CYTOTOXICITY OF EFFECTOR CELLS OF PERIPHERAL BLOOD, LYMPH NODES, AND SPLEEN IN HODGKIN"S DISEASE

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Immunologic investigations of Hodgkin's disease (HD) have revealed defects in the T-cell component of the immunity system: depression of the response to dinitrochlorobenzene, delayed rejection of a skin allograft, a disturbed proliferative response to alloantigens and mitogens, and depression of interferon and interleukin-2 production [1]. However, the cytotoxic activity of the lymphocytes of such patients has received little study. As several workers have shown, and as our own data have confirmed [3], killer T cells capable of causing specific lysis of autologous tumor cells have been discovered in the peripheral blood of patients with malignant neoplasms. Since tumor cells have not been definitively identified in HD and are not accessible for study, to investigate the functional activity of patients' lymphocytes we have modified and used the technique of lectin-dependent cytotoxicity [2], based on the fact that T cells, possessing lytic potential, can kill any target in the presence of phytohemagglutinin (PHA), concanavalin A (con A), or succinyl-con A [5]. Thus the method can be used to detect the general pool of cytotoxic T lymphocytes in any population of lymphoid cells.

The aim of this investigation was to study lectin-dependent and natural cytotoxicity of lymphocytes from the peripheral blood, spleen, and lymph nodes (LN) of 83 patients with HD and 50 healthy blood donors.

EXPERIMENTAL METHOD

Altogether 83 patients with HD (37 men and 46 women) aged from 17 to 57 years were investigated. Of the total number, 10 were in stage I of the disease, 38 in stage II, 24 in stage III, and 11 in stage IV. Blood was taken from the donors in the blood transfusion department of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. Post-traumatic spleens were obtained from the N. V. Sklifosovski Moscow Emergency Aid Research Institute.

Mononuclear cells (MNC) wre isolated from peripheral blood and spleen by Böyum's method [6].

T lymphocytes were obtained by purification of MNC from adherent cells on a column with nylon wadding by the method of Julius et al. [10]. The proportion of T cells was determined by the indirect immunofluorescence method using monoclonal antibodies ("Ortho Diagnostic System"). The resulting suspensions contained 91.3 ± 6.6% of cells with markers of OKT3 T lymphocytes; the number of $OKB7^+$ and $OKM1^+$ cells did not exceed 1-2%.

To assess cytolytic activity, K562 and HeLa target cells (TC), labeled with $Na_2{}^{51}CrO_4$ were used. K562 cells were introduced into round-bottomed 96-well plates at the rate of 10^4 TC per well. HeLa cells were labeled the day before the experiment and seeded in 96-well flat-bottomed microplates at the rate of 2×10^4 TC per well. Lymphocytes were added to K562 TC in the ratio of 50:1, 25:1, and 12:1. To assess lectin-dependent cytotoxicity, HeLa cells

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were treated with con A in a concentration of 100 μg/ml in the course of 3 h before addition of effectors in the ratio of 12:1. After incubation for 18 h at 37°C in an atmosphere of 5% CO_2, supernatant was taken from each well to determine radioactivity. The percentage of lysed TC was calculated by the equation:

$$\text{cytolysis} = \frac{\text{experimental} - \text{spontaneous yield}}{\text{total lysis} - \text{spontaneous yield}} \times 100\%$$

where experimental yield is the yield of isotope from TC on the addition of lymphocytes, spontaneous yield denotes release of isotope by intact TC, and total lysis signifies the total quantity of isotope incorporated into TC.

EXPERIMENTAL RESULTS

As Table 1 shows, cytotoxicity of lymphoid cells was increased about three-fivefold in the presence of lectin. On average, lectin-dependent cytotoxicity of T lymphocytes in the patients and healthy blood donors was identical, but depending on the stage of the disease, a definite difference could be detected: cytolytic activity of T lymphocytes was 1.5 times higher in stage Ia than in the control, in stage IIa-IIIb it was the same as in the control, and only in stage IV was it significantly lowered by half compared with the control (Fig. 1). These data may indicate quite high activity of killer T cells, which was reduced only in the terminal stage of the disease. Since the severity of the course of HD is largely determined by the histologic type of the disease, we distributed data on the cytotoxicity of T lymphocytes from the blood and spleen in accordance with their morphologic criteria. As will be clear from Fig. 2, the cytolytic activity of T lymphocytes in patients with a benign lymphohistiocytic variant was 1.5 times higher than in healthy blood donors, in nodular sclerosis it coincided with the control data, it was a little depressed in the mixed-cell version, and reduced by half compared with the control in patients with the diagnosis of lymphatic cachexia, a sign of a malignant course of the disease. The study of cytolytic activity of splenocytes obtained after removal of the spleen from 22 patients, on the day of operation, and of five post-traumatic spleens revealed no significant difference (Figs. 1 and 2). However, a comparative study of affected and intact areas of the same spleen from 11 patients revealed a significant decrease by 50% of cytotoxicity in pathological foci.

