ANTIGEN-REACTIVE CELLS IN THE SPLEEN OF MICE WITH METHYLCHOLANTHRENE-INDUCED SARCOMA

L. V. Yakimenko and Yu. A. Umanskii

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Experiments on BALB/c mice showed a progressive decrease not only in the number of antibody-forming cells, but also in the number of antigen-reacting cells, the preceding stage of antibody formation, during chemical carcinogenesis induced by a single injection of methylcholanthrene. The number of antibody-forming cells in the spleen of the BALB/c mice fall by 6-6.5 times and the number of antigen-reactive cells by 3-4 times during the development of methylcholanthrene sarcoma.

Experiments have shown a decrease in the number of antibody-forming cells (AFCs), detected by the method of local hemolysis in gel [9], during chemical carcinogenesis induced by various polycyclic hydrocarbons.

One of us (L. V. Yakimenko [3]) has shown that the decrease in the number of AFCs in BALB/c mice during methylcholanthrene-induced carcinogenesis is most marked in the pretumor period (3 months after injection of methylcholanthrene) and during development of the blastoma.

The decrease in the number of AFCs in the spleen of the experimental mice could be the result of direct damage to the AFCs or damage to their precursors. Support for the second view is given by earlier observations [1-3].

For instance, whereas the number of nucleated cells in the spleen in BALB/c mice 1 month after injection of methylcholanthrene was reduced by only 23%, the number of AFCs detectable on the 4th day after immunization with 5×10^7 sheep's red cells intraperitoneally was reduced by 55%. During the development of methylcholanthrene-induced sarcoma the number of nucleated cells in the spleen during this period was reduced by only 10% compared with their number in the spleen of control animals of the same age group, whereas the number of AFCs was reduced by 6.5 times.

Much evidence has now been obtained to show that interaction (cooperation) between three cell systems is necessary for the immune response to take place: besides the macrophages, providing the antigenic stimulus, and the antibody-forming cells synthesizing immunoglobulins, a further group of cells must also participate – the antigen-recognizing and antigen-reactive cells (ARCs). These cells stimulate the undifferentiated precursors of the AFCs, which arise from the bone marrow and cannot themselves react to the antigen, to receive the antigenic stimulus and to differentiate into mature plasma cells, capable of producing specific immunoglobulins [10-15].

On the basis of the investigations of Groves et al. [6], Mitchell and Miller [12], and Shearer and Gudkowicz [13, 15], showing that AFC formation takes place as the result of interaction between the AFC precursors and ARCs, and that the number of AFCs is directly dependent on the ratio between the number of AFC precursors and the number of ARCs, it was postulated that a decrease in the number of AFCs in the spleen of BALB/c mice during the development of blastomas induced by methylcholanthrene would be accompanied by a decrease in the number of ARCs.

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TABLE 1. Antigen-Reactive Cells in Spleen of BALB/c Mice during Development of Tumor Induced by Methylcholanthrene (M ± m)

Group of animals	Number of animals	Weight of spleen (in mg)	Number of nucleated spleen cells		Number of ARCs		
			in entire organ	per mg weight of spleen	to 5×10^6 spleen cells	in entire organ	per mg weight of spleen
Intact (control)	11	210 ± 29	580,4 ± 79·10⁶	3,04 ± 0,39·10⁶	11,9 ± 0,91	1477 ± 280	7,03 ± 1,09
Experimental	12	421,2 ± 53 <0,01	874,3 ± 114·10⁶ <0,05	2,12 ± 0,16·10⁶ <0,05	4,04 ± 0,52 <0,001	613,4 ± 103 <0,05	1,64 ± 0,27 <0,001

It was accordingly decided to study the ARC population in the spleen of mice with a methylcholanthrene-induced sarcoma.

