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INDUCTION OF TUMORICIDAL ACTIVITY OF HUMAN AND MURINE PERITONEAL MACROPHAGES BY ANTITUMOR CHEMOTHERAPY

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Cytostatics used in the tumor chemotherapy are traditionally related to immunodepressants. Recently the heterogeneity of action of chemotherapeutic agents on the immune system has been demonstrated. For instance, the immunomodulating effects of alkylating agents (cyclophosphamide, melphalan), antimetabolites (mercaptopurine, 5-fluorouracil), antitumor antibiotics (doxorubicin, bleomycin), plant alkaloids (vinblastine, vincristine), platinum compounds, etc., have been discovered [1, 4, 6]. There is evidence that cytostatics can activate cells of the mononuclear phagocytic system [3, 4, 5, 7]. It has been shown, in particular, that the presence of macrophages potentiates the cytotoxic action of chemotherapeutic drugs on tumor cells cultured in agar diffusion chambers [2].

The aim of this investigation was to study the ability of cytostatics (platidiam, cyclophosphamide, 5-fluorouracil, adriamycin, and aclarubicin) to induce tumoricidal activity of human and murine peritoneal macrophages (PM).

EXPERIMENTAL METHOD

Human peritoneal cells were isolated from ascites fluid obtained from patients with disseminated ovarian carcinoma, during therapeutic paracentesis. The cells were washed and suspended in medium RPMI-1640 with 10% fetal serum, glutamine (2 mM), HEPES (10 mM), and gentamicin (50 μg/ml). The cells were counted, their viability determined (with the aid of 0.1% trypan blue solution), after which they were introduced into flat-bottomed wells of 96-well panels at the rate of $5 \cdot 10^5$ PM per well. After incubation for 2 h at 37°C in 5% CO_2 the monolayer of adherent cells (autologous tumor-associated PM – ATPM) were vigorously washed and incubated for the next 18 h with the chemotherapeutic agents or with medium (control), after which the adherent cells were again washed and used as effectors. Nonadherent cells (autologous tumor target cells – ATTC) were collected and cultured for 18 h under the same conditions as the PM, after which they were added to the ATPM in the ratio of 1:5-1:50. The ATPM were incubated with ATTM for 48 h, after which the number and viability of the ATTM were determined with 0.1% trypan blue solution. The cytotoxic index (CTI) was calculated by the formula:

$$\text{CTI} = (A/A_1 - B/B_1) \cdot 100\%,$$

where A and B denote the number of living tumor cells in the wells after culture in the presence of macrophages (B) and in their absence (A); A_1 and B_1 denote the initial number of cells.

To obtain murine PM the peritoneal cavity of DBA/2 and (C57BL/6 × DBA/2)F_1 mice (five mice were used in each variant of the experiment) was washed with cold (on ice) medium 199 with 10% fetal serum, heparin (10 U/ml), and monomycin (20 U/ml). The peritoneal cells were washed 3 times with the same medium (without heparin), centrifuged, counted, made up to a concentration of $2 \cdot 10^6$ PM/ml, and transferred in a volume of 0.2 ml ($4 \cdot 10^5$ PM/well) into flat-bottomed wells of panels. The subsequent stages of obtaining the PM monolayer and its processing were the same for

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TABLE 1. Cytotoxic Activity (in %) of Human ATPM Treated with Chemotherapeutic Agents

Agent	Number of cases	Dose, μg/ml	Effector cells/target cells			
			50:1	20:1	10:1	5:1
Platidiam	19	2,0	81,3±1,9	73,3±1,9	54,4±4,1	35,8±4,6
Cyclophosphamide	8	70,0	42,5±3,6	40,3±5,2	33,1±4,7	29,7±3,0
Adriamycin	8	0,4	31,6±4,5	29,3±4,1	14,7±2,3	15,8±3,5
5-Fluorouracil	16	10,0	7,2±2,8	8,6±2,4	4,8±3,4	7,9±3,3

TABLE 2. Kinetics of Cytotoxic Activity of ATPM Treated with Platidiam

Dose of platidiam, μg/ml	ATPM/ATTM	CTI, in %		
		after 24 h	after 48 h	after 72 h
0,2	10:1	19,6±4,1	28,4±6,7	34,1±12,4
2,0	10:1	33,6±3,8	78,3±8,9	79,7±6,9

human and murine PM. Next, syngeneic or semisyngeneic P388 lymphatic leukemia cells, adapted to conditions of culture in vitro and suspended in medium RPMI-1640 with corresponding additives, were added to the murine PM monolayer in the ratio of 1:20-1:50. In each well 1 μCi of ^{3}H-thymidine was added 18 h before the end of the cytotoxic reaction to evaluate DNA synthesis in the leukemic target cells, after which the cells were transferred, with the aid of a 12-channel "Dynatech" harvester to strips of glass-fiber filter paper (Whatman), washed with cold physiological saline and 5% TCA solution, and then treated with ethanol. The filters were dried and placed in flasks with scintillation fluid. The radioactivity of the samples was determined on a "Mark III" β-scintillation counter. The cytostatic action of PM was estimated as the cytostatic index (CSI):

$$CSI = \frac{CPM_c - CPM_e}{CPM_c} \cdot 100\ \%,$$

where CPM_c denotes the number of counts per minute in the control and CPM_e the same in the experiment.

