MIGRATION ACTIVITY OF SYRIAN HAMSTER PERITONEAL EXUDATE CELLS UNDER NORMAL CONDITIONS AND AFTER SUPPRESSION OF THEIR NATURAL TUMOR RESISTANCE

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UDC 616-006.04-092.9:612.112.2

KEY WORDS: resistance; peritoneal exudate; tumor.

Much has been published on the important role of macrophages in defense of the body against tumors and their metastases [4, 7, 11]. At the same time, we know that a developing tumor can weaken the defensive powers of the host, in particular, through its action on macrophages [5, 8, 9, 13]. The direct inhibitory action of tumor cells on macrophages has been conclusively demonstrated in experiments *in vitro*. For instance, several workers [6, 12, 14] have demonstrated depression of monolayer formation by macrophages, stimulation of their migration, and inhibition of chemotaxis and antitumor cytotoxicity under the influence of extracts and supernatants of tumor cells. Deichman et al. [1], in the writers' laboratory, showed that injection of inactivated tumor cells into hamsters depresses their natural resistance (NR) so much that transplantable tumors can grow from single cells. Since one effector of NR is the macrophage, it was interesting to study changes in its activity when NR is depressed. Among the functional characteristics of macrophages, their migration activity, one of the important factors in the cytotoxic and phagocytic activity of macrophages, is particularly interesting.

The aim of this investigation was to determine the characteristics of peritoneal exudate cell (PEC) migration in Syrian hamsters under normal conditions and when their NR was depressed.

EXPERIMENTAL METHOD

To depress NR of the Syrian hamsters, inactivated cells of the following hamster tumors were used: GT-11B (spontaneous hepatocellular carcinoma), E-1 (sarcoma induced by virus SV-40), or HETR MLN-8 (a metastatic variant of hamster embryonic cells spontaneously transformed *in vitro* – HETR). As the control, HETR and HE (normal hamster embryonic cells), not suppressing NR in the animals [1, 2], were injected.

All types of cells listed above were inactivated by heating at 56°C for 1 h and irradiating in a dose of 10,000 rads; the E-1 cells in addition were lyophilized. Suspensions of inactivated cells were injected into normal adult Syrian hamsters intraperitoneally in a single dose of between $1 \cdot 10^7$ and $4 \cdot 10^7$ cells. PEC were harvested 9-13 days after injection into the experimental hamsters, and also intact animals, by washing out the hamster's peritoneal cavity once with 35 ml of 0.5% lactalbumin hydrolysate with 10% heated bovine serum, 100 units/ml monomycin, and 10 units/ml heparin. PEC were tested for their ability to migrate from microcapillary tubes, using a micromodification of the capillary test suggested by Suslov and Chernousov [3]. A special feature of our conduct of the test was that migration ability of PEC of animals treated by various preparations of inactivated cells was investigated without the addition of specific antigens or factors to the test system. PEC from each hamster were introduced into 4 or 5 capillary tubes and the mean individual migration zone (MIMZ) and the mean group migration zone (MGMZ) were measured separately for the control and experimental groups of animals.

The size of the PEC migration zones was measured by projecting them on paper under standard magnification, followed by tracing the outlines of the projection zones, cutting them out, and weighing. Individual and group migration indices (IMI and GMI) were calculated by the equations:

Laboratory of Antitumor Immunity, Research Institute of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 3, pp. 334-336, March, 1984. Original article submitted July 15, 1983.

0007-4888/84/9703-0323$08.50

TABLE 1. Migration Activity of PEC from Animals Treated with Inactivated HETR MLN-8 Cells

No. of hamster	Preliminary treatment of hamsters*	Index of migration activity of macrophages		
		MIMZ†	MGMZ	variability of IMI
1—4	—	5,0±0,69 ; 5,6±0,2; 4,7±0,18; 4,8±0,30	5,0±0,20	0,94—1,12
5—12	+	3,1±0,20‡ ; 10,3±0,50; 13,0±0,63; 13,9±0,69; 14,8±0,66; 14,9±1,13; 16,3±1,70; 18,1±1,21	13,5±0,78	0,62 2,06—3,62

*HETR MLN-8 cells, inactivated by heating and irradiation, were injected intraperitoneally into hamsters Nos. 5-12 in a dose of $2.2 \cdot 10^{-7}$ per hamster 10 days before their PEC were taken.

