In addition, diffuse hyperplasia of the lymphoid tissue with obliteration of the normal pattern was observed in the spleen of the mice. Lymphoid infiltration also was found in the kidneys and, less frequently, in the liver and lungs, evidence of the formation of lymphatic leukemia. Similar changes also were observed in some of the intact animals.

Injection of DANA into mice, just as into rats, thus led to the development of multiple tumors in the lungs. The neoplasms developed in all animals after a relatively short time; the first adenomas were formed as early as 3-4 months after the beginning of the experiments. Subcutaneous injection of DANA can accordingly be recommended for the induction of experimental lung tumors in mice.

It must also be pointed out that some of the animals developed proliferative changes in the kidneys and urinary bladder (carcinoma of the bladder in two cases and a malignant tumor of the kidney in one case). Possibly by reducing the dose of carcinogen the toxic effect would be diminished and a more selective action of DANA on the lungs would be obtained.

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CHANGES IN THE IMMUNOLOGIC STATUS REFLECTED IN THE PHYSICOCHEMICAL CHARACTERISTICS OF CELLS OF THE IMMUNOCOMPETENT SYSTEM DURING CARCINOGENESIS

E. N. Stepanova, G. P. Airapet'yan, and I. N. Maiskii

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The state of the cell population of the lymph nodes and thymus was studied by the fluorescent probe method in rats with sarcoma of the thigh induced by dimethylbenzanthracene. Statistically significant changes were found in the uptake of the negatively charged probe 1-aniline-8-naphthalene-sulfonate (ANS) in cell suspensions depending on the stage of development of the sarcoma. In the early stages of carcinogenesis cell forms with a less hydrophobic surface and containing fewer binding sites were observed to appear. It is suggested that these cells belong to the immature category. It is concluded that the fluorescent probe method is suitable for recording changes in the immunologic status of the organism during carcinogenesis.

KEY WORDS: chemical carcinogenesis; lymphocytes; fluorescent probes.

Changes in the functional behavior of lymphocytes [6], in the morphological composition of the lymph nodes and thymus [4, 5], and also in the fine surface structure of these cells during carcinogenesis [3] has been studied and described. On the other hand, it is stated in the literature that cells at different stages of differentiation or function are characterized by a definite density of their surface negative charges [8], de-

Scientific-Research Laboratory of Experimental Immunobiology, Academy of Medical Sciences of the USSR, Moscow.

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tected by the method of cell electrophoresis. The writers showed for the first time [1, 7] that changes of this type in cells of the lymphoid population during sensitization can be investigated by means of the fluorescent probe method [2].

The object of this investigation was to study some of the physicochemical properties of cells of the immunocompetent system during a change in the immunologic status of the animal during chemical carcinogenesis.

EXPERIMENTAL METHOD

A sarcoma of the thigh was induced with 9,10-dimethyl-1,2-benzanthracene (DMBA) in male Wistar rats. At least 10 animals were used in each experiment. Lymphocytes were isolated from peripheral and regional lymph nodes and the thymus on Ficoll-Verografin with a density of 1.077. The cells in the suspensions were counted and their protein concentration determined by Lowry's method.

Negatively charged 1-aniline-8-naphthalene-sulfonate (ANS), in an initial concentration of $5 \cdot 10^{-3}$ M, was used as the fluorescent probe. Fluorescence was recorded on the MPF-3 (Hitachi) fluorescence spectrophotometer. Fluorescence of ANS was excited by radiation with $\lambda = 370$ nm and the maximum of the fluorescence occurred at $\lambda = 470$ nm. The experimental results were expressed in relative units of intensity of fluorescence per milligram protein of the cell suspension (I). To assess the surface properties of the lymphocytes, double reciprocal coordinates were used on the basis of the following equation [10]:

$$\frac{1}{I} = \frac{\overline{K}}{I_\infty} \cdot \frac{1}{[ANS]} + \frac{1}{I_\infty}$$

where I is the relative intensity of fluorescence with a certain concentration of ANS; $\overline{K}$ is the mean binding constant for ANS; I_∞ the theoretical fluorescence during binding of the maximal number of ANS ions with 1 mg protein of the cell suspension (the number of binding sites for ANS ions); [ANS] is the molar concentration of the dye.

