nection attention is drawn to the fact that the course of leukemia was more severe, and resistant to modern cytostatic agents [2], in patients with OMML and OMoL, on whose blast cells Ig were found.

Proof of the presence of antibodies on the surface of blast cells in these forms of leukemia necessitates the study of their specificity and the attempt, with the aid of these antibodies, to isolate and study the original antigens of the leukemic cell. The dynamics of Ig on the surface of malignant cells in the course of the leukemic process also is interesting. All these matters are undoubtedly important in connection with the elucidation of the complex mechanisms of antileukemic immunity and the explanation of various aspects on interaction between malignant cells and the immune system of the body. In the writers' view the results of the present investigation provide a basis for the development of new methods of immunologic treatment of leukemia.

LITERATURE CITED

1. R. S. Samoilova and T. I. Bulycheva, in: Pathogenesis, Clinical Picture, and Treatment of Leukemias [in Russian], Leningrad (1971), p. 65.
2. R. S. Samoilova and T. I. Bulycheva, Probl. Gematol., No. 10, 33 (1974).
3. S. C. Bansal and H. O. Sjögren, Int. J. Cancer, 9, 490 (1972).
4. M. Cikes, J. Immunol. Meth., 8, 89 (1975).
5. B. Gerstl, L. F. Eng, and J. W. Bigbee, Cancer Res., 37, 444 (1977).
6. J. U. Gutterman, E. M. Hersh, G. M. Mavligit, et al., Natl. Cancer Inst. Monogr., 37, 153 (1973).
7. F. Loor, L. Forni, and B. Pernis, Eur. J. Immunol., 2, 203 (1972).
8. M. Ran and I. P. Witz, Int. J. Cancer, 6, 361 (1970).
9. C. M. Richman, L. Chess, and K. A. Yankee, Blood, 51, 1 (1978).
10. O. Tönder, E. C. Krishman, W. R. Jewell, et al., Acta Pathol. Microbiol. Scand., Sect. C, 84, 105 (1976).
11. E. Sobczak and C. de Vaux Saint Cyr, Int. J. Cancer, 8, 47 (1971).
12. E. S. Vitetta, J. Klein, and W. J. Uhr, Immunogenetics, 1, 82 (1974).
13. I. P. Witz, S. Kinamon, M. Ran, et al., Clin. Exp. Immunol., 16, 321 (1974).

ACTION OF RABBIT ANTI-MOUSE BRAIN SERUM ON SELF-MAINTAINING RAUSCHER ERYTHROLEUKEMIA CELLS

E. B. Mechetner and É. N. Rozinova

UDC 616.155.191.5-092.9-085.373

It was shown previously that the cell population of different strains of self-maintaining Rauscher erythroleukemia consists of two types of cells, morphologically identical with proerythroblasts and erythroblasts [4, 5]. Clonal analysis of erythroleukemic strains maintained by passage *in vivo* (strain RAL) and *in vitro* (strain K-2), revealed the presence of erythroleukemic colony-forming units (ECFU) in the composition of the tumor population, with the property of unlimited self-maintenance and the ability to differentiate into cells of both the types described above [3]. The ECFU were thus identical with or a part of the population of erythroleukemic stem cells (ESC).

Erythroblast antigen (AGEB), specific for all morphologically identifiable cells of the erythroid branch of hematopoiesis, except erythrocytes of mice and other animals, has been found on the surface of ECFU [2, 9]. It has also been shown that splenic CFU (CFU-s) of mouse bone marrow do not carry AGEB on their surface [3]. On that basis it has been suggested that ESC closely resemble committed unipotent erythroid precursors of the BFU-e (erythroid burst-

KEY WORDS: rabbit anti-mouse brain serum; self-maintaining strain of Rauscher erythroleukemia; stem cells.

Laboratory of Immunochemistry and Diagnosis of Tumors and Laboratory of Immunology of Oncogenic Viruses, Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 4, pp. 476-478, April, 1981. Original article submitted August 28, 1980.

0007-4888/81/9104-0516$07.50

TABLE 1. Action of Unexhausted RAMBS (—) and RAMBS Exhausted with Erythrocytes and Thymus (+) on Different Target Cells in the Cytotoxic Test

Target cells	Experimental conditions: RAMBS + complement	II
AKR thymocytes	+	95
	—	0
F_1 thymocytes	+	95
	—	3
AKR splenocytes	+	92
	—	5
F_1 splenocytes	+	97
	—	2
K-2	+	96
	—	—2
	Antibodies against AGEB + complement	90

TABLE 2. Action of RAMBS on Clonogenic Cells of RAL Strain in Colony Growth Inhibition Test (M ± m)

Expt. No.	Experimental conditions	Number of ECFU per 10^4 cells injected	II
1	A	2,77±0,358	0
	B	2,78±0,900	
2	A	12,8±2,12	4
	B	13,4±2,40	
3	A	5,60±1,63	—18
	B	4,75±1,86	

Legend. A) RAL + RAMBS + complement; B) RAL + NRS + complement.

forming unit) and CFU-e (erythroid colony-forming unit) type of proerythroblasts which, as a result of tumor transformation, have acquired the property of self-maintenance *in vivo* and *in vitro*.

