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Bone marrow morphology during haematopoietic stem cell mobilisation with cyclophosphamide in mice

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The aim of the study was to examine the morphology of the bone marrow of mice after stimulation with cyclophosphamide (Cy). The experimental mice were given a single intraperitoneal injection with 250 mg/kg bw cyclophosphamide. After 2, 4 and 6 days of experiment the femurs were obtained for morphological study. On the 2nd day after the mobilisation of the mice with Cy destruction of the bone marrow was observed with a decrease in the haematopoietic compartment and an increase in the area occupied by sinusoids filled with erythrocytes. Erythrocytes were located among the haematopoietic cells, which indicated that the endothelial barrier had been disrupted. On the 4th day after treating the mice with Cy, repair processes in the bone marrow were conducted, including macrophages. The cells filled with haemosiderin migrated from the extravascular compartment of the bone marrow into the lumen of the sinusoids. There were proliferating cells among the haematopoietic cells. On the 6th day the morphology of the bone marrow was similar to the morphology of that in the control mice. However, more haematopoietic cells were visible compared to the control bone marrow. The presence of an increased number of leucocytes in the sinusoid lumen in comparison with the control suggested that at that time the migration of haematopoietic cells from the bone marrow had been initiated.

key words: bone marrow morphology, cyclophosphamide, haematopoietic cells, sinusoids

INTRODUCTION

Haematopoietic stem cells can be mobilised from the bone marrow into the peripheral circulation in response to a wide number of agents such as chemokines, haematopoietic growth factors or haematopoietic cytokines [1]. Moreover, chemotherapy with cyclophosphamide (Cy) has been used clinically to enhance this process [3]. Cy is a DNA alkylating agent, with myelosuppressing and immunomodulatory properties. Cy-induced haematopoietic stem cell mobilisation is commonly used for clinical transplantation and experimental procedures. It has been shown that bone marrow transplantation is a standard method to reconstitute haematopoiesis and in bone marrow failure following myeloblastive therapy in patients with a variety of cancers and haemato-oncological malignances [5]. Recently studies have reported that autologous transplantation of haematopoietic bone marrow stem cells can be beneficial in the treatment of cardiological and neurological diseases [4, 9]. However, the mechanism of the mobilisation of haemato-

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poietic cells is poorly understood. Thus, the aim of the study was to investigate changes in the morphology of the bone marrow following mobilisation of haematopoietic cells by the stimulation of mice with cyclophosphamide.

MATERIAL AND METHODS

The experiment was performed on pathogen-free, 5-week-old, mature female inbred Balb C mice (Polish Academy of Sciences, Wroclaw, Poland). The animals were randomly divided into control and experimental groups. The animals were maintained under standard laboratory conditions in a 12 h/12 h light-dark cycle at 21°C. The mice experimental group were injected intraperitoneally with a single dose (200 mg/kg bw) of cyclophosphamide (Endoxan, Asta Medica). Cyclophosphamide was diluted in a solution of saline, according to the manufacture's recommendation. The mice of control group were injected with a phosphate-buffered saline (PBS) equivalent in volume to the cyclophosphamide in mice of the experimental group. The experiment was terminated after 6 days. The mice were sacrificed by lethal anaesthesia with sodium pentobarbitone and the femurs were collected for morphological studies. The bone fragments were decalcified and embedded in paraffin and the slides were stained with H-E.

The experiment received the approval of the Local Ethical Committee.

