PERIPHERAL BLOOD T LYMPHOCYTES OF PATIENTS WITH CHRONIC B CELL LYMPHATIC LEUKEMIA EXPRESS Ia-LIKE ANTIGENS

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Human Ia-like antigens are serologically polymorphic histocompatibility antigens, controlling intercellular interactions in immunological reactions of graft survival and sensitivity to diseases [9]. They are expressed in healthy human peripheral blood on B lymphocytes and monocytes and are absent on granulocytes. Expression of Ia-like antigens on activated T cells has been described [5, 6]. As regards resting cells it is considered that a small part of the T-cell population (5-10%) is antigen-positive [5, 6]. A small population of Ia^+-T cells has been detected in the parafollicular zones of the tonsil and in reactively hyperplastic lymph nodes [8]. The question of expression of Ia-like antigens on leukemic T cells has not been unequivocally answered: the view is held that antigens are expressed on early precursors of T cells and leukemia cells developing from them [7]. In the investigation described below expression of Ia-like antigens was established on T cells of chronic B cell lymphatic leukemia (B-CLL) by comparison with T cells from healthy blood donors.

EXPERIMENTAL METHOD

Mononuclears were isolated from the peripheral blood of 11 patients with B-CLL and 19 healthy blood donors in a Ficoll-Verografin density gradient (ρ = 1.076). T cells were isolated by the method of E-rosette formation with sheep's red blood cells (SRBC), treated with 0.5 unit of *Vibrio cholerae* neuraminidase, produced by the Gor'kii Research Institute of Epidemiology and Microbiology, Ministry of Health of the RSFSR. For this purpose 0.5 ml of a suspension of mononuclears ($5 \cdot 10^6$-$6 \cdot 10^6$ cells/ml) was mixed with 0.5 ml of a 1% suspension of SRBC and with 0.5 ml of embryonic calf serum (Flow Laboratories), adsorbed with SRBC. The mixture was incubated at 37°C for 20 min, centrifuged at 1000 rpm for 3 min, and incubated at 4°C for 18 h. The residue was resuspended and the cells separated in a Ficoll-Verografin gradient (ρ = 1.076). Cells in interphase (the E^--fraction) repeatedly formed from 0 to 8.5% of E-rosettes, whereas in the residue (the E^+-fraction) 0.7 ± 0.2% of B cells containing surface immunoglobulins (SIG) were found (Table 1). The SRBC were lysed with hypotonic solution, pH 7.2. Ia-like antigens were determined on E^+-cells by the indirect immunofluorescence test using IKO-1 monoclonal antibodies (MCA) [1], antigens of undifferentiated blast cells were determined with the aid of IKO-02 MCA [2], antigens of NK cells by means of IKO-11 MCA [3], and antigens of early precursors of T lymphocytes were determined with the aid of IKO-10 MCA [4]. Expression of SIG was determined in the direct immunofluorescence test, using polyvalent rabbit serum against human immunoglobulins. Fluorescence was counted on the Opton III photomicroscope.

EXPERIMENTAL RESULTS

In the peripheral blood of 11 patients with B-CLL, with an absolute leukocyte count of between $16 \cdot 10^9$/liter and $300 \cdot 10^9$/liter and a relative lymphocyte count of 89-99%, E^+-cells accounted for 15.7 ± 3.5% (Table 1). In six of 11 patients Ia-like antigens were expressed on E^+-cells. Expression of Ia-like antigens on T cells of these patients varied from 20 to 100% of antigen-positive cells. Expression of Ia-like antigens on the T lymphocytes of the

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TABLE 1. Expression of Antigens on T Cells of Peripheral Blood of Patients with B-CLL, Isolated by the E-Rosette Formation Method

Patient	Peripheral blood leukocytes, 3×10^9 cells/liter	Lymphocytes, %	E^+-cells, %	Immunological markers of E^+-lymphocytes		
				Ia-like antigens, %	antigens of NK-cells, %	SIG, %
G	27,8	87	7,0	100,0	0	0
A.	131	98	25,0	0	0	0
K.	18	89	35,0	20,0	1,0	1,5
Shch	89	95	18,0	4,95	8,6	0
L-va	16	97	8,0	30,5	3,2	1,9
L-v	132	90	3,5	34,0	0	0
B-va	71	94	5,0	24,0	0	3,0
B-ko	300	98	18,0	59,0	0	0
O.	18	96	34,0	3,0	0	0
D	300	99	2,0	0	0	0
V	23	89	17,0	8,2	15,2	1,0
$M \pm m$	102,3±32,3	93,8±1,3	15,7±3,5	25,7±8,5	5,2±1,1	0,7±0,2

