During the first days of hemoperfusion very slight positive shifts were observed in the blood levels of T and B lymphocytes. Marked stimulation of the rosette-forming capacity of the neutrophils and restoration of the lysozyme level were noted. Taken as a whole, these observations point to an increase in total functional activity of the neutrophils, and they are in agreement with data published previously [8-10].

Thus in acute destructive pancreatitis inhibition of the mechanisms of immunity, and mainly of its T-cell component, takes place. The most favorable and rapid immunocorrective action on these mechanisms is exhibited by UV irradiation of the blood and the use of a xenogeneic spleen.

Hemoperfusion, incidentally, has a normalizing, and even stimulating, effect on the parameters of immunity in the later period after the beginning of treatment (the 3rd and 7th days).

REFERENCES

1. V. M. Buyanov et al., Vestn. Khir., No. 9, 28 (1989).
2. E. V. Gembitskii et al., Revmatologiya, No. 3, 3 (1987).
3. I. I. Dzerzhinskaya, Immunologiya, No. 1, 64 (1982).
4. V. G. Dorofeichuk, Lab. Delo., No. 1, 28 (1968).
5. V. I. Midlenko, Vestn. Khir., No. 2, 33 (1982).
6. L. V. Poluektov et al., Vestn. Khir., No. 21, 92 (1982).
7. Zh. Sundetov, "Correlation of humoral factors and immunity," Author's Abstract of Dissertation for the Degree of Candidate of Sciences, Aktyubinsk (1973).
8. L. D. Taranenko et al., Vestn. Khir., No. 2, 35 (1990).
9. V. S. Savel'ev et al., Acute Pancreatitis [in Russian], Moscow (1983).
10. V. I. Filin, Acute Diseases and Injuries of the Pancreas. Textbook for Physicians [in Russian], Meditsina, Leningrad (1983).
11. A. B. Tsypin et al., Treatment of Septic Diseases by Connection to a Xenogeneic Spleen: Technical Recommendations [in Russian], Moscow (1988), p. 18.

EXPRESSION OF TRANSCOBALAMIN II RECEPTORS OF THE PLASMALEMMA OF HUMAN BLOOD LYMPHOCYTES STIMULATED BY MITOGENS

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KEY WORDS: transcobalamin II receptor; human blood lymphocytes; endocytosis of Co-cyanobalamin; stimulation of division.

In mammalian cells cobalamin-dependent methionine synthetase is the key regulatory mechanism of formation of the pool of folate coenzymes that are essential for synthesis of the purine and pyrimidine bases of RNA and DNA [2, 10]. Embryonic, normal proliferating, and tumor cells take up exogenous precursors of the cobalamin coenzymes from the surrounding medium [1, 3]. Interaction of a complex of cobalamin and a blood plasma transport

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protein transcobalamin-II (TC-II) with receptors of the cell surface membrane [4, 9] is of decisive importance for penetration of precursors of the cobalamin coenzymes into the cytoplasm. The intensity of uptake of the precursor of the cobalamin coenzymes and their biosynthesis increase in healthy human blood lymphocytes, after blast transformation by phytohemagglutinin (PHA) [12]. However, there is no information on the mechanisms of activation of cobalamin transport during stimulation of division of normal hematopoietic cells. In this connection, we studied expression of TC-II receptors in the plasmalemma of healthy human blood lymphocytes and endocytosis of ^{57}Co-cyanocobalamin under the influence of the mitogens PHA and concanavalin A (con A)

EXPERIMENTAL METHOD

Lymphocytes were isolated from healthy human blood by centrifugation in Ficoll-Verografin density gradient (1.062 g/ml). The cells were resuspended in Eagle's medium with the addition of plasma of the same donor and mitogens PHA or con A (from "Sigma") were added. Lymphocytes cultured under the same conditions without the mitogen served as the control. The action of the mitogens on blood lymphocytes was assessed during culture for 72 h by the double label method: incorporation of ^{3}H-thymidine into DNA and of cyantobalamir (^{57}Co-CNCbl) in order to assess the efficacy of the main stages of cobalamin transport. For this purpose, 1 h before analysis of the TC-II receptors, ^{3}H-thymidine was added to a cell suspension ($1.5 \cdot 10^{6}$ cells/ml) at the rate of 40 kBq/ml (USSR/CIS preparation, 780 TBq/mole, 0.064 g/liter). After selection of the cells for counting radioactivity on a β-counter, the rest of the specimen was used to determine receptors. TC-II receptors and endocytosis of cobalamin were estimated by an enzymic method, using a special test system containing TC-II, saturated with ^{57}Co-CNCbl ("Amersham," Great Britain, 389 kBq/ml, 0.0477 μg/ml) [4]. The ability of the lymphocyte to take up cobalamins in the absence of TC-II during blast transformation also was studied. For this purpose, the lymphocyte suspension was incubated for 15 min in medium with ^{57}Co-CNCbl (0.025 μg/ml), washed 3 times to remove free radioactivity by portions of fresh medium, and counted on a γ-counter.

