(Fig. 1). This result is in agreement with data in the literature and it evidently indicates the presence of several binding sites for the neuropeptide, with different dissociation constants [4].

Reactivity of the cells, recorded as accumulation of cAMP in the lymphocytes, showed a similar fall with age (Table 2). A decrease was observed both in the background cAMP level and in the adrenalin-induced increase of cAMP after exposure of the cell to adrenalin.

Thus during ontogeny the sensitivity of mouse lymphocytes to neurotransmitters falls uniformly, except in the newborn period; this may be one mechanism of the change in immunity in old age observed both in man and in animals.

The main cause of the decrease in the number of receptors to neurotransmitters in old age may be, for example, depression of cellular metabolism, leading to slowing of resynthesis of receptors on the cells.

LITERATURE CITED

1. M. M. Gol'dshtein and V. I. Dontsov, Byull. Éksp. Biol. Med., No. 10, 86 (1982).
2. M. M. Gol'dshtein, Byull. Éksp. Biol. Med., No. 10, 500 (1987).
3. R. V. Petrov, Immunology [in Russian], Moscow (1982).
4. P. V. Sergeev and N. L. Shimanovskii, Receptors [in Russian], Moscow (1987).
5. A. D. Ado and M. M. Gol'dshtein (M. M. Goldstein), Brain Behav. Immun., 2, 79 (1988).
6. J. W. Hadden and E. M. Hadden, Fed. Proc., 32, 1022 (1973).
7. J. P. McManus, J. F. Whitfield, and T. Yondale, J. Cell. Physiol., 77, 103 (1971).
8. G. F. Shreiner and E. R. Unanue, J. Immunol., 114, 802 (1975).

PHENOTYPE AND FUNCTION OF LYMPHOCYTES FROM COLITIS PATIENTS

A. L. Burmistrova and L. Ya. Ébert

UDC 616.345-018.73-07:616.155.32-076.5

KEY WORDS: subpopulations of lymphocytes; cytochemistry; heterologous graft versus host reaction

Research by many workers aimed at studying the immune status of patients with nonspecific ulcerative colitis (NSUC) have yielded evidence of changes in the functional powers of T lymphocytes [8, 13]. The writers showed previously [1] that in NSUC there is disproportion between the ratios of subpopulations posessing helper/suppressor activity: T_G^+/T_G^-, theophylline-sensitive/theophylline-resistant (tps-1/tpr-1) lymphocytes. In severe forms of pathology not only are changes found in the proportions of alternative subpopulations, but unity between the parameters tps-1 and T_G^+, and trp-1 and T_G^- is absent: a decrease in tpr-1 and an increase in tps-1 coexist with an increase in T_G^- and a decrease in T_G^+. In accordance with data obtained in [4, 12], trp-1 and T_G^- and tps-1 and T_G^+-lymphocytes belong to immunoregulatory subpopulations and possess a common range of functions. The functional capacity of T_G^- and T_G^+ was assessed previously [2].

The aim of the present investigation was to test the functional activity of tpr-1 and tps-1 with the aid of the local heterologous graft versus host reaction (GVHR) and to determine the cytochemical status of the lymphocytes, i.e., methods which were used to study T_G^+ and T_G^--lymphocytes.

EXPERIMENTAL METHOD

Mononuclear cells were obtained from human peripheral blood in the usual way [6] by gradient centrifugation. To obtain the lymphoid population, monocytes were removed from a mononuclear suspension by adsorption on plastic Petri dishes for 60 min. Nonadherent cells were added to an equal volume of sheep's erythrocytes (3% suspension made up in medium 199 with 40% fetal calf serum – FCS) and the ordinary E-RFC test was carried out, followed by

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. 110, No. 11, pp. 517-519, November, 1990. Original article submitted February 16, 1990.