At least three samples were determined in each variant of the experiment and each experiment was repeated at least 3 times. The results were subjected to statistical analysis by Student's t test and by the nonparametric Wilcoxon–Mann–Whitney nonparametric test.

EXPERIMENTAL RESULTS

After incubation for 18 h with platidiam, cyclophosphamide, and adriamycin, ATPM had a marked cytotoxic action on the ATTM (Table 1). The cytotoxicity of the ATPM, treated with these chemotherapeutic agents, clearly depended on the ATPM/ATTM ratio and decrease with a decrease in that ratio from 50:1 to 5:1. Platidiam was most able to induce the tumoricidal properties of ATPM, followed by cyclophosphamide, and then by adriamycin. 5-Fluorouracil had no activating effect on ATPM. The concentrations of the cytostatics used corresponded to their blood levels in cancer patients receiving therapeutic doses of these agents. It was shown in the case of ATPM treated with platidiam that the cytotoxic action of ATPM on ATTM was enhanced with an increase in the duration of incubation of the effectors with the target cells. The cytotoxicity of ATPM became evident after 24 h, it reached a maximum after 48 h, and remained at that level until 72 h (Table 2).

The use of murine PM showed that the antitumor anthracycline antibiotic aclarubicin induces tumoricidal activity of these effector cells on direct contact with them in vitro. PM activated by aclarubicin had a marked cytostatic action on both syngeneic and semisyngeneic P388 tumor cells. Figure 1 shows that the effect was greatest when aclarubicin was used in a dose of 0.01-1 μg/ml. Lower (under 0.0001 μg/ml) and higher (over 10 μg/ml) doses of the antibiotic had no activating effect on murine PM.

Aclarubicin stimulated the tumoricidal properties of PM in vivo also. For instance, PM obtained from the F_1 mice receiving a preliminary intraperitoneal injection of aclarubicin in a dose of 2.5 mg/kg, had a cytostatic action on the tumor target cells (Fig. 2). PM from mice receiving aclarubicin 1-4 days before harvesting of the cells had the strongest cytostatic action, whereas administration of the anthracycline 10 days beforehand had virtually no activating action on PM.

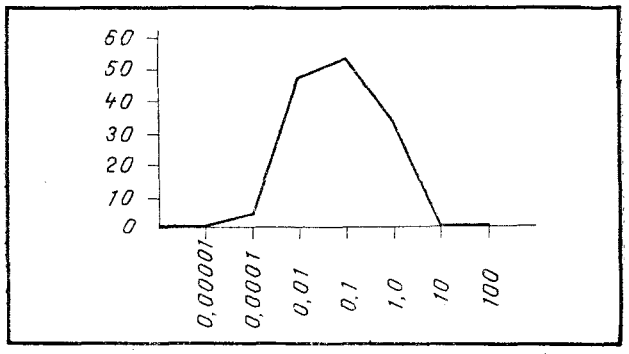

Fig. 1. Dependence of cytostatic action of murine macrophages on aclarubicin concentration in vitro. Abscissa, concentration of drug, μg/ml; ordinate, here and in Fig. 2: CSI, %.

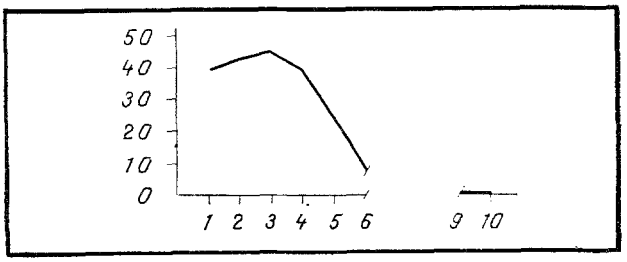

Fig. 2. Kinetics of activating effect of aclarubicin on murine macrophages in vivo. Abscissa, number of days after addition of drug and before obtaining macrophages.

The cytostatics can thus induce tumoricidal activity of human and murine PM both in vitro and in vivo. During incubation of PM with cytostatics in vitro the possibility cannot be ruled out that the drug may have penetrated into the effector cells, and subsequently been liberated into the surrounding medium, which could account for its cytotoxic action on tumor cells. However, Cuellar and co-workers [3] found no residual amounts of the drug in PM from mice receiving aclarubicin, lysates of effector cells having no effect on proliferation of the tumor target cells in vitro. Our results obtained in vivo may also be evidence in support of the activating effect of the antitumor agents of PM. The cytostatics were able to activate both intact murine PM and human ATPM, further confirmation of the role of these effector cells in the mechanism of the antitumor surveillance reaction. Of the five chemotherapeutic agents used, four (platidiam, cyclophosphamide, adriamycin, aclarubicin) had an activating action on PM, whereas 5-fluorouracil was inactive. The reasons for this are not clear and they may probably be associated with differences in the cellular pharmacokinetics and metabolism of each of them. The important point is that the doses of platidiam, cyclophosphamide, adriamycin, and 5-fluorouracil used corresponded to their blood levels in patients receiving chemotherapy, and the dose of aclarubicin given in vivo (2.5 mg/kg) had a therapeutic action on mice with tumors. Further study of the action of cytostatics on effector reactions of antitumor immunity may help to achieve synergism between their cytotoxic and immunomodulating effects.

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