DETECTION OF ANTIBODIES IN CHEMICAL CARCINOGENESIS

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The serum of rats receiving dimethylaminoazobenzene with their food for 60-100 days was found to contain antibodies against their liver antigens in the passive hemagglutination reaction. Antibodies against the liver antigens of normal rats or of rats receiving the carcinogen for 4, 15, and 30 days could not be detected.

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Previous investigations [1-5] have shown that after administration of hepatotropic carcinogenic substances to experimental animals, abnormal antigens are formed in their liver tissue. In the early stages of carcinogenesis, these abnormal antigens possess the specificity of the carcinogen used. It is possible that these abnormal carcinogen–protein antigenic complexes may cause antibody formation. The formation of such antibodies during administration of carcinogenic substances has not previously been investigated. However, according to the immunologic concepts of Green [7-9], antibody-formation following administration of chemical carcinogens plays an important role in the genesis of malignant tumors.

The object of the present investigation was to study the possibility of antibody formation against abnormal rat liver antigens during hepatocarcinogenesis.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 120-140 g. The animals received the hepatotropic carcinogenic azo-dye dimethylaminoazobenzene with their food at the rate of 10 mg daily. The animals were sacrificed 4, 15, 30, 60, and 100 days after the beginning of feeding with the carcinogen by decapitation and the presence of antibodies in their blood serum was investigated (previously the antigenic structure of the liver of rats receiving dimethylaminoazobenzene had been studied at the same periods).

The blood sera of 10-15 rats at the same stage were mixed and investigated as a single specimen. At each stage 2 or 3 samples of serum were used. The antibodies were determined in the sera by Boyden's passive hemagglutination method. Antigens for the reaction were obtained by saline extraction of a 20% suspension of liver tissue of the experimental and normal rats ground with glass sand. The liver was first washed thoroughly with physiological saline. The saline extracts of the tissues thus obtained were dried lyophilically. A solution of antigen in mixed buffer was used in the reaction (9 mg antigen in 1 ml solvent).

Antigens from the liver and blood serum of normal animals were used as the control. In addition, the technical control for Boyden's reaction consisted of tanninized sheep's erythrocytes not loaded with antigen.

EXPERIMENTAL RESULTS

The control sera did not give positive results in the passive hemagglutination reaction either with antigens of normal liver or with liver antigens of the experimental animals or with erythrocytes not loaded with antigen. Sera of the experimental animals did not react with antigen of normal rat liver or with erythrocytes not loaded with antigen.

The sera of animals receiving the carcinogen for 4, 15, and 30 days likewise did not react with the liver antigens of these rats.

After administration of dimethylaminoazobenzene to rats for 60 and 100 days, antibodies could be detected in their serum, the antibody titer rising until the 100th day of the experiment (from 1:4 to 1:64 on the 60th day of the experiment).

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It was next decided to study whether the antigens and, correspondingly, the antibodies found on the 60th and the 100th day of the experiment are identical. Sera of animals receiving the carcinogen for 60 days gave a positive cross reaction with liver antigens of rats on the 100th day of the experiment in dilutions of 1:18-1:32. The sera of animals receiving dimethylaminoazobenzene for 100 days likewise reacted with liver antigens of rats receiving the carcinogen for 60 days, and also in dilutions of 1:18-1:32.

Consequently, the crossed reaction observed between the sera and liver antigens of animals on the 60th and 100th days of the experiment are evidence of identity of the antigens and antibodies formed at these stages of the experiment.

As the results show, no autoantibodies against normal liver antigens could be found in the sera of normal rats in the passive hemagglutination reaction. In the first 30 days of feeding the animals with dimethylaminoazobenzene, no antibodies likewise could be detected against liver antigens in the blood serum of the experimental rats. On the 60th and 100th days of the experiment, autoantibodies appeared in the serum of the experimental animals, their titer being 1:4 on the 60th day and rising to 1:64 on the 100th day. Sera of animals on the 60th and 100th days of the experiment gave a positive cross reaction with liver antigens of rats at these stages of the experiment. This indicates identity of the liver antigens of the animals at these times and also identity of the antibodies formed against them.

As was pointed out above, abnormal antigens found in the liver of animals after administration of hepatotropic carcinogens possess the specificity of the carcinogen used in the early stages of carcinogenesis.

It has been shown experimentally [6] that the content of bound hepatotropic carcinogen (dimethylaminoazobenzene) in the liver of rats reaches a maximum on the 30th day after the beginning of administration of the carcinogen. Consequently, at this period of the experiment antibody formation could be expected. However, no antibodies were found before the 60th day of the experiment. How can the absence of antibodies against abnormal liver antigens of the experimental animals until the 60th day of the experiment be explained?

We (T. A. Korosteleva, L. L. Khundanova, Z. M. Chistyakova) have found by studying the phagocytic activity of blood leukocytes of rats receiving dimethylaminoazobenzene that on the 15th-30th day from the beginning of administration of the carcinogen a considerable decrease in phagocytic activity of the leukocytes is observed, to recover on the 60th-100th day of the experiment. The phagocytic activity of the blood leukocytes is an index of the state of the reticulo-endothelial system, the tissue cells of which participate in antibody formation. After the 15th-30th day of administration of the carcinogen it is possible that phagocytosis of the antigen, converting it into the "immunogenic" form, is depressed as a result of the toxic action of the carcinogen. In addition, the function of the antibody-forming cells themselves may be depressed under these circumstances. As a result of this, antibodies either are not formed whatsoever against the abnormal liver antigens on these days of the experiment, or they are formed in such small quantities that they cannot be detected by the reaction used in these experiments.

Another possibility is that the antibodies formed in such small quantities are bound by the corresponding antigen, and antibodies were found in the serum of the rats only after they had accumulated to excess. Finally, it may be postulated that on the 60th-100th day of the experiment, new antigens appeared in the liver of the experimental animals, which were not present in the earlier stages of the experiment. Against these antigens the body produces the antibodies which we detected.

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