0007-4888/90/0011-1540$12.50

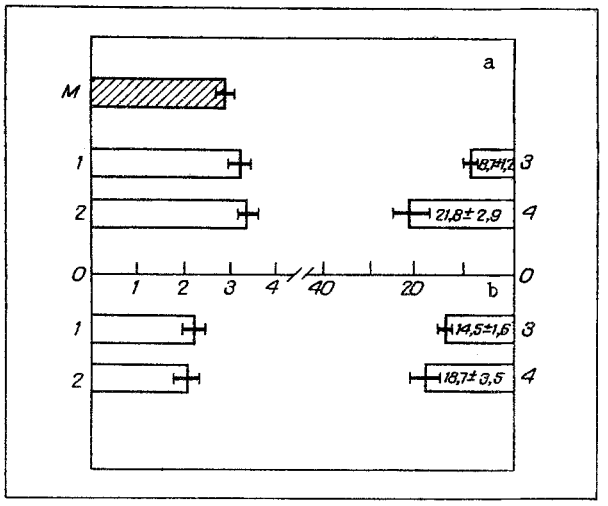

Fig. 1

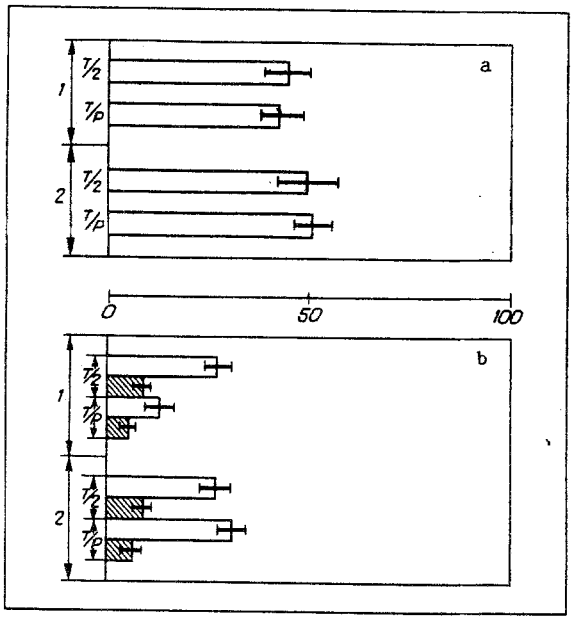

Fig. 2

Fig. 1. Effect of action of tpr- and tps-lymphocyte subpopulations on development of local GCHR and distribution of Fc_G-positive cells among them. a) Healthy individuals; b) patient with NSUC. M) Mononuclears; 1) tpr-1 added to mononuclears; 2) tps-1 added to mononuclears; 3) percentage of Fc_g-positive cells among tpr-1; 4) percentage of Fc_G-positive cells among tps-Abscissa, index of GVHR on left, percentage of Fc_G-positive cells on right.

Fig. 2. Nonspecific esterase activity in lymphoid subpopulations. a) Diffuse, finely granular distribution, weak degree of activity; b) unshaded columns) distribution of enzyme as a distinct spot; shaded columns) coarsely granular, with a high degree of activity. Abscissa, percentage of positively stained cells. 1) Donors' cells, 2) cells from patients with NSUC.

gradient centrifugation on Ficoll-Veregrafin (1.077). The RFC formed were freed from erythrocytes by hypotonic lysis with ammonium chloride (0.83% solution). The isolated T-lymphocytes were incubated at 37°C for 1 h with theophylline, 3 mg/ml ("Serva," USA), and this was followed by the ordinary rosette-formation test (as when isolating T lymphocytes). Separation of the cells into tpr-1 and tps-1 was carried out by gradient centrifugation [12]. Lymphocytes from the interphase constituted a tps-1 subpopulation, those from the residue – tpr. After the isolated cells had been counted, the EA-RFC test was carried out [9] with each subpopulation to determine the number of cells carrying receptors for the FC-fragment of IgG (Fc_G–R); bovine erythrocytes loaded with rabbit antibodies of the IgG class were used in this case (the blood sera were generously provided by Professor N. A. Kraskina).

To assess the functional activity of the isolated lymphocyte subpopulations the local heterologous GVHR was carried out, with assessment of the results on the basis of the index of lymph node enlargement, based on the method suggested in [12]. The mononuclear cell suspension collected from several healthy blood donors, containing $5 \cdot 10^6$ cells in a volume of 40 ml, was injected subcutaneously into the right footpad of recipient mice (CBA, weight 25-30 g, obtained from the "Stolbovaya" and "Rappolovo" nurseries, Academy of Medical Sciences of the USSR); a suspension of syngeneic spleen cells was injected into the left footpad in the same dose. At each point 5-8 animals were used. The animals were given cyclophosphamide in a dose of 100 mg/kg body weight 24 h before the experiment. The GVHR was tested 5 days after injection of the cells, with evaluation of enlargement of the lymph nodes, determined as the ratio of the number of cells in the popliteal nodes of the right and left limbs. The effect of tpr-1 and tps-1 on development of the GVHR was estimated as the arithmetic mean, error of the mean, and coefficient of significance between GVHR with injection of mononuclears from a pool of donors and GVHR following injection of a combination of cells: mononuclears from a pool of donors and tpr-1 of patients or healthy individuals, and mononuclears from a pool of donors and tps-1 from patients or healthy individuals. The doses of cells injected were chosen beforehand: to $5 \cdot 10^6$ mononuclears from a pool of donors were added $(3\text{-}5) \cdot 10^4$ cells from patients of healthy donors, and this mixture of cells was injected into the animal.

