CHANGES IN THE IMMUNOLOGICAL SPECIFICITY OF TISSUE PROTEINS DURING CARCINOGENESIS

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Investigations have shown that carcinogenic substances, when introduced into the organism, form complexes with cell proteins and modify the immunological specificity of the proteins, thereby performing the function of a hapten [2-4,6]. However, these changes in the antigenic properties of the tissue proteins are not characteristic of the process of carcinogenesis, for similar changes in the immunological specificity of the proteins are also found after introduction of noncarcinogenic compounds into the body [5,7]. The specific action of carcinogenic substances on tissue is evidently more closely associated with the appearance of antigens whose immunological specificity is independent of the presence of a carcinogenic hydrocarbon in their molecule. In this respect, some interesting investigations have been carried out [3], in which the antigens of the liver of mice have been studied by means of the reaction of anaphylaxis with desensitization in various stages of carcinogenesis caused by orthoaminoazotoluene. While still in the precancer stage, characterized morphologically by diffuse hyperplasia of the liver tissue and by the formation of hepatocytic adenomas, as antigen possessing specificity to orthoaminoazotoluene and an antigen analogous to the hepatoma antigen could be detected.

The object of the present investigation was to determine the order of appearance in the process of carcinogenesis of these two types of antigens, whose specificity is or is not associated with the presence of a carcinogenic substance in the protein molecule.

EXPERIMENTAL METHOD AND RESULTS

The pharyngeal tonsils of dogs were used as test object. The reasons for this choice were as follows: 1) the tonsils of dogs have a common antigenic complex with human tonsular tissue [1]; 2) the response reaction of the lymphoid tissue of the tonsil to carcinogenic hydrocarbons has not previously been investigated; 3) the tonsils are accessible for observation; 4) the effect of tissue incompatibility between animals on the experimental results was eliminated.

Experiments were carried out on 15 dogs aged 4-6 months. All the animals received an injection of 10 mg of 9,10-dimethyl benzanthracene (DMBA) in the form of an emulsion in mineral oil, into the right tonsil. To prevent the changes associated with the solvent from influencing the result of the experiment, mineral oil alone was injected into the other tonsil.

On the 10th and 30th days after injection of the carcinogen, attempts were made to detect changes in the immunological specificity of the protein, which may or may not have been determined by the presence of DMBA in the protein molecule. For this purpose, at these times bilateral tonsillectomy was performed on the animals. Unabsorbed mineral oil with carcinogen was removed by excision. Next, a saline extract was prepared of the tonsillar tissue by the usual method. Part of the extract from healthy tonsillar tissue was mixed with DMBA and the mixture was incubated for 24 h at 4° and pH 7.2.

Specific antigens were detected by the method of sensitization with desensitization in guinea pigs weighing 300-350 g. The animals were sensitized by a single subcutaneous injection of 2 mg of antigen from the "pathological" tonsil. The animals of the control group were sensitized by the same dose of antigen from the "healthy" tonsil.

Change in Immunological Specificity of Proteins of Tonsillar Tissue 10 and 30 Days after Injection of 9,10-dimethybenzanthracene

Experiment No.	Day after injection of DMBA	Number of guinea pigs	Sensitization			Desensitization				Testing for completeness of desensitization				Reacting injection			
			PAG	HAG	Reaction	PAG	HAG	HAG+DMBA	Reaction	PAG	HAG	HAG+DMBA	Reaction	PAG	HAG	HAG+DMBA	Reaction
			In mg			In mg				In mg				In mg			
1	10-th	3	—	2	—	—	6	—	++,+++	—	2	—	—	—	4	—	—
	30-th	3	—	2	—	—	6	—	+++	—	2	—	—	—	4	—	—
2	10-th	3	2	—	—	—	6	—	+++	—	2	—	—	4	—	—	++
	30-th	3	2	—	—	—	6	—	+++	—	2	—	—	4	—	—	++, +++
3	10-th	3	2	—	—	—	6	—	+++	—	2	—	—	—	—	4	++, +++
	30-th	3	2	—	—	—	6	—	+, +++	—	2	—	—	—	—	4	+, ++
4	10-th	3	2	—	—	—	—	6	+++	—	—	2	—	4	—	—	—
	30-th	3	2	—	—	—	—	6	+++	—	—	2	—	4	—	—	++
5	10-th	3	2	—	—	6	—	—	+++	2	—	—	—	—	—	4	—
	30-th	3	2	—	—	6	—	—	+++	2	—	—	—	—	—	4	—

Legend: PAG — antigen isolated from "pathological" tonsil; HAG — from "healthy."

The guinea pigs were considered to be sensitized 21 days after injection of the antigen. Desensitization, testing for the completeness of desensitization, and the reacting injection were carried out, depending on the experimental conditions, with the use of antigen from the "pathological" tonsil, from the "healthy" tonsil, and with a mixture of antigens from the "healthy" tonsil and carcinogen. The first desensitizing dose of antigen was injected subcutaneously in a dose of 6 mg and the second (next day) intravenously in a dose of 2 mg.

