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EFFECT OF "MEDIUM-SIZED MOLECULES" OF UREMIC SERUM IN HEMATOPOIESIS IN INTACT MICE

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KEY WORDS: chronic renal failure; uremic serum; hematopoiesis.

An important role in the regulation of erythropoiesis is played by erythropoietin, which is produced mainly in the kidneys. It has been suggested that besides erythropoietin, there is also a humoral regulator with the opposite action – an inhibitor of erythropoiesis. Their interaction probably maintains the erythron system at the physiological level. A disturbance of the excretory functions of the kidneys in chronic renal failure (CRF) is accompanied as a rule by anemia, of uncertain pathogenesis. It has been shown that the main stage in the development of this anemia is a disturbance of erythropoietin production [3, 4]. Meanwhile it has been observed that substances with medium molecular weight (MMW) accumulate in the blood of these patients. It has been suggested that these substances may inhibit erythropoiesis. However, the mechanism of inhibition of erythropoiesis by these substances is not clear, and it may be due both to toxic metabolites and also to a specific inhibitor of erythropoiesis. The writers showed previously that uremic serum inhibits erythropoieses in intact and polycythemic mice, and likewise that it has no inhibitory or cytotoxic action on the pluripotent stem cell [1, 2].

The aim of this investigation was to study the effect of the MMW fraction from patients with CRF on hematopoiesis in intact animals.

EXPERIMENTAL METHOD

Twenty patients (children and adolescents aged from 3 to 17 years) with CRF in the terminal stage of kidney disease (creatinine 11.4 ± 3.2 mg %, urea 91.4 ± 25.7 mg %) were investigated. All the children had anemia: hemoglobin concentration 6.2 ± 2.0 g %, hematocrit index 18.6 ± 2.0 %, both kept at a constant level by transfusions of red cells (on average 0.9 transfusion of 300 ml per month). The patients were being treated by hemodialysis 3 times a week, for 3-4 h each time, on the NGAK-4 capillary dialyzer (1.3 m^2).

The MMW fraction was separated from the serum of the patients with CRF by ultrafiltration on XM-50 disks (from Amicon), and the ultrafiltrate was then fractionated on Sephadex G-25 (from Pharmacia, Sweden). Fractionation was carried out on a 2.6 × 100 cm column (from LKB) in the presence of 0.02 M NaCl solution, pH 7.4, and extinction was measured at 210 nm. The resulting MMW fraction (3000-800) was lyophilized. The MMW fraction was injected into intact mice weighing 25 g over a period of 2 days in a total dose (as protein) of 2.5 mg per mouse (concentration 130 mg %), in accordance with the following scheme: 0.5 ml subcutaneously, interval of 5 h, 0.5 ml intraperitoneally. The absolute number of myelokaryocytes per femur and the morphological composition of the bone marrow were determined in the animals before injection and 1, 2, and 4 days after the last injection of the MMW fraction. Marrow from the femur was suspended in 2 ml of 3% acetic acid solution; the homogeneous bone marrow suspension was introduced into a white cell mixing chamber. The absolute number of myelokaryocytes was counted in a Goryaev's chamber. Bone marrow was obtained from the other mouse femur and films were prepared from it to study its morphological composition; the total myelogram was derived from

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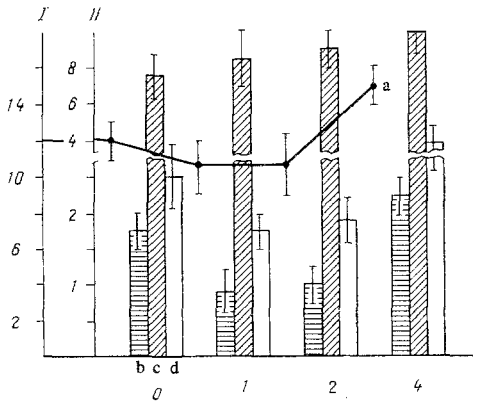

Fig. 1. Effect of MMW fraction on absolute number of myelokarcytes (a), erythropoietic series (b), granulopoietic series (c), and lymphopoietic series (d) in intact mice. Abscissa: time (in days); ordinate: I) total number of myelokaryocytes (10^6/femur); II) number of myelokaryocytes (10^6/femur).

TABLE 1. Partial Erythrogram of Mouse Bone Marrow after Injection of MMW Fraction (M ± m)

Cells ($\cdot 10^{-3}$/femur)	Time of investigation, days			
	0	1	2	4
Proerythroblasts and basophilic erythroblasts	120±99	60±50	110±90	223±200
Polychromatophilic erythroblasts	1400±152	660±300*	770±400*	1430±590
Normoblasts	230±141	220±180	110±56	560±400*
Total no. of cells of erythropoietic series	1750±550	910±430*	990±500*	2223±820

*$P < 0.05$ compared with day 0.

differential counting of 400 bone marrow cells, and the percentages were converted to absolute numbers. Each group contained six to eight mice.

