CHARACTERISTICS OF LYMPHOCYTE SUBPOPULATIONS ISOLATED FROM PERSONS IN NORMAL HEALTH AND WITH NONSPECIFIC ULCERATIVE COLITIS

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UDC 616.348-002.44-07:616.155.32-008.1-07

Key words: lymphocyte subpopulations; cytochemistry; xenogeneic graft versus host reaction.

Information has now been obtained on deviations in the immune status of patients with nonspecific ulcerative colitis (NUC) [15]. Some particularly interesting work has been done on disturbances in immunoregulatory mechanisms: depression of the total number of T lymphocytes [2]; increased helper activity and a decrease in the number and activity of suppressor T cells [8]; the presence of thymic disturbances [16]. It was shown previously [1] that many immature postthymic cells, characterized as autorosette-forming [10], theophylline-dependent lymphocytes [4], accumulate in the peripheral blood of patients with NUC; the level of T_G^+-cells among the E-RFC falls, but both the number of 0-lymphocytes and expression of receptors for IgG on their membrane increase. However, no direct relationship is known to exist between phenotype and function of T cells [13].

To determine the degree of agreement between the phenotypic and functional manifestation of the lymphocyte subpopulations in normal individuals and in the development of a pathological process associated with inflammatory changes in the colonic mucosa, in the investigation described below activity of isolated cells was evaluated by the local xenogeneic "graft versus host reaction" (GVHR) and by determination of the cytochemical status of the lymphocytes.

EXPERIMENTAL METHOD

Mononuclear cells were obtained from human peripheral blood by the method in [7] during gradient centrifugation. To obtain a lymphoid population from the mononuclear suspension, monocytes were removed by adsorption on plastic Petri dishes for 60 min. Nonadherent cells were bound with an equal volume of sheep's red blood cells (3% suspension, made up in medium N199 with 40% embryonic calf serum – ECS) and the E-RFC test was carried out in the usual way, and was followed by gradient centrifugation on Ficoll-Verografin (1.077). The RFC thus formed were freed from erythrocytes by hypotonic lysis with ammonium chloride (0.83% solution). Next, the EA-RFC test was carried out with the isolated T lymphocytes, using cow's erythrocytes sensitized with rabbit antibodies of the IgG class to them (the rabbit antibodies were generously provided by Professor N. A. Kraskina, to whom the authors are grateful). After the end of the EA-RFC test gradient centrifugation was repeated and T_G^+ cells were isolated from the residue by hypotonic lysis; cells classed as T_G^- were found in the interphase layer. The 0-lymphocytes were obtained from nonadherent cells, exhausted with E-RFC and EAC-RFC [9].

To estimate the functional activity of the isolated lymphocyte subpopulations the local xenogeneic GVHR was carried out, using the increase in size of the lymph nodes, determined by the method in [14], as the index of evaluation of the results. A mononuclear cell suspension, obtained from several healthy blood donors and containing $5 \cdot 10^6$ cells in a volume of 40 ml, was injected subcutaneously into the right foot pad of CBA recipient mice weighing 25-30 g, obtained from the "Stolbovaya" and "Rappolovo" Nurseries, Academy of Medical Sciences of the USSR; a suspension of syngeneic spleen cells in the same dose was injected into the left foot pad. From five to eight animals were used at each point. The animals were given cyclophosphamide 24 h before the experiment in a dose of 100 mg/kg body weight. The GVHR was read 5 days after injection of the cells and the index of enlargement of the lymph nodes determined, as the ratio of the number of cells in the popliteal

Chelyabinsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 9, pp. 318-320, September, 1989. Original article submitted December 25, 1988.

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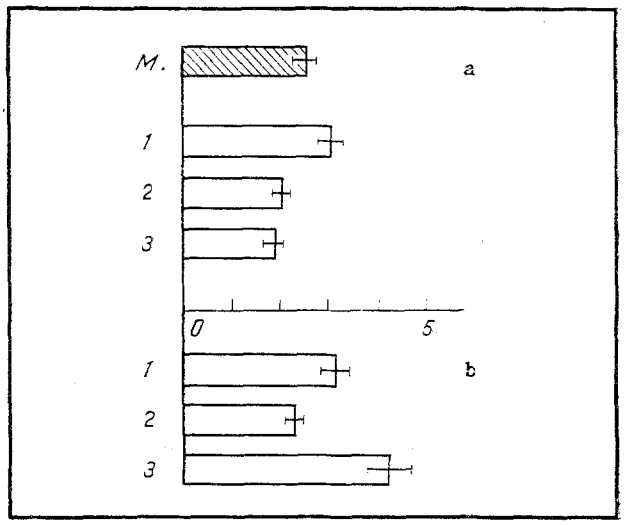

Fig. 1. Effect of various cell combinations isolated from healthy individuals (a) and patients with NUC (b) on development of local GVHR. Abscissa, index of enlargement of lymph nodes of recipient mice; M) mononuclears; 1) T_G^- lymphocytes were added to mononuclears; 2) T_G^+ lymphocytes were added to mononuclears; 3) 0-lymphocytes were added to mononuclears.