We next obtained affected LN from 34 patients, but succeeded in isolating lymphoid cells from only 14 of these LN, so that it was impossible to assess the cytotoxicity of the lymphocytes at different stages of the disease. It was also impossible to obtain LN from healthy blood donors. On average, lectin-dependent cytotoxicity of the MNC population was 9.9 ± 3.5%, falling after removal of the adherent cells to 4.38 ± 1.4%, or 3.4 and 8 times respectively lower than activity of MNC and T lymphocytes of the blood and 3.2 and 6.4 times lower respectively than the effect of the patients' splenocytes (Table 1).

TABLE 1. Lectin-Dependent and Natural Cytotoxicity of Effector Cells from Blood, Spleen, and LN of Patients with HD

TC	Effector cells	Blood donors		Patients					
				blood		spleen		LN	
		$M \pm m$	p_1	$M \pm m$	p_2	$M \pm m$	p_3	$M \pm m$	p_4
HeLa*	MNC	11,8±1,8	>0,05	12,5±1,5	>0,05	17,4±2,3	>0,05	23,4±7,4	>0,05
	T lymphocytes	7,67±1,3	>0,05	6,75±1,67	<0,05	12,6±1,8	<0,05	29,0±4,97	<0,05
HeLa+ con A	MNC	34,3±2,5	>0,05	33,5±2,7	>0,05	32,4±2,37	<0,05	9,87±3,5	<0,05
	T lymphocytes	38,4±3,4	>0,05	35,2±4,1	>0,05	27,9±2,8	<0,05	4,38±1,4	<0,05
K562**	MNC	47,2±3,06	<0,05	22,3±2,8	<0,05	40,4±4,2	<0,05	7,56±3,29	<0,05
	T lymphocytes	61,7±3,8	<0,05	35,6±3,4	>0,05	37,2±5,4	<0,05	10,6±3,8	<0,05

*Effector to TC ratio – KM 20:1.
**Effector to TC ratio – KM 50:1.
Legend. p_1) Significance of difference in cytotoxicity of blood cells from donors and patients, p_2) patients' blood and spleen, p_3) patients' spleen and LN, p_4) patients' blood and LN.

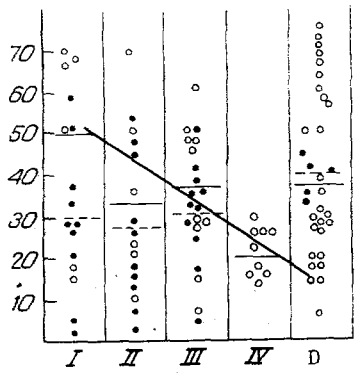

Fig. 1

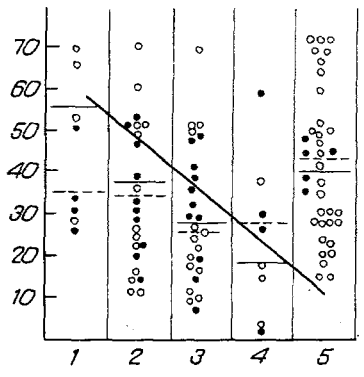

Fig.2

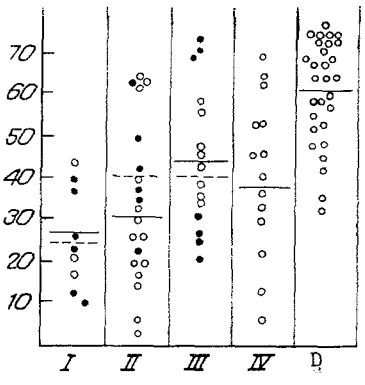

Fig 3

Fig. 1. Lectin-dependent cytotoxicity of T lymphocytes from blood (empty circles) and spleen (filled circles) at different stages of lymphogranulomatosis. Ordinate, percentage cytolysis of TC; abscissa, here and in Fig. 3: groups of patients, distributed according to stages of the disease, and donors (D).

