In the present writers' opinion, the results described in this paper do not contradict the hypothesis of the derepressing action of ATG on hematopoietic presursors in forms of HA in which the number of immunologically active T lymphocytes in the bone marrow is increased.

Analysis of the structure of the bone-marrow lymphocyte pool in the 15 patients investigated by the writers showed that the predominant cells in it were B lymphocytes and "null" cells; T cells formed only a very small proportion of the lymphocytes. It can be tentatively suggested that the number of target cells was insufficient for the derepressing action of ATG in the cases investigated.

LITERATURE CITED

1. B. V. Afanas'ev, A. Yu. Zaritskii, and T. S. Zabelina, Fiziol. Cheloveka, No. 2, 301 (1976).
2. J. Ascensao, R. Pahwa, et al., Lancet, 1, 669 (1976).
3. A. J. Barrett, A. Faille, F. Saal, et al., Path. Biol., 26, 35 (1978).
4. A. Faille, A. J. Barrett, N. Balitrans, et al., Br. J. Haemat., 42, 371 (1979).
5. E. Gluckman, A. Devergie, A. Faille, et al., Exp. Hemat., 6, 679 (1978).
6. H. Haak and J. Velde, Br. J. **Haemat.**, 35, 671 (1977).
7. R. Hoffman. E. Lanjani, J. Lutton, et al., Blood, 48, 1003 (1976).
8. W. Kagan and J. Ascensao, Proc. Natl. Acad. Sci. USA, 73, 2890 (1976).
9. J. Kaplan, S. Inoue, and M. Ottenbreit, Nature, 271, 458 (1978).
10. J. Singer, K. Donney, and E. Thomas, Blood, 54, 180 (1979).
11. B. Speck, Transplant. Proc., 10, 131 (1978).
12. B. Speck, E. Gluckman, H. Haak, et al., Clin. Haematol., 7, 611 (1977).

PROBABLE NATURE OF THE CELL POPULATION RESPONSIBLE FOR SPLENIC COLONY FORMATION

O. V. Semina, T. N. Semenets,
A. A. Yarilin, and A. M. Poverennyi

UDC 612.411.014.3:612.6

Rabbit anti-mouse brain serum (RAMBS) has been shown [8] to be capable of cross-reacting with hematopoietic stem cells, resulting in a sharp decrease in the ability of bone marrow treated with this serum to form splenic exocolonies. In many laboratories of the world this method has been used to obtain further information about stem cells. It is now held that four antigens of hematopoietic cells can be distinguished with the aid of RABMS; these include T-cell antigen and antigen of pluripotent stem cells, which disappears as a result of differentiation, leading to commitment, to granulopoiesis [9] for example.

Investigations [4, 5] have shown that the ability of bone marrow to form colonies, if inhibited by RAMBS, can be substantially restored by the addition of syngeneic thymocytes. On the basis of these data the present writers suggested that RAMBS may perhaps inactivate not stem cells directly in the bone marrow, but cells of another population, present in the bone marrow and essential for normal colony formation. This hypothesis is in good agreement with the results of recent investigations showing that certain definite intercellular interactions are essential for the proliferation and differentiation of pluripotent stem cells, and in addition, it must be emphasized that these processes are thymus-dependent [1, 2].

The investigation described below was devoted to a study of certain characteristics of the population essential for exogenous splenic colony formation, and inactivated by RAMBS.

EXPERIMENTAL METHOD

Male CBA, C57BL, and (CBA × C57BL)F_1 mice aged 2.5 months were used. The **recipient** mice were irradiated with ^{60}Co γ rays in a dose of 8.5 Gy. Colony-forming activity of

KEY WORDS: splenic colony formation; T lymphocytes; precursor cells.

Research Institute of Medical Radiology, Academy of Medical Sciences of the USSR, Obninsk.

(Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 4, pp. 485-487, April, 1981. Original article submitted August 15, 1980.

