MECHANISM OF ACTION OF STEM CELL INHIBITION FACTOR ON EXOGENOUS HEMATOPOIETIC COLONY FORMATION IN MOUSE SPLEEN

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KEY WORDS: stem cell inhibition factor; exogenous hematopoetic colonies.

The existence of a soluble factor secreted by lymphoid cells after treatment with antilymphocytic globulin, and with the ability to inhibit the development of exogenous colonies in the spleen of lethally irradiated mice after transplantation of syngeneic bone marrow treated with this factor (called stem cell inhibition factor – SCIF) in vitro, into them was discovered previously [2, 3]. It has been shown that SCIF does not affect the direction of stem cell differentiation, for the relative numbers of erythroid, myeloid, and megakaryocytic foci remained within normal limits despite a general reduction in the number of colonies [1].

In this investigation an attempt was made to discover some possible mechanisms of the action of SCIF on the formation of hematoepoietic foci in the spleen of lethally irradiated mice.

EXPERIMENTAL METHOD

Male (CBA×C57BL/6)F_1 hybrid mice weighing 20-22 g were obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR.

SCIF was obtained and tested as described previously [2]. Bone marrow cells treated with supernatant from thymocytes incubated with normal rabbit globulin was injected into control mice. The time course of regeneration of erythropoiesis was studied by the method described in [4]. The mice were killed 6 h after receiving an injection of ^{59}Fe (citrate, specific activity 0.2 mCi/ml) in a dose of 0.5 μCi and incorporation of label into the spleen and bone marrow (1 femur) was counted on a gamma-spectrometer (Nuclear Chicago, USA).

The fractions of stem cells settling in the spleen (CFUs) (f) was determined by the method in [16] and calculated by the equation:

$$f = \frac{A}{B} \cdot 100\%,$$

where A is the number of CFUs found in the spleen of secondary recipients, multiplied by the dilution factor (in this case by 3, for the spleen cell suspension from intermediate recipients was divided into three parts for injection into the final recipients); B denotes the number of CFUs in the primary transplant.

To determine the number of CFUs in the S phase of the cell cycle, bone marrow cells 2 h after treatment with SCIF were incubated with hydroxyurea (from Serva, West Germany) in a final concentration of 1 mg/ml for 2 h in medium 199 and 2.5% fetal calf serum and 1% HEPES (37°C, 5% CO_2).

The colony-forming ability of the transplant was estimated by the usual method [18]. Cells were injected into lethally irradiated (8.0 Gy) mice in the course of 3 h after irradiation. Colonies were counted on the 8th-9th day.

EXPERIMENTAL RESULTS

The study of the time course of regeneration of erythropoiesis in lethally irradiated mice showed that treatment with SCIF leads to delay of the beginning of restoration of erythropoiesis in the spleen (i.e., the be-

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TABLE 1. Effect of Treatment of Bone Marrow Cells with SCIF in Vitro on Exogenous Colony Development in Spleen of Mice Irradiated in a Dose of 8.1 Gy

Type of treatment of bone marrow cells	Number of experiments	Number of animals	Number of hematopoietic foci in spleen (M ± m)	Inhibition of colony formation, per cent
CS	3	28	9,54±0,96	—
SCIF	3	27	2,89±0,39	69,7

Legend. Number of bone marrow cells injected was $0.5 \cdot 10^5$. Incubation with SCIF for 1 h at 37°C. Here and in Tables 2 and 3: CS) control supernatant obtained after treatment of thymus cells with normal rabbit globulin.

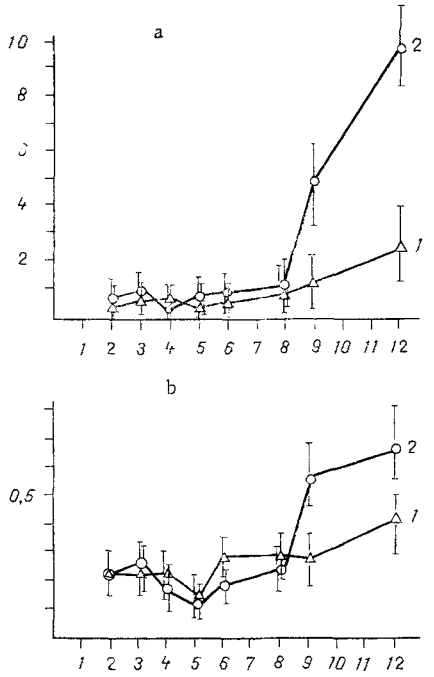

Fig. 1. Dynamics of ^{59}Fe uptake into spleen (a) and bone marrow (b) after irradiation of mice in a lethal dose (8.0 Gy) and injection of 10^5 bone marrow cells. Abscissa, time after irradiation and transplantation of hematopoietic tissue (in days); ordinate, ^{59}Fe incorporation (in percent of activity injected). 1) SCIF, 2) control supernatant. Combined results of 3 experiments shown; each point represents data for at least 4 mice.

beginning of erythroid differentiation in proliferating CFUs), recorded as incorporation of ^{59}Fe, by about 3 days, compared with the control (Fig. 1). Incorporation of the isotope in bone marrow remained stable for 8 days after irradiation and it began to rise after the 9th day in the control and the 12th day in mice receiving bone marrow cells treated with SCIF (Fig. 1). Data on inhibition of SCIF for postradiation restoration of erythroid hemato-