Fig; 2. Lectin-dependent cytotoxicity of T lymphocytes from blood (empty circles) and spleen (filled circles) in different histologic variants of lymphogranulomatosis. abscissa: 1) lymphohistiocytic, 2) nodular sclerosis, 3) mixed-cell variant, 4) lymphatic cachexia, 5) healthy donors; ordinate, percentage cytolysis of TC.

Fig. 3. Natural cytotoxicity of T lymphocytes from blood (empty circles) and spleen (filled circles) at different stages of lymphogranulomatosis. Continuous horizontal lines indicate mean values of cytolysis of TC interacting with T-lymphocytes from blood; broken horizontal lines denote mean data for cytolysis of TC, interacting with splenic lymphocytes. Ordinate, percentage of cytolysis of K562 cells.

Activity of natural killer cells of the blood in the MNC population and among T lymphocytes purified from adherent cells was significantly higher, by 2 and 1.7 times respectively, in the donors compared with the patients. However, the level of natural cytotoxicity of the blood and spleen showed no correlation with the stage of the disease (Fig. 3) or with the histologic variant. Natural cytotoxicity of the splenocytes did not differ from that in the healthy spleen, but was considerably suppressed in the MNC population and in the T-lymphocytes of the affected LN. The writers showed previously that crossed stimulation of patients' lymphocytes by cells from patients with the same disease, with peripheral blood cells from healthy donors, or PHA is characterized by marked suppression of the proliferative response to the mitogen, whereas the response to stimulation by the patients' alloantigens is only slightly reduced, and only in the terminal stage of the disease, possible evidence of the high intensity of specific antitumor immunity [4]. These data are in agreement with results obtained in the present investigation: the cytotoxic activity of the patients' lymphocytes was higher than or equal to that of healthy blood donors, but was sharply reduced in stage IV of the disease. Conversely, natural cytotoxicity was only half as high as that of healthy blood donors and did not correlate with the stage of the disease or its histologic variant. Other workers have obtained similar results [8, 9].

The nature of tumor cells in lymphogranulomatosis has not yet been elucidated. Most investigators consider tht they are Sternberg-Reed cells (SRC). It has been shown that with respect to various cytochemical and marker characteristics SRC are similar to antigen-presenting cells and, in particular, they adsorb antibodies to human Ia-like antigen. In pathological foci and in the zone of infiltration surrounding SRC, T lymphocytes predominate, and as has been shown by the use of monoclonal antibodies, mainly helper T cells and activated lymphoid cells are associated around SRC [7].

On the assumption that SRC do in fact belong to the class of antigen-presenting macrophages, they may play the role of antigenic stimulus, inducing permanent proliferation of normal lymphoid cells.

It can accordingly be concluded from these data that antitumor immunity in patients with lymphogranulomatosis is not low, and is depressed only in the terminal stage of the disease.

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IMMUNOHISTOCHEMICAL IDENTIFICATION OF RESERVE CELLS OF THE ENDOCERVICAL CANAL BY MONOCLONAL ANTIBODIES EE_{21-06d}

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The mucous membrane of the cervical canal is lined with cylindrical epithelium, beneath which, on the basement membrane, may lie reserve (or cambial) cells, capable of differentiating into either glandular or stratified squamous epithelium [1, 2]. Although the epithelial nature of the reserve cells can be taken as proven, the precise mechanism of their formation is not known [8]. In recent years research into proteins of the cytoskeleton of epithelial cells (cytokeratins, prekeratins) has been conducted on an extensive scale in biology and medicine, with the aid of monoclonal antibodies (McAb). After the compiling of a catalogue of cytokeratins, in which 19 polypeptides have been distinguished [7], it was shown that cells of different epithelia differ from one another in the set of expressed cytokeratins [3, 7]. Previously prekeratins Nos. 7, 8, 18, and 19, characteristic of simple epithelium, and also cytokeratins of squamous-cell type Nos. 5 and 17 were found in the reserve cells. Cytokeratins Nos. 7, 8, 18, and 19 are present in the cylindrical cells of the cervical mucous membrane, but prekeratins Nos. 1, 2, 4, 10, 13, 14, 15 of squamous-cell type are not presented [3, 7, 8].

The aim of this investigation was to detect reserve cells with the aid of new McAb against a complex of cytokeratin polypeptides, which are markers of squamous-cell differentiation. For this purpose we used original McAb EE_{21-06d} (IgG_1k), obtained by G. Serre by the hybridoma method after immunization of mice with extract of human callus. These antibodies react with five cytokeratin polypeptides Nos. 1, 2, 9, 10, and 11, characteristic of stratified squamous epithelium [7].

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