COMPARISON OF SUPPRESSOR AND CYTOTOXIC ACTIVITY OF BLOOD MONONUCLEARS DURING ADAPTIVE IMMUNOTHERAPY OF CANCER PATIENTS WITH LYMPHOKINE-ACTIVATED KILLER CELLS WITH A LOW DOSE OF RECOMBINANT INTERLEUKIN-2

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Tumor growth in man is accompanied by weakening of immunologic protection of the individual. Suppressor activity of T-cells and monocytes in the blood and spleen of cancer patients is increased [6, 7, 9, 13]. However, more than 50% of patients preserve the cytotoxic activity of natural and tumor-specific killer cells, which destroy the cells of the intrinsic tumor [10]. It has been shown that one way of strengthening antitumor immunity may be the adaptive immunotherapy (AIT) of patients with interleukin-2 (IL-2) and lymphokine-activated killer cells [8]. At the Oncologic Scientific Center, Academy of Medical Sciences of the USSR, 7 patients with disseminated melanoma of the skin and with carcinoma of the kidney have been treated by LAK cells together with recombinant interleukin-2 (RIL-2) of Soviet origin [4].

In the present investigation the cytotoxic and suppressor activity of peripheral blood mononuclears (MN), determined at different times of AIT, was studied in cancer patients.

EXPERIMENTAL METHOD

The cytotoxic activity of MN was determined by the standard method [3] and suppressor activity was determined by our own new method of assessment of suppression, effected by centrifugation of peripheral blood MN in a Percoll gradient [1, 2]. Six melanoma patients (4 men aged 33, 35, 38, and 47 years; nos. 2, 5-7, and 2 women aged 53 and 40 years; nos. 1 and 4) and also one woman with carcinoma of the kidney (nos. 3) were given AIT in a schedule described in [8]. In the first 2-3 days of the week taken from the patients by the method of leukopheresis; MN were isolated and cultured in a concentration of (1-3) $\cdot 10^6$ cells/ml of medium RPMI-1640, containing 2% of heated fetal calf serum, 2% of 0.1 M HEPES-buffer, 1% 200 mM L-glutamine, 40 μg/ml of gentamycin, and 1000 U/ml of RIL-2, in the course of 48 h, to produce generation of LAK cells. The cells were cultured in roller bottles with a capacity of 3 liters (New Brunswick Scientific, USA) on an apparatus from the same firm, rotating the bottles at a speed of 0.5 rpm. On subsequent days of the week the patients were given 200 ml of physiological saline containing (2.5-13.6) $\cdot 10^9$ autologous LAK cells with 75,00 U activity of RIL-2 (one course of AIT), twice or three times intravenously. Depending on the patients' clinical state they were given 1-5 weekly courses of AIT. Blood was taken by leukopheresis from the patients 2 days before the beginning of each course of AIT, on a "Travenol" CS-3000 blood separator (USA), and blood was taken from a vein one day after the end of the course of treatment. MN was obtained from the leukocyte mass and peripheral blood by centrifugation on a Ficoll—Verografin gradient with density 1.077 g/cm^3. The isolated MN were washed twice and resuspended in medium RPMI-1640. To determine suppressor activity. (25-50) $\cdot 10^6$ MN were isolated from the patients' peripheral blood and layered above a

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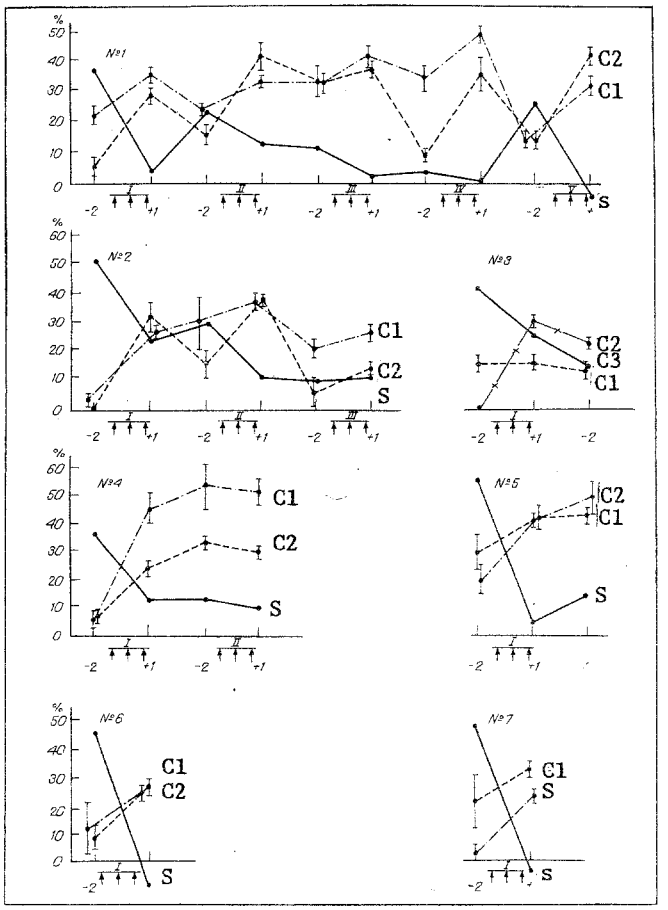

