A trace of contractions of the leech muscle under the influence of various concentrations of ACh before (a) and after incubation with normal rabbit serum (b) and with serum containing AAChR antibodies (c), is illustrated in Fig. 1. It will be evident that these antibodies blocked the ACh receptors of the smooth muscles of the leech and prevented ACh from acting on them.

Average amplitudes of contraction of the leech dorsal muscle under the influence of various ACh concentrations, before and after incubation of the preparation with AAChR antibodies, are shown in Fig. 2.

The results thus show that AAChR antibodies obtained by immunizing a rabbit with antigenic material from motor-denervated skeletal muscles of the hind limbs of BALB/*c* mice, had a marked atropinelike action on the dorsal muscle of the leech, an object highly sensitive to ACh. Our data also confirm the familiar view that ACh receptors do not possess species-specificity [5-7]. In fact, antibodies against ACh receptors of motor-denervated muscles of BALB/*c* mice blocked ACh receptors of motor-denervated muscles of an animal as far removed as regards its species from the mouse as the leech. By way of preliminary conclusion it can be stated that this action of AAChR antibodies can also be observed on other such well-known laboratory objects as the heart and lungs of the frog.

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ROLE OF ADHESIVE CELLS IN THE MECHANISM OF NATURAL CYTOTOXICITY REACTIONS

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A definite role in the regulation of natural killer (NK) cell activity is played by other lymphocyte populations and also by monocytes, macrophages, neutrophils, and eosinophils [15]. Some of these cells participate directly in natural cytotoxicity (NCT) reactions, whereas others mediate regulatory effects on NK cells, without exhibiting any natural killer properties of their own [9].

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Monocytes, like macrophages, can take part in NCT reactions [9]. It has been suggested that acceleration of tumor growth does not take place in nude mice in which NK cells are blocked by antibodies to asialo-GM due to the work of monocytes or macrophages [13]. Monocytes play a definite role in the ability of mononuclear cells (MNC) to respond to interferon. The cells themselves in this case produce little interferon; their main role in the production of this important factor of NK cell activation is initiating [8]. In the context of regulation of NK cell activity, monocytes are regarded as modulators of the level of function of the NCT system [16]. Their effects depend essentially on the incubation time and the ratio between the numbers of cells in the experimental system.

Macrophages are essential in culture to maintain the normal level of proliferation of large granular lymphocytes, including NK cells. It has been suggested that macrophages supply antigens to NK cells [1]. Regarding the effect of macrophages on NCT of lymphocytes, most workers are of the opinion that they suppress the cytotoxic activity of NK cells [14].

It follows from the facts stated above that for NCT reactions to take place cooperative relations are necessary between the various cell populations; the character of the role and contribution of individual cell fractions in the realization of the basic functions of NK cells (cytotoxic and interferon-producing) have not been studied. In particular, the regulatory effects of adhesive cells (AC) on NK cell activity under normal conditions and also in various pathological states in particular have remained virtually unstudied. AC constitute a cell population obtained from a suspension of MNC by adhesion to plastic. This fraction contains monocytes, macrophages, T_γ-lymphocytes, and up to 6% of NK cells. It is by studying the role of AC in the regulation of NK cell function that the mutual influence of individual blood cell populations can be analyzed in an experimental system closely resembling conditions pertaining in the body.

EXPERIMENTAL METHOD

The peripheral venous blood of five healthy donors of both sexes, aged from 19 to 44 years, six patients with multiple sclerosis (MS) of both sexes aged from 24 to 36 years, with the cerebrospinal form of the disease and with a remittant course of the disease (stage of exacerbation); and four women with systemic scleroderma (SSD), aged from 24 to 54 years, was investigated. The patients with SS and MSD were under the care of colleagues in the Departments of Neurology and Neurosurgery of the No. 1 Faculty of Internal Medicine and the Departments of Dermatology and Venereology of the N. I. Pirogov Second Moscow Medical Institute.

The cytotoxic activity of the NK cells was assessed by the radiometric method of Hashimoto and Sudo (1971) in the modification of Rykova et al. (1981). NK cell activity was determined on a suspension of MNC, obtained from the peripheral venous blood of healthy subjects and patients in a one-step Ficoll–Paque density gradient ("Pharmacia Fine Chemicals," Sweden), d = 1.077 g/cm^3, during centrifugation (400g, 45 min, 20°C). A suspension culture K-562 of human erythromyeloblasts, labeled with ^{3}H-uridine in a dose of 3 μCi/ml, was used as the target cells (TC).

For the cytotoxic test, cells selected from the interphase ring were washed twice with buffered physiological saline during centrifugation (400g, 10 min, 20°C). The isolated and washed suspension of peripheral blood MNC was resuspended in 1 ml of complete nutrient medium (CNM), based on RPMI-1640 ("Amimed," Switzerland), of the following composition: RPMI-1640 88 ml, embryonic calf serum (from the N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR) 12 ml, glutamine 200 mM, gentamicin 10 μg/ml. After the number of isolated lymphocytes had been counted in a Goryaev chamber, a similar concentration of lymphocytes was prepared, containing $10 \cdot 10^6$ cells in 1 ml of CNM. The initial concentration of TC was $10 \cdot 10^4$ in 1 ml of CNM.