TABLE 2. Number of CFUs (in percent) in S Phase of Cell Cycle under Normal Conditions and 2 h after Treatment of Bone Marrow Cells with SCIF

Type of treatment	Number of colonies in spleen (M ± m)	Number of cells in S phase, per cent
CS	10,7±0,85 $\left(\frac{2}{32}\right)$	6,6
CS + HU	10,0±0,77 $\left(\frac{2}{30}\right)$	
SCIF	1,95±0,36 $\left(\frac{2}{21}\right)$	
SCIF + HU	0,75±0,14 $\left(\frac{2}{28}\right)$	61,5

Legend. Incubation with SCIF lasted 1 h, incubation with hydroxyurea (HU) lasted 2 h at 37° C. In irradiation control there were fewer than 0.15 endogenous colonies per spleen. Number of bone marrow cells injected was 10^5. Here and in Table 3 numerator gives number of experiments, denominator – number of mice.

poiesis in the spleen correlate with the inhibitory action of the factor on exogenous colony formation, determined in parallel tests (Table 1).

Exogenous colonies in the spleen are known to be formed mainly from CFUs of the graft which, at the time of transplantation, are in the G_0 phase of the cell cycle [5]. Hence it follows that if treatment of the bone marrow cells with SCIF stimulates the emergence of CFUs into the S phase, this may lead to partial loss of colony-forming ability by the graft. To determine the number of CFUs in the S phase, treatment with hydroxyurea, which inhibits ribonucleotide reductase activity [17], so that deoxyribonucleotide formation ceases and the cell cannot begin to synthesize DNA, was used. It was found (Table 2) that 2 h after treatment with SCIF, more than 60% of CFUs entered the phase of DNA synthesis. The possibility cannot be ruled out that the rapid stimulation of emergence of CFUs into the S phase may be one cause of inhibition of hematopoietic colony development after transplantation.

Some procedures (injection of vinblastin and endotoxin [9], or 5-fluorouracil [8], and treatment with neuraminidase [14, 19], are known to disturb the migration of transplanted CFUs into the spleen, thus reducing the f fraction. A study of the ability of CFUs from bone marrow treated with SCIF to migrate into the spleen showed (Table 3) that 2 h after transplantation the value of f, which was 15.8% in the control, fell to 4.2%. This may be evidence that pretreatment with SCIF somehow prevents CFUs from settling in the spleen. Treatment of CFUs with neuraminidase (but not with elastase, pronase, papain, or trypsin) had a similar action, with the result that the value of f was reduced and proliferation of CFUs was delayed [7, 19]. Injection of 5-fluorouracil, which mainly damages cells in the S phase, into donor mice is known to delay proliferation of transplanted CFUs by 3 days; subsequently, moreover, with an increase in the time elapsing after irradiation, the number of exogenous colonies increases: Whereas on the 10th day after transplantation one or two colonies appeared in the spleen, on the 13th day there were more than 20 of them, whereas in the control the number of colonies in the period from the 9th to the 13th day did not change [10, 15]. On this basis the authors cited [10] postulated the existence of a "pre-CFUs" population, resistant to 5-fluorouracil and giving rise to CFUs after transplantation. According to another point of view, CFUs require a certain length of time to repair injuries after exposure to the cytostatic, and as a result their proliferation in the spleen commences after a delay [15]. Recent investigations have shown that in fact it is perhaps only exogenous colonies, detectable 12-13 days after transplantation, that develop from stem cells, whereas hematopoietic foci recorded in the earlier stages are transient in character, and are evidently formed from a more highly differentiated progeny, which has preserved a sufficiently high stem potential [12]. A study of the effect of SCIF on colony formation also showed [1] that the number of exogenous hematopoietic foci increases rapidly on the 14th day after transplantation, and as a result the inhibitory effect of SCIF is neutralized. Under these circumstances, besides large raised colonies, small discrete foci

TABLE 3. Determination of Fraction for Bone Marrow CFUs

Type of treatment of bone marrow cells	Type of recipients	Number of cells injected	Number of hematopoietic foci in spleen (M ± m)	f, %
CS	R_3	1/3 of spleen R_2	$15,3 \pm 1,0 \frac{2}{21}$	15,7
CS	R_1	$5 \cdot 10^4$	$7,4 \pm 0,6 \frac{2}{17}$	
SCIF	R_3	1/3 of spleen R_2	$2,0 \pm 0,3 \frac{2}{23}$	
SCIF	R_1	$5 \cdot 10^4$	$3,1 \pm 0,4 \frac{2}{21}$	4,8

Legend. Spleen cells removed from R_2 recipients into which $2 \cdot 10^6$ bone marrow cells had been injected 2 h previously.

also developed. A similar pattern was observed during proliferation of CFUs from mice receiving 5-fluorouracil [10, 11]. After treatment with SCIF the duration of the lag-period, which usually does not exceed 48 h [6], also increased, after which the transplanted CFUs began to proliferate in the recipient. The switching of a considerable proportion of transplanted CFUs into the phase of DNA synthesis and also the decrease in value of the f fraction may also be facilitated by inhibition of colony formation after treatment with SCIF. The two last effects are perhaps interconnected: The ability of CFUs to colonize the spleen has been shown to depend on the phase of the cell cycle [13], and a sharp decrease in the value of f for CFUs entering the cycle after synchronization by a cytostatic has been demonstrated [8]. Probably the mechanisms described above are not the only cause of the inhibitory effect of SCIF on colony formation. A further investigation is planned to study the other possible mechanisms.

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