THE SIMPLIFICATION OF THE ANTIGENIC STRUCTURE OF LIVER PROTEINS DURING EXPERIMENTAL CARCINOGENESIS

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(Received September 23, 1957. Presented by Active Member AMN SSSR, L. A. Zil'ber)

During the last decade Zil'ber et al. [2, 14] demonstrated the presence in tumors of specific antigens, not found in normal tissues. The most important technic in the domonstration of these tumor antigens was the application of the anaphylactic reaction with desensitization in guinea pigs.

The application of this technic to the study of the antigenic composition of livers during carcinogenesis brought to light a number of new facts. Thus, it was possible to demonstrate the presence of an antigen with the specificity of orthoaminoazotoluene (OAAT) in the early stages of action of this compound [3]; a specific antigen in induced and transplanted hepatoma was found [4, 5, 1]; an antigen similar to that of hepatoma was also identified in the precancer stage [1]. At the same time there appeared reports dealing with the simplification of tissue antigen structure in the process of carcinogenesis. Such results were claimed by Weiler [11-13], who employed complement fixation, for liver tumors in rats and kidney tumors in hamsters. Seligmann, Grabar and Bernard [10] employed precipitation in gels to demonstrate the simplification of antigenic structure in leukemia cells compared to normal leucocytes. Using the same technic, Parnes and Abelev [7] found simplified antigenic structure in tissues affected by leucosis.

The present work deals with the demonstration of simplified antigenic structure in the liver during experimental carcinogenesis, using the anaphylaxis and desensitization technic.

EXPERIMENTAL METHODS

Guinea pigs were used throughout. The antigens used were protein fractions, obtained from the tissues investigated by extracting in slightly alkaline (ca. pH 8) water, rejecting the fraction which precipitated on subsequent acidification to pH 6, and collecting the fraction which precipitated when pH was further lowered to 4.5. A batch of 4-5 mice was used in each antigen preparation. Guinea pigs were sensitized by a subcutaneous injection of from 3.5 to 10 mg of antigen protein from livers of normal C_3HA strain mice. The protein content was determined by the Kjeldahl method, in the analytical laboratory of this Institute (head of laboratory: A. D. Chinaeva). In order to eliminate possible differences in experimental results due to the different blood content of hepatomas and normal livers, some of the organs were perfused with physiological saline prior to excision. Desensitizing agents were introduced intravenously 1.5-2 months after sensitization. The antigens used in desensitization were prepared by the method described above, from livers of mice which were receiving periodical paintings with a 1% solution of OAAT in benzene over a period of 2-2.5 months (pre-cancer stage); livers from animals bearing OAAT-induced hepatoma, or a transplanted hepatoma, were also used. Protein from livers of normal C_3HA mice was introduced as a resolving antigen.

All antigens were checked prior to experiment for possible toxicity, by intravenous injection into normal

TABLE

A Comparison of Normal Liver Antigens With Those of Precancerous Liver and Hepatoma

	Sensitization			Desensitization (check for completeness)				Resolving introduction						
	normal liver antigen	number of guinea pigs	dose, mg protein	Antigen		dose, mg protein	reaction	normal liver antigen	dose, mg protein	no. of guinea pigs which gave reaction				
										—	+	++	+++	++++
1	№ 97	4	6.77	№ 94	Mouse liver in the precancer stage (after 3 mos of painting with OAAT)	7.4—9.2	—	№ 976	7—9	—	2	2	—	—
2	№ 113	5	3.5—6.3	117		7.2—8.5	—	№ 124	7—8.5	1	3	1	—	—
3	№ 159 after perfusion	5	7.7—10.2	176		7.6—10.0	—	№ 159 after perfusion	7.6—9.4	—	1	4	—	—
4	№ 97	4	6.77	№ 95	Induced malignant hepatoma	8.8	—	№ 976	9.1	—	4	—	—	—
5	№ 157 after perfusion	5	7.57	161		7.53	—	№ 159 after perfusion	7.5	—	1	4	—	—
6	№ 124	6	3.4—4.2	123в	Induced non-malignant hepatoma	4.3	—	№ 127	4.3	1	3	2	—	—
7	№ 144	5	6,2	154		6.5	—	№ 144	6.5	—	4	1	—	—
8	№ 136 bearing transplanted hepatoma	6	5.5	135	Transplanted hepatoma	5.5	—	№ 136 bearing transplanted hepatoma	5.5	—	2	4	—	—
9	№ 144	5	6.2	152	as above	3.6—6.2	—	№ 144	3.7—6.2	—	—	4	1	—
10	№ 148	5	8.3	156	» »	8.8	—	№ 153	8.9	—	1	2	2	—
11	№ 157 after perfusion	5	7.5	160	» »	7.5	—	№ 157 and 159 after perfusion	7.5	1	2	2	—	—
12	№ 159 after perfusion	3	6.8—8.3	167	» »	6.8—8.5	—	№ 159 after perfusion	6.8—8.5	—	—	1	2	—
13	№ 173 after perfusion	2				9.5		№ 190 after perfusion	2.5	—	2	—	—	—
		3	5.1	189	» »	9.5	—	№ 190	4.9	—	—	2	—	1
	Total number of guinea pigs used	63	—	—	—	—	—	—	—	3	25	29	5	1

