DETECTION OF EMBRYONIC α-GLOBULIN BY AGGREGATE HEMAGGLUTINATION IN SERUM OF PATIENTS WITH CERTAIN TYPES OF CANCER

A. M. Olovnikov and V. S. Tsvetkov

UDC 616-06.6-07:616.153.962.4-074

A new method of detection of embryonic α-globulin (aggregate hemagglutination), more than 10,000 times more sensitive than the precipitation reaction, is described. By means of this method, embryonic α-globulin could be detected in the blood of patients with hepatocellular cancer and chorionepithelioma, in whom this protein could not be detected by the precipitation reaction.

* * *

Investigations by Abelev and co-workers [1-3], Tatarinov and co-workers [7, 8], and Uriel and co-workers [9] have shown that the blood of animals and man with hepatocellular carcinoma of the liver contains a specific embryonic α-globulin (α_F-globulin). This protein can be used as an indicator of the presence of a malignant tumor. The gel-diffusion reaction can be used to identify α_F-globulin in sera. The sensitivity of precipitation is such that α_F-globulin can be determined if its content is not less than 1-3 μg/ml.

The writers have previously [4-6] suggested a method of aggregate hemagglutination to determine the concentration of antigens. This method is extremely sensitive, more than 10,000 times more sensitive than the precipitation reaction. The essence of the method is that erythrocytes used to determine antigens by agglutination are sensitizedby antibodies preliminarily incorporated into three-dimensional aggregates. This enables the active centers of the antibodies to be separated in space from the surface of the erythrocyte, thus making the active centers accessible to the antigen determinant [4]. The use of this method to detect α_F-globulin could result in a sharp increase in the sensitivity of determination, and it thus could be used as a method for the much earlier diagnosis of human liver carcinoma, teratoblastoma, and other tumors secreting this protein into the blood stream.

This paper gives the first results indicating that this method of aggregate hemagglutination can be used, in principle, to detect α_F-globulin in cases when it cannot be detected by the ordinary methods.

The monospecific antisera used in the investigation were obtained by immunizing rabbits with human embryonic α-globulin, isolated by electrophoresis in polyacrylamide gel,* injected into a lymph gland.

To sensitize the erythorcytes, 1 ml of an 8% suspension of washed formalinized erythrocytes in 1% $NaHCO_3$ solution was treated with a freshly prepared cold (4°) solution of a stable tetrazonium salt (a 4% solution of diaminodiphenylamine tetrazotate was used in the form of the substance "Diazol Black S," batch No. 7 dated 1964). After stirring for 30 sec, the residue was washed twice with cold 0.1 M acetate buffer solution, pH 4, in volumes of 8-10 ml, and once with cold 0.85% NaCl solution, pH 4-5. The residue of erythrocytes, washed to remove unfixed tetrazotate, was treated with 1 ml of a solution of previously ag-

* These sera were kindly presented by A. I. Gusev and A. K. Yazova, whose generosity is hereby acknowledged.

Laboratory of Chemistry and Synthesis of Antibodies and Laboratory of Immunochemistry of Cancer, Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow.
(Presented by Academician of the Academy of Medical Sciences of the USSR O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 68, No. 12, pp. 102-104, December, 1969. Original article submitted June 26, 1969.

TABLE 1. Detection of α_F-Globulin in Serum of Patients with Hepatomas and Certain Other Types of Human Cancer, in Pregnant Women and Healthy Donors

Serum	Diagnosis	Titer of α_F-globulin: determination by agar diffusion method	Titer of α_F-globulin: determination by aggregate hemagglutination method	No. of determinations
D 1	Donor	0	0	3
D 2	Donor	0	0	4
D 3	Donor	0	0	4
D 4	Donor	0	0	2
D 5	Donor	0	0	2
NA-1 ascites	Hepatocellular carcinoma	1:128	1:320 000	15
SB-1	Pregnancy	0	1:160	3
TB	Pregnancy	0	1:160	1
2306	Cirrhosis	0	0	2
2182	Cirrhosis, followed by development of hepatocellular carcinoma	0	1:20	3
2240	Hepatocellular carcinoma	0	0	1
1952	The same	0	0	1
1794	" "	0	0	1
1936	" "	0	1:160	2
1967	" "	0	1:80	2
2/A/Africa	" "	0	1:160	1
155/A/Africa	" "	0	1:160	1
1969	Chorionepithelioma	0	1:40	1
2000	Chorionepithelioma	0	1:160	1
1998	Chorionepithelioma	0	1:80	1
2015	Seminoma	0	0	1

gregated proteins of a specific α_F-antiserum in 2% $NaHCO_3$ solution, and the mixture was incubated for several hours at 37°, after which a suspension of sensitized erythrocytes of the required concentration was prepared.