RESULTS AND DISCUSSION

The present study revealed that on the 2nd day after the intraperitoneal injection of cyclophosphamide (Cy) bone marrow destruction was observed. There was an increase in the area occupied by dilated sinusoids filled with erythrocytes and a decrease in the haematopoietic compartment in the bone marrow (Fig. 1). The presence of erythrocytes among the haematopoietic cells indicated that the continuity of the sinusoid wall had been broken and the integrity of the endothelium barrier had been lost. Our observation confirmed the study performed by Shirota and Tavassoli [8]. They presented alternations to bone marrow endothelium induced by cyclophosphamide [8]. We found that on the 4th day after treatment with Cy the morphology of the bone marrow was changed (Fig. 2). The haematopoietic compartment was increased and a proliferation of haematopoietic cells was observed. Moreover, we noted the presence of cells filled with haemosiderin — macrophages among the haematopoietic cells and in the lumen of vessels. This is probably a mechanism for the removal of erythrocytes as a consequence of an increased permissiveness of the endothelial barrier. A similar finding of the penetration of macrophages into the lumen of vessels has also been reported previously [8]. In our study the morphology of the bone marrow was similar to that of the control mice on the 6th day after Cy but there was an increased number of haematopoietic cells (Fig. 3, 4). The lumen of sinusoids was filled with leucocytes. Our observation indicated that the mobilisation and the releasing of leucocytes from the haematopoietic compartment to the marrow sinusoids were initiated on the 6th day. The process of cell migration induced with Cy was likely to be connected with the disturbance of bone marrow endothelial cells and transient abolition of the blood-bone marrow barrier.

The molecular basis of haematopoietic cell mobilisation from the bone marrow caused by cyclophosphamide include two main pathways. The first of these is the decrease in VCAM-1 expression in the stromal cells and direct cleavage of VCAM-1 by neutrophil proteases accumulated in the bone marrow [2]. The other is the SDF-1/CXCR4 chemotactic axis [2], which can be sensitised by C3a anaphylatoxin [7]. It is postulated by Ratajczak et al. [6] that the axis is involved not only in chemo-attracting circulating haematopoietic stem/progenitor cells but also in CXCR-positive muscle stem/progenitor cells [6]. A decreasing gradient from the extravascular compartment of the bone marrow toward the lumen of the vessels induces migration of haematopoietic cells from the bone marrow.

CONCLUSIONS

We suggest that the mobilisation of mice with cyclophosphamide results in the migration of haematopoietic cells from the bone marrow into the circulation and that the process is initiated on day 6 following stimulation with Cy.

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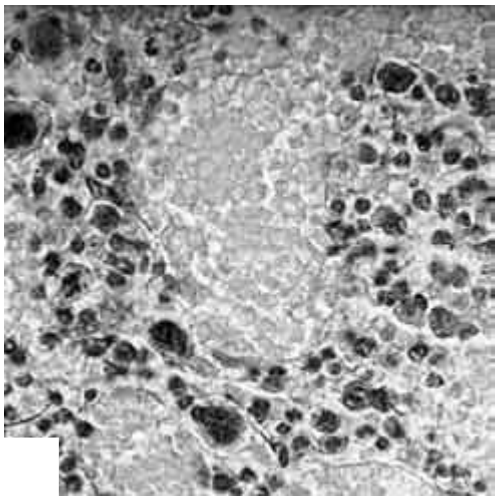

