POSSIBLE ROLE OF T CELLS IN THE REGULATION OF HEMATOPOIESIS IN SITU

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It can now be taken as established that T cells and their products have a modulating action on hematopoiesis under experimental conditions, by modifying the self-maintaining ability of hematopoietic stem cells and also their proliferative and differentiating potential [2, 4, 5, 8]. However, most of the data on the action of T cells on hematopoiesis have been obtained under nonphysiological conditions: in the absence of syngeneity [2, 4], with the use of modified bone marrow [5, 13], or on mixing of heterogeneous cell populations in vitro [3, 11]. There is virtually no convincing evidence in the literature on the physiological role of T cells in the regulation of hematopoiesis. Data on successful correction of disturbances of medullary hematopoiesis in patients with aplastic anemia after elimination of autoreactive T lymphocytes may be taken as the exception [7, 15].

The aim of this investigation was to establish the possibility of physiological negative regulation of normal hematopoiesis by T cells in situ, for which purpose an approach based on elimination of medullary suppressor T cells by means of antiserum against allelic products of the specific marker of suppressor T lymphocytes (I-J antigen) was used.

EXPERIMENTAL METHOD

Inbred mice of lines CBA ($H = 2^k$), AKR ($H = 2^k$), A/Sn ($H = 2^{k/d}$), and CC57BR ($H = 2^b$), obtained from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR, were used.

Antisuppressor alloantiserum of $(3R \times DBA/2)F_1$ mice against Con A-blasts of 5R mice (anti-I-J^k) was obtained and generously provided by Dr. A. S. Apt (Central Research Institute of Tuberculosis, Ministry of Health of the USSR). The specific antisuppressor activity of the serum was tested relative to a number of parameters [1].

The basic scheme of the experiments was as follows: the femoral bone marrow was removed from the mice, homologous suspensions were obtained, and cells were incubated with anti-I-J-serum (10^6-$5 \cdot 10^6$ cells in 0.2 ml of HEPES-buffered medium) for 30 min at 4°C. Fresh guinea pig complement was then added (1:4) and the mixture was incubated for 30-60 min at 37°C. The cells were then washed by centrifugation (or not washed — this did not affect the results) and their viability was determined by studying incorporation of trypan blue: the number of dead cells as a rule did not exceed 10%. The cells were diluted to the required concentration and the number of stem cells (CFUs) was counted by the method of exocolonization of the scheme of lethally irradiated syngeneic recipients. The mice were autopsied 7-8 or 11-12 days after irradiation, the spleens were fixed in Bouin's solution, and the number of macroscopic surface colonies was counted. Background endocolonization did not exceed 0.1 colony per spleen. In some experiments spleen cells were tested by a similar scheme. Bone marrow cells were injected in a dose of $5 \cdot 10^4$, and spleen cells in a dose of $5 \cdot 10^5$ per mouse.

The fraction f of CFUs migrating into the spleen and forming colonies was determined by the classical method [12]. Syngeneic bone marrow cells ($5 \cdot 10^4$) were injected intravenously into primary recipients (R_1). Recipients of the second group were injected with $2 \cdot 10^6$ bone marrow cells, and 2 h later these mice were killed, suspensions of their spleen cells were prepared, and these were injected, in a dose of one-third of a spleen, into recipients of the third group (R_3). The mice were killed on the 8th day and the number of exogenous colonies counted. The fraction f was determined by the equation:

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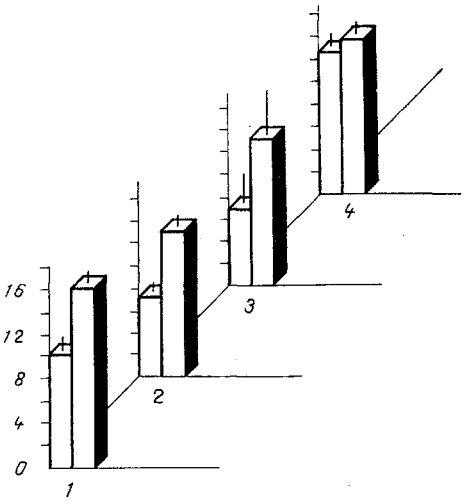

Fig. 1. Effect of treatment of bone marrow cells with anti-I-J^k-serum on formation of hematopoietic colonies in spleen of mice of various lines. Vertical axis, number of colonies in spleen. Column on left — mean number of colonies formed from CFUs of bone marrow not treated with antiserum; column on right — after treatment with antiserum and complement. 1) AKR, 2) CBA, 3) A/Sn, 4) CC57BR mice.

$$f = \frac{R_3 \cdot 3}{R_1 \cdot 40} \cdot 100,$$

where R_1 denotes the mean number of colonies in the spleen of the R_1 mice; R_3 the mean number of colonies in the spleen of the R_3 mice; f the fraction of CFUs of the transplant migrating into the spleen and giving rise to colonies (in %).

EXPERIMENTAL RESULTS

In the experiments in which bone marrow cells of CBA, AKR, and A/Sn mice were treated with anti-I-J^k serum and subsequently transplanted into lethally irradiated syngeneic recipients, an increase in the number of exogenous colonies was found in all cases 7-8 days after transplantation compared with the colony-forming ability of the control bone marrow (treated with normal serum or complement). When bone marrow cells from CC57BR mice were treated in the same way no difference from the control could be detected (Fig. 1). The stimulating effect was preserved, at least partially, when colonies were counted after 11-12 days.

Under similar experimental conditions, when spleen cells were used, the tendency toward stimulation was preserved when the number of exogenous colonies was counted 7 days afrer transplantation (Table 1). If the number of colonies was counted 11-12 days after transplantation, treatment with antiserum did not cause any increase in the number of exogenous colonies.