EXPERIMENTAL RESULTS

Exposure to PHA (10 μg/ml) led to transformation of healthy human blood lymphocytes into lymphoblasts. The intensity of incorporation of ^{3}H-thymidine into the cellular DNA increased, and after 72 h of culture it was 6 times higher than the control level (3500 and 600 cpm respectively). A few TC-II receptors (150 per cell) were discovered on the surface membrane of cells of the original culture of blood lymphocytes. During culture of the lymphocytes without mitogen (control) and with the addition of 20% plasma of the same donor, the number of TC-II receptors increased (up to 460 per cell). Maximal expression of receptors was observed on the membrane of PHA-transformed blood lymphocytes after 72 h of culture. Under these circumstances, the number of specific receptors on blast-transformed cells was twice as great as in control cells at the same times of culture, and 6.5 times greater (970 per cell) than on the surface membrane of mature lymphocytes [5].

Similar results were obtained during the action of con A on lymphocytes. Blast transformation of lymphocytes in vitro was observed during the action of con A for not less than 10 min, and with an increase in the duration of exposure (to 20 min) to the mitogen the intensity of incorporation of ^{3}H-thymidine into DNA and expression of the membrane TC-II receptors increased. Expression of TC-II receptors on the lymphoblast membrane also was studied for different concentrations of mitogen ranging from 15 to 200 μg/ml. No significant increase in the number of TC-II receptors could be observed after 72 h of culture of the lymphocytes and with the concentration of con A in the medium under 75 μg/ml (Fig. 1). However, with an increase in concentration of the mitogen in the medium, expression of the membrane TC-II receptors increased sharply. The optimal concentration of con A was 125 μg/ml, and with a higher concentration of the mitogen no further increase in the number of receptors on the lymphoblast membrane could be observed. Under conditions of induced DNA synthesis in the cells, expression of their surface TC-II receptors increased during culture from very few (150) to 30,000 per cell (Fig. 2). Thus exposure to mitogens led to marked expression of TC-II receptors on the plasmalemma of the blast-transformed lymphocytes. The stimulating action of PHA and con A on mature lymphocytes differed only in the degree of expression of the TC-II receptors. The reason evidently was that to stimulate the lymphocytes the minimal concentration of PHA was used, and it

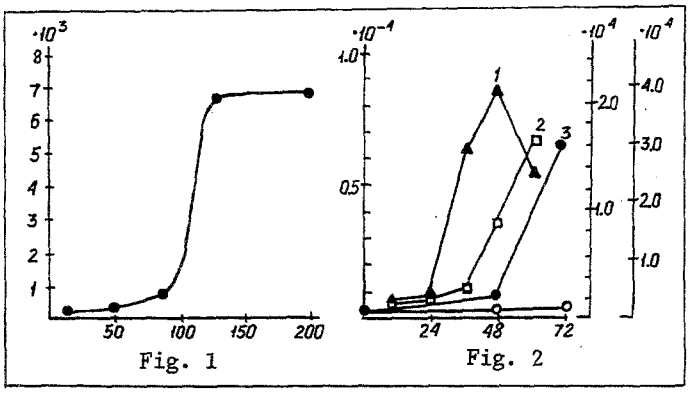

Fig. 1 Fig. 2

Fig. 1. Effect of different concentrations of con A on expression of TC-II receptors of the surface membrane of human blood lymphocytes. Abscissa, con A concentration in medium (in μg/ml), exposure 15 min; ordinate, number of TS-II receptors per cell after 72 h of culture.

Fig. 2. Entry of ^{57}Co-cyanocobalamin into cytoplasm during transformation of mature blood lymphocytes by con A and expression of TC-II surface membrane receptors. Abscissa, duration of culture of lymphocytes (in h); ordinate, on left (1): rate of entry of ^{57}Co-CNCbl not bound with TC-II (in pg/ml) into cytoplasm; in middle (2): incorporation of ^{3}H-thymidine in DNA of cells (in cpm); on right (3): number of TC-II receptors per cell.

