Migration of lymphoid cells to the bone marrow of rat following eradication of cells in DNA synthesis and in mitosis[1,2]

Z. Ben-Ishay, F. Reichert and S. Sharon

Department of Anatomy and Embryology, Hebrew University-Hadassah Medical School, P.O.B. 1172, Jerusalem (Israel), 15 March 1978

Summary. Eradication of replicating bone marrow cells of rat by means of combined administration of single doses of hydroxyurea and vinblastin is followed within 9-10 h by an inflow of lymphoid cells of extramedullary origin in the range of 13,200,000/femur. The rat bone marrow with a high content of lymphoid cells was previously shown to be concentrated in stem cells. The factor(s) which convey the 'information' of decrease of replicating marrow cells to extramedullary sites is at present unknown.

Previous studies in the rat indicated that administration of DNA-synthesis inhibitors results in a significant reduction of bone marrow cells in the S-phase of the cycle[3,4]. 9 h following administration of single doses of hydroxyurea (HU) (400 mg/kg) to rats, the total nucleated cells per femur are reduced by half, 65% of which are represented by lymphocytes. Upon these data and based on total counts of nucleated cells per femur, it was calculated that the lymphocyte marrow portion of rat becomes enlarged by several million cells per femur, 9-10 h after administration of a single dose of HU[5]. The lymphocyte-enriched bone marrow resulting from HU administration was also shown to be concentrated in stem cells[5]. It thus becomes of interest to establish whether the additional lymphocytes originate from replication of marrow cells or whether they represent an incoming stream of cells of extramedullary origin. In an attempt to answer this question, rats were administered simultaneously with a single i.p. injection of HU (400 mg/kg) and vinblastin (VIN) (1 mg/kg). Hydroxyurea, a potent DNA-synthesis inhibitor, kills marrow cells in the S-phase of the cycle, while vinblastin kills cells in mitosis. Should the enlarged marrow lymphoid compartment result from activation and replication of previously quiescent lymphocytes, they would be killed by the toxic effect of vinblastin.

The data obtained in the present investigation clearly indicate that, following a significant reduction of marrow replicating cells, there is an influx of lymphocytes from some extramedullary source(s).

Female rats of the Hebrew University strain weighing 100-120 g were administered i.p. 2 separate injections: 1 of hydroxyurea (400 mg/kg) and 1 of vinblastin (1 mg/kg). 9 h following HU and VIN injections, the rats were sacrificed and suspensions of bone marrow cells from each femur were separately prepared in M199 tissue culture medium. A total of 20 absolute counts of nucleated marrow cells were performed in 10 rats. Smears of bone marrow cells were stained with Giemsa and differential counts of 500 cells/femur were done. Similar total and differential marrow cell counts were performed in 6 normal rats of same sex, strain and weight as the experimental group of animals.

Several specimens of bone marrow tissue of 1. normal rats, 2. 9 h following a single injection of HU, 3. 9 h following a single injection of VIN, 4. 9 h following combined administration of HU and VIN, were processed for electron microscopy and semi-thin sections were examined by light microscopy.

Upon examination of semi-thin sections of HU-treated rats, it was noted that the bone marrow contains large numbers of necrotic cells as compared with the normal marrow (figures 1 and 2). In a previous study it was reported that the erythroid compartment is in particular reduced by administration of a single dose of HU[5]. On the other hand, 9 h following administration of single doses of VIN (1 mg/kg) to rats, a large majority of marrow cells are killed in mitosis (figure 3). Combined administration of HU and VIN results in a hypocellular marrow of a lymphoid appearance (figure 4). The HU and VIN treated marrow contains few cells blocked in mitosis contrary to the bone marrow of rats treated with VIN only. The explanation for this fact may be related to the specific killing effect of HU of cells in the S-phase preventing further cycling to the stage of mitosis.

