were then seeded into a fresh nutrient medium [9] - a finding that is another good indication that adhesive properties are altered in laser-irradiated cells.

In summary, the present results show that a brief (10 sec) exposure of He-Ne laser radiation entails complex and protracted temporal (over 3 h) variations in the strength of intercellular adhesion.

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Primary Interaction of "Latent High-Avidity Antibodies" from Preparations of Gamma-Globulin is Probably Determined by the Presence of a Cofactor

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UDC 616.5-002.525.2-07:616.153.962.4-078.33-073.916

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 6, pp. 624-625, June, 1993
Original article submitted January 23, 1993

Key Words: *immunoglobulins; latent high-avidity antibodies to DNA; Farr test*

The presence of high-avidity autoantibodies against native DNA (nDNA), which can be revealed by the Farr test (radioimmunoassay with precipitation by 50% ammonium sulfate in the presence of a coprecipitant), is an important criterion of systemic lupus erythematosis [3, 7].

Previously we isolated a fraction of high-avidity proteins from commercial preparations of human γ-globulin, whose interaction with nDNA may be revealed with the Farr test. This property was explained by the presence of latent natural DNA-reactive antibodies in the total pool of γ-globulins which are released from the complex during ion-exchange chromatography [6].

In the present study the role of different isotypes of immunoglobulins in newly detectable nDNA-binding activity was investigated.

MATERIALS AND METHODS

Commercial preparations of human γ-globulin (or immunoglobulin) were used in the experiments. The methods for isolation of the nDNA-binding fraction and determination of nDNA-protein inter-

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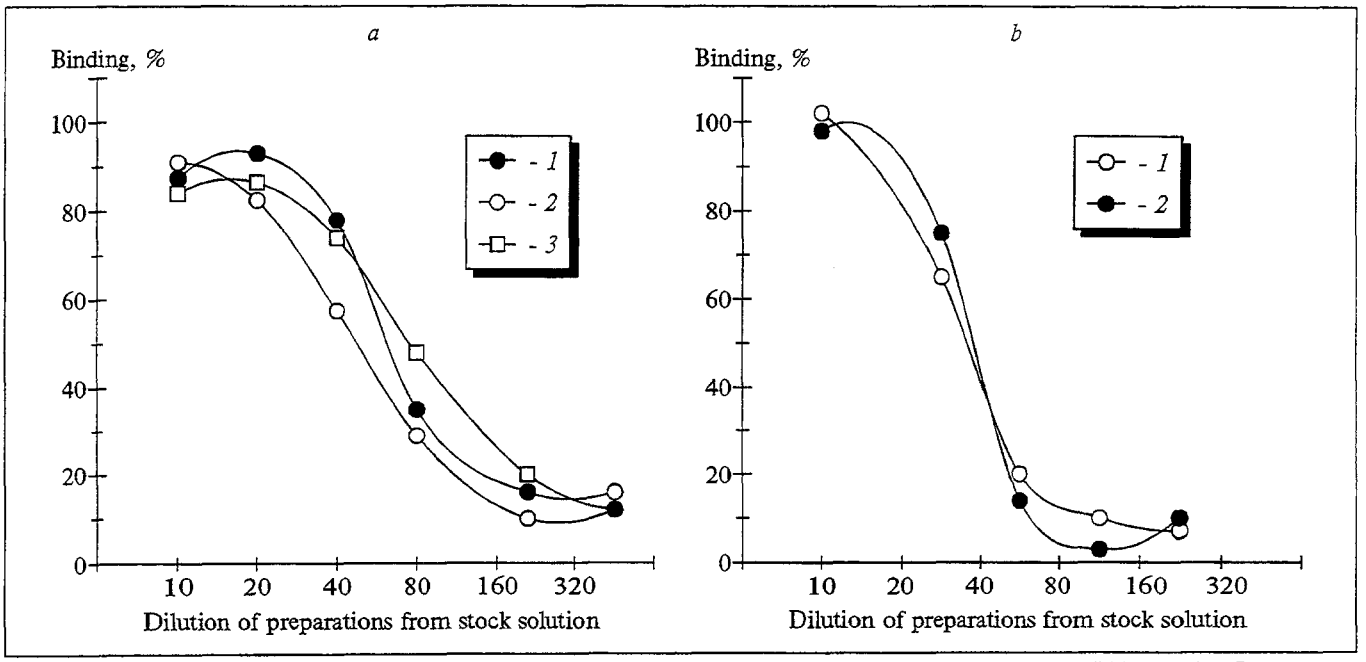

Fig. 1. Effect of different classes of human serum Ig on the interaction between AaP fraction and nDNA in the Farr test. a: 1) AaP fraction treated with Sepharose CL-4B (concentrations: IgG 180.5 and IgA 627.1 μg/ml); 2) the same treated with Protein A-Sepharose CL-4B (concentrations: IgG <0.3, IgA 300.6 μg/ml); 3) the same treated with Immobilized Jacalin (concentrations: IgG 173.2, IgA 292.8 μg/ml); b: 1) original AaP fraction, 2) AaP fraction treated with 2-mercaptoethanol.

action after Farr were the same as described earlier [6]. ^{3}H-DNA was prepared according to a modified method [4] and treated and tested as described previously [2].

