INTERACTION BETWEEN CELLULAR AND SERUM FACTORS OF IMMUNITY IN THE COURSE OF CHEMICAL CARCINOGENESIS

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UDC 616-006.6-092.9-02:615.277.4]-07:[616.15+616-008.9]-097.078.7

The effect of serum components on manifestation of the cellular reactions of immunity during chemical carcinogenesis was investigated by studying inhibition of migration of peritoneal macrophages from the capillaries. The level of inhibition of migration of the macrophages was higher and inhibition was observed in a larger percentage of cases when macrophages were cultured on autologous serum of animals with induced tumors than when normal sera were added to this test system. In cases in which the ability of the macrophages to migrate was increased, the serum of normal animals facilitated this phenomenon more than the serum of rats with induced carcinogenesis. The cellular reactions of immunity were sharply reduced at the 6th month of carcinogenesis regardless of the action of serum factors.

KEY WORDS: peritoneal macrophages; induced tumors; migration of macrophages; chemical carcinogenesis.

The role of serum factors in the manifestation of the cellular reactions of immunity to malignant neoplasm is of great interest. Investigations have shown that serum components prevent the harmful action of cells of the immunocompetent system on the tumor [2, 3, 5-8]; whereas other workers have noted the inconstancy of this phenomenon [9-11, 13-15].

Inhibition of migration of macrophages from the capillaries provides the basis for an interesting test for simulating and observing interaction between the humoral and cellular factors of immunity in experiments in vitro.

By the use of this test the writers were able to study the cellular reactions of immunity and to examine the effect of autologous sera on their manifestation during chemical carcinogenesis.

EXPERIMENTAL METHOD

Tumor formation was induced in Wistar rats by intramuscular injection of 3 mg of the carcinogen DMBA in 0.5 ml peach oil. Observations on tumor development were carried out for 6 months, and in the course of each month no fewer than 12-15 experimental animals and the same number of controls were tested.

Cells of the peritoneal exudate were obtained 48 h after injection of 10 ml of 10% Difco peptone into a rat. The peritoneal exudate cells of normal animals acted as the control. All procedures with macrophages were carried out in an ice bath, using equipment treated with "Siliclad" (Clay-Adams Inc., New York). After washing with Eagle's medium with the addition of antibiotics (penicillin 100 units/ml, streptomycin 100 μg/ml) the cells were resuspended in medium No. 199 and counted. The optimal concentration was $60 \cdot 10^6$ cells/ml. The suspension contained from 15 to 20% of lymphocytes, sufficient to produce

Research Laboratory of Experimental Immunobiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 78, No. 11, pp. 86-89, November, 1974. Original article submitted March 29, 1974.

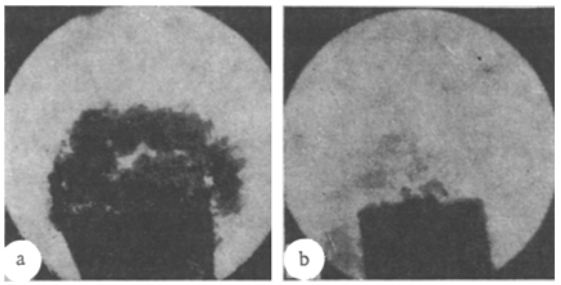

Fig. 1. Zone of migration of macrophages from normal rat and rat with chemical carcinogenesis: a) normal rat; culture in the presence of normal serum and antigen; b) rat with chemically induced sarcoma; culture in the presence of antigen from tumor and autologous serum.

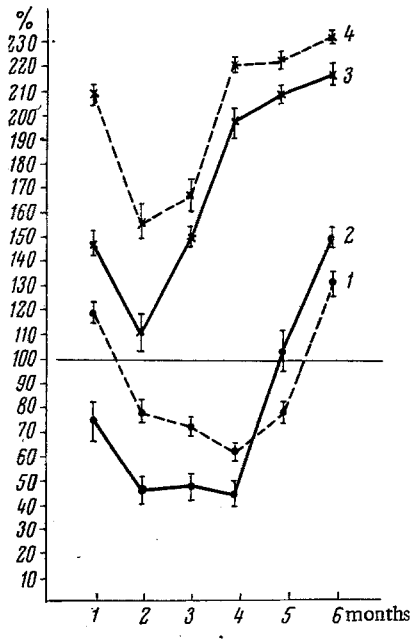

Fig. 2. Effect of serum components on inhibition of migration of macrophages during chemical carcinogenesis: 1) predomination of inhibition of macrophages grown on normal serum; 2) predominance of inhibition of migration of macrophages grown on autologous serum; 3) increased migration of macrophages grown on autologous serum; 4) increased migration of macrophages grown on normal serum. Abscissa, period of carcinogenesis (in months); ordinate, percentage migration of macrophages.

the factor inhibiting migration of the macrophages, for according to David [4] a lymphocyte concentration of 2.5% was enough to satisfy this requirement.