Fig. 1. Evaluation of effector functions of blood lymphocytes from cancer patients in the course of AIT. Abscissa, number of courses of AIT (I-V) and times of determination of suppressor and cytotoxic activity of MN, two days before (-2) and 1 day after (+1) end of each course of AIT; ordinate, suppressor (S) and cytotoxic (C) activity of MN in 7 patients with melanoma (Nos. 1, 2, 4-7) and carcinoma of the kidney (No. 3). Testing cytotoxicity of MN against MeI-1 (C1), K562 (C2), and HeLa (C3) cells.

stepwise Percoll gradient with step densities of 1.056, 1.067, and 1.077 g/cm^3. After centrifugation of the gradient fractions concentrated on the boundary between Percoll layers with density of 1.056-1.067 g/cm^3 (fraction 1, enriched with suppressors) and with density of 1.067-1.077 g/cm^3 (fraction 2, enriched with precursors of cytotoxic T cells) were collected. Unfractionated MN ($5 \cdot 10^6$), MN from fractions 1 and 2 of the gradient, and also a mixture of $5 \cdot 10^6$ MN of fraction 1 and $5 \cdot 10^6$ MN of fraction 2 were cultured in complete medium RPMI-1640, containing 1 μg/ml of phytohemagglutinin (PHA). MN from fraction 1 was treated with mitomycin C before combined culture with cells from fraction 2 of the gradient. After culture for 48 h, in all versions of the experiment the cytotoxic activity of the killer cells was determined against tumor target cells (TC), labeled with ^{51}Cr. The percentage of suppression was estimated by the formula:

$$(a - b)/a \cdot 100\%,$$

where a and b denote the cytotoxic effect of MN from fraction 2 and a mixture of cells from fractions 1 and 2 of the gradient respectively. To determine the cytotoxic activity of MN we used MeI-1, K562, and HeLa tumor cells. The K562 and HeLa $(0.5\text{-}4.0) \cdot 10^6$ TC were incubated in medium containing 50-200 μCi $Na_2{}^{51}CrO_4$ for 1-2 h at 37°C, washed three times, and resuspended in medium RPMI-1640. Labeled TC (10^4) were mixed with $5 \cdot 10^5$ effector lymphocytes and incubated on 96-well plates in a volume of 0.2 ml for 18 h at 37°C and in an atmosphere with 5% CO_2. Each reaction was

carried out in triplicate. At the end of incubation the samples were centrifuged for 7 min at 300*g* and half the supernatant was withdrawn to determine radioactivity. Spontaneous release of label was measured in samples containing TC only; the maximal yield was measured in samples containing TC with the addition of a 1% solution of SDS. Specific release of ^{51}Cr was estimated in percent by the equation:

$$\text{Percentage cytotoxicity} = [\text{Release of } ^{51}Cr \text{ in expt.} - \text{Spontaneous release of } ^{51}Cr]/[\text{Max. release of } ^{51}Cr - \text{Spontaneous release of } ^{51}Cr] \cdot 100.$$

Spontaneous release of the isotope in the control tests was 20-25%. For statistical analysis of the results the mean and standard error of the mean were calculated. The significance of differences between the mean values was determined by Student's table.

EXPERIMENTAL RESULTS

To administer AIT with 75,000 activity units of RIL-2 we used LAK cells with cytotoxic activity against MeI-1, HeLa, and K-562 cells, averaging 50-54%. The high antitumor activity of the LAK cells was connected with shortening of their cultivation time to 48 h, for we showed previously that after 72 h the cytotoxicity of the LAK cells was reduced by 1.5-2.5 times as a result of activation of suppressors of antitumor immunity by RIL-2 [5].

