FUNCTION TESTING OF LYMPHOKINE-ACTIVATED KILLER CELLS FROM NORMAL SUBJECTS AND PATIENTS WITH INTERLEUKIN-2-DEPENDENT IMMUNODEFICIENCY

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The phenomenon of induction of lymphokine-activated killer cells (LAK cells) is characterized by acquisition by lymphocytes, after culture in vitro in the presence of interleukin-2 (IL-2), of the ability of lyse target cells (TC), resistant to the cytotoxic action of natural killer cells (NKC) [9, 10]. LAK cells have been shown to be large granular lymphocytes with a well developed lysosomal system and with the Leu 7^- Leu 11^+ phenotype [12, 14]. LAK cells are a unique cell population and differ both from cytotoxic T lymphocytes and from NKC, whereas at least some NKC precursors can undergo differentiation into LAK cells [11]. Monocytes are not precursors of LAK cells, but their presence in the culture is essential for effective LAK formation [7].

The biological role of LAK cells is unknown, but it has been shown that LAK cells have an antitumor action in vivo. Attempts have been made to treat patients with metastasizing tumors by injecting LAK cells alone or together with IL-2 [8, 15]. However, some unsolved problems remain with respect to the nature of precursors of LAK cells, the mechanisms of their antitumor action, possible ways of controlling LAK activity, and the use of LAK as a diagnostic test in clinical immunology.

The aim of the present investigation was accordingly to develop methods of testing the functional activity of LAK cells and the use of such a test when investigating patients with IL-2-dependent immunodeficiency.

The writers showed previously that in patients with an eye lesion caused by infection with herpes simplex virus (ophthalmic herpes – OH), production and the response of lymphocytes to IL-2 are depressed and a deficiency is present in the NKC system, expressed as depression of the function and a decrease in the frequency of NKC precursors in the peripheral blood [3]. It was therefore decided to use the test developed previously to evaluate the functional activity of LAK cells in patients with OH.

EXPERIMENTAL METHOD

Mononuclear cells (MNC) were isolated from peripheral blood by Boyum's method [2]. NKC activity was determined in the 14-h microcytotoxic test against TC of lines K-563 (NKC-sensitive) and P-815 (NKC-resistant), labeled with ^{3}H-uridine [4, 5]. Cytotoxicity was evaluated by measuring the level of residual radioactivity

$$CI = (1 - (E/C)) \times 100\%$$

where CI denotes the cytotoxic index, E the number of counts in the test cell [well contained TC + effector cells (EC)]; C the number of counts in the cell containing TC only. NKC activity was estimated by the serial dilutions methods with effector/target ratio of 100:1, 50:1, 25:1, 12:1, and 6:1 [5].

Estimation of LAK activity involved two stages:

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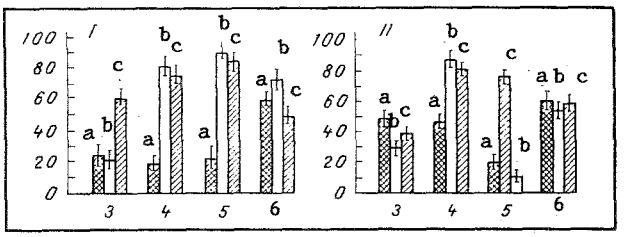

Fig. 1. Changes in cytotoxicity depending on times of culture of MNC from healthy blood donors with rIL-2 different concentrations, when P-815 (I) and K-562 (II) cells were used as TC. Abscissa, period of culture, days; ordinate, CI, %. Columns indicate concentration of rIL-2, U/ml: a) 50, b) 100, c) 200.

Stage I – Induction of LAK Cells

MNC were cultured in vitro in the presence of recombinant IL-2 (rIL-2, from "Biogen," USSR), which was used in doses of 50-200 U/ml. The cells were cultured in siliconized penicillin flasks in complete RPMI-1640 medium containing 10% fetal serum, 2 mM HEPES, 100 U/ml of pencillin and 100 μg/ml streptomycin, and 1% glutamine. The concentration of MNC in the cultures did not exceed $1 \cdot 10^6$/ml. The volume of culture medium in the flasks was 4 ml. The cells were cultured in a moist atmosphere with 5% CO_2 at 37°C for 3-7 days.