By extrapolating $1/I \rightarrow 0$ we obtain the intersection of the curve with the axis $1/[ANS] = -K$. Extrapolation of $1/[ANS] \rightarrow 0$ gives $1/I = 1/I_\infty$.

To determine changes in the quantum yield of fluorescence of ANS double reciprocal coordinates also were used: $1/I = f(1/C_p)$, where C_p is the quantity (in mg) of protein in the cuvette.

On extrapolation of $1/C_p \rightarrow 0$ to the 1/I axis a value reflecting a change in the quantum yield of fluorescence of ANS is obtained.

All the data were subjected to statistical analysis by the t criterion, with a level of significance of differences of $P \leq 0.05$.

EXPERIMENTAL RESULTS

The immunological control of the course of chemical carcinogenesis was based on the cellular reactions of immunity described previously [6]. The results of incorporation of ANS into cells of the lymph nodes and thymus were similar in type (Table 1). For instance, prior to 2.5 months of carcinogenesis a decrease in the incorporation of ANS into cells of both lymph nodes and thymus was recorded. However, a significant decrease in fluorescence of the probe was observed as early as the 2nd month for thymus cells, and rather later – 2.5 months – for lymphocytes. No fully formed sarcomas were present in the animals at these times. Starting with 4 months and until death of the animals a significant increase was found in the incorporation of ANS, and this was accompanied by an increase in the intensity of its fluorescence. This fact was observed both for cells of the lymph nodes and for thymocytes. Some workers [4, 5] have described morphological changes in the lymph nodes and thymus during carcinogenesis induced by DMBA and in other malignant neoplasms. Before the appearance of the first foci of tumor growth, hyperplasia was the predominant process in the lymph nodes and thymus, but in the later stages, at the time of fully formed tumors, this was replaced by complete plasma cell reconstruction of the lymphoid organs.

To study the fine details of changes in the surface properties of the lymphocytes in the early stages of carcinogenesis, double reciprocal coordinates were used (Figs. 1 and 2).

It will be clear from Fig. 1 that the number of binding sites for the dye per unit of protein was reduced in the cell suspension obtained from the lymph nodes and thymus of the experimental animals compared with

TABLE 1. Intensity of Fluorescence of ANS in Cells of Lymph Nodes and Thymus during Carcinogenesis

Cells studied	Period of carcinogenesis, months					
	1	2	2½	4	5	7
Cells of lymph nodes	I_e =16,24 I_n =8,104	I_e=5,88 I_n =8,104	I_e =2,88 I_n =8,104	I_e=23,98 I_n =8,104	I_e=27,23 I_n =8,104	I_e=15,56 I_n=8,104
P	≤0,05	> 0,05	≤0,05	≤0,05	≤0,05	≤0,05
Cells of thymus	I_e =9,02 I_n =5,65	I_e =1,31 I_n =5,65	I_e =0,3 I_n =5,65	I_e=11,31 I_n =5,65	I_e=9,79 I_n =5,65	I_e=18,54 I_n =5,65
P	> 0,05	≤0,05	≤0,05	≤0,05	≤0,05	≤0,05

Legend. I_e and I_n) Intensity of fluorescence of ANS in cells of experimental and normal rats, respectively.