†In 7 of the 8 experimental animals the value of MIMZ was significantly greater than the value of MGMZ in animals of the control group ($P < 0.001$).

‡The value of MIMZ for hamster No. 5 was less than the value of MGMZ in the control ($P < 0.01$).

TABLE 2. Migration Activity of PEC from Animals Treated with Various Inactivated Cells (Normal and Tumor)

Parameter	Inactivated cells injected									
	HE†		HETR†		HETR MLN-8‡		GT-11B‡		E-1‡	
	—	+	—	+	—	+	—	+	—	+
Number of hamsters in group	4	4	8	15	8	16	5	8	14	16
MGMZ	4,3±0,23	4,4±0,39	5,8±0,19	5,7±0,30	5,8±0,19	14,7±1,5	4,7±0,22	12,9±0,44	5,6±0,25	14,5±1,12

*Cells inactivated by heating and irradiation were injected intraperitoneally into hamsters in doses of $1 \cdot 10^7$-$4 \cdot 10^7$ cells per hamster 9-14 days before their PEC were taken.

†Value of MGMZ in experiment did not differ significantly from value of MGMZ in control ($P > 0.1$).

‡Value of MGMZ in experiment differed significantly from value of MGMZ in control ($P < 0.0001$).

$$IMI = \frac{\text{weight of MIMZ in experiment}}{\text{weight of MIMZ in control}}; \quad GMI = \frac{\text{weight of MGMZ in experiment}}{\text{weight of MGMZ in control}}.$$

EXPERIMENTAL RESULTS

The results of an experiment carried out with PEC of hamsters receiving an injection of inactivated HETR MLN-8 cells in a dose of $2 \cdot 10^7$ cells per hamster 10 days before the peritoneal exudate was obtained. Values of MIMZ for 7 of the 8 experimental hamsters were significantly higher than in intact hamsters: MGMZ values in these animals were 2.7 times greater ($P < 0.001$) than MGMZ in the control. Staining the migration zones showed that the PEC migration front was formed by macrophages.

It must be emphasized that when migration zones of PEC from a large group of control intact hamsters (males and females aged from 2 to 12 months) were compared no significant differences could be found in the size of their migration zones. Only in 1 of the 94 animals tested was MIMZ significantly greater than MGMZ for the control group of animals.

Experiments similar to those illustrated in Table 1 also were carried out with PEC of animals receiving inactivated HE, HETR, E-1, and GT-11B cells. Table 2 gives values of MGMZ for each experiment and the corresponding control values of MGMZ. According to previous results [1, 2], HE and HETR cells do not suppress NR in repeated transplantation tests on animals treated with the corresponding types of cells. It will be clear from Table 2 that PEC of hamsters treated with HE and HETR cells did not differ in their migrating ability from PEC of the control animals. By contrast, inactivated GT-11B and E-1 cells, like HETR MNL-8 cells, depress NR and potentiate significantly the migration activity of PEC.

In hamsters receiving cells of tumor strains (GT-11B, E-1, and HETR MLN-8) beforehand, migration activity of PEC was thus found to be increased compared with intact animals and animals receiving HETR and HE cells. The mechanism of enhanced migration of PEC in these experiments is not yet clear.

In our opinion enhanced migration of macrophages in these experiments was not connected with the local inflammatory reaction of PEC to injection of inactivated cells, for enhancement of migration was not observed in animals treated with inactivated HETR and HE cells. Meanwhile the possibility cannot be ruled out that inactivated tumor cells, by contrast with inactivated HETR and HE cells, secrete factors modifying migration activity of macrophages and (or) attracting macrophages into the peritoneal cavity from other organs, differing in mobility. Since not only NR, but also ability to induce specific antitumor immunity [1] was depressed in hamsters treated with inactivated tumor cells, the possibility cannot be ruled out that T suppressors which, according to data in the literature [10], can produce a factor stimulating macrophage migration, appear in such animals.