However, the data so far obtained did not rule out the possibility that ESC are in fact transformed stem cells which have lost the ability to differentiate into all other branches of hematopoiesis than the erythroid. To exclude or confirm this possibility it was necessary to determine whether ESC possess surface antigens specific for CFU-s. To examine this problem, it was decided to use rabbit anti-mouse brain serum (RAMBS), which reveals S-antigen(s) on the surface of CFU-s [8] which has lost its antibody activity against committed precursor cells and differentiated hematopoietic cells after appropriate adsorption [6, 10, 12, 13].

The object of the investigation was to study the action of RAMBS on ESC and their progeny.

EXPERIMENTAL METHOD

Mice of line AKR and (C57BL/6 × CBA)F_1 hybrids (CBF) were used. Irradiation was carried out on the IPK apparatus (Central Institute of Blood Transfusion). The dose was 950 rads, the dose rate 23 rads/min, and the source ^{137}Cs. Self-maintaining strains of erythroleukemia transformed by Rauscher leukemia virus were used: strain RAL, transplanted in AKR mice, and a clonal suspension culture of K-2 erythroleukemic cells [4, 5].

The RAMBS was obtained by Golub's method [8] with modifications. A rabbit was immunized subcutaneously with brain homogenates of C57BL/6 mice 5 times at intervals of 15 days (first immunization, with Freund's complete adjuvant). Blood was taken 7 days after the last immunization. The immune serum was exhausted successively by erythrocytes and thymocytes of AKR and CBF mice. Erythrocytes and serum were mixed in the ratio 1:2, thymocytes and serum in the ratio 1:1. The cells were incubated with RAMBS for 1 h at 37°C and overnight at 4°C. Completeness of exhaustion was tested by hemagglutination and cytoxicity test [1]. The inhibition index (II) was calculated by the standard formula.

The splenic colony growth inhibition test after Till and McCulloch [12] was carried out as follows. Femoral marrow cells were washed out with CCM medium (RPMI-1640 with 2.5% HEPES and 1% BSA) and mixed with RAMBS in different dilutions or with normal rabbit serum (NRS) in a dilution of 1:2. The number of nucleated bone marrow cells in the sample was $3 \cdot 10^6$ in 0.2 ml medium and the volume of serum was 0.3 ml. The cells were incubated with serum for 1 h at 37°C and sedimented by centrifugation. The residues were treated with 1.5 ml of fresh guinea pig complement (1:10) and incubated for 1 h at 4°C. The cells were then diluted with Eagle's medium with 20% bovine serum and injected intravenously in a dose of $2 \cdot 10^5$ cells into groups of 10-12 irradiated mice. II was calculated by the formula:

$$\left(1 - \frac{N_{exp} - N_{end}}{N_{nrs} - N_{end}}\right) \times 100\%,$$

where N_{exp} is the number of CFU-s in the given series per 10^5 injected cells; N_{nrs} is the number of CFU-s in the control; N_{end} the number of endogenous CFU-s.

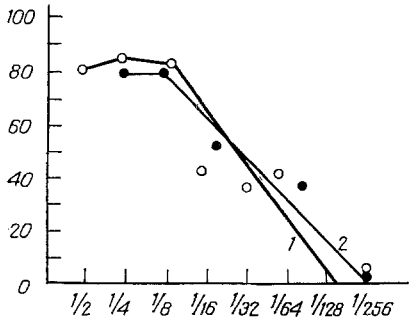

Fig. 1. Inactivation of CFU-s of CBF_1 (1) and AKR (2) mouse bone marrow by RAMBS. Abscissa, dilution of RAMBS; ordinate, inactivation index of CFU-s (in %).

The method of obtaining erythroleukemic colonies in the spleens of unirradiated AKR mice was fully described previously [3]. The colony growth inhibition test was carried out by the scheme described above. The number of cells in the sample was $3 \cdot 10^5$ and the dose $2 \cdot 10^4$ per mouse. II was calculated by the formula:

$$(1 - \frac{N_{exp}}{N_{nrs}}) \times 100\%,$$

where N_{exp} is the number of ECFU in the given experimental series; N_{nrs} the number of ECFU in the control.

