LITERATURE CITED

1. Immunomodulators in Infectious Pathology [in Russian], Moscow (1988).
2. G. B. Kirillicheva, V. V. Mit'kin, N. V. Bogomolova, et al., Abstracts of Proceedings of an All-Union Symposium on Interaction between the Immune and Nervous Systems [in Russian], Orenburg (1990), p. 36.
3. V. L. Pustovalov, G. I. Vasil'eva, and A. K. Kiseleva, Pathological Physiology, Immunology and Allergology [in Russian], Saratov (1984), pp. 8-12.
4. G. A. F. Seber, Linear Regression Analysis, Wiley, New York (1977).
5. E. E. Baker, H. Soniner, L. L. E. Foster, et al., J. Immunol., **68**, No 2, 131 (1952).
6. J. Dornand, C. Remininiae, and J. C. Mani, J. Biochem., **509**, 425 (1977).
7. T. C. Montie, D. B. Montie, and D. E. Wennerstrom, Microbiology 1975, Washington (1975), pp. 278-282.

CHARACTERISTICS OF PERIPHERAL BLOOD LYMPHOID SUBPOPULATIONS OF SHIGELLOSIS PATIENTS

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The writers showed previously [1, 2] that in inflammatory diseases of the large intestine characterized by the common nature of the principal symptoms of the disease (exacerbation of a chronic recurrent form of nonspecific ulcerative colitis – NUC – and the acute form of bacterial dysentery – BD), destabilization of immune homeoctasis is observed, one manifestation of it being a disturbance of relations of immune regulatory lymphocytes both with one another and with other immunocompetent cells. Changes such as a decrease in the total number of T-l, $T\gamma^-$, and theophylline-sensitive lymphocytes and an increase in the number of T-active lymphocytes and immature postthymic cells (theophylline-dependent), in NUC and BD are common in character. The differences between them are: 1) in BD there is no increase in the number of O lymphocytes (O-l) and lymphocytes forming rosettes with autologous erythrocytes; 2) in 20% of patients with BD the number of $T\gamma^+$ lymphocytes in a mononuclear suspension exceeds the number of E-RFC (in patients with NUC the percentage of $T\gamma^+$-l is inversely proportional to the severity of the patient's condition); 3) during determination of the content of $T\gamma^-$-l and $T\gamma^+$-l and of theophylline-resistant and theophylline-sensitive lymphocytes (tpr-l and tps-l) in an enriched T-l cell suspension, an equivalent distribution of $T\gamma^+$-l and tps-l was observed in patients with BD, but there was no correspondence between them in NUC [1].

There is no doubt that disturbances of relations between immunoregulatory subpopulations of lymphocytes involve changes in the character of their action on mutually subordinate systems, and in turn, this determines the course of the pathological process, its outcome, and the efficacy of ways and means of immunocorrection. Particular features of the phenotype and function of lymphocytes during an exacerbation in patients with NUC were examined by the writers previously [3, 4].

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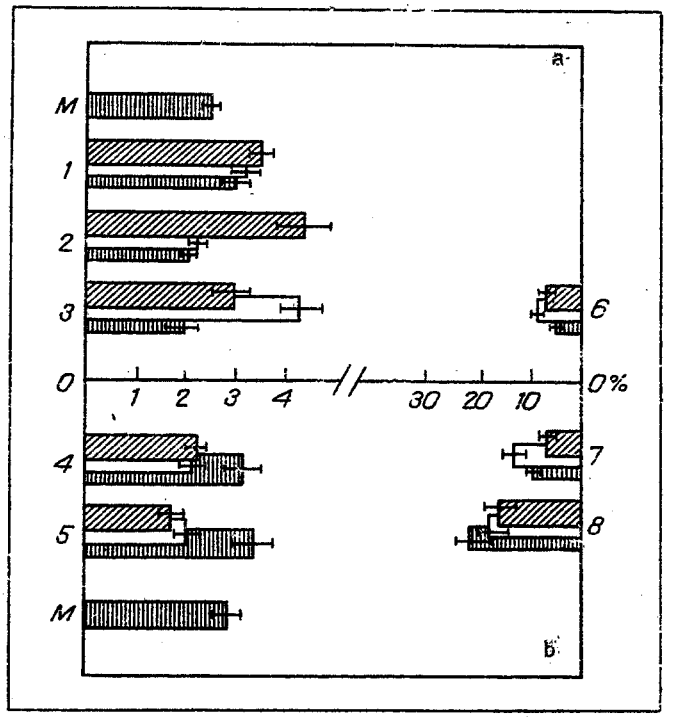