0007-4888/81/9104-0528$07.50

TABLE 1. Effect of Different Sera on Colony-Forming Ability of Bone Marrow (M ± m)

Treatment of bone marrow	Number of mice	Number of colonies per 10^5 injected bone marrow cells
—	23	11,7±0,9
RAMBS	31	1,4±0,3
RAMBS exhausted by thymus	28	5,5±0,8
RAMBS exhausted by brain	28	8,8±1,4
Anti-θ-serum	24	9,0±1,1
NRS	23	9,3±0,9

TABLE 2. Effect of Treatment of Thymus Cells on Their Ability to Abolish the Action of RAMBS on Colony-Forming Ability of Bone Marrow (M ± m)

Treatment of bone marrow	Thymus cells (2.0×10^7 cells per mouse)	Number of mice	Number of colonies per 10^5 injected bone marrow cells
—	—	30	12,2±0,9
RAMBS	—	33	1,6±0,5
"	No treatment	30	8,4±0,8
"	Anti-θ-serum	23	6,3±1,0
"	Anti-θ-serum + complement	20	6,0±1,1
"	RAMBS	21	3,4±0,7
"	Thymus from mice treated with Con A	20	1,6±0,3

the bone marrow cells was determined by the method of splenic exocolonies [12]. RAMBS was obtained as described previously [7]. A 100% level of cytotoxicity for thymocytes was achieved in dilution of 1:40 and the working dilution was 1:2. Exhaustion of RAMBS by thymocytes was carried out by incubating 1 ml serum with $4 \cdot 10^8$ cells, washed beforehand, for 2 h at 37°C. RAMBS was similarly exhausted with brain (1.5 g brain tissue to 1.5 ml serum). Anti-θ-serum was obtained by immunization of AKR mice with thymocytes of CBA mice. A cytotoxicity of 100% against thymocytes was maintained in a dilution of 1:40 and the working dilution was 1:2. Bone marrow cells and thymocytes were treated with RAMBS and anti-θ-serum as described in [4, 5]. Concanavalin A (Con A, from Calbiochem, USA) was injected intraperitoneally into the donors in a dose of 300 μg/mouse in 0.2 ml physiological saline 2 days before removal of the thymus. Intact thymocytes ($2\cdot 10^7$ cells per mouse) were injected intravenously into the recipients 30-40 min before injection of bone marrow cells.

EXPERIMENTAL RESULTS

The experimental results (Table 1) show that RAMBS, exhausted by brain tissue, did not reduce the number of splenic colonies. Serum exhausted by intact thymus cells had a much weaker action on the colony-forming ability of bone marrow than RAMBS. After treatment of the bone marrow suspension with anti-θ-serum the number of colonies formed was practically the same as in the control.

The results for the action of anti-θ-serum on thymus cells are particularly interesting (Table 2). Treatment of intact thymocytes with anti-θ-serum in the presence and in the absence of complement reduced the ability of these cells to increase colony formation by bone marrow treated with RAMBS only very slightly, although the thymus is known to contain mainly cells rich in θ-antigen. Treatment of thymocytes with RAMBS under these same conditions led to the almost total loss of their effect in restoring colony formation. Probably both the thymus and bone marrow contain practically identical cell populations exhibiting a helper effect with respect to stem cells during colony formation. It can be tentatively suggested that these cells either are θ-negative or have low density of θ-antigen on their surface. Probably there are very few of them in the thymus, so that very many thymocytes are needed to abolish the effect of RAMBS on splenic colony formation (optimal ratio 1:200).

In the writers' view, the cells possessing helper properties relative to stem cells are precursor cells of thymocytes. They are present in both bone marrow and thymus. According to data in the literature, one population of precursors of lymphocytes with identical markers (TdT and θ-) in fact exists, in both bone marrow and thymus [10]. Data showing that after exhaustion of RAMBS by thymocytes the reaction with the latter disappears, whereas that with precursors of thymocytes is preserved, although in a lower titer [6], confirm sufficiently well the hypothesis put forward above regarding the existence of a population of thymocyte precursor cells capable of splenic colony formation. This may also perhaps explain the small decrease in the ability of bone marrow to form colonies after treatment with RAMBS, exhausted by thymocytes (see Table 1).