Before the MNC and TC were distributed among the wells of 96-well round-bottomed plastic micropanels ("Nunc," Denmark), the suspension of TM was treated with the enzyme pancreatic ribonuclease ("Reanal," Hungary) in a dose of 5 μg/ml [4]. The MNC and TC were then distributed among the wells of the micropanels in a volume of 0.1 ml into each well, after which the MNC were incubated with TC for 14 h, at 37°C in a humid atmosphere containing 5% CO_2. TC incubated under the same conditions as the experimental samples, but without effectors, were used as the control in the cytotoxic test.

At the end of the period of incubation the contents of the wells were deposited on glass-fiber filters ("Whatman," England) with a pore diameter of 2.5 μm, with a 12-channel "Dynatech" biological fractions harvester (England).

Residual radioactivity on the filters was counted by means of a "Beckman" LS-2800 scintillation counter (USA). Each sample was counted in 5 ml of toluene scintillator (composition: toluene 1 liter, PPO 5 g, POPOP 0.1 g) for 2 min. Each test was carried out in two or three parallel wells of the micropanels.

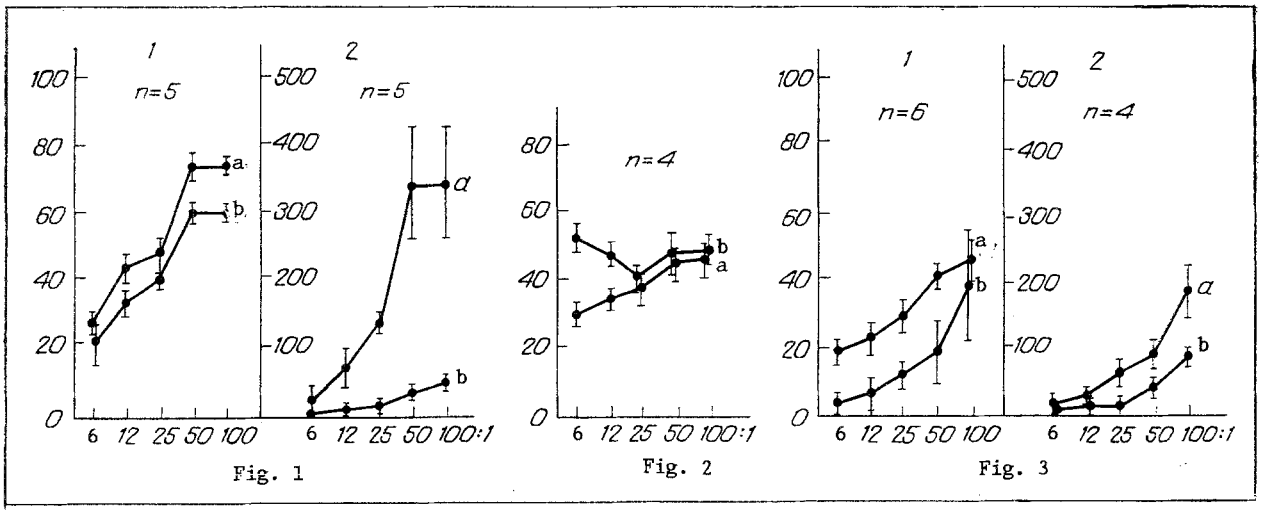

Fig. 1

Fig. 2

Fig. 3

Fig. 1. Changes in cytotoxic (1) and interferon-producing (2) NK cell activity in NK cell:TC system after removal of adhesive cells. Abscissa, ratio E:T. Ordinate: 1) CI, %; 2) interferon, U/ml.

Fig. 2. Increase in cytotoxic activity of NK cells in patients with SSD after removal of adhesive cells. Abscissa, ratio E:T. Ordinate, CI, %. a) Control, b) experiment.

Fig. 3. Effects of removal of adhesive cells from suspension of peripheral blood MNC from patients with MS. Abscissa, ratio E:T. Ordinate: 1) CI, %; 2) interferon, U/ml. 1) Cytotoxic activity of NK cells, 2) interferon-producing activity of NK cells. a) Control, b) experiment.

As a measure of the cytotoxic activity of the NK cells, the cytotoxic index (CI) was calculated by the formula:

$$CI = \left(1 - \frac{\text{no. counts in exptl. well}}{\text{no. counts in control}}\right) \cdot 100\ \%.$$

The interferon-producing activity of the NK cells was determined in supernatants of cultures of interacting cells (NK cells + TC), taken from the wells of the micropanels at the end of the incubation time, by estimating the protective effect on a monolayer culture of human fibroblasts, infected with 100 CPD of vesicular stomatitis virus [6]. Production of interferon α was tested as its stability within the pH range 2.0-7.0 and on eating (56°C, 30 min).

AC were removed from the suspension of peripheral blood NMC by adhesion to plastic Petri dishes ("Nunc," Denmark), treated with embryonic calf serum, for 45 min at 37°C. AC accounted for 10-15% of the MNC.

