DISCOVERY OF AN ANTIGEN CONTAINING 3-HYDROXYANTHRANILIC ACID IN THE BLOOD SERUM OF PATIENTS WITH TUMORS IN VARIOUS SITUATIONS

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Investigation of an unusual antigen containing the carcinogenic tryptophan metabolite 3-hydroxyanthranilic acid as hapten (3-HAA-antigen) showed that it is present in the blood serum of most patients with malignant tumors in different situations and in different stages of the disease. The 3-HAA-antigen was found only rarely in patients with noncancerous diseases and was not found in the blood of healthy donors or of patients with benign tumors. The importance of the results for the diagnosis of cancer irrespective of its situation is discussed.

KEY WORDS: malignant tumors; 3-hydroxyanthranilic acid; immunodiagnosis of tumors.

In previous investigations the writers showed the presence of an unusual antigen, distinguished by the fact that it contains the carcinogenic tryptophan metabolite 3-hydroxyanthranilic acid (3-HAA-antigen), in the blood serum of animals in the early stages of carcinogenesis [1], in patients with carcinoma of the bladder [2], and also in workers exposed to the risk of development of occupational bladder carcinoma [3].

The appearance of this antigen in the blood is evidently due to an increase in the 3-hydroxyanthranilic acid level in the body and its interaction with the blood serum proteins. This hypothesis is supported by much evidence in the literature of a pathologically high excretion of carcinogenic tryptophan metabolites in the urine observed in patients with bladder carcinoma and with malignant neoplasms in other situations [5, 6], and also by reports of the interaction between exogenous carcinogenic compounds and blood serum proteins [4].

In the investigation described below the presence of 3-HAA-antigen was studied in the blood serum of patients with tumors in different situations.

EXPERIMENTAL METHOD

3-HAA-antigen was determined in patients' blood in the Laboratory of Immunology of Carcinogenesis, Research Institute of Oncology, Ministry of Health of the USSR. Patients admitted to that Institute with tumors in different stages of development were studied.

The search for 3-HAA-antigen in the patients' blood serum was made with the aid of specific rabbit antibodies against 3-HAA as the hapten, by the method of counter diffusion in 0.6% agarose.

To obtain antibodies against 3-HAA, a preparation of chemically pure 3-HAA synthesized by the method developed by the Swiss firm of Hoffman-La Roche, was used. The 3-HAA was diazo-coupled with horse serum proteins and rabbits were immunized with the azoproteins thus obtained. The presence of antibodies against 3-HAA in preparations of the immune sera was tested in reactions of these sera with azoproteins containing different protein carriers coupled with the same hapten (3-HAA).

As the control, for every patient's serum tested, a parallel test was carried out with the serum of a donor of the same blood group.

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TABLE 1. Determination of 3-HAA-antigen by Counter Diffusion Method in Blood Serum of Patients and Donors

Group No.	Disease		Discovery of 3-HAA-antigen
1	Malignant tumors	Carcinoma of the stomach	46/47
		Carcinoma of the colon and rectum	83/86
		Carcinoma of the bladder	42/42
		Carcinoma of the kidney	34/34
		Carcinoma of the prostate	15/15
		Carcinoma of the penis	5/5
		Ovarian tumors	9/9
		Carcinoma of the uterus	3/3
		Carcinoma of the lung	6/6
		Melanoma	9/9
		Leukemias	6/6
			258/262 P<0.001
2	Precancerous diseases	Gastric ulcers	8/11
		Gastric polyps	9/11
		Gastritis	5/7
		Polyps of colon and rectum	20/21
		Papillomas of the bladder	61/61
			103/111 P< 0.01
3	Benign tumors (dermoid cysts of the rectum, cysts of the spleen and kidney, and epididymis, polyps of the urethra, adenomas of the prostate)		0/21
4	Nontumor diseases (colitis, proctitis, cystitis, pyelonephritis, etc.)		3/98
5	Healthy donors		0/123

Legend. Numerator shows number of patients in whose serum 3-HAA-antigen was found; denominator shows number of patients tested.

The clinical diagnosis was compared with the results of immunoserological determination of 3-HAA-antigen after the clinical diagnosis had been finally established and confirmed by histological tests.

For the statistical analysis of the results the criterion t was determined by Van der Waerden's method.

EXPERIMENTAL RESULTS

An immunoserological investigation was carried out to detect 3-HAA-antigen in the blood sera of 262 patients with malignant tumors in different situations and 111 patients with precancerous diseases. Groups of healthy donors and of patients with benign tumors, and also a group of patients with diseases other than tumors, acted as the controls.

The results of the tests are compared with the histologically confirmed final clinical diagnosis in Table 1 and Fig. 1.

The presence of 3-HAA-antigen in the patients' blood serum was recorded visually from the appearance of a distinct precipitation line in the agarose, in which it appeared as the result of interaction between antibodies of the immune serum against 3-HAA as the hapten and the 3-HAA-antigen of the patients' serum. The reaction

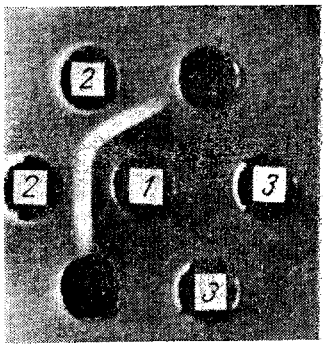

Fig. 1. 3-HAA-antigen in blood serum of patient with malignant degeneration of an adenomatous polyp of the stomach: 1) rabbit immune serum containing antibodies against 3-HAA as the hapten; 2) patient's serum; 3) serum of healthy donor.

was assessed as positive only in the presence of a negative control (absence of a precipitation line on interaction between the same immune serum and the blood serum of healthy donors) in a parallel test which accompanied the investigation of every patient's serum (Fig. 1). It will be clear from Table 1 that 3-HAA-antigen was not found in any of the 123 donors. By contrast, in tests on patients with malignant tumors (group 1) and precancerous diseases (group 2), 3-HAA-antigen was discovered in the overwhelming majority of cases. Statistical analysis confirmed the significance of the difference in the frequency of discovery of 3-HAA antigen in patients with malignant tumors ($P < 0.001$) and with precancerous diseases ($P < 0.01$) than in subjects with no tumors.

The high frequency of discovery of 3-HAA-antigen in patients with malignant tumors and precancerous diseases and its absence in donors, in patients with benign tumors, and in most patients with diseases other than tumors demonstrates the great diagnostic value of determination of 3-HAA antigen in these patients.

The nature of the phenomenon of the appearance of 3-HAA-antigen in the blood of cancer patients has not been finally established. All that can be confidently said is that this antigen is not secreted by tumor cells, for it continues to be discovered in the patients' blood after surgical removal of the tumor. Its appearance is evidently connected with disturbances of metabolism which not only accompany tumor growth but also precede it. In this connection it is interesting to note that in many patients with polyps of the colon and stomach in whose serum 3-HAA antigen was found, histological investigation showed the presence of rapidly proliferating adenomatous cells. These observations, together with the results of earlier experiments in which the writers demonstrated the appearance of 3-HAA-antigen in the serum of animals in the early stages of carcinogenesis induced by benzidine, are of considerable interest in connection with the future development of methods of detecting persons with increased risk of developing malignant tumors.

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