guinea pigs. No reaction to the antigens was observed in any case with these animals. A total number of 13 experiments were carried out with 63 guinea pigs, 12 various normal antigens being tested.

EXPERIMENTAL RESULTS

The results have been presented in the Table. It will be seen that in 3 experiments (14 guinea pigs) the antigens used were obtained from livers at the pre-cancer stage, in 4 other experiments (20 guinea pigs) they were obtained from induced hepatoma, and in 6 others (29 guinea pigs) – from transplanted hepatoma. As a rule, the guinea pigs reacted to the resolving introduction of normal liver proteins with an anaphylactic reaction of a strength + or ++. When compared with transplanted hepatoma, guinea pigs responded, in some cases, with a reaction of strength +++, or even ++++ (death). The results were not affected by the source of the antigen, and both the sensitizing and resolving antigens could be derived from different groups of mice. Thus, no difference could be detected between the results of experiments 2, 5, 6, 10 and 13, where antigens from a variety of preparations were used, and those of experiments 1, 3, 4, 7, 8, 9 and 12, where the same antigens were used throughout. The blood content of the various preparations was also without any apparent effect on the results. In experiment 10, during desensitization, and prior to the introduction of the antigen from transplanted hepatoma, the guinea pigs were given two lots of 6 mg each of serum proteins from hepatoma-bearing mice. In experiments 3, 5, 11, 12 and 13 the livers of the normal mice were perfused with physiological saline prior to preparation of the antigens; nevertheless, the results obtained in this experimental series did not differ from those in other corresponding groups.

It is noteworthy that antigenic differences between normal liver and transplantable hepatoma were observed only in cases when the material was obtained from the same animals (Experiment 8). Positive results were obtained in experiment 13, where the resolving antigen was added in doses 2 and 4 times lower than those employed in the test of completeness of desensitization.

This seems to indicate that the situation did not simply involve quantitative differences in the content of normal components in hepatoma and normal livers.

The results thus show that the normal mouse liver contains antigens (or antigen) which cannot be demonstrated in livers of mice bearing hepatoma, whether induced or transplanted, or in mouse livers in the pre-cancer stage.

The antigenic simplification of tumor tissue may be contemplated as the immunological demonstration of its well known biological and morphological de-differentiation; it may well prove to be an important link in the pathogenesis of a malignant growth. The importance of this phenomenon may be better realized in the light of the recently advanced "exclusion theory" of Miller and Miller [6], which has been supported by other authors [8, 9]. On the basis of the observed presence in the precancer liver of a protein-bound dye, and the absence of bound dye in hepatoma, Miller and Miller advanced a working theory, consisting of the following:

In the process of carcinogenesis the azo dye – paradimethylaminoazobenzene – is bound by proteins which play an important role in "the reaction of the cell to the action of growth-regulating intracellular factors". Successive cell generations contain decreasingly smaller amounts of this protein until, finally, cells are formed which are completely devoid of growth-regulating proteins. These cells respond with uninterrupted division to the continuous flow of nutritive substances, and finally become malignant.

Considering the fact that the tumors are devoid of certain antigens which are present in normal tissues, and that they contain specific tumor antigens which normal tissues do not contain, it is feasible that in the process of carcinogenesis there takes place not merely a loss of normal proteins, but a complex transformation of the antigenic structure of the tissue.

Some antigens (and consequently, proteins) disappear while others appear. It is difficult to state, on present evidence, whether the process involves a transformation of one protein, or a complete substitution of an old protein by the new one. It is even difficult to determine which antigen is expended in the simplification of the tissue structure of the tumor. For instance, Weiler [11], on the basis of his experimental evidence, concluded that both hepatoma and kidney tumor lost their tissue-specific antigen. It is the present author's opinion that the solution of this problem requires further investigation with the application of various immunological methods.

SUMMARY

The simplification of the antigenic structure has been revealed during the experimental carcinogenesis in mouse liver at the precarcinogenic stage, induced with orthoaminoazotoluene in transplanted and induced hepatoma. These experiments were performed with the aid of the anaphylactic reaction following desensitization.

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