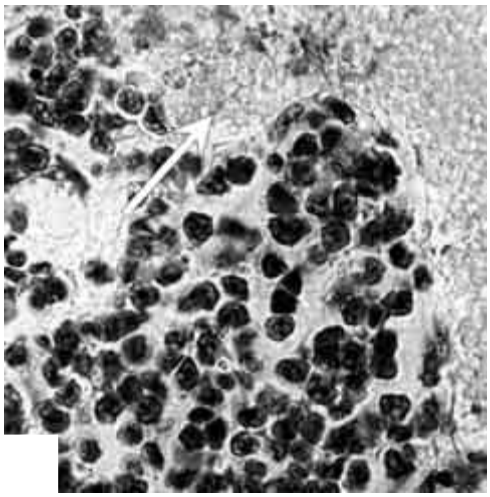

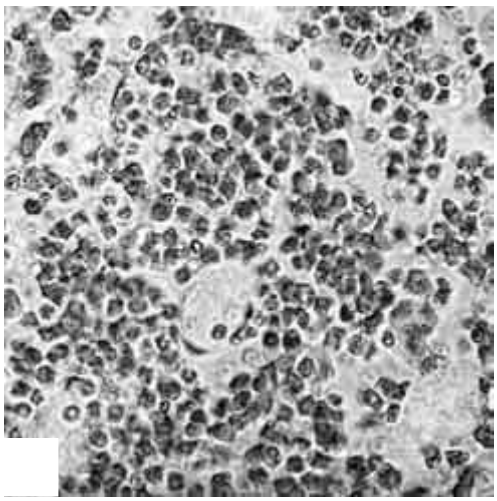

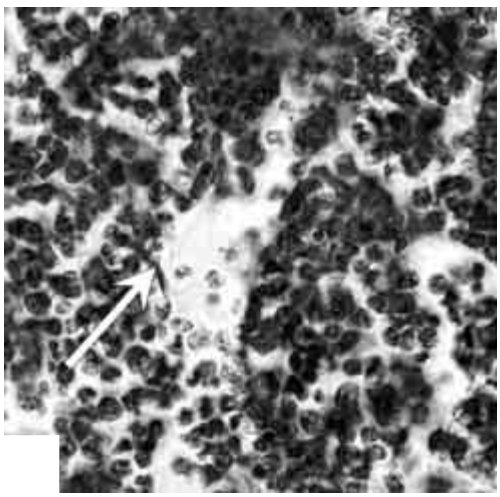

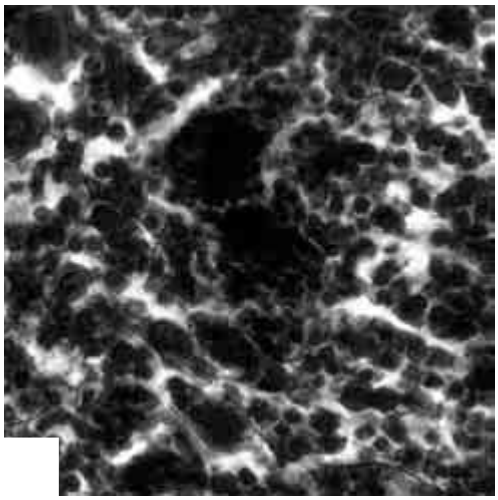

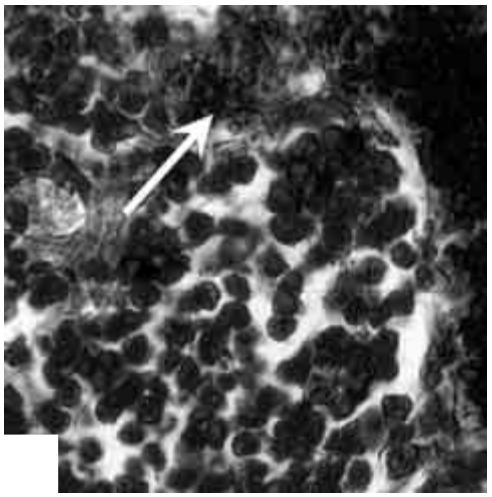

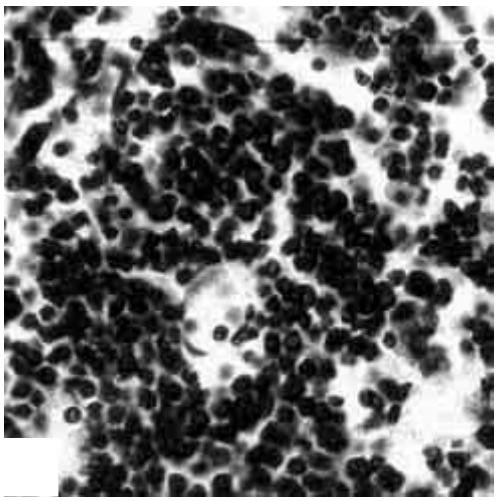

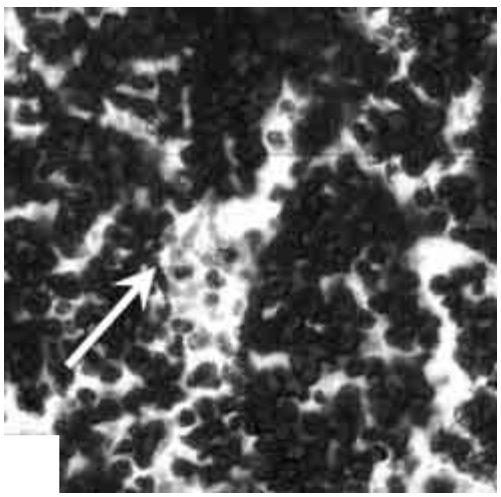

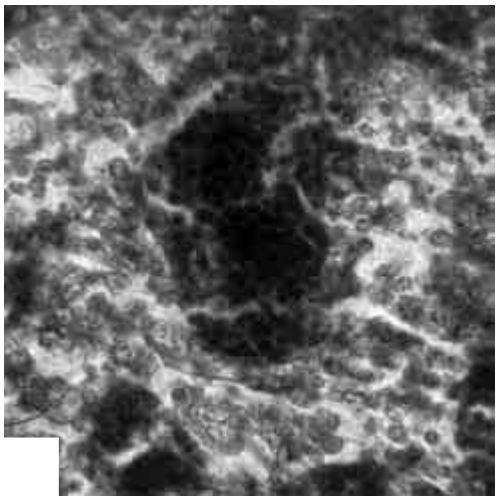

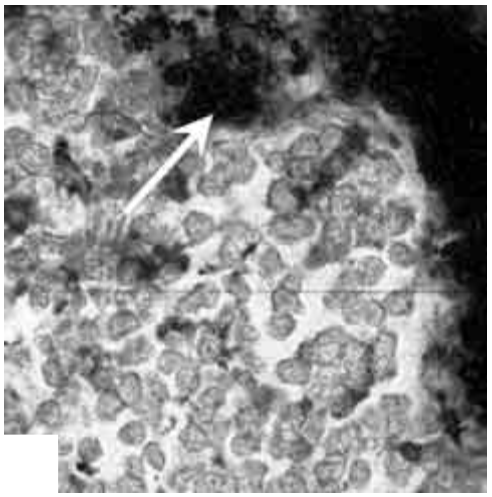

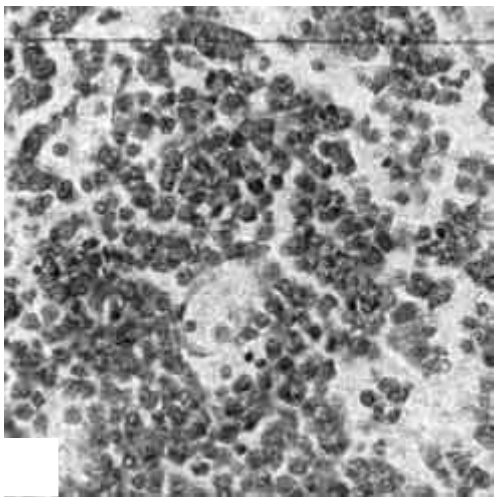

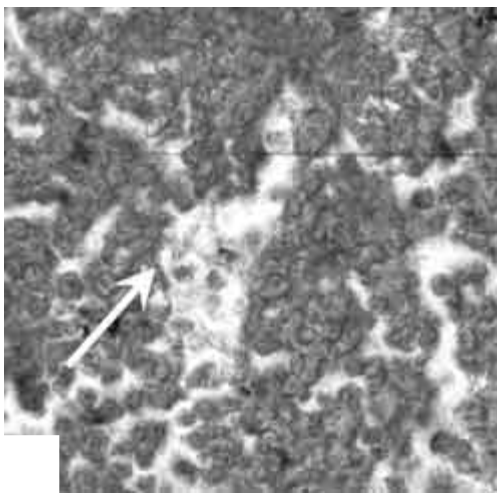

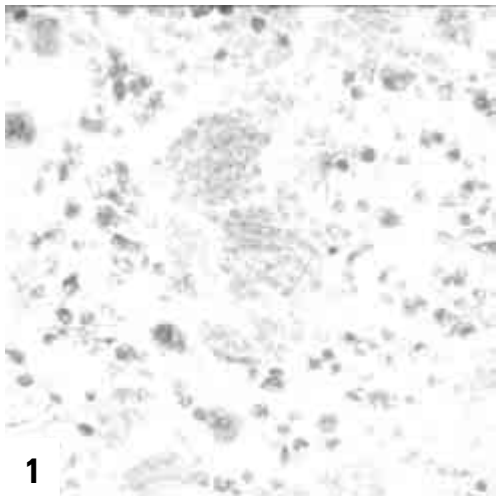