Anaphylactic shock was produced by the intravenous injection of the antigen in a dose of 4 mg. The severity of the shock was determined from the animal's behavior, the change in the clotting time of the blood and the fall of body temperature.

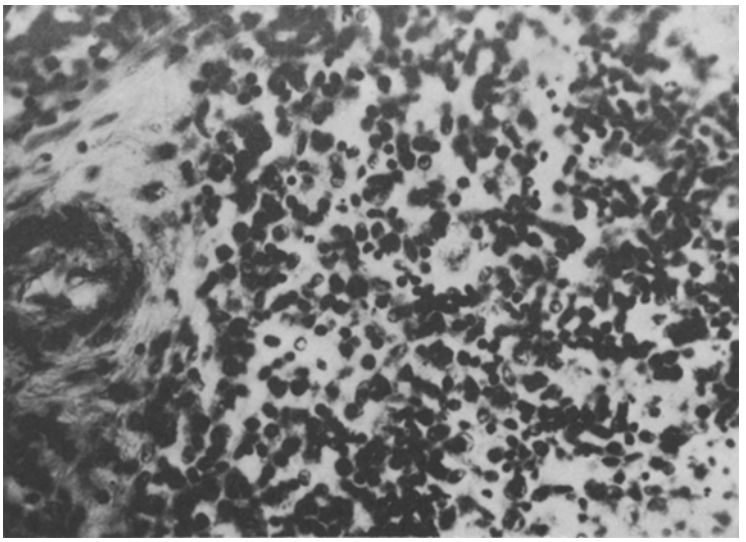
The results of the investigations of the antigenic properties of the tonsillar proteins 10 and 30 days after injection of DMBA are shown in the table.

It is clear from the table that the guinea pigs sensitized with antigen from the "healthy" tonsil were completely desensitized by this same antigen (see table, experiment No. 1). Consequently, desensitization of the experimental animals to antigens from the "healthy" tissue was complete.

The animals sensitized with antigen from the "pathological" tonsil and desensitized with antigen from the "healthy" tonsil responded by anaphylactic shock in all the experimental conditions to injection of antigen from the "pathological" tonsil (see table, experiment No. 2) as reacting injection. This demonstrates that a specific antigen was present in the "pathological" tonsil. However, no conclusion can be drawn from the results obtained regarding the origin of the specific antigen found in the "pathological" tonsil. The appearance of this antigen may have been due, on the one hand, to the presence of DMBA in the protein molecule, or on the other hand, to profound changes in the metabolic processes in the cell. This problem was solved by performing the next two series of experiments. The guinea pigs in these experiments were also sensitized with antigen from the "pathological" tonsil.

In the experiments of series 3, the desensitization was carried out with antigen from "healthy" tissue and for the reacting injection, a mixture of antigen from "healthy" tissue and DMBA, and anaphylactic shock was caused by antigen from "pathological" tissue. In these experiments, a negative reaction was obtained on the 10th day of the investigation, and a positive reaction on the 30th.

Consequently, 10 days after injection of DMBA into the tonsillar tissue, only one specific antigen was detected, resulting from the formation of a complex between protein and carcinogen. On the 30th day of the investigation, an additional antigen was formed in the "pathological" tonsil, the specificity of which was unrelated to the presence of DMBA in its molecule. The appearance of this antigen was evidently due to profound metabolic disturbances in the cells, and to the processes of conversion of normal into malignant cells, as shown by the morphological changes in the "pathological" tonsil. For example, whereas 10 days after the injection of DMBA, no significant changes were



Tonsil one month after injection of carcinogen. Diffuse hyperplasia of reticuloendothelium with formation of plasma cells and epithelioid cells. Hematoxylin-eosin. Objective 20 ×, ocular 10 ×.

observed in the histological structure of the tonsillar tissue, after one month a well-marked diffuse hyperplasia of the reticulo-endothelial cells appeared, with the formation of lightly stained cells and with the presence of plasma cells and endothelioid cells (see figure). The germ centers of the follicles showed signs of irritation, and the lymphoid cells were, to a large extent, replaced by small, more lightly stained cells resembling polyblasts.

In the regional cervical and submandibular lymph glands, activation of the reticulo-endothelial cells was observed, as shown by their swelling and slight proliferation, with the formation of solitary polyblasts and plasma cells or small clusters of these cells.

No significant changes were found in the tissue of the opposite tonsil. The changes observed were similar to those undergone by lymphoid tissue in the course of immunogenesis.

Hence, the process of carcinogenesis may be subdivided into a nonspecific stage, when complex compounds are formed between the proteins and the carcinogenic substance, and a specific stage during which the immunological response reaction of the tissue takes place to this complex, and profound modifications of the metabolism develop in the proliferating cells.

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