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Bone marrow morphology during haematopoietic stem cell mobilization with G-CSF in mice

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The aim of the work was to examine the morphology of the bone marrow of mice during stimulation with G-CSF. Experimental Balb C mice were daily injected subcutaneously with 250 μg/kg b.w. G-CSF (Neupogen). After 2, 4 and 6 days of the experiment femurs were obtained for morphological study. On day 2 of the mobilization the amount of haematopoietic cells in the bone marrow increased and dilatation of the sinusoids was observed. Only single leukocytes were observed in the lumen of the vessels. There were numerous leukocytes in the lumen of the sinusoids on day 4 of the mobilization. The morphology of the bone marrow on day 6 was similar to that of the control. Mobilization of mice with G-CSF resulted in migration of haematopoietic cells from the bone marrow and the process is most pronounced on day 4.

Key words: bone marrow morphology, sinusoids, haematopoietic cells, G-CSF, mobilization

INTRODUCTION

Haematopoiesis is sustained by the continuous replenishment of circulating cells with newly formed cells. This process is possible due to of the presence of pluripotent haematopoietic stem cells in the bone marrow. Mobilization of haematopoietic stem and progenitor cells from the bone marrow into the circulation has been shown to be induced clinically or experimentally in animal models by a wide number of molecules such as cytokines, chemokines or chemotherapeutic agents [3]. After mobilization haematopoietic stem and progenitor cells are released into the periphery. However, the mechanism of mobilization of the cells in not fully understood and the morphological structure of the bone marrow during mobilization is not well known. The aim of the study was to evaluate the morphology of bone marrow during mobilization of haematopoietic stem and progenitor cells by repetitive, daily stimulation of mice with G-CSF.

MATERIAL AND METHODS

The experiment was performed on pathogen-free 5-week-old mature female inbred Balb C mice (Polish Academy of Sciences, Wrocław, 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 in the experimental group were injected subcutaneously with

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250 μg/kg bw G-CSF (Neupogen — La Roche, Switzerland) every day of the experiment. The mice of the control group were injected with a phosphate-buffered saline (PBS) in the same volume as the G-CSF 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 study. Fragments of bones were decalcified and embedded in paraffin, and the slides were stained with H-E [1].

The experiment received the approval of the Local Ethical Committee.

RESULTS AND DISCUSSION

The first sign of stimulation with G-CSF was an increase in the number of haematopoietic cells in the bone marrow (Fig. 1). This was the expected effect in view of the fact that the chemokine possesses the physiological property of being a main regulator of the proliferation, differentiation and survival of neutrophils [2]. Stimulation with G-CSF induced dilatation of the sinusoid lumen, in which single leukocytes were visible (Fig. 1). On day 4 of G-CSF mobilization the lumen of the sinusoids was filled with numerous leukocytes (Fig. 2). This indicated that most of the haematopoietic cells had left the bone marrow by this

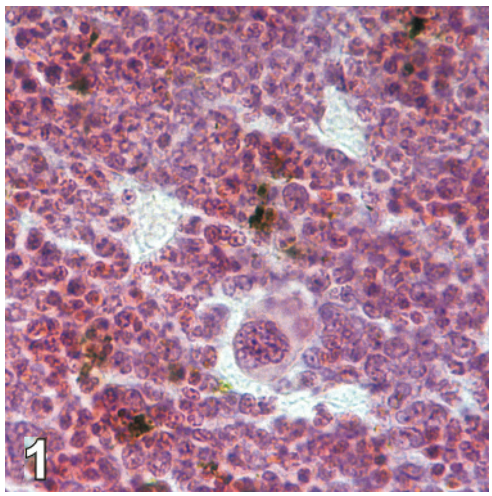

Figure 1. The bone marrow morphology of mice after day 2 of stimulation with G-CSF. Slight dilatation of the sinusoids and a few leukocytes in the lumen of capillaries. Scale bar, 100 μm.