EXPERIMENTAL RESULTS

A wide range of antibodies directed against mouse cells was found in the unexhausted RAMBS, including four types of antibodies against hematopoietic cells: T cells, B cells, CFU-s (for details, see [10]). Unexhausted RAMBS agglutinated erythrocytes of AKR and CBF mice in a dilution of 1:32 and was highly toxic for thymocytes and splenocytes of mice of these lines (Table 1). The serum had to be exhausted so that it did not contain antibodies directed against antigens common for CFU-s and other hematopoietic cells (tissue-specific, H-2, etc.). The RAMBS, exhausted by the scheme described above, did not agglutinate AKR and CBF erythrocytes in a dilution of 1:1 and was virtually nontoxic for AKR and CBF thymocytes and splenocytes (Table 1).

Exhausted RAMBS* suppressed 80-85% of CFU-s of both lines of mice in dilutions of between 1:2 and 1:10; on further dilution of the RAMBS its activity was reduced (Fig. 1). Curves of inactivation of CFU-s of AKR and CBF mice were similar. RAMBS thus contained antibodies specifically (within the limits of sensitivity of the method) suppressing CFU-s.

Since the action of RABMS on target cells was evidently due to contact between opsonized antibodies of the CFU-s and the reticuloendothelial system *in vivo*, and although it was connected with the action of complement *in vitro* [10], cells of strain RAL, **capable of forming splenic** tumor colonies *in vivo* were used for subsequent analysis. Treatment of RAL cells with

*The word "exhausted" is henceforward omitted.

TABLE 3. Action of RAMBS and of RAMBS Exhausted by K-2 Cells on Activity of CFU-2

Expt. No.	Experimental conditions	Number of CFU per 10^5 injection cells	Number of endogenous colonies	II
1	A	3,85±0,568		83
	B	3,60±0,402	0,0625±0,0625	84
	C	22,1±2,24		—
2	A	0,497±0,287		96
	B	0,497±0,319	0	96
	C	12,4±1,66		—
3	A	3,11±0,976		75
	B	3,73±0,488	0,500±0,224	69
	C	10,9±0,856		—

Legend. A) Bone marrow + RAMBS + complement; B) bone marrow + RAMBS K-2 + complement; C) bone marrow + NRS + complement.

RAMBS did not reduce the number of ECFU compared with the control (Table 2). It follows from Table 1 that K-2 erythroleukemic cells, maintained by passage *in vitro* and similar to RAL cells in their surface antigen spectrum [2], containing AGEB on their surface, were not inactivated by RAMBS.

The RAMBS was exhausted twice (ratio of volume of cells to volume of RAMBS 1:1; 1 h at 37°C and overnight at 4°C) with K-2 cells. RAMBS exhausted by K-2 cells did not lose its cytotoxic properties against CFU-s of AKR mice (Table 3).

The question of the nature of the target cell for the transforming action of Rauscher and Friend viruses is currently being widely discussed [11, 14]. Most workers are inclined to the view that these viruses, which infect cells of all branches of hematopoiesis and CFU-s, transform erythroid precursor cells *in vivo* and *in vitro*.

The results of the present experiments support the view that RAMBS does not contain antibodies against ESC of strain RAL or their progeny. Consequently, ESC do not carry S-antigen(s) specific for CFU-s on their surface. This conclusion is in good agreement with those drawn previously on antigenic differences between CFU-s and ESC of strain RAL for AGEB [3]. Further investigations are needed for the more accurate identification of the level of differentiation of ESC of strains RAL and K-2.

LITERATURE CITED

1. B. D. Brondz, Byull. Éksp. Biol. Med., No. 5, 64 (1964).
2. E. S. Ievleva et al., Probl. Gematol., No. 11, 40 (1979).
3. E. B. Mechetner et al., Probl. Gematol., No. 9, 51 (1979).
4. É. N. Rozinova et al., Probl. Gematol., No. 9, 46 (1977).
5. Z. N. Tikhonova et al., Probl. Gematol., No. 9, 42 (1977).
6. S. S. Adler et al., Blut, 37, 211 (1978).
7. S. S. Adler et al., Blut, 39, 61 (1979).
8. E. S. Golub, J. Exp. Med., 136, 369 (1972).
9. E. S. Ievleva et al., Int. J. Cancer, 17, 798 (1976).
10. F. C. Monette, Blood Cells, 5, 175 (1979).
11. J. P. Okunewick, Biomedicine, 26, 152 (1977).
12. J. E. Till and E. A. McCulloch, Radiat. Res., 14, 213 (1961).
13. G. J. Van den Engh and E. S. Golub, J. Exp. Med., 139, 1621 (1974).
14. G. J. Van den Engh et al., in: Experimental Hematology Today, New York (1978), p. 28.