PLASMA LEVEL OF AUTOANTIBODIES TO THROMBIN IN HEALTHY SUBJECTS DETECTED BY AN IMMUNOCHEMICAL ANALYZER

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The writers showed previously that healthy human and animal blood plasma contains autoantibodies to thrombin and to factor Xa, which can inhibit the enzymic activity of the procoagulant and eliminate it from the blood stream [1]. Autoantibodies were detected by the passive hemagglutination method and by immunoassay (ELISA) [2]. Antibody levels were found to undergo regular changes in coagulopathies of varied genesis and this may provide a definite diagnostic test of the individual's hemostatic potential. Meanwhile the great urgency of the problem necessitates a ceaseless search for new methods of rapid and effective determination of autoantibodies and blood clotting factors with the aim of improving diagnosis, prognosis, and correction of coagulopathic states. The solution to these problems has become particularly urgent in recent years in connection with the appearance of new instruments for immunologic investigation, namely immunochemical analyzers. This paper describes the results of determination of autoantibody levels using the Beckman nephelometric analyzer.

EXPERIMENTAL METHOD

Plasma from healthy blood donors was used as the test substrate. The degree of dilution of the plasma was assigned by the computer system of the instrument and varied from 1:64 to 1:256. Human thrombin (from the Leningrad Institute of Hematology and Blood Transfusion) was used as the antigen. The antigen concentration in each test was between 0.5 and 0.016 mg/ml, and was determined by a calibration curve (dose—effect), also drawn by the computer of the instrument. Since the thrombin contained accompanying proteins (IgG, IgM, IgA), correction factors were used for the final calculation of the autoantibody levels. The principle of operation of the immunochemical analyzer is laser nephelometry.

In each determination the calculated quantity of thrombin in 42 μl of diluted buffer was added to 600 μl of the same buffer. The mixture was incubated for 5 min at room temperature, then centrifuged at 15,000 rpm in a refrigeration centrifuge at 4°C, and again introduced into the analytical a refrigeration centrifuge at 4°C, and again introduced into the analytical system of the instrument. The same specimen of plasma was analyzed 3 times. Pooled plasma from the donors was used in most investigations. The result was expressed in absolute figures and the percentage consumption of the total amount of immunoglobulins IgG, IgM, and IgA was calculated.

EXPERIMENTAL RESULTS

According to previous data [3] autoantibodies to blood clotting factors belong to the IgG class. These observations were made by the method of affinity chromatography (with sepharose 4B-thrombin or sepharose 4B-factor Xa as the immunosorbent). The eluted proteins were analyzed by rocket immunoelectrophoresis, using antisera against IgG, IgM, and IgA, antithrombin-III, α_2-macroglobulin, α_1-antitrypsin, etc. A positive result was obtained only with anti-IgG-serum. However, the method described above could not rule out the possibility of the presence of autoantibodies in other immunoglobulin fractions, which could not be detected by rocket immunoelectrophoresis because they were present in below-threshold concentrations.

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TABLE 1. Concentration of Autoantibodies to Thrombin

Thrombin concentration, mg/ml	Absolute concentration of autoantibodies in fractions, mg/ml			Concentration as a percentage of total autoantibodies		
	IgG	IgM	IgA	IgG	IgM	IgA
0,5	0,75	0,17	0,17	11,4	14,9	12,3
0,25	0,63	0,1	0,17	11,1	6,9	12,3
0,125	1,17	0,1	0,19	20,7	6,9	13,7
0,075	0,85	0	0	15,0	0	0
0,037	0,77	0	0	13,6	0	0
0,016	0,65	0	0	11,5	0	0

The results obtained with the immunochemical analyzer are given in Table 1.

The results in Table 1 show that autoantibodies to thrombin were recorded in all immunoglobulin fractions. However, the concentration of IgG-antibodies was highest. The dose–effect curve showed that the highest concentration of autoantibodies was observed to thrombin in a concentration of 0.125 mg/ml. Later, with a decrease in the thrombin concentration, the concentration of detectable autoantibodies also fell, but they ceased to be detected in the IgM and IgA fractions.

The concentration of autoantibodies to thrombin in the IgG fraction was comparable with the results obtained by affinity chromatography [3].

The discovery of autoantibodies belonging to the IgM-fraction is not unexpected. IgM are known to be the commonest immunoglobulins, playing the role of anticoagulants in inhibitor-dependent forms of hemophilia [4]. Moreover, we know from the general principles of pathology that manifestation of a pathological process is based on the extraordinary intensification of an existing physiological mechanism.

Thus the use of nephelometric analysis confirmed yet again the presence of autoantibodies to active blood clotting factors in healthy individuals. Further investigations will determine the relative importance of physiological anticoagulant-immunoglobulins in the genesis of coagulopathic disturbances.

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