As an additional measure, nonspecific esterase was determined in the different lymphocyte subpopulations (α-naphthyl-AS-acetate esterase) [5]. Depending on the distribution of the enzyme, the following types of staining were distinguished: diffuse finely granular with a weak degree of activity, coarsely granular with a high degree of activity, and a compact distinct spot.

Isolation of the lymphoid subpopulations and the study of their properties in the GVHR and according to their cytochemical characteristics were used to investigate 10 donors and eight patients with nonspecific ulcerative colitis (chronic recurrent form, average degree of severity, period of exacerbation).

EXPERIMENTAL RESULTS

The results of the GVHR, reflecting the interdependent influence of cells participating in different combinations, are summarized in Fig. 1. Addition of healthy human lymphocytes to the mononuclear pool of donors gave the effect of stimulation of GVHR: weak on testing tpr-1 and significant ($p < 0.05$) on testing tps-1. In the study of cells isolated from patients with NSUC, the lymphocytes were found to possess an alternative form of action on development of the GVHR compared with healthy donors, i.e., they had a distinct inhibitory effect ($p < 0.01$) on addition of tps-1 to the mononuclear pool and a weaker effect on addition of tpr-1 ($p < 0.05$). This was probably connected with the presence of cells capable of suppressing the suppressors due to their own activation among the tps-1 [11].

Consequently, in healthy individuals and in partients with NSUC tpr-1 and tps-1 exhibit diametrically opposite properties in relation to GVHR formation. Meanwhile the study of activity of T_G^+ and T_G^- showed [2] that, independently of the state of the donor of the cells (healthy individuals and patients with NSUC) T_G^+ possessed weak inhibitory activity, T_G^- possessed stimulating activity in respect to GVHR. It is possible that our results do not contradict data in [4, 11] on the common properties of T_G^+ and tps-1 and T_G^- and tpr-1, but differences between them are due to the polyfunctional nature of the subpopulations and the presence of different proportions of effector cells and regulator cells in the patients and healthy individuals [3].

We next determined the relative number of Gc_G-R-carrying cells among tpr-1 and tps-1 (Fig. 1). It was shown that in NSUC a significant increase was observed in the Fc_G-R-positive cells among tpr-1. We know that the relationship to theophylline and the connection with Fc_G-R enable the cells to be placed in different functional groups [10]: lymphocytes carrying Fc_G-R within the tps-subpopulation possess increased natural killer-cell activity, whereas among tpr-1 there are inducers of suppression. The increase in the number of Fc_G-R-positive lymphocytes within tpr-cells points to a change in the functional properties of the subpopulation (an increase of activity), and this is reflected in metabolic processes.

Analysis of the distribution of nonspecific esterase in tpr-1 and tps-1 showed (Fig. 2) that in NSUC the percentage of cells with a diffuse distribution of the enzyme is significantly ($p < 0.05$) increased among tpr-1 but the number of cells with staining of the enzyme in the form of a compact spot is reduced among tpr-1 and tps-1 (the difference compared with the group of healthy donors is not significant for tpr-1). It can be concluded from data in [7, 14], relating to the cytochemistry of lymphoid populations, that in NSUC disparity is observed between the form of distribution of the enzyme and the affiliation of the tpr-1 to the helper group. These changes probably point to the manifestation of alternative functional powers of the tpr-1 under certain conditions. This conclusion is in agreement with data on the increase in the number of Fc_G-R-positive cells among them and the inhibitory character of their effect on the development of the local heterologous GVHR. Evidence of the existence of dualistic powers in immunoregulatory (h/s) lymphocytes is given by the results obtained by many investigators [15] and, in particular, on the manifestation of cytotoxic properties by $OKT4^+$ lymphocytes, due to the method of activation of these cells.

Thus quantitative variations of lymphoid subpopulations in NSUC coexist with disparity of their phenotype and function.