EXPERIMENTAL METHOD

Experiments were carried out on 12 BALB/c mice in which a sarcoma was induced at the age of 1.5 months by methylcholanthrene. Tumors developed in the animals after 3.5-6 months. Eleven intact BALB/c mice of the same age as the experimental animals acted as the control. The experimental and control mice were used as donors in the quantitative study of the ARCs by the method of Kennedy et al. [10]. The recipients were 46 BALB/c mice aged 4-5 months: 2 recipient mice to each donor. These mice were exposed to whole-body γ-ray radiation in a dose of 700 R ("Focus" apparatus). With this dose the recipient mice lost their ability to respond by antibody production to the injection of sheep red cells. The absence of an immunologic reaction of the recipient mice was verified by Jerne's direct test with the spleen cells of irradiated mice immunized with sheep red cells. A suspension containing 5×10^6 spleen cells of isologous donor mice and 1×10^8 thoroughly washed sheep red cells, suspended in 0.5 ml Hanks' solution, was injected intravenously into the inactive recipient mice 1 h after irradiation. The recipient mice were killed 4 days later and their spleens were removed and quickly frozen, after which longitudinal serial sections about 300 μ in thickness were cut on a microtome. These sections were thawed in Hanks' solution and carefully mounted on a previously prepared agar bed in Petri dishes and then incubated for 1 h at 37° C. After the dishes had been dried, 2.5 ml guinea pig serum in a dilution of 1:2.5 was poured on the surface, and the sections of the spleen were carefully removed. The dishes with complement were again incubated for 1 h under the same conditions. After the end of incubation the number of foci of hemolysis were counted. According to the recommendations of Kennedy et al. [10], the term foci of hemolysis was applied to zones of hemolysis about 0.5-1.0 mm in diameter. If large areas of lysis up to 3-4 mm in diameter are found on imprints of serial sections of the spleen, Kennedy at al. suggest that they are counted as two or more confluent hemolytic foci. In the present investigations no areas of hemolysis larger than 2 mm in diameter were found. These areas of lysis were assessed as two confluent hemolytic foci. Foci of hemolysis the size of ordinary plaques, detected by the local hemolysis in gel method and consisting of single AFCs separated from a group, were disregarded.

Armstrong and Diener [4, 5] showed that each focus of hemolysis is a reaction of one ARC to antigenic stimulation, and for that reason the number of foci of hemolysis was taken as the number of ARCs.

EXPERIMENTAL RESULTS

The number of ARCs for every 5×10^6 nucleated cells in the intact BALB/c mice was 11.9 ± 0.91, and if expressed per milligram weight of the spleen, 7.03 ± 1.09 (Table 1). The number of ARCs in BALB/c mice has not been studied previously (according to the literature), but in C57BL mice Kennedy et al. [10] found 11.5 ARCs to 1×10^7 nucleated spleen cells, while Shearer and Gudkowicz [13-15] give lower figures – 1 ARC to 3×10^6 thymocytes in C57BL mice and 1 ARC to 1×10^7 thymocytes in C3H mice.

During the development of the induced tumor in the BALB/c mice the number of ARCs, calculated per 5×10^6 injected nucleated cells, fell to 4.04, i.e, by 2.9 times ($P < 0.001$), and if expressed per milligram weight of spleen, the number fell to 1.64, i.e., by 4.3 times ($P < 0.001$).

The reason why the number of ARCs was expressed per milligram weight of the organ was because during methylcholanthrene carcinogenesis the weight of the spleen was increased by twice or more, so that it is more objective to express the number of ARCs per milligram weight of the organ.

During the development of an induced tumor in inbred mice a decrease was thus observed in the number of ARCs in the spleen of the experimental animals with the developing tumor. The number of AFCs, moreover, fell much more sharply (by up to 6-6.5 times) [3] than the number of ARCs (by 3-4 times). The decrease in the number of AFCs during chemical carcinogenesis can be assumed to be the combined result of the depressant action of the carcinogen and of the developing tumor both on the AFC and on the preceding stage of antibody formation – the ARC.

Blocking of the reticulo-endothelial system by colloidal carbon considerably reduces both the number of AFCs and the number of ARCs [7] in the spleen of experimental mice. Meanwhile injection of adjuvants [8] leads to proliferation of ARCs and a consequent intensification of antibody formation in response to the injection of antigen.

Consequently, the ARCs are among the most labile components of antibody formation, and are highly vulnerable in chemical carcinogenesis. Damage to this stage of antibody formation during chemical carcinogenesis leads to a decrease in the number of AFCs in the immunocompetent organs of mice with a developing tumor.

These experiments thus showed that the number of antigen-reactive cells in the spleen of BALB/c mice is reduced by 3-4 times during development of a methylcholanthrene-induced tumor.

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