EXPERIMENTAL RESULTS

The results showing the effect of the medium-sized molecules of uremic serum to hematopoiesis in intact mice at different times after their administration are given in Fig. 1. The absolute number of myelokaryocytes varied within normal physiological limits. The lymphocyte count was virtually unchanged. The number of granulocyte precursor cells was increased from the first day and remained high until the end of the time of observation. The number of cells of the erythroid series fell to its lowest level on the 1st day (by 47%, $P < 0.05$), it remained low on the 2nd day (reduced by 42%, $P < 0.05$), but on the 4th day it was back to normal.

A study of the erythrograms (Table 1) on the 1st day revealed a marked decrease in the number proerythroblasts and basophilic erythroblasts (50%), but the number of normoblasts was unchanged. On the 2nd day the number of immature cells of the erythropoietic series was back to normal, but the number of normoblasts was reduced (48%). By the 4th day the numbers of all these cells were back to normal.

The results are evidence that the MMW fraction isolated from the serum of patients with CRF specifically inhibits erythropoiesis in intact animals but does not affect other bone marrow cells. It can be concluded from the dynamics of changes in the erythrogram that the MMW fraction acts on the stages of early erythroid precursors, possibly on the stages of the erythropoietin-sensitive cell (ESC). The ESC is known not to differ morphologically, and differentiation is the only way of showing its development from the pluripotent bone marrow stem cell [6, 7]. It has been shown that erythropoietin increases the rate of differentiation of the ESC into proerythroblasts. Erythroblasts began to appear in the spleen of polycythemic mice, with inhibited erythropoietin production, 24 h after injection of erythropoietin, and the number of reticulocytes in the blood was increased on the 3rd-4th days. The authors cited concluded that erythropoietin increases erythrocyte production by increasing the rate of differentiation of stem cells into the erythron [5].

Our own observations, revealing a decrease in the number of proerythroblasts and basophilic erythroblasts 24 h after injection of the MMW fraction suggest that it acts on the ESC. These data are in agreement with the results of studies of the effect of uremic serum on erythropoiesis in polycythemic mice [1]. Those investigations showed that on the 1st day after injection of erythropoietin the number of erythroblasts in the bone marrow was increased by 100%, but when uremic serum was injected together with erythropoietin there was no stimulation.

The results of investigations on intact mice thus demonstrated the specificity of action of the MMW fraction isolated from the serum of patients with CRF on erythropoiesis. Its effect is exhibited on the early stage of the erythron, for inhibition of cells of the erythroid series was observed 24 h after injection, with a maximal decrease on the 2nd day. The results of this investigation indicate an important role of substances with MMW in the pathogenesis of nephrogenic anemia.

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EFFECT OF IMMOBILIZATION OF MICE DIFFERING IN RADIOSENSITIVITY AND OF SCREENING PART OF THEIR BONE MARROW ON SURVIVAL RATE AND SPLENIC COLONY FORMATION AFTER IRRADIATION

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KEY WORDS: bone marrow; screening; immobilization; colony formation in spleen; irradiation.

Counting colonies in the spleen is regularly used to judge recovery of hematopoiesis in mice after irradiation. It is considered that direct correlation exists between the survival rate of the animals and the number of colonies formed in the spleen (within the dose range inducing a hematopoietic syndrome) [6, 7]. However, investigations have shown that changes in survival rate under the influence of protective agents are not accompanied by any corresponding increase (or decrease) in the number of developing splenic endocolonies [1, 8]. The characteristics of correlation between survival and colony formation have been described when age changes in survival rate in mice were compared with colony development after irradiation, and also when colony formation was compared in mice of radioresistant and radiosensitive lines [3, 5]. Restoration of hematopoiesis in radioresistant mice, in which a low level of colony formation was observed, was explained by the authors cited as a result of the greater regenerative activity of the stem cells [4].

To determine the more exact limits to the use of splenic colony counting as a test of survival of mice and to reveal the effects of various factors increasing survival of irradiated mice, the investigation described below was carried out to study dependence of survival rate and splenic endocolony formation on dose in relatively radiosensitive noninbred albino mice and in mice of the radioresistant (CBA × C57BL)F_1 line when their survival was increased by two different methods: by restricting the animals' mobility (immobilization) by tying them to a frame during irradiation, and by screening part of the bone marrow (one leg).

EXPERIMENTAL METHOD

Male noninbred albino mice (700 animals) and (CBA × C57BL)F_1 hybrids (1000 mice) aged 3 months were used in the investigation. Irradiation was given on the RUM-17 apparatus in doses of 4.1-11.4 Gr (dose rate 1.0 Gr/min; voltage

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