was not removed from the culture medium; this could be the cause of accumulation of a factor inhibiting proliferation in the medium [8]. Stimulation of the lymphocytes by con A was carried out under optimal conditions, under which expression of the TC-II receptors was more marked. The particular features of cobalamin transport in lymphocytes induced to divide by the mitogen must be emphasized. In the early stages of activation of division, during stimulation by con A, endocytosis of radioactive cobalamin, not bound with TC-II, considerably preceded the peak of ^{3}H-thymidine incorporation into cellular DNA. Since the plasma transport protein TC-II is an essential intermediary in cobalamir endocytosis, a different possibility of their uptake by transformed lymphocytes was analyzed In the absence of TC-II, the entry of a physiological quantity of labeled cobalamin into the cytoplasm of the stimulated lymphocytes had already been activated by the 12th hour of culture (Fig. 2). Under these circumstances the rate of passage of ^{57}Co-CNCbl into the cytoplasm reached a maximum after the 42nd hour of culture, and subsequently decreased. The most probable alternative path of cobalamir transport into the cytoplasm is evidently a type of diffusion, for at these times of activation of cell division the number of TC-II membrane surface receptors was still not significant, and the regulatory mechanism of their expression was gradually involved, and reached its peak toward the end of the 3rd day after exposure to the mitogen. The highly specific and infective path of cobalamir transport into proliferating cells, using TC-II receptors, rules out any accidental penetration of substances of a different nature into the cytoplasm. The question arises, why in the early stages of induction of cell division under the influence of the mitogen is a different mechanism of cobalamir transport, not mediated through TC-II, also suitable. It may be that nonspecific transport enables the cell to be saturated with the cobalamins that are essential for the beginning of synthesis, to take place rapidly, although with a lower level of selectivity. The order of involvement of fundamentally different mechanisms of cobalamin transport probably reflects the harmonized working of genes associated with activation of the proliferative activity of the cells [7].

Thus cells embarking on the mitotic cycle switch on mechanisms inducing expression of surface receptors to TC-II Leukemic cells, as the writers showed previously, express a constant number of TC-II receptors in the duration of the mitotic cycle, and uses them to carry out internalization of the [TC-II + cobalamin] complex in the phase of DNA synthesis [6]. Expression of TC-II membrane surface receptors as an important parameter of proliferating cells can therefore serve as a marker of proliferation. This conclusion is confirmed by data showing a sharp decrease in the

number of TC-II membrane receptors of the K-562 and HL-60 cell lines of human leukemia during their chemically induced differentiation by arabinoside-cytosine and dimethyl sulfoxide [11].

REFERENCES

1. Yu. V. Vares and N. V. Myasishcheva, Current Problems in Experimental Chemotherapy of Tumors [in Russian], Vol. 2, Chernogolovka (1987), p. 127.
2. G. K. Gerasimova, M. Balinska, O. D. Golenko, et al., Byull. Éksp. Biol. Med., **91**, No. 1, 57 (1981).
3. N. V. Myasishcheva, O. D. Golenko, L. E. Kuznetsova, et al., Vopr. Med. Khim., No. 5, 622 (1977).
4. A. E. Oreshkin and N. V. Myasishcheva, Éksp. Onkol., **11**, No. 2, 45 (1989).
5. A. E. Oreshkin and N. V. Myasishcheva, Biology of the Tumor Cell [in Russian] (1990), p. 77.
6. A. E. Oreshkin and N. V. Myasishcheva, Byull Éksp. Biol. Med., **106**, No. 7, 85 (1990).
7. R. Basserga, P. M. F. Ming, Y. Tsutui, et al., (No details given), New York (1977), p. 409.
8. G. A. Granger, E. C. Laserna, W. P. Kolb, et al., Proc. Nat Acad Sci USA, **70**, 426 (1973).
9. C. A. Hall, P. D. Colligan, and J. A. Begley, J. Cell Physiol., **133**, 187 (1987).
10. F. M. Huennekens and P. N. Digirolamo, Advances in Enzyme Regulation, ed. by G. Weber, Oxford (1976), p. 187.
11. D. W. Jacobsen, T. Amagasaki, and R. Green, Biomedicine and Physiology of Vitamin B_{12}, ed by J. C. Linnell and H. R. Bhatt, London (1990), p. 293.
12. N. V. Myasishcheva, E. V. Quadros, D. M. Matthews, et al., Biochim. Biophys. Acta, **588**, 81 (1979).

ACTIVITY OF HUMAN NATURAL KILLER CELLS UNDER DIFFERENT EXPERIMENTAL CONDITIONS

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KEY WORDS: natural killer cells; experimental conditions.

The traditional radiometric methods of estimating activity of human natural killer (NK) cells presuppose the use of nutrient media prepared with the addition of serum derived from various sources. Depending on the conditions of adaptation of a culture of K-562 cells, the traditional target cells (TC) for testing NK activity in vitro, additives to the medium may include: fetal calf serum (FCS) [6, 8, 14], bovine serum [2], human blood group IV serum [8], autologous plasma [1], and autologous serum [4]. In order to obtain high titers of production of cytotoxic NK factors, it has been shown that the cells must be incubated in serum-free medium [6]. A marked increase in NK activity was recorded in previous studies [1, 4] in the presence of autologous plasma and autologous serum respectively. The basis for this change in activity of NK cells when incubated under nonstandard experimental conditions is provided by the connection, which several workers have noted, of the level of natural cytotoxicity (NCT) with functioning of the HLA system [9, 11, 12], which is confirmed by the high activity of the cells obtained with the use of a xenogeneic model [7]. Meanwhile, the absence of any such connection was concluded from other investigations [5, 10, 15], and it was shown in [8] that for lymphocytes to realize their NK activity in the peripheral blood, a source of serum is not essential.

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