Fig. 1. Effect of immunoregulatory subpopulations of lymphocytes on development of local xenogeneic GVHR and distribution of Fcγ-R-positive cells (Fcγ-R^+) among them: a) action of $T\gamma^-$-l, Tγ-l, and O-l (M denotes mononuclears; 1) $T\gamma^-$-l added to mononuclears; 2) $T\gamma^+$-l added to mononuclears; 3) O-l added to mononuclears; 6) percent of Fcγ-R cells among O-l); b) action of tpr-l and tps-l (M – mononuclears; 4 – tpr-l added to mononuclears; 5 – tpr-l added to mononuclears; 7 – percent of Fcγ-R^+ cells among tpr-l; 8 – percent of Fcγ-R^+-cells among tps-l). Obliquely shaded columns – lymphocytes from patients with BD; unshaded columns – lymphocytes from patients with NUC; horizontally shaded columns – lymphocytes from healthy donors. Abscissa: on left – index of GVHR, right – per cent of Fcγ-R^+-cells.

The aim of this investigation was to determine functional activity of lymphocytes of patients with BD: $T\gamma^+$-l, $T\gamma^-$-l, tps-l, tpr-l, and O-l, in the local xenogeneic graft-host reaction (GVHR), which we have used to study similar lymphocyte subpopulations isolated from patients with NUC.

EXPERIMENTAL METHOD

Mononuclear cells were isolated from human peripheral blood in the usual way by gradient centrifugation (density 1.077). All the methods which we used were described in detail previously [2-4]. To obtain a lymphoid population from a mononuclear suspension, monocytes were removed by adsorption on plastic Petri dishes for 60 min. The usual E-RFC test was set up [6] with nonadherent cells, and this was followed by gradient centrifugation on Ficoll–Verografin. The RFC formed were freed from erythrocytes by hypotonic lysis (with 0.83% ammonium chloride solution). Next, the EA-RFC test was carried out with the isolated T cells [3]. After the end of the EA-RFC reaction, gradient centrifugation was repeated, and $T\gamma^+$ were isolated from the residue by hypotonic lysis, cells classed as $T\gamma^-$ being contained in the interphase layer. O-l were obtained from nonadherent cells, exhausted by E-RFC and EAC-RFC [7]. To obtain tpr-l and tps-l the isolated T-l were incubated at 37°C for 1 h with theophylline (3 mg/ml, from Serva, USA), after which the usual rosette-formation test was carried out. Fractionation of the cells into tpr-l and tps-l was carried out by gradient centrifugation [8]. After the isolated cells had been counted, the EA-RFC test was carried out with each subpopulation to determine the number of cells carrying receptors for the Fc-fragment of IgG (Fcγ-R). To assess the functional activity of the isolated lymphocyte

subpopulations, the local xenogeneic GVHR was carried out, with evaluation according to the index of lymph node enlargement [3, 4, 8]. Activity of the different lymphocyte subpopulations was determined by addition of $3 \cdot 10^4$-$5 \cdot 10^4$ cells from patients or healthy individuals to $5 \cdot 10^6$ mononuclears from a pool of healthy donors, and injection of this mixture of cells subcutaneously into the right footpad of recipient mice (CBA mice weighing 25-30 g). The following cell combinations were used: 1) mononuclears from a pool of donors: 2) mononuclears from donors and $T\gamma^+$ from patients or healthy individuals; 3) mononuclears from donors and $T\gamma^-$ from patients or healthy individuals; 4) mononuclears from donors and O-l from patients or healthy individuals; 5) mononuclears from donors and tpr-l from patients or healthy individuals; 6) mononuclears from donors and tps-l from patients or healthy individuals. At each point 5-8 animals were used. $T\gamma^+$, $T\gamma^-$, and O-l were isolated and their properties studied in material from 10 donors and eight patients with BD. During isolation of tpr-l and tps-l, and performance of the local xenogeneic GVHR with them, peripheral blood from 10 donors and eight patients with BD was used.