Capillary tubes 0.65 mm in diameter and 7 cm long were filled with the resulting suspension, sealed at the free end, and centrifuged at 800 rpm for 2 min. The capillary tubes were then cut at the line separating the supernatant from the sedimented peritoneal cells and secured to the bottom of a receiver into which medium No. 199 with antibiotics and containing 15% autologous rat serum, obtained on the day of the experiment before removal of the peritoneal exudate, was poured. The antigen consisted of twice-washed cells of an autologous tumor and the control of normal tissue cells. Their number in the culture medium was not less than 10% of the number of macrophages. Because of the considerable variability of the indices each test was repeated 8 times. After incubation for 24 h the zone of migration was measured planimetrically and the percentage migration of the macrophages calculated as the quotient of the migration index in the experimental series divided by that in the control, multiplied by 100. Inhibition of migration of the macrophages was deemed to be present if this percentage was below a 100, whereas values above a 100% denoted increased ability of the macrophages to migrate. The statistical analysis of the results was carried out by Student's method.

EXPERIMENTAL RESULTS

The first observation to be made was that autologous antigens of chemically induced sarcomas had a sensitizing action strong enough to evoke a response of cellular immunity. The zone of migration of macrophages obtained from a normal rat and grown in the presence of normal antigen and normal serum is compared with the zone of migration of macrophases taken from a rat at the 4th month of carcinogenesis, with the addition of autologous tumor cells and autologous serum, in Fig. 1a and b. The zone of migration in the control was statistically significantly larger than the zone of migration of the macrophages in the experimental series, which was considerably inhibited, in agreement with the results of earlier investigations [1].

Data on the character of action of the serum factors in inhibition of migration of the macrophages at different stages of chemical carcinogenesis are summarized in Fig. 2.

Two test systems were compared. The first consisted of the ratio between the migration activity of macrophages obtained from a rat at a certain period of carcinogenesis and cultured with autologous tumor cells and with autologous serum taken on the day of the experiment, and the migration of macrophages of a control rat in the presence of normal antigen and normal serum. In the second test system all the components of the ratio were the same, except that macrophages of this experimental rat were cultured on normal serum.

It will be clear from Fig. 2 that the migration activity of the macrophages during progressive growth of the tumor depended not only on the period of carcinogenesis, but also on the presence of the serum components that participated in the experiments in vitro.

In the case in which culture of macrophages from rats with induced carcinogenesis was carried out on autologous tumor serum, the level of inhibition of migration of the macrophages was higher than in experiments in which normal serum was added to this same system.

Similar results were obtained when the experiments reflected in the upper part of the graph in Fig. 2 were compared: in the presence of tumor serum the increase in migration was less marked than after culture on normal serum.

Analysis of the relative percentages of cases with inhibition and stimulation of migration activity of the macrophages from the 1st to the 5th month of carcinogenesis showed that in the presence of serum from rats with induced tumors, inhibition of migration of the macrophages occurred in 61% of experiments, stimulation of migration in 28%, and no statistically significant difference in 11% of cases. The use of sera of normal rats in the system indicated above altered these figures. Inhibition of migration of the macrophages was observed in only 44% of cases, stimulation in 43%, and no difference in 13% of the experiments.

It is interesting to note that by the 6th month of carcinogenesis, when the tumor had attained a large size, a sharp decrease in cell reactivity occurred and increased migration of the macrophages was observed independently of the serum components.

Tests were also carried out to study the effects of different fractions of immunoglobulins isolated on Sephadex G-200 from the sera of rats in the 3rd-4th month of carcinogenesis, when the cellular reactivity was at its highest. These tests showed that the 4S-fraction, consisting chiefly of albumins, possessed the most active inhibitory properties in the test systems described above. At the same time, the soluble factor inhibiting migration of macrophages is also known [12] to occur in the albumin-containing peak.

It thus follows from the results described above that the role of serum components in the manifestation of the cellular reactions of immunity during malignant growth is highly complex and requires further clarification on various models.

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