological appearance (Fig. 7). The mere presence of granulopoietic cells in the spleen does not signify an extramedullary haematopoiesis, since these cells may also occur in the spleen under physiological conditions (Moeschlin 1947). In conjunction with erythroblasts, however, granulopoietic precursor cells in the spleen constitute clear evidence of extramedullary haematopoiesis (Fischer et al. 1970). It seems noteworthy that in the present study splenic haematopoiesis was observed in the majority of otherwise healthy controls – a result that differs significantly from earlier studies (Fischer et al. 1970; Fresen 1960). Moreover, splenic haematopoiesis was present in all patients with cirrhosis of the liver

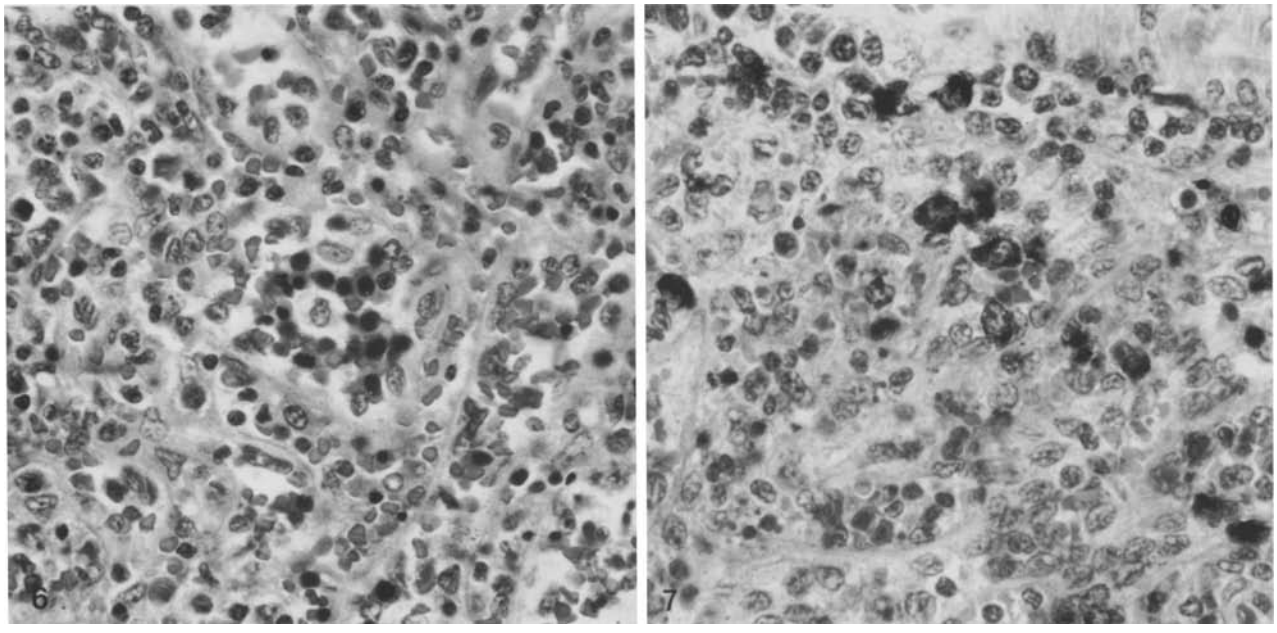

Fig. 6. Splenic erythropoiesis: an erythron-like structure consisting of erythroblasts within a splenic sinus. Giemsa, × 400

Fig. 7. Splenic granulopoiesis: Promyelocytes, myelocytes and metamyelocytes are scattered within the pulp cords. ASDCL-method, × 400

that we studied – an association that has not been studied systematically before. Our results show that there exists a correlation between the extent of connective tissue contents of the liver and the degree of bone marrow haematopoiesis (Fig. 1): progressive liver damage appears to cause an expansion and stimulation of a morphologically normal haematopoiesis (Berman et al. 1949). This could be due to an insufficient degradation of factors stimulating haematopoiesis, e.g. erythropoietin or bacterial endotoxins, or to an increased splenic pooling (see below) and destruction of blood cells. Indeed, there exists a correlation between the increase in splenic weight and the expansion of bone marrow haematopoiesis. Since only one vertebral body was examined, the data obtained for bone marrow haematopoiesis do not pretend to be representative. However, since there were striking differences between patients and controls they do permit interindividual comparisons between both groups.

We could not demonstrate a direct relationship between splenic weight and splenic haematopoiesis. Our results may be explained at least partly by the fact that splenic haematopoiesis results from the entrapment of circulating hematopoietic precursor cells in the splenic red pulp and their subsequent proliferation (Haas 1978; Fliedner et al. 1982; Arnold et al. 1983). It is well known that haematopoietic precursor cells are detectable in the peripheral blood even in healthy individuals, albeit at a very low level. Unquestionably, they are able to colonize the spleen that seems to offer a suitable microenvironment (Von Melcher and Lieschke 1981). Our observation of a low-level splenic haematopoiesis in healthy controls would seem to reflect this situation. In contrast, patients with cirrhosis of the liver mostly suffer from anaemia and from elevated serum levels of bacterial endotoxins. These factors both result in a stimulation of bone marrow erythro- and granulopoiesis (Wintrobe et al. 1981) with a concomitant increase in the number of circulating haematopoietic precursor cells. Moreover, the portal hypertension observable in the majority of cases with cirrhosis of the liver causes structural changes in the spleen. These in turn may decrease the speed of the blood passing through the splenic sinuses and thus cause an increased splenic pooling of mature blood cells as well as of haematopoietic precursor cells. In some cases even a progression to hypersplenism resulting in peripheral pancytopaenia and bone marrow hyperplasia may be noted.

In humans both mechanisms – stimulation of bone marrow haematopoiesis and increased splenic pooling favor the development of splenic haematopoiesis that in animals may be induced by endotoxins alone (Staber and Metcalf 1980). However, this does not apply to splenic thrombopoiesis; at autopsy megakaryocytes in the spleen of haemato-

logically normal individuals in most cases are only detectable after prolonged periods of circulatory failure. It seems important that in our study splenic thrombopoiesis could not be found in either patients or controls. In contrast the occurrence of megakaryocytes is highly typical for patients whose bone marrow is either severely compromised, e.g. in malignant tumours (Falk et al. 1987) or in osteopetrosis (Freedman and Saunders 1981), or highly activated, e.g. in severe anaemias (Stutte 1985). In these conditions splenic haematopoiesis may develop in order to compensate for bone marrow failure, while splenic erythro- and granulopoiesis in patients with cirrhosis of the liver appear to result from both bone marrow hyperplasia and increased splenic pooling of circulating haematopoietic precursor cells.

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