LITERATURE CITED

1. A. L. Burmistrova, Immunologiya, No. 6, 64 (1988).
2. A. L. Burmistrova, L. Ya. Ebert, and S. N. Teplova, Byull. Eksp. Biol. Med., No. 9, 18 (1989).
3. I. M. Dozmarov, Usp. Sovrem. Biol., 107, No. 2, 195 (1989).

4. F. G. J. Hayhoe and D. Quaglino, Haematological Cytochemistry, London (1980).
5. A. A. Yarilin, Cellular Factors in the Regulation of Immunogenesis [in Russian], Novosibirsk (1985), p. 24.
6. A. Boyum, Scand. J. Clin. Lab. Invest., 21, Suppl. 97, 77 (1968).
7. J. Fehr and O. Scherer, Blut, 44, 201 (1982).
8. J. Goronzy, C. M. Weyand, and I. Waase, Clin. Exp. Immunol., 61, 593 (1985).
9. D. Horwitz and M. A. Garett, J. Immunol., 118, 1712 (1977).
10. G. M. Peterman, G. R. Klimpel, and C. Altman, Clin. Immunol. Immunopath., 28, 1 (1983).
11. B. Shohat, J. Cohen, R. Fogle, and A. Zizov, Cancer (Philadelphia), 48, 923 (1981).
12. B. Shohat and H. Joshua, Thymus, 4, 323 (1982).
13. S. R. Targan, M. F. Kagno, M. D. Brogan, and F. Shanahan, Ann. Intern. Med., 106, 853 (1987).
14. D. T. Umetsu, H. H. Jabara, R. H. Dekrnyff, et al., Am. Ass. Immunol., 140, 4211 (1988).
15. N. Wada, A. M. Preier, and C. Criscolli, Pediat. Res., 15, 1266 (1981).

DEPENDING OF STROMAL CFU-f COLONY FORMATION ON STIMULATING EFFECT OF HEMATOPOIETIC CELLS

N. V. Latsinik, A. Ya. Fridenshtein, Yu. F. Gorskaya, I. L. Moskvina, and Yü Hung-Cheng

UDC 612.119.014.42.08

KEY WORDS: bone marrow, CFU-f, colony-stimulating factors

CFU-f colonies are formed in cultures of bone marrow cells [7, 9, 13, 21] and consist of fibroblasts, which synthesize collagen of types I and III but do not synthesize Willebrand's factor VIII or angiotensin convertase, and they do not possess Fc- and C-receptors or marker antigens of macrophages [4, 6, 12]. These features distinguish fibroblasts of stromal colonies from other adhesive bone marrow cells: macrophages and endothelial cells. If the serum concentration is sufficient to create a high PDGF concentration (not less than 10 ng/ml) in the culture medium the formation of CFU-f colonies does not require any additional growth-stimulating influences. Meanwhile, if the density of explanation of bone marrow cells is low, the efficiency of colony formation (ECF-f) is low and can be raised if irradiated bone marrow cells are added to the cultures [3, 6, 10]. This suggested that hematopoietic cells stimulate the development of stromal colonies. The investigation described below confirmed that for the formation of CFU-f colonies it is in fact necessary that the cultures contain blood platelets or nonadhesive bone marrow cells. It was found that, under these circumstances, the colony-stimulating activity of these cells is not dependent on their content of PDGF.

EXPERIMENTAL METHOD

Single-cell suspensions of marrow cells were prepared by pipeting bone marrow from the femora of CBA mice through a Pasteur pipet or by trypsinization for 60 min in 0.25% trypsin solution on a magnetic mixer [5, 11]. The cell suspensions thus obtained were filtered through a nylon filter and, after washing twice, the cells were explanted into plastic flasks with an area of 25 cm^2. Testing for CFU-f colonies [6, 9, 10, 13] was carried out in two modifications: by culturing the complete population of bone marrow cells (BMC) or adhesive bone marrow cells (ABMC). ABMC cultures were obtained by changing the medium and removing nonadherent cells 2 h after bone marrow explanation, after which the adherent cells were washed three times and the flasks were filled with fresh culture medium. At this stage, irradiated (feeder) calls or growth factors PDGF, EGF, or IL-3 were added to some of the cultures. Mechanically disaggregated BMC, and spleen, thymus, and lymph node cells from CBA mice and guinea pigs, and also leukocytes and platelets isolated from the blood of guinea

N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 11, pp. 519-522, November, 1990. Original article submitted February 16, 1990.