The study of the complement-dependent action of antiserum on transplanted hematopoietic cells thus demonstrated an increase in the number of colonies formed from CFUs after treatment with antiserum compared with the control transplant. The effect of the antiserum was specific toward CFUs of mice with a well-marked I-J^k allele: CBA (H = 2^k), AKR (h = 2^k), and A/Sn (K^k-$A^kJ^kE^kC^dS^dL^dD^d$), and it was not observed in mice with the H = 2^b haplotype (CC57BR). In the writers' view stimulation of the efficiency of colony formation is connected with elimination of suppressor cells expressing the I-J marker by the action of the antiserum. However, to make sure that the stimulating effect could not be explained by other, nonspecific causes, a series of additional controls was set up. In the first place, it was necessary to determine whether the fraction f of the transplant is changed by the action of the antiserum.

It was found (Table 2) that the ability of CFUs, treated with anti-I-J-serum to migrate into the spleen and to give rise to colonies was maintained at the normal level, namely 12.2% in the control and 12.7% after treatment with antiserum. Another probability to be tested was an increase in the endogenous background under the influence of the antiserum. To test this hypothesis antiserum was injected into recipients without transplantation of hematopoietic cells. Endocolony formation under these circumstances did not exceed 0.2 per spleen, i.e., it could not have affected the results.

TABLE 1. Effect of Preliminary Treatment of Spleen Cells with Anti-I-J^k-Serum on Exogenous Colony Formation in Spleen of Syngeneic Mice 7 and 11-12 Days after Transplantation

Line of mice	Expt. No.	Treatment with antiserum	No. of colonies (M ± m) after 7 days	No. of colonies (M ± m) after 10-11 days
CBA	1	—	6,0±0,8 (10)	4,6±0,9 (6)
		+	9,0±1,3 (10)	3,7±0,8 (7)
	2	—	11,3±1,2 (9)	5,0±0,4 (5)
		+	15,3±1,4 (10)	5,1±0,7 (7)
	3	—	5,4±0,8 (11)	—
		+	11,2±1,7 (10)	
AKR	1	—	7,8±2,6 (6)	—
		+	16,5±3,8 (12)	

Legend. By summation of results of three experiments done on CBA mice the following values are obtained: control (without antiserum treatment) 7.4 ± 1.0; after treatment 11.8 ± 1.1 (t = 2.98). Number of mice given in parentheses.

TABLE 2. Effect of Treatment of Bone Marrow Cells from CBA Mice with Anti-I-J^k-Serum on Fraction f of Transplant

Bone marrow cells	Recipient	Number of colonies (M ± m)	Fraction f, %
Normal	R_1	5,3±0,5	12,2
	R_3	8,6±1,5	
Treated with antiserum	R_1	8,3±1,0	12,7
	R_3	14,0±1,5	

The results thus indicate that elimination from native hematopoietic tissue of cells expressing the specific marker of suppressor T cells, namely I-J antigen, leads to stimulation of the colony-forming ability of CFUs. By contrast with experiments to study T-cell regulation of hematopoiesis in which exogenous syngeneic and nonsyngeneic T cells and (or) their products were used [2, 4-6], in the present investigation T-cell populations of hematopoietic tissue were subjected to negative selection. We know that the number of T cells in bone marrow is small, although it may increase during stress. T cells expressing the I-J marker are present in bone marrow in an even lower concentration. With respect to the absolute number of these cells in the lympho-hematopoietic organs, the first place is occupied by the spleen, followed in decreasing order by lymph nodes, thymus, and bone marrow, although with respect to the relative concentration of these cells among the total T-cell population the order is almost reversed: bone marrow, spleen, lymph nodes, and thymus [10]. It is perhaps due to the lower concentration of $I\text{-}J^+$ cells in the spleen that treatment of splenocytes with anti-I-J-serum in the presence of complement leads to stronger stimulation of colony formation. The absence of effect of the antiserum in this case on testing 12 days after transplantation is most likely to be due, not to the fact that splenic CFUs are less sensitive to the action of the regulator than mature colony-forming precursors, producing colonies after 7 days, but to the fact that the CFUs concentration in the spleen was much lower than that of 7-day colony-forming cells. Evidence that this is so is given by the decrease in the number of colonies formed from the splenic transplants when the animals were autopsied after 7 and 11-12 days, respectively, whereas during an analogous investigation of colony formation by bone marrow cells no decrease in the number of colonies was observed on the 12th day (Tables 1 and 2), in agreement with data in the literature [6].

The functions of suppressor T cells may evidently be wider than has hitherto been considered, and not limited purely to control of lymphoid populations [9]. For instance, some disturbances of hematopoiesis and, in particular, aplastic anemia are linked with activation of

autoreactive suppressor T cells [7, 15]. Activity of suppressor T cells rises sharply in mice with experimental tuberculosis [1, 14], and these animals develop a transient anemia due to inhibition of erythropoiesis by suppressor T cells, against the background of compensatory stimulation of granulocytopoiesis, which plays an important role in the development of the chronic process [8]. Evidently there are at least two subpopulations of regulatory T cells, which exert a helper and suppressor action on proliferation and differentiation of CFUs, and thus participate in the homeostatic regulation of hematopoiesis. Under normal conditions these populations are balanced (which evidently accounts for the unsuccessful attempts to discover the effect of the unfractionated T-cell pool on hematopoiesis in a syngeneic system), but their homeostatic equilibrium may be upset during stress, thereby resulting in pathology of hematopoiesis.

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