Figure 1. The bone marrow of mice after the 2nd day of stimulation with cyclophosphamide. Dilated sinusoid lumens filled with erythrocytes. Erythrocytes among the cells of the haematopoietic compartment. Scale bar, 100 μm.

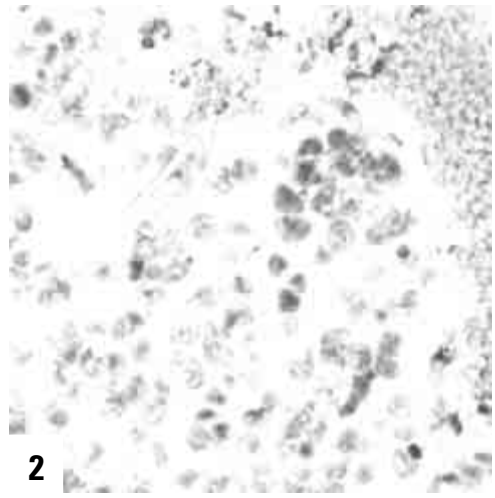

Figure 2. The bone marrow of mice after the 4th day of stimulation with cyclophosphamide. The increased area occupied by the haematopoietic compartment. The presence of haemosiderin-filled cells in the intravascular compartment (arrow). Scale bar, 100 μm.

