The results obtained with experimental sarcoma 45 thus indicate that repeated fractional combined local SHF hyperthermia with x-ray irradiation, irrespective of the order in which the factors are applied, can result in the practically complete absorption of the tumor in the logarithmic stage of its growth.

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ORIGIN OF IMMUNOGLOBULINS BOUND WITH SURFACE MEMBRANES OF MALIGNANT CELLS IN SOME NONLYMPHOID FORMS OF ACUTE LEUKEMIA IN MAN

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In malignant neoplasms of varied tissue origin, in both man and animals, the tumor cells acquire the ability to bind on their surface antibodies against various classes of immunoglobulins (Ig), mainly IgG [5, 9, 11, 14]. Malignant cells of tumors of hematopoietic tissue — acute human myelo-, myelomono-, and monoblastic leukemias (OML, OMML, and OMoL respectively) possess the same property [1, 2, 6]. No Ig have been found on membranes of immature cells of the normal myelomonocytic series of hematopoiesis [10], and for that reason the fact that anti-Ig-antibodies are bound by blast cells in these forms of acute leukemia is difficult to explain and is of considerable interest.

The object of the present investigation was to study structure-binding antibodies against different classes of Ig on the surface membranes of Ig-positive [2] blast cells in certain forms of acute leukemia in man.

EXPERIMENTAL METHOD

Peripheral blood leukocytes from four patients with OMML and two patients with OMoL, taken in the acute period of the disease before the beginning of cytostatic chemotherapy, and preserved in a viable state at −196°C were studied. The leukocyte preparations contained 53-95% of blast cells.

Ability of the membrane to bind anti-Ig-antibodies was estimated by the direct immunofluorescence test (DIT) [2], using goat antisera against human IgG, IgM, IgA, and IgD, labeled with fluorescein isothiocyanate (**Behringwerke, West Germany**).

The presence of receptors for the Fc-fragment of IgG (FcR) on the surface of the cells was determined by rosette-formation with sheep's red blood cells sensitized with rabbit antierythrocytic antibodies [7].

KEY WORDS: leukemia; immunoglobulins.

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TABLE 1. Immunologic Characteristics of Blast Cells in Peripheral Blood of Patients with OMML and OMoL

Patient's initial	Type of leukemia	Number of cells, %					
		blast	cells binding labeled anti-Ig				cells with FcR
			anti-IgG	anti-IgM	anti-IgA	anti-IgD	
A.	OMML	95	52	5	0	0	13
B.	OMML	88	65	6	0	0	51
L.	OMML	93	30	0	0	0	20
K.	OMML	53	42	8	0	0	62
M.	OMoL	66	65	10	0	0	49
Ch.	OMoL	70	84	24	0	0	32

Spontaneous elution of structures binding anti-Ig-antibodies was studied by incubation of the cells in RPMI-1640 medium with the addition of calf embryonic serum (from Difco, USA) at 37°C for 20 h, with a suspension density of $1 \cdot 10^6$ cells/ml. After incubation the cells were tested repeatedly in the DIT and rosette formation test.

Immune complexes were determined in the culture fluid after incubation of the cells by the complement fixation test (CFT) in a microradiomodification [4].

After incubation for 20 h the Ig-positive cells were tested for the presence of intracellular Ig. For this purpose the cells were treated with NP-40 detergent (from BDH, England) in the presence of trasylol; the resulting lysate was centrifuged at 35,000g for 30 min and tested by analytical differential electrophoresis in polyacrylamide gel followed by analysis of the gels on the DMU densitometer (Japan).

Ig specifically bound with the surface antigens of the cell membrane were eluted from the cells with glycine buffer, pH 2.8 ($1 \cdot 10^6$ cells to 1 ml buffer, 30 min, at 4°C) and the eluate was investigated in the double immunodiffusion test in agarose gel with antisera against different classes of human Ig. After acid elution the cells were quickly washed with culture medium and retested in the DIT.

The intensity of protein synthesis and, consequently, the metabolic activity of the cells, was estimated by the degree of incorporation of ^{3}H-leucine by the cells.

EXPERIMENTAL RESULTS

Between 13 and 62% of cells in preparations of the patients' leukocytes carried FcR on their surface. Meanwhile the DIT showed that 30-84% of cells of all six patients tested bound anti-IgG-antibodies, and cells of five of them also bound anti-IgM-antibodies. In no case did the blast cells bind anti-IgA- or anti-IgD-antibodies. No correlation was found between the number of FcR- and Ig-positive cells in each patient (Table 1). These results show that binding of labeled antibodies did not take place on account of the FcR present on the cells. In all probability these antibodies reacted with IgG and IgM molecules contained on the surface of the blast cells. Accordingly further investigations were carried out with the aim of studying two hypotheses: either malignant cells in these forms of leukemia synthesize Ig and contain them on their surface as a membrane component, or the Ig detected were autoantibodies against surface antigens of blast cells.