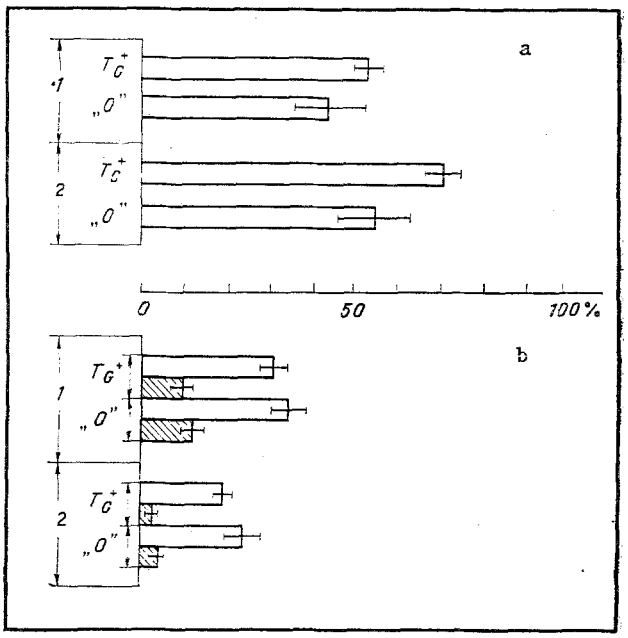

Fig. 2. Nonspecific esterase activity in lymphocyte subpopulations. Abscissa, % of positively stained cells; panel a: diffuse fine-grain distribution of weak degree of activity (here and in panel b; 1) cells obtained from healthy donors; 2) cells from patients with NUC); panel b: unshaded rectangles denote distribution of enzyme as a distinct stain, shaded rectangles — coarse-grain distribution of high degree of activity.

nodes in the right and left limbs. Activity of the different lymphocyte subpopulations was determined by addition of $3 \cdot 10^4$-$5 \cdot 10^4$ cells of patients or donors to $5 \cdot 10^6$ mononuclears from the donors' pool [14] and injection of this mixture of cells into the animal. The following cell combinations were used: mononuclears from the donors' pool, donors' mononuclears + T_G^+ cells from patients or healthy individuals, donors' mononuclears and T_G^- cells from patients or healthy indi-

viduals, and donors' mononuclears and 0-lymphocytes from patients or healthy individuals. Nonspecific esterase (L-naphthyl-AS acetate-esterase) also was determined in the different lymphocyte subpopulations [5, 6]. On the basis of distribution of the enzyme the following types of staining were distinguished: diffuse fine-grain distribution of a high degree of activity; coarse-grain distribution of a high level of activity, a clear compact stain. Isolation of the lymphocyte subpopulations and the study of their properties in the GVHR and their cytochemical characteristics were carried out in order to investigate 12 healthy blood donors and eight patients with NUC (chronic recurrent form, moderately severe course, period of exacerbation).

EXPERIMENTAL RESULTS

The results of the GVHR with respect to the action of different cell combinations obtained from the peripheral blood of patients with NUC and healthy blood donors, are summarized in Fig. 1. Addition of lymphoid subpopulations (T_G^+, T_G^-, 0) isolated from healthy individuals to the mononuclear suspension gave stimulation of the GVHR when T_G^- were added and suppression with T_G^+ and 0-lymphocytes, although the data were significant only in the latter case ($p < 0.05$).

In NUC a similar tendency was observed in the effect of T_G^+ and T_G^- lymphocytes on the development of GVHR. In relation to the 0-population opposite results were obtained: a distinct stimulation effect ($p < 0.01$). Such a marked degree of functional activity of the 0-lymphocytes is contrary to the lowering of activity of natural killer cells in NUC observed by Stenina [3]. It is perfectly possible that this is a question of different subpopulations, because 0-lymphocytes, like NK cells, which may be included in the 0-class, are heterogeneous and, consequently, they may differ in activity during realization of their effector functions.

Analysis of the distribution of the enzyme in lymphocytes during NUC (Fig. 2) showed that the lowest level of activity was present among T_G^+ and 0-lymphocytes. The fall of enzyme activity in the cells may be evidence of their low degree of maturity [12], although the suggestion of a stimulating action of the antigen on them likewise cannot be ruled out [11]. It can be tentatively suggested that the first suggestion may apply with a high degree of probability to T_G^+ lymphocytes, the second to 0-lymphocytes, taking into account the results of the GVHR.

Our data probably confirm the conclusions of Moretta and co-workers [13] that in certain types of pathology the sphere of activity of the small lymphocyte subpopulation (helper – cytotoxic) is widened, and it is possible to speak of the heterogeneity and the potential properties of the 0-population of lymphocytes depending on whether the initial state is one of health or pathology.

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