Heterophilic Antibodies to Connective Tissue Antigens in the Blood Sera of Rheumatic Patients Do Not Cross-React with Group A *Streptococcus* Antigens

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Heterophilic antibodies reacting with antigens of interstitial connective tissue of bovine myocardium were found in the sera of patients with rheumatic fever. These antibodies were referred to class IgG. Immunologic specificity of the reaction with these antigens was confirmed in experiments with $F(ab')_2$ fragments from IgG isolated from the sera of rheumatic patients. Heterophilic antibodies were not adsorbed by various antigens of group A *Streptococcus,* nor were they isolated in a column with immunosorbent prepared on the basis of nontype-specific streptococcal antigens. The reaction of patients' sera was not inhibited by monoclonal antibodies to nontype-specific antigens cross-reacting with antigens of myocardial interstitial connective tisSue.

Key Words: *heterophilic antibodies; rheumatic fever; streptococcal cross-reacting antigens*

Autoantibodies to muscle fiber antigens in rheumatic patients have been studied in detail [14,15], but the presence in these patients of autoantibodies to connective tissue antigens is still a matter of debate. Our previous studies by indirect immunofluorescence revealed heterophilic antibodies reacting with antigens of interstitial connective tissue (ICT) of bovine and other animals' myocardium [3,5]. These antibodies did not react with antigens of human myocardial ICT.

Heterophilic antibodies (i.e., antibodies detected only in a heterologous tissue system) have been found in humans in various diseases: infectious, autoimmune, tumor [5,13]. Cross-reacting antigens of microorganisms may be among the factors conducive to their appearance. For example, antigens cross-reacting with ForsSman's antigen and heterophilic transplantation antigen are found in enterobacteria, pneumococci, and group C streptococci [5]. Experiments have shown that cross-reacting antigens of group A *Streptococcus* can induce the production of heterophilic antibodies in animals. Heterophilic antibodies to human myocardial ICT antigens have been found in the sera of rabbits immunized with nontype-specific (NTS) streptococcal antigens; these antibodies did not react with homologous myocardial structures [7]. Monoclonal antibodies to NTS antigens cross-reacting with human myocardial ICT antigens have been obtained on mice [1].

The aim of this study was to detect cross reactions between heterophilic antibodies in the sera of rheumatic patients and group A streptococcal antigens; for this purpose rheumatic patients' sera were adsorbed with preparations of various streptococcal antigens and antibodies were isolated from patient sera using an immunosorbent based on NTS antigens.

MATERIALS AND METHODS

Blood sera of patients with rheumatic fever in the active and inactive stage of the disease were tested. The sera were obtained from the Institute of Rheumatology and N. I. Pirogov Moscow Medical University. IgG were isolated from the sera by ion-exchange chromatography on DEAE-cellulose.

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$F(ab')_2$ fragments were obtained by IgG treatment with pepsin [12] followed by gel filtration on a column packed with Sephadex G-200. A group A *Streptococcus* culture was grown in broth with casein hydrolysate. For the preparation of NTS antigens, washed pellet of heat-killed type 29M bacterial cells was subjected to extraction with a 3.5 M solution of KCNS [8]. The resultant extracts were dialyzed against normal buffered saline (pH 7.0) and lyophilized. Immunosorbent was prepared on the basis of porous cellulose balls [6] by taking 40 mg of NTS fraction proteins. Ready adsorbent was loaded onto the column and 3-5 ml of serum (a pool consisting of 2-3 sera) were layered onto it. Glycine-HCl buffer (pH 2.8) was used for antibody elution. The antibody preparations were lyophilized or concentrated with polyethyleneglycol. Streptococcal cell walls and cytoplasmic membranes were obtained by differentiated centrifugation [2]. Polysaccharides A and A-variant were obtained by formamide extraction [9]. Antibodies to NTS antigens in patient sera were detected by enzyme immunoassay (EIA). NTS antigens were placed in wells in a concentration of 10 μg/ml. Antibodies to myocardial ICT antigens were detected in heart tissue slices by indirect immunofluorescence using pure antibodies to human IgG [3].

RESULTS

Rheumatic patients' sera tested on myocardial slices reacted with fibroblasts and capillary cellular elements of ICT (Fig. 1). In the active phase of the disease the reaction with ICT antigens was detected in 51.3±3.3% of cases, that is, 3 times more frequently than in patients with inactive rheumatism and 4 times more frequently than in donors (Table 1).

Using monospecific FITC-labeled sera to human IgG, IgM, and IgA (manufactured by the N.F.Gamaleya Research Institute of Epidemiology and Microbiology), we revealed that the heterophilic antibodies belonged to class IgG. Moreover, IgG fractions isolated from antibody-containing sera of rheumatic patients intensively cross-reacted with myocardial ICT cells. Immunologic specificity of the reactions with myocardial ICT antigens was confirmed in experiments with IgG $F(ab')_2$ fragments isolated from patient sera. Examination of 5 lots of $F(ab')_2$ fragments showed that in concentrations of 0.5 to 1.0 mg/ml all of them reacted with ICT antigens. Hence, the reactions of rheumatic patients' sera with myocardial ICT cells are caused by the active center of the antibodies.

