LATENT AUTOANTIBODIES TO CARDIOLIPIN IN HEALTHY HUMAN SERUM

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The writers have postulated a possible way of regulation of humoral immunity through blocking of the immunologic activity of polyspecific autoantibodies by charged biopolymers contained in blood serum [1]. Definite evidence in support of this hypothesis can be obtained by the discovery of immunologic activity of autoantibodies to autoantigens after separation of intermolecular complexes.

In the present investigation a pool of healthy human serum was fractionated relative to charge by ion-exchange chromatography, and relative to molecular size by dissociation of immune complexes.

EXPERIMENTAL METHOD

Serum proteins were fractionated on QAE-Sephadex A-50 ("Pharmacia") as described previously [2]. The two fractions obtained were dialyzed against phosphate-buffered saline (PBS), containing 0.01 M phosphate and 0.15 M sodium chloride (pH 7.2), and concentrated by ultrafiltration to the original volume.

The serum was fractionated by gel-filtration on a column (2.6 × 80 cm) with Sephadex G-200 ("Pharmacia"), proteins being eluted: a) with 0.1 M acetate buffer, pH 4.05, b) PBS, and c) 0.01 M carbonate buffer, pH 9.2. The fractions were neutralized and tested for the presence of autoantibodies of the IgG and IgM classes to cardiolipin (CL) by enzyme immunoassay (EIA). Fractions responding to the same peak on the elution profile were pooled, dialyzed against PBS, and concentrated by ultrafiltration to the applied volume. Immunoglobulins were concentrated by Mancini's method, using reagents from the Research Institute of Epidemiology and Microbiology, Ministry of Health of the RSFSR, Gor'kii.

Binding of autoantibodies with CL and EIA were carried out as follows. Into each well on a panel ("Nunc") was added 25 μliters of a solution of CL in ethanol (Khar'kov Bacterial Preparations Factory) with a concentration of 100 μg/ml, and the sample was evaporated to dryness overnight at 4°C. After washing with PBS, to each well was added 75 μliters of a 10% solution of bovine serum albumin in PBS in order to block nonspecific binding sites, after which the panel was incubated for 1 h at room temperature, and again washed with PBS. To each well was then added 50 μliters of the test specimen, and the panel was incubated for 1.5 h at room temperature and washed with PBS. The presence of autoantibodies of the IgG and IgM classes was demonstrated with the aid of conjugates of antibodies to IgG or IgM with peroxidase ("Sevac"), the conjugates being added to the wells in a volume of 50 μliters and in the corresponding dilution. Dilutions of the test samples and conjugates were made with PBS containing 10% bovine serum. The substrate was o-phenylenediamine ("Reakhim"). Enzyme reaction continued for 30 min at room temperature and optical density was measured at 492 nm after the reaction had been stopped by addition of 1 M sulfuric acid solution.

To obtain quantitative data on the concentration of autoantibodies to CL, a set of standards with known concentrations of anti-CL antibodies was used (generously provided by Dr. E. N. Harris, The Rayne Institute, St. Thomas's Hospital, London). The concentration of autoantibodies to CL was expressed in GPL or MPL, the unit of GPL (MPL) being taken to be the binding activity of affinity antibodies with a concentration of 1 μg/ml of IgG (IgM) [6].

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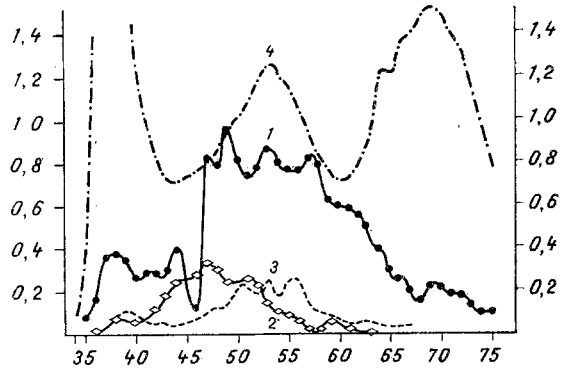

Fig. 1

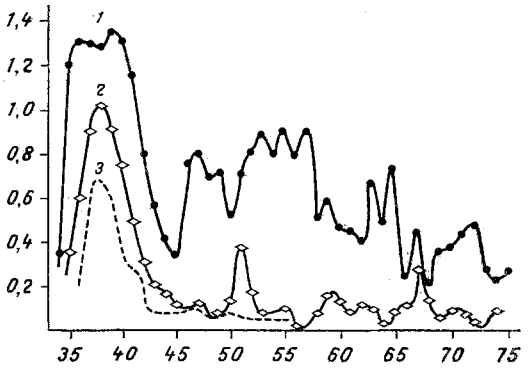

Fig. 2

Fig. 1. Interaction of cardiolipin, immobilized on the solid phase, in EIA with IgG contained in various fractions of pooled human serum after gel-filtration through Sephadex G-200 at different pH values: 1) 4.05, 2) 7.2, 3) 9.2; 4) elution profile. Abscissa, Nos. of fractions; ordinate: on left – optical density at 492 nm, on right – at 280 nm.

Fig. 2. Interaction of cardiolipin immobilized on the solid phase in EIA with IgM contained in different fractions of pooled human serum after gel-filtration through Sephadex G-200 at different pH values: 1) 4.05, 2) 7.2, 3) 9.2. Abscissa, Nos. of fractions; ordinate, optical density at 492 nm.