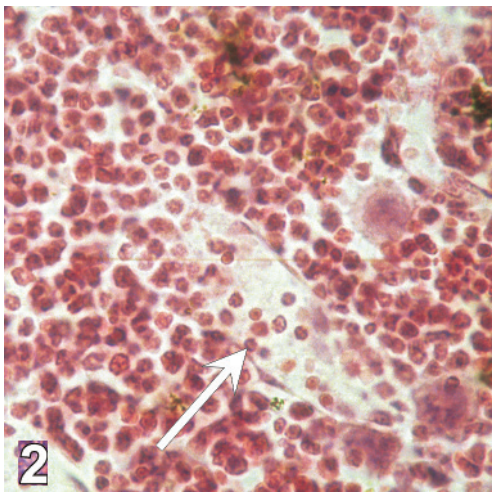

Figure 2. The bone marrow morphology of mice after day 4 of stimulation with G-CSF. The dilatation of sinusoids and numerous leukocytes in the lumen of capillaries (arrow). Scale bar, 100 μm.

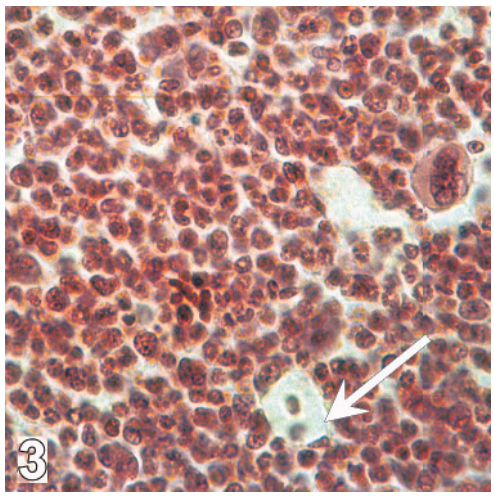

Figure 3. The bone marrow morphology of mice after day 6 of stimulation with G-CSF. The morphology is similar to that of the bone marrow of control mice. Note, the small number of leukocytes in the lumen of the sinusoids (arrow). Scale bar, 100 μm.

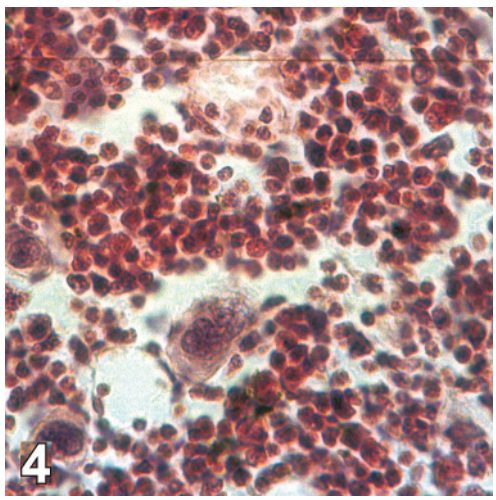

Figure 4. The bone marrow of the control group of mice. Scale bar, 100 μm.

time. The migration of the cells induced by G-CSF had finished by day 6. The morphology of the bone marrow after 6 days of stimulation (Fig. 3) was similar to that of the control mice (Fig. 4). Only isolated leukocytes remained in the lumen of the sinusoids (Fig. 3).

Mobilization of mice with G-CSF releases haematopoietic stem cells from the bone marrow into the circulation. There are different interactions between the haematopoietic cells and the stromal cells as well as the extracellular matrix in the normal bone marrow. The crucial role in the migration of haematopoietic stem cells is played by a stromal-derived factor (SDF-1) secreted by the bone marrow stroma. SDF-1 acts through a specific receptor — CXCR4, expressed in haematopoietic stem and progenitor cells [3] as well as some other cells, such as cell lines derived from muscle and neural tissue [6, 7]. SDF-1 is the only ligand for the receptor. The chemotactic response of haematopoietic cells to SDF-1 is sensitised by C3a anaphylatoxin [8]. Mobilization with G-CSF is accompanied by an increase in granulocyte precursors and mature granulocyte in the bone marrow. Moreover, a large number of active neutrophil serine proteases is released by neutrophils [4]. Neutrophil proteases released in the bone marrow induce the cleavage of the CXCR4 receptor on haematopoietic cells and its ligand SDF-1 [4]. As a consequence, the concentration of SDF-1 in the bone marrow is decreased [5]. The proteolytic cleavage of CXCR4 or SDF-1 by neutrophil proteases leads to the complete inactivation of the SDF-1-CXCR4 signalling axis [4].

In conclusion, we suggest that mobilization of mice with G-CSF results in the migration of haematopoietic cells from the bone marrow into the circulation and that the process is most pronounced on day 4 after stimulation. The cell traffic has finished by day 6.

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