CONTENT OF Θ^+-LYMPHOCYTES IN REGIONAL AND DISTANT LYMPH GLANDS OF C^3H/Sn MICE DURING THE DEVELOPMENT OF PRIMARY METHYLCHOLANTHRENE SARCOMAS

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The development of a primary methylcholanthrene sarcoma in C^3H/Sn mice is accompanied by a marked decrease in the percentage of Θ^+-lymphocytes in cell suspensions obtained from lymph glands regional relative to the tumor compared with distant lymph glands or the lymph glands of control animals.

KEY WORDS: sarcoma; regional lymph glands; T-lymphocytes.

The development of chemically induced tumors in animals is accompanied by various reactive changes in the immunocompetent system of the tumor-bearing animals [2-4, 14]. However, the degree of involvement of the various components of the lymphoid system in this response differs. It depends on the functional and topographic connection of the particular immunocompetent organ with the dynamics and the site of development of the chemically induced tumor in the animal. The study of the functional state of the regional and distant lymph glands relative to the tumor is thus of great interest.

One approach to the investigation of the state of lymph glands is by analysis of the ratio between the various cells contained in them (lymphoid and nonlymphoid).

Considering the important role of lymphocytes in antitumor immunity and the different functions of the T- and B-cells in the immune response to tumors [7, 8, 11], in this investigation an attempt was made to study the ratio between the numbers of cells carrying the Θ-antigen (the antigenic marker of the T-lymphocytes) on their surface [10, 12] and cells on which this antigen cannot be found (Θ^--cells), in cell suspensions from the corresponding lymph glands.

EXPERIMENTAL METHOD

Inbred C^3H/Sn mice (males) were used. Sarcomas were induced by intramuscular injection of 1 mg 20-methylcholanthrene (MC) in 0.1 ml apricot oil (experimental group) into the right thigh. Animals of the same age and sex, not receiving MC, constituted the control group.

The animals with a tumor were killed. The regional (inguinal) and distant (opposite inguinal) lymph glands were weighed and cell suspensions prepared from them. For this purpose, lymph glands carefully freed from adipose tissue and washed in buffered (0.1 M phosphate buffer, pH 7.4) Hanks's solution, were carefully teased apart with thin needles. The resulting suspensions were washed 3 times (5 min at 1000 rpm) in the same salt buffer. After washing the number of cells in 1 ml was counted and the ratio between the numbers of living and dead cells in the suspension calculated. Suspensions were prepared in the same way from the right and left inguinal lymph glands of normal (control) animal. All the operations involved in preparing the suspensions were carried out in the cold.

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TABLE 1. Weight of Lymph Glands and Content of Θ^+-Cells in Regional and Distant Lymph Glands of C^3H/Sn Mice with MC Tumor and of Control Animals (results of cytotoxic test)

Mouse	Ratio of weight of regional lymph glands to weight of distant glands	Cytotoxic index of lymph glands		
		regional	distant	control
1	5	0,50	0,75	0,74
2	3	0,42	0,61	0,68
3	2,7	0,51	0,63	0,73
4	1,4	0,37	0,62	0,68
5	2,1	0,51	0,63	0,79
6	4,6	0,42	0,82	0,77
7	3,2	0,55	0,84	0,79
8	2,6	0,39	0,65	0,73
9				0,70
10				0,78
11				0,79
M ± m		0,46±0,02	0,69±0,03	0,74±0,01
Mean weight		17,0±2,42	6,12±1,10	6,18±0,50

Anti-Θ-serum of AKR mice (anti-C^3H), obtained and tested by the method of Reif and Allen [12], was used as the test serum. The serum was first heated for 45 min at 56°C. It was found to be highly specific. Its titer (cytotoxic index) was 0.50 for C^3H thymocytes in dilutions of the serum between 1 : 512 and 1 : 1024.

The working dilution of the antiserum and normal mouse serum (1 : 4) was prepared in buffered Hanks's solution immediately before the experiments. The cytotoxic test was carried out in the micromodification [5]. A pool of fresh guinea pig sera, nontoxic for lymphocytes, in a dilution of 1 : 3 was used as the source of complement. All tests were carried out with the same batch of antiserum and complement. The results were expressed as the cytotoxic index.

EXPERIMENTAL RESULTS

In 100% of the mice receiving MC sarcomas were induced, and their weight varied between 1.3 and 4.9 g. All the tumor-bearing animals showed the same type of response (hyperplasia) to development of the tumor (Table 1). The cytotoxic index for cells of the regional lymph gland fell significantly compared with the control ($P < 0.001$) during the development of the tumor.

The significant decrease in the cytotoxic index during the test with suspensions of the regional lymph gland reflected a decrease in the content of Θ^+-cells (T-lymphocytes) and a relative increase in the number of Θ^--cells. The distant lymph glands did not undergo such considerable changes. The differences in the degree of hyperplasia of the regional lymph gland evidently correlated not with the size of the tumor, but with the degree of immunogenicity and the rate of growth of the primary tumor. This conclusion is confirmed by results [13] obtained with C^3H/HeJ mice in experiments with transplantation of syngeneic MC sarcomas (transplanted strains) differing in their immunogenicity and rate of gorwth. The fastest and most intensive hyperplasia of the regional lymph gland were caused by strains with low immunogenicity and a high rate of growth. Differences in the degree of hyperplasia of the regional lymph gland in the present experiments can possibly be attributed to these same factors.

Histologically the hyperplasia of the regional lymph glands in the C^3H mice during growth of an MC sarcoma is characterized by plasmacytosis and histiocytosis [9, 13]. Similar changes are also observed in BALC/c mice [1]. However, it is impossible to judge changes in the ratio between the number of Θ^+- and Θ^--lymphocytes in the lymph glands from the histological data for there are no precise morphological differences between these cells. Preparation of a suspension from lymph gland cells for such an analysis to be carried out cancels out the topographic differences in the distribution of the cells in the lymph gland tissue and can be used only to study the general tendency of the ratio between the Θ^+- and Θ^--cells. Incidentally, during teasing apart of the gland (as shown a morphological study of the cell composition of the suspensions), mainly cells not participating in the structure of the gland stroma and only loosely connected with it, i.e., lymphocytes, pass into suspension.

Hyperplasia of the lumph glands in C^3H/Sn mice in response to the development of a primary MC sarcoma is thus accompanied by a decrease in the relative number of Θ^+-lymphocytes. This change in the ratio between the numbers of Θ^+- and Θ^--cells could reflect the lymphadenopathy that develops under the influence of large doses of tumor antigen and that promotes the growth of chemically induced sarcomas in C^3H/Sn mice [13].

The decrease in the relative number of Θ^+-cells (T-lymphocytes) may indicate an immunodepressant effect of the tumor on the lymph gland and inhibition of the cellular immune responses to tumor antigens. On the other hand, the relative increase in the number of Θ^--cells (most of them in the system used were B-lymphocytes and their descendants – plasma cells) may evidently reflect the relative stimulation of antibody formation, possibly aimed at producing so-called blocking antibodies [6], that promote tumor growth.

These hypotheses require direct verification in experiments to study the functional activity of the Θ^+-and Θ^--cells of the corresponding lymph glands during the development of MC-sarcomas. The problem of which factors (specific tumor antigens, toxic products of tumor metabolism, genetic features of the tumor-bearing animal, or the presence of oncornaviruses) affect the ratio between the number of Θ^+-and Θ^--cells in the lymph glands and their functional activity during the development of MC-sarcomas still remains an open problem. Further investigations will be carried out to study these problems.

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