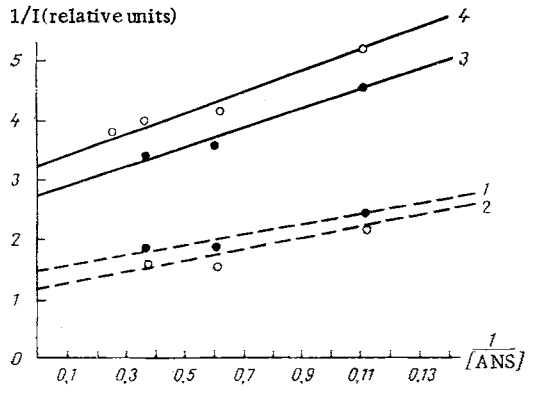

Fig. 1

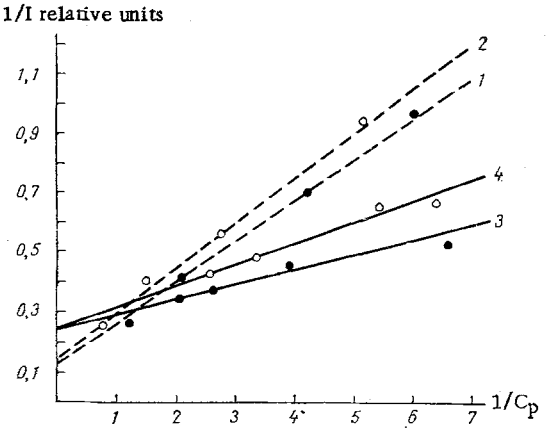

Fig. 2

Fig. 1. Determination of number of binding sites for ANS in suspension of lymphocytes obtained from experimental and normal animals. Here and in Fig. 2: 1) intensity of fluorescence of ANS in cells of normal lymph nodes; 2) in cells of normal thymus; 3) in cells of lymph nodes during carcinogenesis; 4) in cells of thymus during carcinogenesis.

Fig. 2. Intensity of fluorescence of ANS as a function of protein concentration in suspension of lymphocytes obtained from experimental and normal animals.

the control, for the curve reflecting the intensity of fluorescence of ANS in the lymphocytes during carcinogenesis intersected the ordinate significantly higher than in the control.

To discover on account of what the reduction in the number of binding sites took place in the protein of the cell suspension, the expression for the quantum yield of fluorescence was used. As Fig. 2 shows, extrapolation $1/C_p \rightarrow 0$ gives a higher value of $1/I_{(\text{relative units})}$ than of $1/I_n$. This is evidence of a decrease in the quantum yield of fluorescence of ANS in the lymphocytes of the experimental animals, possibly in connection with the less hydrophobic character of the surface of lymphocytes from the rats developing tumors. The decrease in the number of binding sites for ANS ions can thus be explained not only by a decrease in the density of negative charges [8], but also by a change in the hydrophobic properties of the surrounding surface on account of a redistribution of the lymphoid population and the appearance of young cell forms. The increase in the intensity of fluorescence of ANS at the stage of appearance of the tumors could be connected with plasma cell reconstruction of the lymphoid organs, for it has been shown that the plasma cell incorporates much more ANS than the lymphocytes [9], on account of an increase in active binding sites due to excessive synthesis of immunoglobulins.

It follows from the facts described above that the fluorescence probe method can be used to determine any possible reconstruction of the organs of immunity and differences in the cell surface properties of the lymphoid population during carcinogenesis.

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INHIBITION OF THE EMBRYOTOXIC AND TERATOGENIC EFFECTS OF METHYLUREA AND SODIUM NITRITE BY VARIOUS SUBSTANCES

V. A. Aleksandrov and F. K. Dzhioev

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After combined intragastric administration of methylurea (MU) and sodium nitrite (SN) into rats on the ninth day of pregnancy death of the embryos and a teratogenic effect were observed, due to the endogenous synthesis of nitrosomethylurea, which has a pathogenic effect. Ascorbic acid and urotropin completely blocked the embryotoxic and teratogenic effects observed after combined injection of MU and SN. Sodium sulfamate reduced the embryotoxic effect considerably and the teratogenic effect to some extent, whereas urea did not prevent the manifestation of the harmful action of MU and SN on the embryo.

KEY WORDS: pregnancy; teratogenic effect; embryotoxic effect; methylurea; sodium nitrite.

The possibility of endogenous synthesis of nitroso compounds, which is now well established, and the wide distribution of their precursors in the human environment make it necessary to look for substances which will prevent the formation of carcinogenic nitrosamines in vivo. Ascorbic acid has been suggested as an inhibitor of the endogenous synthesis of nitroso compounds from amines and nitrite [11].

When seeking substances of this kind it is very convenient to use experiments in which several amines and amides are injected at the same time as sodium nitrite into pregnant animals and then to assess the embryotoxic and teratogenic effects on their fetuses, for experiments of this type take little time and the effect can be clearly described quantitatively [2].

In this investigation the effect of ascorbic acid, urotropin, sodium sulfamate, and urea on the embryotoxic and teratogenic effects of combined administration of methylurea (MU) and sodium nitrite (SN) to rats on the ninth day of pregnancy was determined.

Laboratory of Experimental Tumors and Laboratory of Carcinogenic Agents, N. N. Petrov Scientific-Research Institute of Oncology, Ministry of Health of the USSR, Leningrad.

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