REACTIONS OF NORMAL HUMAN AND RABBIT IMMUNOGLOBULINS WITH HEART VALVE FIBROBLASTS

T. A. Danilova, L. M. Bartova,
A. A. Beglaryan, and R. L. Panurina

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It was shown by the indirect immunofluorescence method that normal human and rabbit sera, and also IgG isolated from them can react intensively with human and bovine heart valve fibroblasts. Investigations with the Fab and Fc fragments of IgG suggest that this reaction may take place because of the Fc fragments of the IgG molecule and that it is connected with the presence of an Fc receptor on heart valve fibroblasts.

KEY WORDS: fibroblasts; immunoglobulin G; Fab fragment; Fc fragment; Fc receptors

In rheumatic fever the pathological changes are located mainly in the connective tissue of the heart. The study of antibodies against connective tissue antigens is therefore very important. The writers' previous investigations showed that sera of patients with rheumatic fever react with cells of the interstitial tissue of the bovine heart and the hearts of other animals [2, 3]. Kaplan and Clemente [10] described reactions of the sera of patients with rheumatic fever with fibroblasts from human heart valves. Similar results were obtained in our own experiments [4], but by contrast with the observations of Kaplan and Clemente, intensive reactions of donors' sera were found with the same heart valve cells. More recently Fc receptors have been found in various tissues [9, 12] and, as several workers have found, reactions of normal sera with these tissues can take place because of the Fc fragments of the immunoglobulin molecule [5, 6].

The object of this investigation was a detailed study of the reactions of normal human and rabbit sera with fibroblasts of human and bovine heart valves and to attempt to elucidate the nature of these reactions.

EXPERIMENTAL METHOD

Sera from healthy adults, from infants under one year of age, and from unimmunized rabbits in dilutions of 1:4-1:256 were studied by the indirect immunofluorescence method. All sera were studied without preliminary absorption with mouse liver powder. Immunoglobulins G were isolated from the human and rabbit sera on DEAE-cellulose.

Pure antibodies against group A streptococcal polysaccharide were obtained from antistreptococcal rabbit sera by the method of Osterland et al., [14], by hydrolysis of the precipitate in acid medium followed by separation of the antibodies on a Sephadex G-100 column [1]. A preparation of antibodies against bovine myocardial fibroblasts was obtained from the sera of patients with rheumatic fever with the aid of an immunosorbent containing bovine connective tissue antigens [3].

To obtain $F(ab')_2$ fragments, human and rabbit IgG were digested with pepsin [13]. The $F(ab')_2$ fragments were isolated from the digest on Sephadex G-200 [8]. A monovalent pepsin Fab' fragment of rabbit IgG was obtained by reduction of bivalent $F(ab')_2$ fragment with 0.01 M 2-mercaptoethanol and purified to remove unreduced protein by chromatography on Sephadex G-200. A papain Fab' fragment of rabbit IgG was obtained by Porter's method [15] from the pepsin $F(ab')_2$ fragment.

Fc and Fab fragments of human IgG were obtained by hydrolysis with papain [14]. The fragments were purified from undigested IgG on Sephadex G-150. The Fc fragment was separated from the Fab fragment by chromatography on DEAE-cellulose followed by adsorption on an immunosorbent prepared from rabbit serum against human Fab fragment, polymerized with the aid of glutaraldehyde [7]. The degree of purification of all the products was tested by the agar precipitation test with various antisera: IgG with serum against rabbit or

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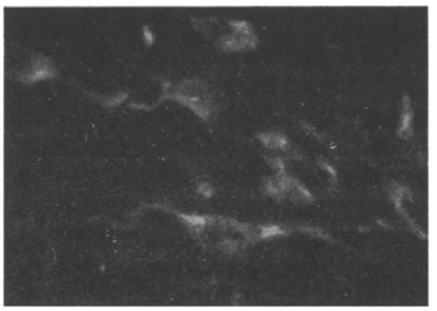

Fig. 1. Reaction of normal rabbit IgG with bovine heart valve fibroblasts. Indirect immunofluorescence method, 120×.

human sera respectively, the Fab fragments with serum against IgG and against the Fc fragment of the IgG molecule; the Fc fragment with serum against IgG and against the Fab fragment.*

The investigations were carried out by the indirect immunofluorescent method, using pure antibodies against human or rabbit IgG, prepared with the aid of an immunosorbent by the method of Avrameas and Ternynck [7] and labeled with fluorescein isothiocyanate. The sample of pure antibodies, as shown by the gel precipitation test, contained antibodies against Fab and Fc fragments of the IgG molecule. The conditions of preparation and labeling of the antibodies were described previously [2].