Intraperitoneal injection of inactivated tumor cells into hamsters thus has evidently a direct or indirect action on activity of peritoneal macrophages and (or) modifies their composition.

Correlation between ability of inactivated tumor cells to stimulate migration activity of PEC and their ability to depress NR (according to results of the transplantation test) will be noted. In this connection it is possible that depression of NR in the hamsters was due to a change in functional activity of the macrophages, including their migration activity. Support for this hypothesis is given also by data in the literature [15] on isolation from tumor cells of a factor depressing various forms of activity of macrophages and leading to enhancement of their migration. The role of T suppressors in the observed changes in macrophage function and in depression of NR requires further investigation.

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EFFECT OF CHEMICAL CARCINOGENESIS ON SYMPATHETIC NERVOUS SYSTEM FUNCTION IN ANIMALS

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UDC 616-006.6-092.9-02:615.277.4]-07:616.839-008.1

KEY WORDS: sympathetic nervous system; carcinogenesis.

In the modern view the process of chemical carcinogenesis embraces mainly two stages: first — circulation of the carcinogen in the body after introduction and the chain of its metabolic conversions, second — binding of metabolites of the carcinogen with the biological substrate of target cells. On entering the cell, these metabolites can bind with all its components: with the cell membrane, cytoplasmic organelles, and nucleus. Numerous investigations have helped to reveal the character of interaction between metabolites of the carcinogen and the substrate leading to malignant transformation of the cells [5, 6]. Another point to note is that when the action of a carcinogen is examined at the cellular level, the possibility that it may also affect other systems of the body, including nervous, vascular, and endocrine systems, must be taken into account. In this connection the question arises: What effect may carcinogens have on overall neurohumoral regulation of trophic processes as a whole, and in particular during the latent period before appearance of a tumor.

Considering that the sympathetic nervous system participates directly in neurohumoral regulation of trophic processes in the tissues, in the investigation described below an attempt was made to study to what degree a chemical carcinogen, introduced into animals, affects its functional state. For this purpose bioelectrical activity of structures of the sympathetic nervous system was investigated in healthy normal rats and in rats treated with the hepatocarcinogen 4-dimethylaminoazobenzene (DAB). This was all the more interesting because previously, in experiments on animals with a transplanted Brown-Pearce tumor, recording bioelectrical activity in different parts of the nervous system showed that tumor development depends on depression of the state of hypothalamic function and a disturbance of transmission of excitation in sympathetic ganglia [7]. Clinical physiological observations also have shown that in patients with malignant tumors, tone of the sympathicoadrenal system is low [8, 9]. Biochemical studies of tissues of malignant tumors of man and animals (mice and rabbits) have revealed a low concentration of noradrenalin, mediator of the sympathetic nervous system, in the tumors themselves and also in the affected organs [2-4].

EXPERIMENTAL METHOD

Experiments were carried out on noninbred rats weighing 150-200 g under pentobarbital anesthesia (50 mg/kg, intraperitoneally). In experiments on 25 healthy rats synaptic transmission of excitation through the inferior mesenteric ganglion (IMG) under normal conditions were studied in situ. In response to stimulation of the preganglionic trunk (intermesenteric tract) action potentials (AP) arising both in the tract itself and in postganglionic nerves (intestinal nerve, innervating the colon, and the ipsilateral hypogastric nerve, innervating the urinary bladder) and other pelvic organs, were recorded. A scheme of IMG of the rat, with

Laboratory of Cell Physiology, Institute of Cytology, Academy of Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR P. N. Veselkin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 3, pp. 337-340, March, 1984. Original article submitted April 12, 1983.

 0007-4888/84/9703-0326$08.50