QUANTITATIVE DETERMINATION OF CIRCULATING ANTIVASCULAR ANTIBODIES IN ATHEROSCLEROSIS BY THE AUTOCOMPLEMENT CONSUMPTION TEST

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UDC 616.13-004.6-07:616.15-097.5:616.13/14-07

KEY WORDS: atherosclerosis; antibodies against collagen; antibodies against elastin.

The detection of circulating antivascular antibodies in patients with atherosclerosis is of considerable interest from both the theoretical and the practical points of view, for we know that the main fibrous proteins of the vascular system – collagen and elastin – can cause the formation of corresponding antibodies in heterologous and autologous systems [3, 10, 11]. Meanwhile the known methods of determining antibodies with the aid of the complement fixation test are not sufficiently sensitive to detect autologous antibodies, and they also present some difficulty for statistical analysis and evaluation of the data.

The object of the present investigation was to develop a modification of the complement fixation test which would allow objective determination of the quantity of circulating antibodies against structural antigens of the human arterial wall in atherosclerosis, which would be highly sensitive and convenient for calculations.

Samples of blood serum from 53 clinically healthy subjects and patients with atherosclerosis were studied. Solutions of collagen and elastin in a concentration of 1 μg/ml, made up from the wall of an atherosclerotic human aorta by the method described previously, were used as the antigens [4].

The complement consumption test, suggested by Chudomel [8, 9] and modified by Ivanov [2], was used as the basis for development of an adequate quantitative method of investigating circulating antivascular antibodies. Advantages of this method include the possibility of using the subject's own complement in the reaction and of obtaining accurate objective quantitative data with the aid of a photoelectric colorimeter (PEC). However, the techniques usually adopted and the methods used to assess the results do not allow for the color of the test serum and of the antigens used, nor for their possible hemotoxic properties.

The suggested modification of the complement consumption test is free from these disadvantages. Its theoretical basis is as follows. In phase 1, the phase of fixation, antibodies of the test blood serum bind with the added antigen in the presence of autocomplement. In the second, hemolytic phase, the residue of complement binds with the added hemolytic system. After removal of erythrocytes the optical density of the experimental sample D_e is measured. The optical density of the control D_c is measured in the same way but without the addition of antigen. On the basis of the results it is possible to calculate what percentage (X) of autocomplement is bound in the test antigen–antibody system:

$$X = \frac{D_c - D_e}{D_c} \cdot 100, \quad (1)$$

This equation is theoretical and can be applied only to the case when all the media tested are completely transparent, which in practice is impossible. To obtain realistic results of the reaction, it is therefore necessary to introduce appropriate corrections.

If the optical density of the medium due to spontaneous (of nonimmune character) disintegration of erythrocytes (the "background density") is designated D_b, and the optical density of the test serum D_{ts}, the true optical density of the control sample D_c will differ from the density D_m, measured on the PEC, by the values D_b and D_{ts}, i.e.

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 12, pp. 690-692, December, 1980. Original article submitted February 25, 1980.

$$D_c = D_{cm} - D_b - D_{ts}. \quad (2)$$

When the optical density of the sample is measured in the experiment it is necessary to take account also of the density due to protein of the antigen itself, and also its possible effect on the hemolytic system, which, allowing for "background" density, is given by $D_{cmag} - D_b$, where D_{cmag} is the density of antigen measured on the PEC in the control. The true optical density of the experimental sample D_e must thus be composed of the measurement of D_{ag} obtained on the PEC without the densities of the serum and antigen and the "background" density, i.e.

$$D_e = D_{ag} - D_b - D_{ts} - (D_{cmag} - D_b) \quad (3)$$

In that case, Eq. (1), allowing for corrections introduced by Eqs. (2) and (3), and after appropriate transformations, assumes the form:

$$X = \frac{D_{cm} - D_{ag} - D_b + D_{cmag}}{D_{cm} - D_b - D_{ts}} \cdot 100. \quad (4)$$

The practical method of conducting the reaction to determine the optical density of the experimental sample is as follows: 1) to 0.25 ml of antigen 0.25 ml of the test serum is added; 2) the mixture is incubated at 37°C for 1 h; 3) 4.5 ml of 0.85% NaCl solution is added; 4) to 0.25 ml of the resulting mixture 0.75 ml of 0.85% NaCl solution and 2 ml of hemolytic system with a 3% suspension of sheep's red blood cells are added; 5) the mixture is incubated at 37°C for 30 min; 6) the red blood cells are sedimented by centrifugation at 3000 rpm for 5 min; 7) to 2 ml of the supernatant 2 ml of 0.85% NaCl solution is added; 8) the optical density of the solution is measured on the FÉK-56M photoelectric colorimeter-nephelometer with a No. 3 filter.

For the necessary control measurements the corresponding components of the reaction must be replaced by an equal volume of 0.85% NaCl solution.

A study of samples of blood serum from 23 clinically healthy subjects by the suggested method showed that on addition of the corresponding antigen anticollagen antibodies bound 4.7 ± 1.9%, and antielastin antibodies bound 29.4 ± 3.5% of the autologous complement. These are probably so-called "normal" autoantibodies participating in protein metabolism and present in all healthy human subjects and animals, in particular antibodies against arterial tissue [5, 7, 11].

During investigation of the blood serum of 30 patients with atherosclerosis anticollagen antibodies bound only 0.8 ± 0.3% whereas antielastin antibodies bound 17.6 ± 2.4% of autocomplement, i.e., in these patients the titers of circulating antibodies against both collagen ($P < 0.05$) and elastin ($P < 0.01$) were reduced.

The decrease in content of circulating antivascular antibodies in atherosclerosis was evidently due to their fixation on the exposed antigenic determinants of the destroyed structural proteins of the atherosclerotic vascular wall. Deposition of antibodies in the tissues in the presence of complement under these circumstances led to the development of an arteriopathy, followed by pathophysiological, morphological, and biochemical changes [1], facilitating further progression of the atherosclerotic process [6, 11].

The method of determining antibodies suggested above on the basis of absorption of autocomplement can thus be used to detect small quantities of circulating antivascular antibodies in patients with atherosclerosis.

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