The table indicates the values of total and differential counts of marrow cells in normal rats and in rats 9-10 h following combined administration of hydroxyurea and vinblastin. The toxic effect of these 2 drugs killed about 50% of total nucleated cells per femur, mainly replicating erythroid and myeloid cells ($p < 0.0005$). The lympoid-cell portion is significantly enlarged following HU+VIN administration (74% as compared to 15% in normal rats). The increase of the lympoid compartment is both relative and absolute: upon calculation it can be concluded that the bone marrow in 1 femur of a normal rat contains 9,000,000 lympoid cells of a total of 60,000,000 nucleated cells. After combined administration of HU+VIN, there are 22,200,000 lympoid cells of a total of 30,000,000 per femur. It results that approximately 13,200,000 lympoid cells per femur must come from an extramedullary source(s) within 9-10 h of HU+VIN administration to rats.

The results of the present investigation indicate a hitherto unknown phenomenon: inflow of lymphocytes to the bone marrow of rat following severe reduction of young type replicating cells. In a previous study, we reported on the occurrence of several additional million lymphocytes per

Total and differential counts of nucleated bone marrow cells of normal and combined hydroxyurea- and vinblastin-treated rats

	Total cells/femur	Differential counts (500 cells differentiated/femur)			
		Erythroblasts	Myeloid cells		Lymphoid cells
			Young types	Nonproliferative types	
6 normal rats	60,000,000 (SD 12,240,000) $p < 0.0005$	47.8% (SD 14.8) $p < 0.0005$	12.3% (SD 8.8) $p < 0.0005$	24.6% (SD 8.8) $p < 0.006$	15.3% (SD 8.1) $p < 0.0005$
10 hydroxyurea- and vinblastin-treated rats	30,100,000 (SD 6,450,000)	4.3% (SD 3.3)	3.7% (SD 2.9)	18.0% (SD 10.0)	74.0% (SD 12.8)

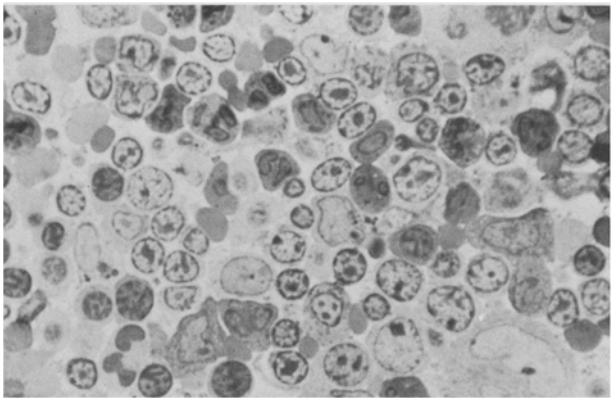

Fig. 1. Normal rat bone marrow.

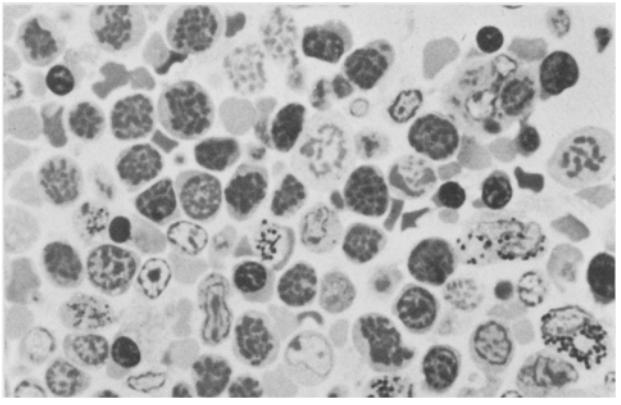

Fig. 3. Rat bone marrow 9 h after a single injection of vinblastin. A large majority of cells are seen blocked in mitosis.

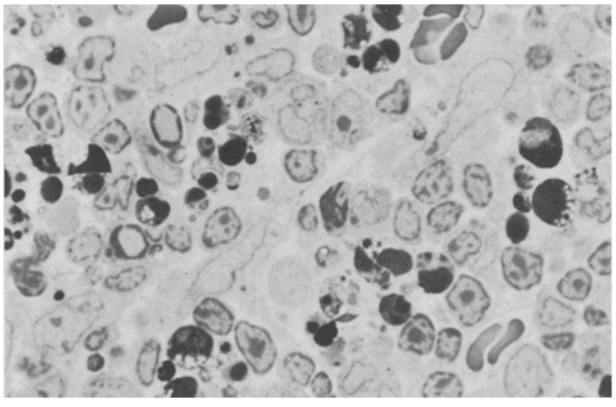

Fig. 2. Rat bone marrow 9 h after a single injection of hydroxyurea. Note extensive cellular necrosis (the dark stained cells).