To test the first hypothesis it was assumed that Ig synthesized by the cell ought to be present constantly on the cell membrane provided that intracellular protein synthesis is preserved and ought to be found in homogenates of cells, such as B lymphocytes, for example [8]. It was found that during short-term culture of blast cells *in vitro* there was a considerable decrease in the number of Ig-positive cells but no significant changes in the number of cells carrying FcR (Fig. 1). Parallel studies of incorporation of ^{3}H-leucine by the cells showed that protein synthesis was undisturbed in them. This points to the absence of regeneration of Ig on the surface of the blast cells, so that their intracellular origin can be ruled out. To confirm this suggestion, an electrophoretic investigation was carried out on lysates of these cells obtained after culture for 20 h. Cells of the lymphoblast-like line D-41, synthesizing Ig and preserving them on the surface during long-term culture, were used as the positive control (this line was obtained in the laboratory by Cand. Biol. Sci. A. S. Skori-

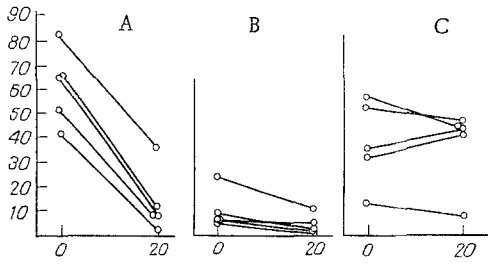

Fig. 1

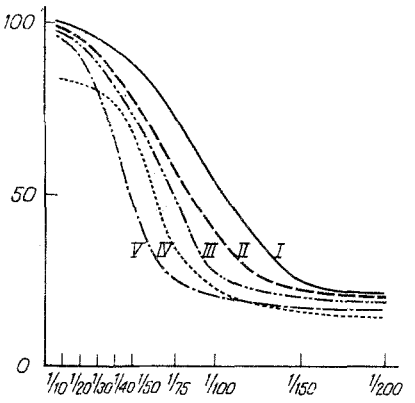

Fig. 2

Fig. 1. Content of Ig- and FcR-positive blast cells before and after culture for 20 h *in vitro*. Abscissa, incubation time (in h), ordinate, number of cells (in %). A) IgG-positive cells; B) IgM-positive cells; C) FcR-positive cells.

Fig. 2. Complement-fixing activity of culture medium after culture of blast cells from patients with OMML and OMoL for 20 h *in vitro*. Abscissa, dilutions of complement; ordinate, lysis of erythrocytes (in %). I) Culture of Ig-blast cells; II) culture of cells from patient M.; III) the same from patient K.; IV) the same from patient Ch.; V) the same from patient A.

kova). Densitometric analysis of the gels after electrophoresis revealed no protein zones in the region of migration of Ig when lysates of leukemic cells were studied, whereas Ig were found in lysates of D-41 cells. The results of treatment of Ig-positive leukemic cells from a patient with OMML with glycine buffer at pH 2.8 (treatment in this way is known to lead to dissociation of antigen–antibody complexes) showed that the cells completely lost their ability to bind labeled anti-Ig-antibodies; however, IgG was found in the eluate by the double immunodiffusion method.

It can be concluded from these results that the Ig found on the surface of leukemic cells are not the product of vital activity of the malignant blast cells but are exogenous in origin, and are evidently antibodies against surface antigens of these cells.

Data in the literature on metabolism of cell membranes and on secretion of antigens from the surface of cells [13] suggest that antibodies against surface antigens of leukemic cells should be eluted into the surrounding medium in the form of a complex with these antigens during short-term cell culture. Analysis of supernatants obtained after culture for four samples of blast cells for 20 h in the CFT showed that their complement-fixing activity was greater than that of material taken after culture of blast cells from a patient with OML, not carrying surface Ig. For instance, the form and arrangement of the curves in Fig. 2 show a significant difference when the experimental and control materials were used. The important point is that complement-fixing activity, directly reflecting the number of immune complexes, correlated positively with the number of malignant cells in preparations of patients' leukocytes.

The results of the investigation thus show that antibodies of IgG and IgM class were found on the surface of blast cells from patients with OMML and OMoL, as well as FcR, and were components of immune complexes with the surface antigens of these cells.

There is as yet no **general agreement** on the nature and biological role of the Ig detected on the surface of malignant cells of human and animal tumors. There is evidence that tumor-bound Ig may exhibit the properties of antibodies against surface antigens of cells or antigen–antibody complexes, for acid eluates of tumor cells possess antitumor activity [12], block the cytotoxicity of immune lymphocytes for malignant target cells *in vitro* [3], and stimulate tumor growth *in vivo* [3].

Autoantibodies discovered in this investigation may be directed either against antigens of malignant cells associated with leukemia or against antigens present also on normal cells of the myeloid series with different degrees of maturity (differential antigens). The possibility cannot be ruled out that these antibodies are blocking antibodies, preventing the action of the effector components of the cellular antitumor immunologic reaction. In this con-

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.

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

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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.

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