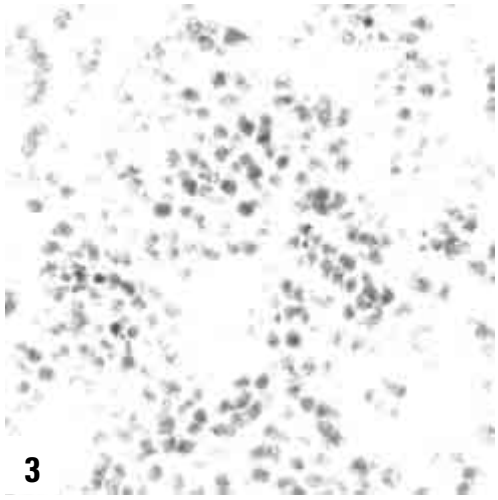

Figure 3. The bone marrow of the control group of mice. Scale bar, 100 μm.

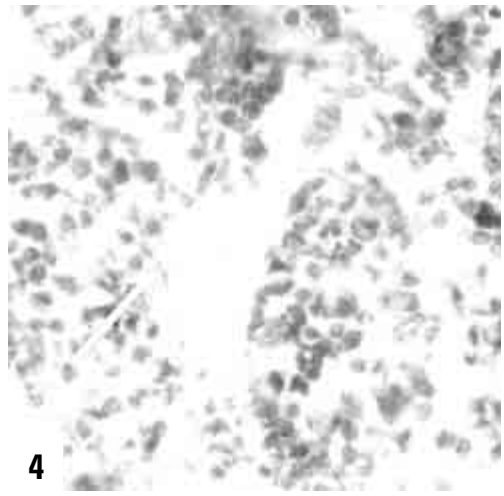

Figure 4. The bone marrow of mice after the 6th day of stimulation with cyclophosphamide. There is an increased number of haematopoietic cells. The presence of leucocytes in the lumen of sinusoids is indicated by an arrow. Scale bar, 100 μm.

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