EXPERIMENTAL RESULTS

It was shown that the concentration of autoantibodies to CL of the IgG class in pooled serum obtained from 100 healthy blood donors was 2.84 ± 0.20 GPL, and the concentration of autoantibodies of the IgM class in the same population was 0.28 ± 0.02 MPL.

The separate the conjecturally pre-existing complexes of autoantibodies to CL with serum biopolymers two approaches were used: fractionation by ion-exchange chromatography on a strong anion exchanger, and gel-chromatography at acid, neutral, and weakly alkaline pH values.

The results of binding of serum immunoglobin fractions obtained by gel-filtration of a pool of blood sera on Sephadex G-200 at different pH values, with CL are given in Figs. 1 and 2. The ability of the immunoglobulins to bind with CL, acquired as a result of gel-filtration, was more marked at pH 4.05 than at pH 7.2 and 9.2. It will be noted that proteins constituting the 3rd peak of the elution profile, with molecular weight of under 100,000 D, react with CL. They are probably fragments of immunoglobulins.

After pooling and concentration of fractions belonging to the corresponding peak on the elution profile (1st pooled fraction with Nos. 35 to 42 inclusive, 2nd pooled fraction with Nos. 46 to 59 inclusive), interaction of the immunoglobulins present in these pooled fractions with CL was studied and compared with that of immunoglobulins from the intact serum pool. The starting point was equality of the IgG or IgM concentrations in the pooled fractions and in the intact sample (with the accuracy of analysis by Mancini's method).

Antibody activity of the IgM class to CL was found to be concentrated mainly in the 1st pooled fraction, and of the IgG class, in the 2nd fraction. If total IgG- or IgM-antibody activities to CL of the pool of sera was estimated after gel-filtration on Sephadex G-200, as follows from Fig. 3, activity rose considerably on elution with buffer at pH 4.05, but increased by a much lesser degree at pH 9.2, whereas virtually no change was found in the case of elution with buffer at pH 7.2. The concentration of anti-CL antibodies in a given pool was determined with the aid of titrated standards as 1.6 GPL and 0.5 MPL, whereas after elution with buffer at pH 4.05 it was 12.6 GPL, and 2.6 MPL in the 2nd and 1st pooled fractions, respectively.

A similar investigation was undertaken with positively (1st fraction) and negatively (2nd fraction) charged fractions after chromatography of a pool of sera through QAE-Sephadex. It was shown by Mancini's method that IgG are eluted in both fractions, but mostly the 1st, IgM mostly in the 2nd. Total antibody IgG- or IgM-activities of serum fractionated on QAE-Sephadex exceeded the analogous value for intact serum (Fig. 3), but this increase was less

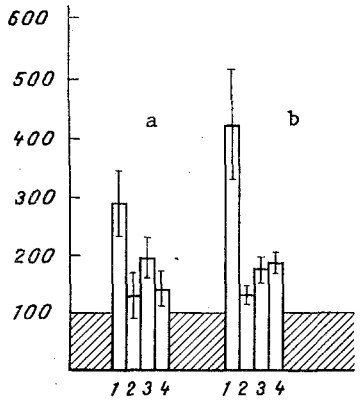

Fig. 3. Total anticardiolipin activity of IgG (a) and IgM (b) classes in pooled serum from healthy blood donors, fractionated under various conditions. 1) Gel-filtration through Sephadex G-200, pH 4.05; 2) gel-filtration through Sephadex G-200, pH 7.2; 3) gel-filtration through Sephadex G-200, pH 9.2; 4) chromatography on QAE-Sephadex. Ordinate, binding (in % of intact blood serum).

than with gel-filtration at pH 4.05, and corresponded approximately to changes observed for elution at pH 9.2

The results are evidence that autoantibodies to CL are present in intact serum in higher concentrations than can be found by ordinary methods. Most of them were shown to occur in a latent form as complexes with other serum components. Deligandization of immunoglobulins has the most favorable effect on fractionation of serum by molecular size under conditions of dissociation of immune and other complexes: to a greater degree elution at pH 4.05, to a lesser degree on elution at pH 9.2, and virtually not at all on elution at pH 7.2. Fractionation on QAE-Sephadex, accompanied by separation of aggregates formed by electrostatic forces, also leads to activation of anti-CL antibody activity.

Consequently, intermolecular interactions in blood serum play an important role in the regulation of humoral immunity and, in particular, in the regulation of activity of antibodies to a widely distributed antigen such as cardiolipin. Disturbances in this system may be the cause of many pathological conditions [4]. The important role of anti-CL antibodies in the development of the intravascular clotting syndrome and in disturbances of the course of pregnancy may be mentioned [3, 5].

Latent antibodies to DNA were discovered by the writers recently in healthy human blood serum [2]. It is interesting to note that antibodies to DNA, including monoclonal, can bind CL [7], and that antibodies induced by injection of CL react with DNA [8]. It can be tentatively suggested that a certain population of immunoglobulins, possessing low specificity, interacts with DNA and CL. As a result of their polyspecificity, these autoantibodies may perhaps be capable of reacting with other structures, carrying a negative charge under physiological conditions.

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