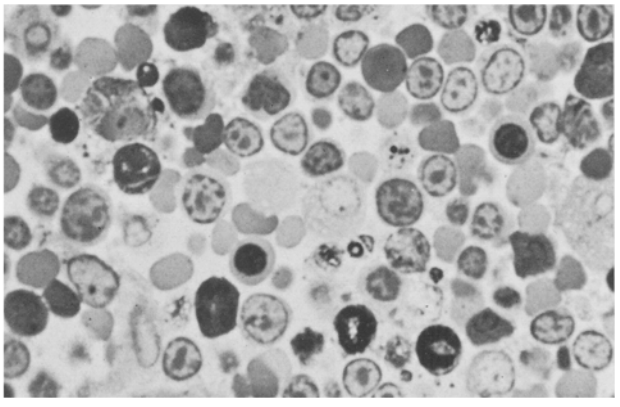

Fig. 4. Bone marrow of a rat which received simultaneously single injections of hydroxyurea and vinblastin 9 h prior to being sacrificed. Note necrotic cells and absence of cells blocked in mitosis.

Figures 1–4 represent light micrographs of rat bone marrow tissue processed for electron microscopy, sectioned 1 nm thick and stained with toluidene blue. × 1100.

femur in HU-treated rats as compared to control animals[5]. The possibility existed that a marked reduction of replicating marrow cells triggers entering of quiescent lymphocytes in cycle. If this were the case, the enlargement of the marrow lymphoid compartment resulted from cellular replication rather than cellular inflow from an extramedullary source(s). In order to test this hypothesis, vinblastin, a specific cytotoxic drug of cells in mitosis, was administered simultaneously with hydroxyurea in the present study. As indicated in the table, combined administration of HU and VIN to rats, presumably resulted in eradication of most bone marrow cells in DNA synthesis and in mitosis, and the addition of approximately 13,200,000 lymphoid cells/femur to the preexistant lymphocyte marrow population. Recently, we reported on the process of committment of uncommitted spleen stem cells of rat 1–3 h following administration of a single dose HU (400/kg)[6]; based upon the observation of an inflow of lymphoid cells to the bone marrow of rats administered HU plus VIN and upon the evidence of committment of spleen stem cells soon after HU administration[6], it becomes highly suggestive that a substance(s), possibly released by the depleted marrow, stimulates committment and migration of extramedullary stem cells. Rencricca et al.[7] ruled out the role of erythropoietin in the process of committment of stem cells following a marked decrease of marrow replicating erythroblasts. Of particular importance is the existence of an extramedullary stem cell pool highly sensitive to low levels of bone marrow myelopoiesis and the process of mobilization of stem cells to 'sites' of normal blood cells production.

Furthermore, it is of interest to note that, at least in rats, administration of restricted doses of HU[5], of VIN or HU + VIN (unpublished observations) produces temporary and reversible bone marrow cells damage and enlargement of the lymphoid compartment. In previous studies it was shown that stem cells are part of the enlarged lymphoid marrow compartment, though we do not equalize lymphocytes with stem cells[3–5]. At present, the observations reported here cannot be extended to man; however, if this were the case, recipients of bone marrow transplants would possibly benefit more from a marrow concentrated in stem cells than from a marrow obtained under the steady state of hematopoiesis.

1 Acknowledgment. This study was supported by a grant from the Chief Scientist Office, Ministry of Health, Israel.
2 Hydroxyurea for this investigation was given as a gift by the Squibb Institute of Medical Research, Princeton, N.J. USA, to which the authors are indebted.
3 Z. Ben-Ishay, Israel J. med. Sci. *11*, 978 (1975).
4 Z. Ben-Ishay, Scand. J. Haemat. *14*, 361 (1975).
5 Z. Ben-Ishay and S. Sharon, Scand. J. Haemat. *18*, 226 (1977).
6 Z. Ben-Ishay, S. Sharon and F. Reichert, Acta haemat. *59*, 88 (1978).
7 N.J. Rencricca, B.S. Morse, F.C. Monette, D. Howard and F. Stohlman, Jr, Proc. Soc. exp. Biol. Med. *149*, 1052 (1975).