In all experiments activity of NKC was determined by utilizing the principle of serial cell dilutions, developed in the Department of Immunology, N. I. Pirogov Second Moscow Medical Institute [5]. NK cell activity was tested over a wide range of ratios between effectors and targets (E:T) in the experimental system (from 100:1 to 6:1). By using the serial dilutions principle it was possible to estimate NK cell function more accurately under normal conditions and also to discover the possible mechanisms of development of an NK cell deficiency in various pathological states.

The experimental results were subjected to mathematical analysis by standard statistical methods. The significance of differences between mean values was determined by Student's t test.

EXPERIMENTAL RESULTS

Figure 1 gives the results of a study of the cytotoxic and interferon-producing activity in MNC fractions possessing NK cell activity. Clearly, removal of AC from the suspension of peripheral blood MNC from healthy blood donors reduced the cytotoxic activity of NK cells on average by 19% relative to the control (Fig. 1, 1). A decrease was uniformly distributed with respect to E:M ratios ($p < 0.05$), except 6:1. The results suggest that AC, about 90% of which consist of monocytes, make a definite contribution to the NCT reaction, by assuming the role of NK cells in vitro. The natural CI of AC in the system in vitro varied between 9 and 13%.

After removal of AC, interferon production fell sharply in the NK cell: TC system (Fig. 1, 2). The effect was observed at all values of functional ratios of E:T, and its mean value was 92.4% ($p < 0.01$). The decrease was uniformly distributed among the E:T ratios, evidence that in healthy donors there are two possible mechanisms of regulation of interferon production by the NK cell fraction of AC: a) AC themselves actively produce interferon in this system, when stimulated by TC; b) without any activating signals from AC, NK cells are unable to maintain the necessary level of mediator production.

Similar results were obtained by Green (1986, [10]), showing that interferon (IFN) production by blood MNC does not take place if there is considerable exhaustion of monocytes. Novikov (1988, [3]) states that macrophages of pleural exudate exhibit weak cytotoxic properties against K-562 TC.

In patients with SSD, after removal of AC from the MNC suspension, the depressed NK cell activity did not change over a range of E:M ratios of 100:1 to 25:1 (Fig. 2). Meanwhile, with lower E:T ratios (12:1 and 6:1), characterizing the number of active forms in the NK cell population, CI increased to values exceeding those even in the group of healthy blood donors, and with E:T ratios of 12:1 and 6:1 it reached 48 ± 2.5 ($p < 0.05$) and 52 ± 3.8 ($p < 0.01$) respectively.

Consequently, in SSD, AC can suppress NK cells, and the mechanism of this suppression is quite complex. The functional activity of NK cells in this disease evidently remains blocked by certain other factors than monocytes and suppressor T cells, whereas the processes of differentiation of NK cells are inhibited due to the effects of the AC fraction.

In patients with MS, after removal of AC from the MNC suspension, the cytotoxic activity of NK cells was reduced on average by half (Fig. 3, 1). The absolute change in the values of CI was from 16 to 24% ($p < 0.05$). This effect was marked at all ratios of E:T tested, except 100:1.

The interferon-producing activity of the NK cells was depressed after removal of AC in patients with MS (as also was the cytotoxic activity), on average by 50% ($p < 0.05$) compared with the control (Fig. 3, 2). The absolute change for individual values of the E:T ratios was between 48 and 96 U/ml.

Consequently, compared with the healthy blood donors, AC of patients with MS exhibit higher cytotoxic activity (values of CI fell after removal of AC not by 20%, but by 50%). The fall in the cytotoxic activity of the NK cells is evidently compensated in this way. Meanwhile in patients with MS the contribution of AC to IFN-α production in the NK cell:TC system is reduced (removal of AC leads to a decrease in IFN production in the NK cell:TC system not by 90%, as in healthy donors, but only by 50%). This effect can be explained also by an increase in IFN production by the NK cells themselves. It is not by accident that Tunkel' and co-workers (1983) [7] observed that the cytotoxic activity of NK cells remains within normal limits in patients with a chronic progressive course of MS and falls sharply if the course of the disease is remittent, in a stage of remission.

Thus in certain pathological states changes take place in the regulating effect of the AC fraction on NK activity of MNC. As our own investigations and those of Gordienko and co-workers [2] showed, this change may differ in direction and may have different effects on the formation of the NK cell deficiency in different pathological states.

An important factor in the study of the regulatory activity of components of the AC fraction is the choice of TC. Itoh and co-workers [12] showed, for instance, that if melanoma cells are used as targets the degree of lysis is the same both for purified (over 99%) monocytes and for NK-cell-enriched lymphocytes isolated from healthy human peripheral blood.

The difference between the possible mechanisms of development of NK cell deficiency in MS and SSD, a condition characterized by the systemic nature of its manifestations and by the presence of immune mechanisms in its pathogenesis, points to the need for a detailed study of levels of involvement of the NCT system in individual diseases, and also the role of deficiency of the population of NK and other cells involved in NCT reactions in general immunopathology and, in particular, in the development of the immunoregulatory imbalance in these patients.

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