INTERACTION BETWEEN T AND B LYMPHOCYTES AND THEIR MIGRATION IN THE IMMUNE RESPONSE DURING METHYLCHOLANTHRENE-INDUCED CARCINOGENESIS

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UDC 616-006.6-092.9-07:612.112.94.017.1

Methylcholanthrene (MC) was injected intramuscularly in a dose of 0.3 mg into (CBA × C57BL)F_1 mice and the ability of their T and B lymphocytes to cooperate during the immune response to injection of sheep's red blood cells and also migration of these cells from the thymus and bone marrow into the spleen were investigated. The results showed that the immunodepressant action of MC is connected with inhibition of processes of migration and cooperation of T and B lymphocytes in the immune response. It is concluded that the immunosuppression developing during carcinogenesis is complex in character and is realized at different stages of immunogenesis.

KEY WORDS: *carcinogenesis*; *T and B lymphocytes — migration and cooperation.*

Previous investigations showed that the immunodepression arising during tumor growth is the combined result of disturbance of the normal regulation of the pool of stem cells, inhibition of their differentiation along the lymphoid pathway, and suppression of the T and B cells under the influence of the malignant tumor and, evidently, of its products [1, 5, 6, 9]. This is observed against the background of the developing (spontaneous carcinoma) or transplanted (carcinoma of the cervix uteri) tumor. Meanwhile, the study of the mechanisms of the disturbances arising in the immune system in the early stages of carcinogenesis, especially induced by a chemical carcinogen, is of the greatest interest.

The object of the present investigation was to determine 1) cooperative interaction between T and B lymphocytes during the primary immune response, 2) migration of B lymphocytes from the bone marrow, and 3) migration of T lymphocytes from the thymus at different periods after injection of the carcinogenic hydrocarbon methylcholanthrene (MC) into mice.

EXPERIMENTAL METHOD

Experiments were carried out on (CBA × C57BL)F_1 mice aged 3-4 months and weighing 22-24 g. The animals were irradiated on the RUM-17 apparatus with an absolutely lethal dose of 800 R ($LD_{100/14}$).

To simulate cooperation between T and B lymphocytes [10] bone marrow cells (B lymphocytes) in a dose of $1 \cdot 10^7$, thymus cells (T lymphocytes) in a dose of $2 \cdot 10^7$, or a mixture of these cells were transplanted into the mice 4-6 h after irradiation. At the same time the animals were given an injection of $2 \cdot 10^8$ sheep's red blood cells (SRBC). On the eighth day after injection of the cells and antigen the number of antibody-forming cells (AFC) in the recipients' spleen was determined by the direct method [8].

During determination of the intensity of migration of the T cells [3, 4] the region of the thymus was protected by a screen during lethal irradiation of the mice. Syngeneic bone marrow cells (B cells) were injected intravenously into the animals 24 h later in a dose of $1 \cdot 10^7$ together with $2 \cdot 10^8$ SRBC. To determine the intensity of migration of the B lymphocytes [2, 6], the animals were irradiated in a lethal dose with part of the bone marrow screened (the hind limbs up to mid-thigh level). After an interval of 24 h $2 \cdot 10^7$ syngeneic thymus cells (T cells) with $2 \cdot 10^8$ SRBC were injected intravenously. On the eighth day after immunization and transplantation of the cells the number of AFC in the spleen was determined. In this way, T and B cells migrating from the thymus or bone marrow (variable) cooperated

Institute of Biophysics, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. D. Gorizontov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 10, pp. 464-466, October, 1977. Original article submitted April 27, 1977.