Stage II – Evaluation of Cytotoxicity of LAK Cells

After the end of incubation the MNC culture was cooled (5 min at 4°C). The cells were washed twice with medium 199 with 10% fetal serum, made up to a concentration of $10 \cdot 10^6$/ml with complete medium RPMI-1640. Cytotoxic activity was assessed against TC of lines K-562 and P-815 in the proportion of effector to target cells (E/T) of 50:1. The microcytotoxic test was conducted in 96-well panels in complete medium RPMI-1640. EC cultured under the same conditions but without rIL-2 served as the control.

EXPERIMENTAL RESULTS

Tests were carried out on 30 normal blood donors (aged 17-40 years) and 12 patients with OH. Preliminary experiments confirmed data in the literature on the resistance of TC of the P-815 line to the cytotoxic action of NKC. MNC freshly isolated from the peripheral blood of healthy donors and patients with OH did not lyse TC of the P-815 line (CI did not exceed 5%). Maximal cytotoxicity (CI 92 ± 4.2%) against line P-815 was obtained by the use of MNC from healthy donors, cultured for 4-5 days with rIL-2 in a dose of 100 U/ml, as EC (Fig. 1, I). The EC obtained under these conditions found an increase in cytotoxic activity against K-562 cells from 67 to 87 ± 5.1% (Fig. 1, II). In control MNC cultures, incubated in the absence of rIL-2, no cytotoxic activity was observed against P-815, whereas against K-562 no significant differences were found compared with freshly isolated cells (CI was 65 ± 2.7 and 63 ± 5.1, respectively). The results of the investigation of LAK-cell function in the group of healthy blood donors enabled optimal conditions to be chosen for the conduct of this test. Larger and smaller doses of rIL-2 and also a decrease or increase in the periods of culture led to reduction of cytotoxic activity (Fig. 1), which does not conflict with data in the literature [13].

Investigation of patients with OH yielded the following results. Natural killer activity of freshly isolated MNC was significantly ($p < 0.05$) reduced with all ratios used (Fig. 2). Freshly isolated MNC likewise had no cytotoxic activity against P-815. To generate LAK cells, MNC from patients with OH were incubated for 5 days in the presence of rIL-2 (100 U/ml). It will be clear from Fig. 2a, b that LAK activity in patients with OH (against P-815) and NK activity (against K-562) from cultures stimulated by rIL-2 were significantly lower than in healthy blood donors (control). The decrease in CI was similar in character and averaged 25-30%.

In herpetic infection a disturbance of function of the effector and regulatory components of the immune system was discovered in the form of: weakening of activity of TC and NKC, reduction of the number of NKC precursors, inhibition of SIF production, disturbance of production of interleukin-1 and IL-2 and of the response to these mediators and, as a

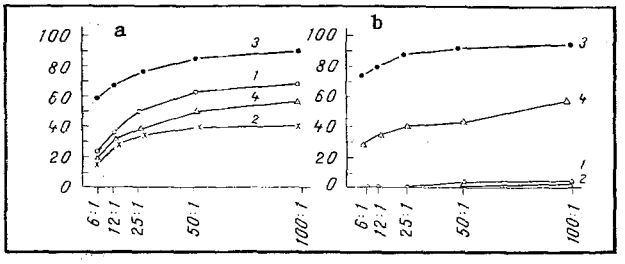

Fig. 2. Assessment of cytotoxicity in healthy subjects and patients with OH. a) Against K-562 TC, b) against P-815 TC. Abscissa, ratio E/T; ordinate, CI, %. 1) Freshly isolated MNC from normal subjects, 2) freshly isolated MNC from patients with OH, 3) EC from normal subjects from cultures with rIL-2, 4) EC from patients with OH from cultures with rIL-2.