Human IgG and IgA were isolated using commercial sorbents: Protein A-Sepharose CL-4B (Pharmacia-LKB, Sweden) and Immobilized Jacalin (Pierce, The Netherlands). In brief, 0.5 ml 50% sorbent suspension in phosphate buffer saline (PBS) containing 0.01 M phosphate and 0.15 M NaCl was added to 1 ml of the protein solution and incubated at room temperature for one hour. The mixture was chemically treated with 0.1 M 2-mercaptoethanol at 37°C for 3 hours to prevent IgM binding to the antigen [5].

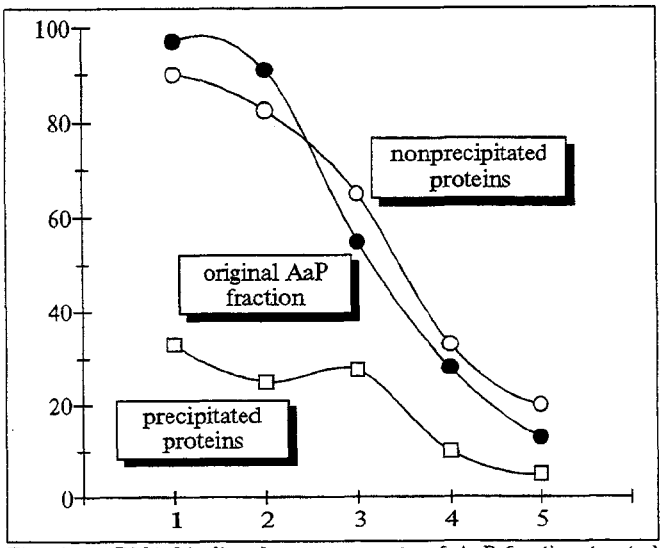

Fig. 2. nDNA binding by components of AaP fraction treated with 50% ammonium sulfate. 1) original AaP fraction; 2) nonprecipitated proteins; 3) precipitated proteins.

The Ig concentration was determined by ELISA. Multiwells (MaxiSorp, Nunc, Denmark) were covered with affinity isolated anti-IgA, anti-IgM or F(ab)'$_2$ fragments of human anti-IgG (Sigma, USA) by incubating the corresponding antibody solutions (10 μg/ml in PBS, 100 μl per well) overnight at 4°C. The wells were then washed three times with PBS containing 0.05% Tween 20 (PBST), blocked with 5% bovine serum albumin (BSA) (Serva, Germany) in PBS, and washed three times with PBST. The standards and experimental solutions in PBST containing 1% BSA (PBST/BSA) were added in a total volume of 100 μl, incubated for 2 hours, washed three times with PBST and then incubated for 2 hours with the corresponding anti-human Ig-peroxidase conjugates dissolved in PBST/BSA: F(ab)'$_2$ anti-IgM and F(ab)'$_2$ anti-IgG (Sigma, USA) 1:1000 or anti IgA 1:4000 (Sevac, Czechoslovakia) in a volume of 100 μl and washed three times with PBST. Ortho-phenylenediamine (Sigma, USA) was used as a chromogen. The optical density was measured with a "Multiskan" reader (Titertek, Finland).

RESULTS

We reported previously that a protein fraction was isolated by means of ion-exchange chromatography

on QAE-Sephadex A-50 in 0.01 M sodium acetate buffer (pH 4.4) (anion-adsorbed proteins, AaP), which can be eluted from the sorbent with the same buffer containing 0.5 M NaCl. These proteins were shown to interact with nDNA in the radioimmune precipitation test [6]. Since the Farr test is considered to reveal the Ig-ligand complexes (generally radioactive), we attempted to determine which class of human serum Ig is responsible for high-avidity nDNA binding. To this end the AaP fraction was treated either with immunosorbents for removing IgG and IgA or chemically for IgM degradation, and then the capacity to nDNA binding was tested. As follows from the results, complete elimination or substantial depletion (in the case of IgG and IgA), as well as chemical modification (in the case of IgM) of either human serum Ig from the AaP fraction cause just minor changes in the nDNA binding in the Farr test (Fig. 1). Moreover, the supernatant obtained after treatment of the AaP fraction with saturated ammonium sulfate (up to 50% saturation) at 4°C (this procedure was shown by ELISA to result in precipitation of 90% total Ig) interacts with nDNA even more actively than the precipitate containing the bulk of Ig (Fig. 2).

These findings allow us to conclude that the AaP fraction contains, apart from Ig, non-immunoglobulin factors which form a primary stable complex with nDNA. This complex may be able to precipitate in half-saturated ammonium sulfate solution either "by itself" or, more likely, as aggregates with any one subpopulation of bovine Ig (co-precipitant), which, in this particular case, acts as high-avidity antibodies. The factor responsible for primary nDNA binding has to be capable of interacting with both nDNA and Ig acting as an "adapter" between nDNA and Ig.

A similar phenomenon has been reported for the interaction between antibodies and cardiolipin occurring in the presence of β_2-glycoprotein I [1].

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