PATHOMORPHOLOGICAL EVALUATION OF ANTITUMOR COMBINATION TREATMENT OF MURINE LYMPHATIC LEUKEMIA BY CYTOSTATICS AND IMMUNOMODULATORS

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Weakening of the side effects of antitumor agents on the body's immune system is an urgent problem in contemporary oncology. In clinical practice cytostatics are used against a background of immunomodulator therapy in accordance with empirically chosen programmes [1, 2, 4]. Experimental studies have shown that small doses of cytostatics can also have an immunomodulating action [3, 5].

The aim of the present investigation was the experimental determination of optimal combinations of the immunomodulators thymalin and reaferon, with one of the frequently used cytostatics, namely nitrosomethylurea (NMU), on a model of murine lymphatic leukemia with the aim of alleviating its immunosuppressive action.

EXPERIMENTAL METHOD

Experiments were carried out on 100 male (C57BL/6 × DBA/2)F_1 mice, inoculated with $2 \cdot 10^6$ murine lymphatic leukemia P388 cells. Depending on the treatment given to the animals they were divided into six experimental groups (15 mice in each group); the control group consisted of 10 mice, which received only the solvent (0.9% sodium chloride solution) by intraperitoneal injection in a dose of 0.2 ml daily for 3 days. Treatment in Groups 2 and 3 began on the 1st day after inoculation of the tumor. Thymalin was injected intramuscularly in a dose of 20 mg/kg daily for 3 days. Animals of Group 3 also received NMU by a single intraperitoneal injection in a dose of 96 mg/kg. Groups 4 and 5 received NMU by a single intraperitoneal injection in different doses: 96 mg/kg and 1/25 of this dose respectively. Treatment of Groups 6 and 7 began on the 1st day after inoculation of the tumor: reaferon was injected intraperitoneally in a dose of $20 \cdot 10^6$ U daily for 3 days. The animals of Group 7 later were given an intraperitoneal injection of 96 mg/kg NMU. The doses of the preparations were equivalent as regards toxicity to those used clinically. On the 7th day after inoculation the animals (5 in each group) were killed by cervical dislocation. The most severely affected organs were taken from each animal for morphological investigation: liver, lungs, spleen. Material was embedded in paraffin wax and histological sections were stained with hematoxylin and eosin. The reaction for acid phosphatase was carried out in frozen sections to reveal macrophages. The relative area of the splenic follicles, expressed as a percentage of the area of Weibel's stereometric grid was determined morphometrically. The bulk density of the macrophages, expressed as fractions of unity, was calculated with the aid of an "Ob"ekt" instrument. The significance of the difference between the relative values was estimated by student's test.

EXPERIMENTAL RESULTS

A significant increase in the survival time of the animals was observed after their treatment with NMU in a dose of equivalent toxicity and a combination of NMU with the immunomodulators (Groups 3 and 4). Injection of immunomodulators

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TABLE 1. Data on Survival Time of Animals with Tumors

Group of animals	1-	2-	3-	4-	5-	6-	7-
Length of survival, days	8,67±0,78	8,30±0,16	13,40±0,7	13,0±0,7	9,2±0,34	9,1±0,25	12,6±0,7
Length of survival, percent of control		−4 %	+55 %	+50 %	+6 %	+6 %	+45 %

TABLE 2. Results of Morphometric Analysis of Relative Area of Splenic Follicles

Group of animals	1-	2-	3	4	5-	6-	7
Area of follicles, %	11,9±1,21	11,8±2,12	15,2±1,16	7,7±0,44	16,9±2,14	15,2±1,02	11,3±1,20
p		>0,05	>0,05	<0,01	>0,05	>0,05	>0,05

TABLE 3. Bulk Density of Macrophages in Spleen

Group of animals	1-	2-	3-	4-	5-	6-	7-
Bulk density of macrophages, fractions of unity	0,17±0,025	0,15±0,009	0,24±0,016	0,19±0,011	0,10±0,008	0,12±0,008	0,16±0,013
p		>0,05	<0,05	>0,05	>0,05	>0,05	>0,05

and NMU in a dose of 1/25 of the equitoxic dose was not accompanied by any significant changes in the survival time of the animals (Table 1).

