USE OF THE LYMPHOCYTE BLAST TRANSFORMATION REACTION TO ASSESS THE STATE OF CELLULAR IMMUNITY

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UDC 616.5-006.443-092:616.155.32-008.1

KEY WORDS: lymphocyte blast transformation reaction; cellular immunity; helper T and suppressor T cells.

Changes in the system of cellular and humoral immunity are observed in various neoplastic diseases [3, 7]. These include Kaposi's sarcoma – a multiple hemorrhagic sarcoma of unknown etiology, in which the tumor process develops especially from cells of the perivascular reticulohistiocytic tissue of the skin. If the disease runs a chronic course it is accompanied by inhibition of cellular immunity, involving the T lymphocyte population [8]. An important criterion reflecting the functional state of lymphocytes is their ability to take part in the blast transformation reaction (LBTR), with the use of mitogens of varied origin. We have used the LBTR to study mitogen-induced proliferation of peripheral blood lymphocytes of patients with Kaposi's sarcoma, admitted for treatment to the central Dermato-Venereologic Institute Ministry of Health of the USSR, in 1984-1985.

EXPERIMENTAL METHOD

Lymphocytes from heparinized blood of 25 patients with Kaposi's sarcoma and 20 normal blood donors were isolated in a Ficoll–Verografin gradient [1]. Next, the cells in a concentration of $5 \cdot 10^6$/ml were resuspended in medium RPMI 1640 with 2 mM L-glutamine, 10% embryonic calf serum, 10 mM HEPES, and 0.1 mg/ml of gentamicin, and introduced into wells of a 96-well panel in a dose of $5 \cdot 10^5$ cells per well. The panels were incubated for 48 h at 37°C in a humid atmosphere with 5% CO_2 in the presence of mitogens. Next, ^{3}H-thymidine was added to each well for 24 h in a dose of 1 μCi. Counting of the radioactivity of the label incorporated into cellular DNA was carried out on a scintillation counter. Five separate wells were used at each experimental point. The results were subjected to statistical analysis by

TABLE 1. Dependence of Lymphocyte Blast Transformation Reaction on Ratio of Helper to Suppressor T Cells, Determined with the Aid of Monoclonal Antibodies OKT4 and OKT8 (M ± m)

Patient	Number of lymphocytes in 1 ml peripheral blood ($\times 10^6$)		OKT4/OKT8	Incorporation of ^{3}H thymidine into lymphocytic DNA (cpm)
	OKT4+	OKT8+		
1. A. L.	0,14	0,38	0,24	6787±1267
2. P. O.	0,11	0,2	0,55	12733±5815
3. S. U.	0,32	0,36	0,88	28383±712
4. B. O.	0,19	0,204	0,93	22851±12151
5. Z. B.	0,33	0,31	1,06	39879±16069
6. P. I.	0,35	0,33	1,06	52997±7357
7. B. E.	0,37	0,34	1,07	53346±15647
8. S. E.	0,44	0,28	1,56	44258±12824
9. Donor I	0,36	0,28	1,3	56178±16664
10. Donor II	0,82	0,41	2,0	62924±17813

D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR, Moscow. Central Dermato-Venereologic Institute, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. M. Zhdanov. [Deceased].) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 4, pp. 469-471, April, 1989. Original article submitted July 22, 1987.

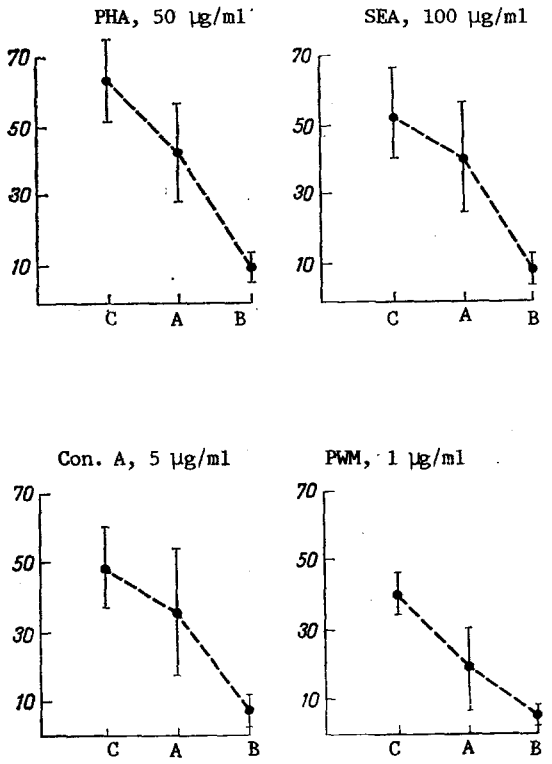

Fig. 1. High and low LBTR in patients with Kaposi's sarcoma. C) Donors (20), A) patients with Kaposi's sarcoma and with high LBTR (16), b) patients with Kaposi's sarcoma with low LBTR (9).

