EFFECT OF ANTITHYMOCYTIC GLOBULIN ON COLONY-FORMING ABILITY OF BONE MARROW IN HYPOPLASIA OF MYELOPOIESIS

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According to one hypothesis on the mechanism of development of hypoplasias of myelopoiesis [8], the onset and progression of the disease are connected with immune suppression of hematopoietic stem cells. It has been suggested that not only humoral antibodies, but also cytotoxic cellular mechanisms participate in the development of bone marrow insufficiency of immunologic nature.

Investigations [2, 7, 8] have shown that lymphocytes of patients with hypoplastic anemia (HA) reduce the colony-forming ability (CFA) of normal bone marrow. The suppressive effect of lymphocytes also has been discovered on precursor cells of the granulomonocytic series [2, 8] and on erythroid precursor cells [7].

At the same time it has been shown [5, 11, 12] that treatment of a bone-marrow cell suspension with antithymocytic globulin (ATG) increases the colony-forming and cluster-forming ability (ClFA) of the bone marrow of patients with HA. This depressant effect of ATG on the bone marrow of patients with HA is exhibited in the presence of complement and is evidently due to blocking of immunologically active lymphocytes responsible for the cytotoxic effect on precursor cells.

The object of this investigation was to study the effect of ATG on CFA and ClFA of bone marrow during hypoplasia of myelopoiesis.

EXPERIMENTAL METHOD

Altogether 38 investigations were undertaken of CFA and ClFA of bone marrow from 15 children with HA. Antithymocytic globulin of Soviet origin (series I, protein content 8 mg/ml), obtained in the laboratory of the Institute of Epidemiology and Microbiology, Ministry of Health of the RSFSR (Dr. Med. Sci. N. A. Kraskina), was used.

The bone marrow puncture material, in a volume of 0.2-0.3 ml, was introduced into a sterile test tube with 1 ml medium No. 199 and 0.1 ml heparin (from Gedeon Richter, Hungary) in a dilution of 1:10. The resulting cell suspension was thoroughly mixed and allowed to stand at room temperature. After standing for 1 h the supernatant layer, contaminated with only a few erythrocytes, was drawn off and transferred to another tube, and the total number of myelokaryocytes in the suspension was counted.

After counting, the cell suspension was diluted with medium No. 199 to a concentration of $1 \cdot 10^6$/ml and poured into four test tubes, each containing 1 ml.

The first tube served as the control for determination of the initial values of CFA and ClFA of the bone marrow. To the second tube 0.1 ml of diluted rabbit serum was added to assess the action of complement on CFA and ClFA. To the third tube 0.1 ml complement and 0.1 ml ATG in the original concentration were added (to give a dilution of 1:10), and to the fourth tube 0.1 ml complement and 0.1 ml ATG in a dilution of 1:5 were added (final dilution 1:50). The tubes were incubated for 1 h at 37°C.

After incubation the contents of the tubes were centrifuged for 10 min at 800 rpm. The cells in the suspension were counted in all tubes and the survival rate of the cells was determined with the aid of trypan blue. The number of myelokaryocytes in all the tubes and in all the tests remained the same as initially ($1 \cdot 10^6$ in 1 ml) and the survival rate was 98%.

KEY WORDS: hypoplastic anemia; antithymocytic globulin; colony-forming ability of bone marrow.

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TABLE 1. Effect of ATG on CFA and ClFA of Bone Marrow

Statistical index	Initial data						After splenectomy					
	control		complement		ATG		control		complement		ATG	
	CFA	ClFA	CFA	ClFA	CFA	ClFA	CFA	ClFA	CFA	ClFA	CFA	ClFA
n	25	25	24	24	25	24	14	14	14	14	14	14
M	1,84	1,92	1,58	1,75	1,72	1,81	1,42	1,92	1,57	2,33	1,64	2,42
$\pm m$	0,55	0,32	0,43	0,34	0,5	0,27	0,3	0,5	0,3	0,38	0,31	0,42
P	—		>0,05		>0,05		—		>0,05		>0,05	

Legend. Calculated per 10^5 cells.

The cell suspension in each tube was cultured in three dishes in a dose of $2 \cdot 10^5$ cells per dish.

CFA and ClFA of the bone marrow were determined by Pike and Robinson's method in the modification of Afanas'ev et al. [1].

EXPERIMENTAL RESULTS

The results are given in Table 1. They show that the initial CFA and ClFA of the bone marrow of patients with HA were very low and that the number of colony-forming and cluster-forming cells was 1.84 ± 0.55 and 1.92 ± 0.32, respectively.

Under the influence of ATG the number of colony-forming and cluster-forming bone marrow cells was unchanged at 1.72 ± 0.5 and 1.81 ± 0.27, respectively ($P > 0.05$).

Similar results were obtained when CFA and ClFA of the bone marrow were investigated 6 months after splenectomy on the patients with HA. The initial values for CFA and ClFA of the bone marrow remained low. The mean number of colonies was 1.42 ± 0.3 and of clusters 1.92 ± 0.5. After treatment of the bone marrow suspension with ATG the number of colony-forming and cluster-forming cells was unchanged (1.64 ± 0.31 and 2.42 ± 0.42, respectively; $P > 0.05$).

No effect of ATG could thus be detected on CFA and ClFA of the bone marrow of patients with HA either in the initial period of the disease or after splenectomy.

There are as yet few data in the literature on the effect of ATG on colony-forming activity of granulomonocytic precursors in hypoplastic anemia. According to Gluckman et al. [5], who studied the bone marrow of 16 patients with HA, an increase in cluster formation was observed in the culture in eight cases after treatment of the bone marrow cell suspension with ATG.

According to Barrett et al. [3], an increase in ClFA of bone marrow after incubation of the cells with ATG was found in three of six patients.

Faille et al. [4] carried out a simultaneous investigation of the clinical efficacy of antilymphocytic globulin and its effect on the colony-forming activity of committed granulocytic bone marrow precursors of the same patients *in vitro*. They found that in nine of 22 patients with severe aplastic anemia the peripheral blood indices were improved and ClFA of the bone marrow in culture was increased. In the remaining patients antilymphocytic globulin had no effect on hematopoiesis either *in vivo* or *in vitro*.

In the present investigation no effect of ATG on the activity of bone-marrow cells *in vitro* was observed in any of the 51 patients with HA studied. Splenectomy did not change the sensitivity of granulocytic colony-forming cells to ATG.

On the basis of these results it is impossible to assess the mechanism of action of antilymphocytic globulin or of ATG on precursor cells in HA.

Since there is evidence in the literature of a repressive action of the lymphocytes of patients with HA on CFA of bone marrow of healthy donors [2, 8, 10], and also on an increase in colony formation in patients with HA after removal of T lymphocytes from the bone marrow [6], it was suggested that ATG has a derepressing effect on precursor cells in HA.

However, treatment of normal bone marrow with ATG also is known to cause a marked increase in colony formation [4, 9], but it is not yet clear to what this action of ATG on precursor cells under conditions of normal hematopoiesis can be attributed.

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.

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PROBABLE NATURE OF THE CELL POPULATION RESPONSIBLE FOR SPLENIC COLONY FORMATION

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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

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