Histological study of the internal organs of the animals on the 7th day of the experiment showed marked generalization of the tumor process in the control group, with the appearance of extensive areas of leukemic infiltration. In the spleen a large part of the pulp was replaced by them. The lymphoid follicles which remained were atrophied, without pale central zones, and were identified with difficulty. Foci of infiltration in the liver were localized mainly around the portal tracts, whereas in the lungs they were distributed along the course of the alveolar septa.

Injection of NMU, irrespective of the dose, caused visible reduction in the size of the foci of leukemic infiltration in the liver and lungs, but in the spleen they remained large. In animals receiving 1/25 of the equitoxic dose of NMU, the splenic lymphoid follicles were numerous and were more distinct than in mice receiving the therapeutic dose. Under the influence of thymalin and reaferon the morphological picture of the organs studied was virtually identical with that in the control group. The combined action of reaferon and NMU did not lead to complete regression of the tumor process in the spleen, as when only the therapeutic dose of NMU was given, although the foci of leukemic infiltration were smaller than in the control. With a combination of NMU and thymalin, the foci of leukemic infiltration in the spleen disappeared, and this was accompanied by morphological signs of intensification of immune reactions: large lymphoid follicles with pale centers, and a histiocytic reaction in the sinuses.

To estimate the effect of the preparations given on the lymphoid tissue and macrophagal system of the spleen, a morphometric analysis was made of the relative area of the splenic follicles and the bulk density of the macrophages.

The morphometric investigation showed that injection of thymalin and reaferon together with 1/25 of the therapeutic dose of NMU led to a very slight increase (not significant) in the relative area of the splenic lymphoid follicles (Table 2). Under the influence of a therapeutic dose of NMU, a statistically significant decrease was observed in the area of the follicles, indicating a depressive effect of the cytostatic on the lymphoid organs. This negative effect disappeared if NMU was injected after the immunomodulators in Groups 3 and 7.

A statistically significant increase in bulk density of the macrophages in the spleen was observed in the group of animals treated with NMU and thymalin (Group 3), i.e., in the group which showed the greatest therapeutic effect (Table 3).

The results of this investigation indicate that a combination of NMU and thymalin, while not leading to complete regression of the leukemic process in the animals with tumors, is nevertheless optimal compared with the other combinations studied. Treatment with NMU in conjunction with thymalin lengthens the survival time of the animals, inhibits tumor growth, while leaving intact the lymphoid tissue of the spleen, and has an immunostimulating action on the macrophagal system, which is currently regarded as one of the leading components of antitumor immunity [6].

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SCANNING ELECTRON MICROSCOPY OF RAT BLOOD MONOCYTES DURING LONG-TERM EXPOSURE TO ETHANOL

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Long-term exposure to ethanol leads to lowering of the body's resistance to unfavorable factors [3, 8, 11]. However, the mechanisms of interaction of the body with ethanol and of maintaining its optimal level of resistance have not been adequately studied. Hence the need for an experimental study of the unfavorable action of ethanol on the structural and functional state of the cellular systems that play an important role in maintaining homeostasis. One such system is that of the mononuclear phagocytes (MPS), a single cell line in the process of differentiation from monocytic bone marrow precursors to tissue macrophages [2, 11]. Great importance is attached to the study of disturbances of the structure and function of the blood monocytes — the only cells of the human MPS accessible for clinical-laboratory investigation.

The aim of this investigation was to study the trend of changes in the structural and functional state of blood monocytes during long-term peroral exposure of rats to ethanol.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing initially 180-230 g and receiving ethanol continuously for 2, 5, and 10 months with their drinking water (ad libitum) in a concentration of 100 g/liter. The reason why females were chosen for the experiments was that according to data in the literature, the unfavorable effect of ethanol develops earlier in females [5]. Six groups of animals were used (six rats in each group), and of this number three groups were controls. The structural and functional state of the blood monocytes was analyzed by scanning electron microscopy of cell monolayers obtained on coverslips during culture of mononuclear cells (monocytes and lymphocytes), isolated from blood by centrifugation in a Ficoll-Verografin density gradient [1, 10], for 1 h. Blood (4 ml) was taken from the abdominal aorta of the rats, anesthetized by intraperitoneal injection of Medinal in a dose of 50 mg/kg. Specimens for scanning electron microscopy were prepared as described previously [6, 7]. The preparations were studied and photographed on a Hitachi N-3010 scanning electron microscope (Japan), with an accelerating voltage of 20 kV.

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