Autologous suppressor activity in the blood of patients Nos. 1-7, determined by gradient centrifugation 2 days before the beginning of AIT with RIL-2 varied from 36.2 to 56.2%.

In the course of AIT the level of suppression in the blood of these patients was observed to fall after the end of each course of treatment (Fig. 1). In patients Nos. 3, 5, and 7, who received only one course of AIT, suppressor activity of MN fell to 15.8%, with the possibility of complete disappearance of the suppressor effect 24 h after treatment. In patients Nos. 4, 2, and 1, who received 2, 3, and 5 courses respectively of AIT, the level of suppression measured in the blood two days before the beginning of each course of treatment gradually declined until the end of treatment, when it reached 10.1-11.5%, whereas in patient No. 1, the suppressor activity of MN 24 h after the end of the 5th course of AIT disappeared.

Lowering of the level of suppression toward the end of AIT correlated with the tendency for the cytotoxic activity of MN to rise in all patients. In patients Nos. 6, 4, 2, and 1, who received 2, 3, and 5 courses of treatment respectively, against the background of weakening of the suppressor activity of MN a statistically significant increase in the cytotoxicity of MN was observed 24 h after the end of each course of treatment against MeI-1 and K562 cells, by 18.8-37.7% ($p < 0.001$).

Lowering the level of suppression in the patients' blood can be explained by a shift of the immunologic balance of the cells during AIT toward strengthening of the cytotoxicity of MN, which takes place on account of the introduction of LAK cells into the patients, as well as activation of precursors of cytotoxic lymphocytes by RIL-2 and lymphokines, secreted by LAK cells.

Potentiation of antitumor immunity during AIT was demonstrated by other workers. For instance, after injection of autologous cells with a low dose of RIL-2 into patients with carcinoma of the kidney, potentiation of the cytotoxic activity of natural killer cells and also of antibody-dependent cytotoxicity against carcinoma of the kidney cells was observed [11]. Intraperitoneal injection of LAK-cells with RIL-2 into 20 patients with a tumor in the peritoneal cavity caused an increase in the Cytotoxic activity of peritoneal exudate cells [12]. Meanwhile no information is yet available on the study of the suppressor activity of MN in the course of AIT of patients with LAK-cells and RIL-2.

Thus when the AIT method is used, levels of cytotoxic and suppressor activity in the blood of cancer patients must be evaluated, for this may be one criterion of the efficacy of treatment.

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SPECIFICITY OF THE TOXIC GENETIC ACTION OF CARCINOGENIC AROMATIC COMPOUNDS ON MUS-MUTANT STRAINS OF *Drosophila*

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Genetic toxic effects of chemical carcinogens are revealed in *Drosophila* in the form of mutations in sex or somatic cells [1] or as their lethal action on larvae. The toxic effect increases to the level of hypersentivity in specially created mutant strains, defective for DNA repair [7]. Among these strains, the mus-mutants of *Drosophila melanogaster* obtained by Henderson and co-workers, and characterized by sensitivity to several direct and indirect action carcinogens [5], are of great interest. Since precarcinogens are activated to genetic toxic metabolites by microsomal monooxygenases [6], it was important to discover if correlation exists between the sensitivity of individual strains to precarcinogens and the activity of these enzymes in them. To use mus-mutants as the test object for screening carcinogens, it is also necessary to know how specific their sensitivity to these agents may be, i.e., to study their effects parallel with the action of noncarcinogenic analogs.

EXPERIMENTAL METHOD

Reagents. Benz(a)pyrene [B(a)P] was obtained from "Fluka AG," Switzerland; benz(e)pyrene [B(e)P] from "Schuchardt," West Germany; 2-acetylaminofluorene(2-AAF)and dimethyl sulfoxide (DMSO) from "Serva," West Germany; pyrene and fluorene were of USSR origin and of the chemically pure grade.

Strains of *D. melanogaster* mus210, mus208^{B1}, mus208^{B2}, and mus205^{B1}, and also the original strain b pr cn/Cy 0, not containing the mus locus, were generously provided by Dr. Henderson.

For the experiments on a standard nutrient medium, 10 homozygous pr cn*/b pr cn* females and 7 or 8 heterozygous b pr cn*/Cy 0 males were transferred into each glass container. After 48 h the parents were removed and 200 μl of an emulsion of the test substances in 10% DMSO solution was applied to the surface of the 2-day *Drosophila* cultures. The

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