Highly intensive reactions with bovine myocardial ICT cells (+++ - ++++) were observed in

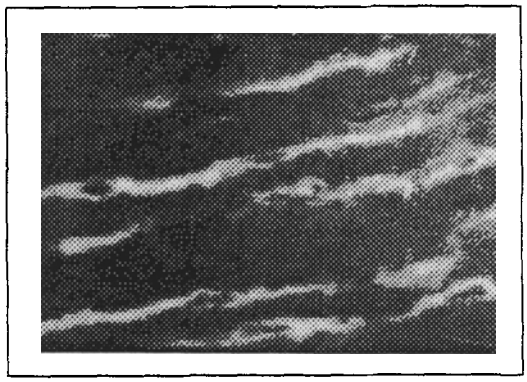

Fig. 1. Reaction between a rheumatic patient's serum and bovine myocardial ICT cells. Obj. 40, homal 3.

22% of sera from patients with active rheumatism. Maximal titers of these sera were 1/64-1/128. Such sera were selected and used for studies of cross-reactivity of heterophilic antibodies.

Table 2 lists the streptococcal preparations and doses used for adsorption of patient sera. As a rule, doses which could remove from the sera antibodies to respective antigen were used for adsorption. Testing of 4 different lots of NTS antigen preparation revealed that none of them eliminated the reaction of patient sera with myocardial ICT cells. The results of serum adsorption with streptococcal cell walls and membranes were negative, too. Polysaccharides of group A streptococcus and A-variant similarly failed to remove heterophilic antibodies from patient sera.

In the next series of experiments rheumatic patient sera containing heterophilic antibodies were passed through a column with NTS immunosorbent. Antibody eluates were tested with bovine heart tissue slices and in EIA in a concentration of 1 mg/ml. Three series of experiments with 3 different pools of sera were carried out. In none of the cases did the isolated antibodies react with the bovine heart tissues. As for the reactions with NTS antigens, all the initial sera reacted in EIA with KCNS extract in 1/400- 1/800 dilutions. Antibody eluates were positive in EIA as well. Hence, antibodies isolated from patient sera on NTS immunosorbent and retaining the capacity to

TABLE 1. Detection of Heterophilic Antibodies to Bovine Myocardial ICT Antigens in the Sera of Rheumatic Patients

Group examined	Number of patients	Number of positive reactions, $\%\pm m$
Healthy donors	128	13.2±2.9
Active rheumatism	226	51.3±3.3*
Inactive rheumatism	97	17.5±3.8

Note. Asterisk shows reliable differences $p<0.01$ in comparison with the inactive rheumatism phase and $p<0.001$ in comparison with donors.

TABLE 2. Results of Adsorption of Heterophilic Antibodies with Various Streptococcal Preparations

Preparation	Antigen dose per ml serum in working dilution	Presence of reaction with myocardial ICT antigens
Cell walls	10–20 mg	+
Cytoplasmic membranes	10–20 mg	+
NTS antigens	10 mg	+
A–polysaccharide	200 μg	+
A–variant of polysaccharide	200 μg	+

interact with a specific antigen did not react with bovine myocardial ICT cells.

Inhibition experiments with monoclonal antibodies similarly confirmed the absence of cross-reactions between heterophilic antibodies in the sera of rheumatic patients and streptococcal NTS antigen. B6/5 monoclonal antibodies were used, obtained as a result of hybridization of splenocytes of a mouse immunized for a long time with NTS antigens and of Sp2/0 myeloma cells (B6/5 antibodies were graciously offered by V. N. Abyzov). These antibodies intensively reacted with human and animal myocardial ICT cells. B6/5 antibodies were layered onto bovine myocardial slice for 1 h at room temperature. After washing, some slices were fixed in acetone at 4°C (in order to better fix the antibodies on the slice) and then patient sera containing heterophilic antibodies were layered on. Five sera from rheumatic patients were not used in these experiments; reactions with bovine myocardial ICT cells were not inhibited in any of the cases.

Hence, sera of rheumatic patients contain heterophilic antibodies reacting with bovine myocardial ICT antigens which do not cross-react with group A *Streptococcus* antigens. The incidence of these antigens was shown to be much higher in the active phase of the disease. These antibodies belonged to the IgG class. Immunologic specificity of the reactions with myocardial ICT antigens was confirmed in experiments with $F(ab')_2$ fragments obtained from IgG isolated from the sera of rheumatic patients. Heterophilic antibodies were not adsorbed by various antigens of group A streptococcus and could not be isolated in a column with immunosorbent based on NTS antigens. Pretreatment of slices of bovine myocardium with B6/5 monoclonal antibodies cross-reacting with myocardial ICT antigens did not inhibit the reaction of heterophilic antibodies. The data indicate that heterophilic antibodies to myocardial ICT antigens in patient sera differ from heterophilic antibodies in rabbit sera and do not cross-react with streptococcal antigens. It is noteworthy that these antibodies are not rheumatism-specific and are detected in a number of autoimmune and cardiovascular diseases [5].

Our previous investigations revealed that the antigenic specificity of heterophilic antibodies is determined by α-D-galactose [4]. α-Galactose is known to be widely prevalent in animal glycoconjugates. In human tissues the epitopes containing α-galactose are mostly latent. However, they may become available under the effect of various factors, such as infection, aging, etc. Terminal α-galactose has been shown to appear on old red cells and in thalassemia and anemia [10]. We obtained preliminary evidence of the appearance of the specific heterophilic determinant on the red cells of patients with active rheumatic fever. There are reports about the appearance of carbohydrate epitopes containing α-galactose on human tumor cells [11].

Although our studies revealed that heterophilic antibodies do not cross-react with streptococcal antigens, their appearance in rheumatic patients may be caused by streptococcal infection. Group A streptococci are known to produce different enzymes: hyaluronidase, neuraminidase, proteinase, etc. These enzymes may promote the "release" of latent determinants on the cell surface and induce the production of antibodies reacting with epitopes carrying terminal α-D-galactose.

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