The products listed above were tested on sections of bovine and human blood group 0 heart valves. Sections 4μ were cut in a cryostat from tissue frozen to −70°C and they were used unfixed. The test product was applied for 30 min at room temperature to sections washed with buffered 0.85% NaCl solution, pH 7.0, for 5 min, and allowed to stand for 18 h at 4°C. Fuller details of the method of treating the sections were described previously [2]. The reaction was read by means of the ML-2 luminescence microscope in blue-violet light and by the LYUMAM I-2 microscope in ultraviolet light. RF-3 film was used for photography.

EXPERIMENTAL RESULTS

During the immunofluorescence study without preliminary absorption with mouse liver powder, all the normal human and rabbit sera tested reacted with human and bovine heart valve fibroblasts. During titration on sections of bovine valves the rabbit sera reacted with fibroblasts to a dilution of 1:256, and on sections of human valves to a dilution of 1:64. Samples of normal rabbit IgG in a concentration of 500 μg/ml also reacted strongly with bovine and human valve fibroblasts – with an intensity of +++ (Fig. 1). The study of different preparations of Fab fragments of rabbit IgG yielded inconsistent results. The reaction was completely preserved when the double pepsin $F(ab')_2$ fragment was used. When the monovalent pepsin Fab fragment or the papain Fab fragment [obtained from the $F(ab')_2$ fragment] was applied to the section the degree of fluorescence was considerably reduced on bovine valve and fluorescence disappeared completely from sections of human valve. The results were unchanged if the concentration of monovalent Fab fragments was increased by 2 to 4 times (up to 2 mg/ml) compared with the IgG concentration.

Donors' sera reacted with fibroblasts to a dilution of 1:64–1:128 on sections of bovine valve and to a dilution of 1:16–1:32 on sections of human valve. Children's sera reacted with valve fibroblasts in the same dilution. As a result of titration of four samples of immunoglobulin isolated from individual donors' sera, inconsistent results were obtained: Minimal protein concentration at which a reaction was observed on sections of human valve was 400 μg/ml–3 mg/ml, and on sections of bovine valve 60–230 μg/ml.

Besides normal sera, the sera of patients with rheumatic fever, rabbit sera against group A streptococcus, and also the preparations of immunoglobulins isolated from sera of both species also had the ability to react with valve fibroblasts. Pure antibodies against streptococcal A polysaccharide, isolated from the sera of animals immunized with group A streptococcus, and antibodies against bovine myocardial fibroblasts, isolated from the sera of patients with rheumatic fever by means of an immunosorbent containing bovine connective tissue antigens were the exceptions.

*The antisera were prepared in the Laboratory of Fluorescent Sera, N. F. Gamaleya Institute of Epidemiology and Microbiology.

During tests of three series of pepsin $F(ab')_2$ fragments obtained from normal human IgG a distinct reaction was observed, especially on bovine valve, with fibroblasts with an intensity of + to +++. When preparations obtained by hydrolysis of IgG with papain were tested, positive results were observed with both the Fab and the Fc fragments. Parallel titration of both preparations on sections showed that a positive reaction with the Fab fragment is observed in a much higher concentration (0.5-1 mg/ml protein) than with the Fc fragment (0.125 mg/ml).

The results suggest that the reaction of normal human and rabbit IgG with heart valve fibroblasts takes place on account of the Fc fragments of the molecule. The positive reaction of the $F(ab')_2$ fragment is evidently due to contamination with Fc fragment or with undigested IgG, which cannot be detected by the immunodiffusion method. Evidence in support of this view is given by results obtained with monovalent Fab fragments. Papain human Fab fragments obtained by hydrolysis of IgG gave a positive reaction with fibroblasts. Meanwhile the reaction of monovalent rabbit Fab fragments obtained from the $F(ab')_2$ fragments, i.e., much more highly purified, was very weak or virtually absent.

The results of the study of sera from healthy children under one year of age, which reacted with valve fibroblasts in the same dilution as healthy donors' sera, confirmed that the reactions observed are unconnected with the presence of antibodies against fibroblasts in the sera.

The question why two preparations of antibodies – those against streptococcal A polysaccharide and against bovine myocardial fibroblasts – did not react with fibroblasts still remains unanswered. The reason may perhaps be that different subclasses of IgG differ in their ability to bind with the Fc receptors on the cells [11]. Preliminary experiments with the use of different subclasses of IgG showed that immunoglobulins belonging to the G_1 subclass bind most intensively with valve cells.

The reactions of normal human and rabbit sera with heart valve fibroblasts are thus evidently connected with the presence of Fc receptors on these cells. These findings must be borne in mind during the study of autoantibodies against antigens of valve fibroblasts in autoimmune processes.

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