 0007-4888/77/8410-1470$07.50

TABLE 1. Cooperation between T and B Lymphocytes of Mice Treated with MC during Immune Response to Injection of Sheep's Red Blood Cells (M ± m)

Time after injection of MC, days	Number of animals	Number of AFC in spleen of irradiated recipients receiving mixture of $1 \cdot 10^7$ bone marrow cells and $2 \cdot 10^7$ thymocytes	P
Control	27	1880±56	—
10	11	328±61	<0,001
15	10	179±58	<0,001
40	12	597±173	<0,01

TABLE 2. Migration of B Cells from Bone Marrow in Mice Treated with MC (M ± m)

Time after injection of MC, days	Number of animals	Number of AFC in spleen of mice irradiated with bone marrow screened and after injection of $2 \cdot 10^7$ thymocytes	P
Control	52	5650±407	—
10	14	2340±50	<0,001
40	27	1950±300	<0,001

Legend. Data on number of AFC in control (here and in Tables 2 and 3) pooled because of equal values at different times of the experiment.

with transplanted B and T cells respectively, the number of which was always standard (constant). Special experiments showed that the number of AFC in the spleen of recipients of the standard dose of B or T cells was entirely determined by the intensity of migration of the T and B cells respectively [4].

Carcinogenesis was induced by injection of 0.3 mg MC in 0.1 ml benzene intramuscularly into the thigh. Treatment with MC in this way leads to induction of tumor formation in 90-95% of mice after 120-140 days. By this time the tumor attains a weight of 8-12 g and, morphologically, it is a rhabdomyosarcoma. The tumor starts to form 45-60 days after the injection of MC. Most animals with tumors die 5-7 weeks after appearance of the tumor. Benzene only was injected into the control mice.

The numerical results were subjected to statistical analysis with calculation of the arithmetic mean (M), the standard error (m), and the significance of differences (P) with the aid of the Student–Fisher criterion.

EXPERIMENTAL RESULTS

Table 1 shows that the ability of thymus and bone marrow cells to cooperate during inhibition of the immune response to injection of SRBC is considerably depressed in mice receiving MC as early as on the 10th day after injection of the carcinogen.

The study of migration of the B cells from the bone marrow gave the following results (Table 2). In the control mice, irradiated with the bone marrow screened, after injection of a standard dose of thymocytes and SRBC more than 5000 AFC accumulated in the spleen through migration of B lymphocytes, cooperating with the transplanted T cells, from the bone marrow into the spleen. Only about 2000 AFC, however, accumulated in the spleen of the experimental mice receiving MC. As Table 2 shows, marked inhibition of migration of B lymphocytes from the bone marrow occurred 10 days after injection of MC.

As regards migration of T cells from the thymus, it will be clear from Table 3 that transplantation of the standard dose of bone marrow cells into irradiated mice with the thymus screened led to production of about 5500 AFC through migration of thymocytes, which play a secondary role during interaction with transplanted B cells, into the spleen. Far fewer AFC formed in the spleen of the experimental mice treated with MC. These findings indicate inhibition of migration of T cells from the thymus in mice with induced carcinogenesis. A substantial decrease in migration of T cells was observed 25 days after injection of MC.

Carcinogenesis induced by MC thus leads to a substantial immunosuppressive effect, the combined result of suppression of cooperation and migration of the T and B lymphocytes participating in immunogenesis. Consequently, the immunosuppression developing after injection of MC is complex in character and is realized at different stages of immunogenesis. It is interesting to note that migration of B cells is inhibited much sooner than migration of T

TABLE 3. Migration of T Cells from Thymus into Mice Treated with MC (M ± m)

Time after injection of MC, days	Number of animals	Number of AFC in spleen of mice irradiated with thymus screened, and receiving $1 \cdot 10^7$ bone marrow cells	P
Control	16	5230±850	—
15	7	4150±690	<0,2
25	7	2770±177	<0,001
40	13	1410±175	<0,001

cells in animals treated with MC. Suppression of cooperation and migration of T and B cells during immunogenesis in mice treated with MC precedes the process of tumor formation considerably in time (i.e., it is observed in the latent period of tumor growth), and is maintained until tumor formation. In animals with established tumors, immunopoiesis is deeply inhibited [6, 9]. The immunodepressive action of MC and of other carcinogenic hydrocarbons is well known [7, 11]. Noncarcinogenic hydrocarbons do not affect the immune response. It can accordingly be concluded that induction of tumor formation by the action of carcinogens takes place against the background of suppression of different stages of immunogenesis: cooperation of T and B lymphocytes and their migration from the organs in which they are generated.

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