TABLE 2. Expression of Ia-Like Antigens on Peripheral Blood T Cells from Healthy Blood Donors and Patients with B-CLL

Diagnosis	Number of positive cases	Frequency of antigen expression, %	Antigen-positive cells, %
B-CLL (n = 11)	6	54	44,6±19,6
Normal blood donors (n = 19)	±19	±100	4,8±0,6

Legend. ±Weakly positive cases.

remaining five patients averaged 3.2 ± 2.2%, which corresponded to the degree of expression of Ia-like antiges in the donors. E^+-cells did not express SIG, antigens of undifferentiated blasts, or antigens of early precursors of T lymphocytes. A small percentage of E^+-cells expressed antigen of NK-cells (5.2 ± 1.1%). The normal blood donors' E^+-cells contained a significantly smaller ($P < 0.001$) percentage of Ia-like antigens (4.8 ± 0.6; Table 2), but did not carry antigens of NK-cells, of undifferentiated blasts, or of early precursors of T lymphocytes.

The investigation thus showed that T cells of half of the patients with B-CLL expressed Ia-like antigens. The intensity of expression of these antigens was heterogeneous and much greater than the expression of Ia-like cells discovered on a very small subpopulation of T cells from normal blood donors. The presence of heterogeneous expression of Ia-like antigens on T cells in B-CLL may be due to various causes, including activation of a certain subpopulation of T cells. Activated T lymphocytes were discovered in some patients with B-CLL. We know that Ia-like antigens are absent on resting T cells and are expressed on activated lymphocytes. This activation may be connected with the fact that one subpopulation of helper T cells contains Ia-like antigens. In mixed lymphocyte culture, therefore, cells responsible for generation of helper activity consist of Ia^+-T cells [6]. On the other hand, in autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus, T-cells express Ia-like antigens. It is possible that the appearance of Ia^+-T cells in B-CLL, just as in the systemic collagenoses, is connected with the appearance of a population of activated T lymphocytes and reflects a disturbance of the adaptive response. However, it is pointless to look for any direct dependence of the disturbance (expression of Ia-like antigens on T cells) discovered in B-CLL and the autoimmune nature of this disease, for it is not found in all cases of B-CLL.

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COMPARATIVE BIOCHEMICAL AND ELECTRON-MICROSCOPIC STUDY OF MEMBRANE DAMAGE TO THE ENDOPLASMIC RETICULUM BY A CHEMICAL CARCINOGEN IN VIVO AND IN VITRO

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It has recently been shown that the unfavorable action of chemical environmental pollutants, including nitroso compounds, has a membrane-damaging effect [5, 7], which is characterized by increased permeability of biomembranes coupled with inhibition of activity of specific enzymes contained in intracellular structures. The study of the membrane-damaging effect on experimental models in vivo and in vitro, using modern biochemical, morphological, electron-microscopic, and other methods of investigation, could shed light on some of the general principles governing metabolic changes and could provide an approach to the solution of an urgent problem of medico-biological importance in the field of hygiene, namely rapid testing of biological effects.

This paper gives the results of a comparative biochemical and electron-microscopic study of the membrane-damaging action of the chemical carcinogen N-nitrosodimethylamine (NDMA), as a result of exposure in vivo and in vitro.

EXPERIMENTAL METHOD

Experiments in vivo were carried out on noninbred mature male albino rats weighing 180-250 g, kept on a standard diet. Tests were carried out during development of the biological effect of NDMA, 12, 24, 48, and 72 h after administration of the carcinogen by the intragastric route (30 mg/kg, 0.75 LD_{50}).

The experiments in vitro were carried out on a culture of human amnion cells, grown in flasks on medium 199. On the 2nd day of culture, during monolayer formation, nutrient medium containing activated NDMA was added to the cell culture in a dose of 2.5, 5, and 10 mg/liter. Activation of NDMA and subsequent treatment of the cellular material followed the description given previously [8].

Activity of the following membrane-bound enzymes of the nedoplasmic reticulum was determined in a liver tissue homogenate and in the culture of human amnion cells: UDP-glucuronyltransferase (UDP-GCT), catalyzing the reaction of the second phase of xenobiotic metabolism, and glucose-6-phosphatase (G-6-P), which plays an important role in glycogenolysis and gluconeogenesis; the methods used were described in [4, 5].

Material for electron-microscopic study was fixed in a 2% solution of glutaraldehyde in phosphate buffer, pH 7.4, followed by fixation in 1% OsO_4 solution. Sections were stained with an aqueous solution of uranyl acetate and with lead hydroxide by Reynolds' method. The sections were examined in the UMV-100 electron microscope with instrumental magnification of between 10,000 and 25,000.

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