EXPERIMENTAL RESULTS

The results of performance of the local xenogeneic GVHR, summarizing the action of the different lymphocytes obtained from peripheral blood from healthy individuals and patients with BD, on development of the reaction are illustrated in Fig. 1. It was found that $T\gamma^+$-l and $T\gamma^-$-l have a stimulating action on GVHR when added to a pool of donors' mononuclears ($p < 0.05$). $T\gamma^-$-l and $T\gamma^+$-l from healthy individuals and patients with NUC had a weakly stimulating and inhibitory action respectively on the development of the reaction. Despite the equivalent and enhanced distribution of $Fc\gamma^+$-lymphocytes among O-l in BD and NUC (Fig. 1), O-l from patients with BD were inert on testing, whereas those from NUC patients possessed a clear stimulating effect. O-l from donors demonstrated an inhibitory action on GVHR ($p < 0.05$).

The effect of tpr-l and tps-l on development of the GVHR was studied. On the addition of tpr-l and tps-l from patients with BD to the pool of donors' mononuclears, just as during work with similar cells from patients with NUC, we observed an inhibitory effect on the formation of the local xenogeneic GVHR, which was clearly defined under the influence of tps-l ($p < 0.01$). In donors, these cells had a stimulating effect on GVHR. With respect to the number of Fcγ-R-positive cells, tps-l from patients with BD and NUC did not differ. Differences were observed during investigation of tpr-l: in patients with BD and healthy donors the number of $Fc\gamma^+$-cells was identical, but in patients with NUC it was significantly increased (Fig. 1).

Consequently, although lymphoid subpopulations $T\gamma^+$-l, $T\gamma^-$-l, tps-l, and tpr-l are capable of exhibiting equivalent immunoregulatory action on mutually subordinate systems, the character of that action is determined by many conditions, among which an important role is made by the relationships of the alternative subpopulations with one another and with the target cells [5]. The possibility cannot be ruled out that the spectrum of target cells determines not so much the phenotype of the immunoregulatory lymphocytes or effector lymphocytes but rather dominance of a concrete function from their polyfunctional spectrum. The change of phenotype may perhaps be a secondary phenomenon, maintaining the degree of involvement or noninvolvement of concrete subpopulations in the immune response, i.e., it maintains its prolonged activation or blocks it.

Hence, in our opinion, mismatching between phenotype and function of lymphoid cells often arises in various pathological processes and must be borne in mind when the immune status of patients is evaluated and ways and means of immunocorrection are chosen.

LITERATURE CITED

1. A. L. Burmistrova, Immunologiya, No. 6, 64 (1988).
2. A. L. Burmistrova, Zh. Mikrobiol., No. 3, 64 (1989).
3. A. L. Burmistrova, L. Ya. Ébert, and S. N. Teplova, Byull. Éksp. Biol. Med., No. 9, 318 (1989).
4. A. L Burmistrova and L. Ya. Ébert, Byull. Éksp. Biol. Med., No. 11, 517 (1990).
5. I. M. Dozmorov, Usp. Sov. Biol., **107**, No. 2, 195 (1989).
6. M. Jondal, G. Holm, and H. Migzell, J. Exp. Med., **136**, 207 (1972).
7. D. Horwitz and M. A. Carett, J. Immunol., **118**, 1712 (1977).
8. B. Shohat and H. Joshua, Thymus, 4, 323 (1982).