Precursors of thymocytes are known to be capable of being induced to differentiate and to exhibit antigenic markers characteristic of mature lymphocytes by mitogens and thymosin [11]. In the present experiments thymus from mice treated with Con A 2 days before removal of the gland completely lost its ability to abolish the action of RAMBS on splenic colony for-

TABLE 3. Formation of Splenic Colonies after Treatment of Bone Marrow with RAMBS and Injection of Thymocytes from Mice of Different Lines (M ± m)

Treatment of bone marrow	Line of mice	Number of mice	Number of colonies per 10^5 cells injected
—	—	64	12,5±1,2
RAMBS	—	57	1,5±0,4
"	(CBA×C57BL) F_1	63	8,3±0,6
"	CBA	50	3,0±0,5
"	C57BL	53	2,1±0,5
—	(CBA×C57BL) F_1	24	13,1±1,3
—	CBA	15	12,4±1,5
—	C57BL	20	13,5±1,7

Legend. (CBA × C57BL)F_1 mice were both donors of bone marrow and recipients.

mation (see Table 2). The writers showed previously [3] that intraperitoneal injection of Con A into donor mice 2 days before removal of their bone marrow, and also treatment of an intact bone marrow suspension *in vitro* with Con A gives the same result: a reduction of the yield of splenic colonies by half compared with the control. This effect was almost completely abolished by additional injection of thymus cells. Possibly this effect of Con A on bone marrow and thymus is due to proliferation and the subsequent differentiation of precursor cells of T lymphocytes responsible for normal colony formation, under the influence of this agent.

The results suggest that the formation of splenic macrocolonies is preceded by direct interaction between stem cells and precursor cells of T lymphocytes. Such an event is perhaps realized at bone marrow level. This suggestion is confirmed to some extent by the results of experiments given in Table 3. The ability of syngeneic and semisyngeneic thymocytes to restore colony formation from bone marrow treated with RAMBS was investigated. Mainly syngeneic thymocytes and, to a much lesser degree, thymocytes of CBA mice, possess this property.

The view that cells possessing a helper effect in relation to colony formation, and the hypothesis regarding their nature are supported by evidence [13] showing that proliferative control over stem cells is effected by the balance between stimulating and inhibiting factors and is short-ranging, i.e., it is located in the hematopoietic tissue itself. The stimulating cells have a density of 1.064-1.072 g/cm^3, and according to data obtained by other workers the peak activity of thymocyte precursors lies in approximately the same region.

LITERATURE CITED

1. I. N. Golovistikov, R. V. Petrov, and R. M. Khaitov, Dokl. Akad. Nauk SSSR, 194, 1208 (1970).
2. R. V. Petrov, R. M. Khaitov, N. V. Aleinikova, et al., Radiobiologiya, 14, 560 (1976).
3. A. M. Poverennyi, O. V. Semina, and A. G. Konoplyannikov, Dokl. Akad. Nauk SSSR, 223, 1248 (1975).
4. A. M. Poverennyi, O. V. Semina, A. A. Yarilin, et al., Radiobiologiya, 18, 545 (1978).
5. A. M. Poverennyi, O. V. Semina, T. N. Semenets, et al., Byull. Éksp. Biol. Med., No. 6, 708 (1978).
6. R. S. Basch and J. L. Kadish, J. Exp. Med., 145, 405 (1977).
7. E. S. Golub, Cell. Immunol., 2, 353 (1971).
8. E. S. Golub, J. Exp. Med., 136, 369 (1972).
9. F. C. Monette, Blood Cells, 5, 175 (1979).
10. P. H. Naylor and A. L. Goldstein, Life Sci., 25, 301 (1979).
11. O. W. Press, C. Rosse, and J. Clagett, J. Exp. Med., 146, 735 (1977).
12. J. Till and E. McCulloch, Radiat. Res., 14, 213 (1961).
13. E. G. Wright and B. I. Lord, Leukemia Res., 3, 15 (1979).