To obtain soluble aggregates, antiserum proteins were also treated with diaminodiphenylamine tetrazotate in a dose one-third less than that producing opalescence or forming a gel-like clot. This dose can be determined by a filtration test on chromatographic paper or by the viscosity or turbidity of the solution of immune globulins [6]. The dose for aggregation of the antiserum proteins by glutaraldehyde was chosen in the same way. Aggregate hemagglutination is carried out on ordinary agglutination plates or in a microtitrator in salt solution with 0.1-0.2% normal lyophilized rabbit serum, in which 1:20 dilutions of the test sera were made up. The patients' sera were not exhausted with erythrocytes and were used in the reaction in dilutions starting from 1:20. After addition of 0.025-0.05 ml of suspensions of sensitized erythrocytes and incubation at room temperature or at 37°, results were recorded. In a positive reaction the bottom of the well was covered by erythrocytes, and in a negative reaction the erythrocytes formed a pinpoint or circular residue in the center of the floor of the well.

Two bifunctional agents—the stable tetrazonium salt (diaminodiphenylamine tetrazotate) and glutaraldehyde—were both found to be suitable for production of 3-dimensional aggregates for sensitizing erythrocytes in the α_F-globulin—rabbit α_F-antiserum system tested in this investigation. Both these reagents were perfectly suitable for use in the aggregate hemagglutination test.

The first results of investigation of sera from patients with various types of cancer by this method are given below. In a series of experiments a positive aggregate hemaglutination reaction was obtained for several "negative hepatomas," i.e., embryonic α-globulin could be found in patients giving negative results by the precipitation method.*

"Negative" hepatomas were transferred to the "positive" class if the aggregate hemagglutination method was used for their determination (Table 1), although some "negative" hepatomas did not give positive reactions. The reason for this may be either absence of synthesis or of α_F-globulin, or because the content of α_F-globulin was very low. In the latter case, a further increase in sensitivity of determination in the aggregate hemagglutination test could lead to the transfer of these hepatomas also into the "positive" class. Further work in this direction is proceeding.

Embryonic α-globulin sometimes appears in the blood in the presence of other types of tumor [3]. It was found in the sera of patients with chorionepithelioma giving a negative precipitation test. In one case of teratoblastoma, the precipitation reaction was assessed as weak (±). The titer in the agglutination reaction was more than 1:40.

Because of the very high sensitivity of the aggregate hemagglutination reaction embryonic α-globulin could be detected in the serum of pregnant women although it was absent in donors' blood. This must be taken into account in the early diagnosis of these types of cancer in clinical practice.

*Thanks are expressed to S. D. Perova and A. I. Khazanov for providing the series of patients' sera tested for absence of α_F-globulin in the agar diffusion reaction.

The results described thus show that the early diagnosis of primary carcinoma of the human liver and of certain other tumors is possible by the aggregate hemaglutination method. Probably this method may also be found suitable for the diagnosis of certain other tumors secreting other antigens (toxohormone, antigen in carcinoma of the stomach, etc.), and also to verify the effectiveness of treatment.

LITERATURE CITED

1. G. I. Abelev, Acta Un. Internat. Cancer, 19, 80 (1963).
2. G. I. Abelev, I. V. Assekritova, N. A. Kraevskii, et al., Internat. J. Cancer, 2, 551 (1967).
3. G. I. Abelev, Cancer Res., 28, 1344 (1968).
4. A. M. Olovnikov, Dokl. Akad. Nauk SSSR, 169, No. 5, 1180 (1966).
5. A. M. Olovnikov, Immunochemistry, 4, No. 2, 77 (1967).
6. A. M. Olovnikov, in: Immunochemical Analysis [in Russian], Moscow (1968), p. 68.
7. Yu. S. Tatarinov, Vopr. Med. Khimii, No. 1, 90 (1964).
8. Yu. S. Tatarinov and A. I. Nogaller, Vopr. Onkol., No. 12, 26 (1966).
9. J. Uriel, B. de Neuchau, M. Stanislawski-Birencwaig, et al., C. R. Acad. Sci., 265, 75 (1967).