Student's test. The following mitogens were used: phytohemagglutinin (PHA; from West Germany, 50 μg/ml), staphylococcal enteroxtoxin A (SEA; from Serva, West Germany; 100 μg/ml), concanavalin A (Con A; from Serva, 5 μg/ml); pokeweed mitogen (PWM; from Serva, 1 μg/ml). Helper and suppressor T lymphocytes were counted by means of monoclonal OKT4 and OKT8 antibodies (Orthon Diagnostic, USA), as indicated in [6].

EXPERIMENTAL RESULTS

In the first series of experiments, peripheral blood lymphocytes from normal donors (20 persons) and patients with Kaposi's sarcoma (25 persons), isolated in a Ficoll–Verografin gradient, were incubated in the presence of mitogens PHA, Con A, PWM, and SEA, and activity of synthesis of cellular DNA was determined. The results of these experiments, illustrated in Fig. 1, show that the patients with Kaposi's sarcoma investigated can be divided into two groups: those with "high" and those with "low" ability of their peripheral blood lymphocytes to take part in the blast transformation reaction. The group with high LBTR (over 12,000 cpm) when incorporation of ^{3}H-thymidine was measured [9] consisted mainly of patients with a chronic, indolent form of the disease, as a rule without complications. The group of "low" LBTR (under 12,000 cpm) included patients with a severe, acute form of the disease or patients with chronic disease during an exacerbation. Patients with "high" LBTR accounted for 64% of the total number in the group (16 patients), whereas those with "low" LBTR acounted for 36% of the total number of patients examined (9). Incidentally, the level of mitogen-stimulated incorporation of labeled precursor into lymphocytic DNA in the group of patients with "high" LBTR was appreciably lower than in the donors of the control group, for all mitogens tested.

In the second series of experiments peripheral blood lymphocytes were divided into two parts. The number of helper and the number of suppressor T cells, per milliliter of peripheral blood, was determined in one part, whereas lymphocytes from the other part were used to perform the LBTR in the presence of PHA. It can be concluded from the data in Table 1 that the number of helper T cells differed in the population of peripheral blood lymphocytes of the eight patients tested. A decrease in the number of helper T cells and an increase in the relative number of suppressor T cells were accompanied by a fall of the level of PHA-

induced LBTR. The two-threefold increase in the number of suppressor T cells compared with the number of helper T cells (in patients Nos. 1-4, for example) was accompanied by inhibition of functional activity of the lymphocytes and lowering of their ability to take part in the LBTR, compared both with the control and with the LBTR of other patients, for whom the ratio OKT4+/OKT8+ was depressed but not reversed.

The results examined above confirm views expressed by some workers [2] on the heterogeneity of Kaposi's sarcoma as a nosological form. Our data on the "high" and "low" proliferative response in patients with Kaposi's sarcoma to the action of the various mitogens are in good agreement with results obtained by Gotlieb et al. [4], who showed that mitogen-induced blast cell production is sharply depressed in patients with the acquired immunodeficiency syndrome (AIDS) with conventionally pathogenic infections, moderately depressed in patients with AIDS complicated by Kaposi's sarcoma, but virtually unchanged compared with normal in patients with lymphadenopathies.

Schroff [7], who studied patients with AIDS and in the prodromal stage of AIDS complicated by Kaposi's sarcoma, emphasizes that the proliferative response is particularly low in groups of patients with a reduced number of helper T cells circulating in the peripheral blood. Our results relating to correlation between mitogen-induced proliferative activity of lymphocytes and the number of helper T cells in patients with Kaposi's sarcoma are in good agreement with Schroff's findings of reduced ability of the peripheral blood lymphocytes of patients with a low OKT4+/OKT8+ ratio of their lymphocytes to take part in LBTR. It can be tentatively suggested that correlation between the degree of mitogen-induced blast cell transformation of peripheral blood lymphocytes and the number of helper T cells reflects not only the inadequate functional activity of the lymphocytes, but also a decrease in the number of mitogen-sensitive or antigen-sensitive lymphocytes.

The combined use of the LBTR and determination of the OKT4+/OKT8+ ratio of the lymphocytes may be used to study the state of T-cell immunity and functional activity of the peripheral blood lymphocytes both in patients with immunodeficiency states and also in experimental immunologic research.

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