result, weakening of proliferation of lymphocytes in response to mitogenic and antigenic stimulation [1, 6]. These observations indicated that herpetic infection and, in particular, ophthalmic herpes can be classed in the group of diseases in whose immunopathogenesis the leading role is played by disturbances in the interleukins – interleukin-dependent immunopathological states system [3]. Data described above, indicating weakening of natural cytotoxicity and disturbance of generation of LAK cells, are in agreement with the theory of interleukin-dependent immunodeficiency states. Weakening of LAK activity of the patients' lymphocytes was evidently due to a disturbance of the response to IL-2, for according to the experimental conditions a standard quantity of exogenous IL-2 was added to the test system. In turn, disturbance of the response may have been the result of blockade of receptors for IL-2, disturbance of signal transmission from receptor to cell – a disturbance of the secondary messenger system – or deficiency of expression of IL-2-receptors on the patients' lymphocytes. On the other hand, since endogenous IL-2 promotes expression of receptors on TC, a disturbance of its production in the patients could perhaps lead to deficient expression of IL-2-receptors, thereby disturbing the response of immunocompetent cells to it and, as a result, reducing LAK cell generation.

A similar, but more marked defect arises in many types of malignant neoplasms which, however, in some cases respond to adoptive immunotherapy with high doses of IL-2 combined with LAK cells. The possibility of using such treatment in patients with the severest manifestations of herpetic infection needs consideration.

Assessment of LAK activity can thus be used to test the functioning of the immune system in patients, but the concrete mechanisms of the disturbance of LAK activity and also the place of this test among methods of evaluating human immune status require further study and more precise defintiion.

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EFFECT OF RADIOGRAPHIC CONTRAST MEDIA ON THE COMPLEMENT SYSTEM IN RATS

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Modern radiographic contrast media (RCM), despite their high diagnostic value, frequently induce side effects which, in some cases, require the implementation of urgent resuscitation measures [5]. An important role in the pathogenesis of the allergic and anaphylactoid reactions which present the greatest risk of administration of RCM, is played by activation of the complement system [6, 8, 11, 12]. Siegel and coworkers [13, 14] and Lasser and coworkers [10] have shown that during radiographic contrast studies in patients sensitive to RCM, the hemolytic activity of complement falls much lower than in insensitive subjects. It is therefore a matter of urgent importance to develop a simple test of sensitivity to RCM, based on preliminary assessment of their effect on the complement system, in small volumes of blood serum in vitro. The possibility cannot be ruled out that one such test may be determination of complement activation by an alternative method [2].

The aim of this investigation was to study activation of the complement system in rats by 50% iodipamide (methylglucamine salt), 76% triombrast, and iodamide-380, by an alternative method in vitro and in vivo.

EXPERIMENTAL METHOD

Experiments were carried out on 280 Wistar rats weighing 150-200 g, kept on the standard animal house diet. The animals were divided randomly into control and experimental groups [3], each consisting of not less than 10 animals.

The experiment consisted of three series. In series I rats of the experimental groups received an injection of the RCM into the caudal vein: 50% iodipamide, 76% triombrast, and 80% iodamide-380 (from the M. V. Lomonosov Kiev Pharmaceutical Chemical Factory), in doses of 1, 2, and 15 ml/kg body weight. Animals of the control group received an intravenous injection of physiological saline (37°C). The rate of injection was 0.1 ml/sec. The animals were killed by decapitation under superficial ether anesthesia after 5-10 min, their blood was allowed to stand (at 4°C) and serum was obtained by centrifugation (400g, 8 min). In experiments of series II serum was obtained from the experimental animals after incubation of the test RMC with the rats' blood (37°C, 10 min). In the experiments of series III the test RMC were incubated with serum from the experimental animals (37°C, 10 min). The concentration of RMC in contact with the blood and serum in the experiments in vitro was $2.5 \cdot 10^{-2}$-$2.5 \cdot 10^{-4}$ M. Intact rat serum served as the control for the experiments in vitro.

The effectiveness of activation of complement by the alternative method in the animals' blood serum (0.1 ml) was determined as the degree of hemolysis of rabbit's red blood cells

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