SERUM ANTIBODIES AGAINST FIBROBLAST ANTIGENS IN RHEUMATIC FEVER PATIENTS

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Previous investigations revealed heterophilic antibodies in the serum of patients with rheumatic fever which reacted with cells of the interstitial connective tissue (ICT) of the bovine heart and the hearts of other animals [1]. However, no antibodies were found against human myocardial ICT antigens [1, 6, 7], despite the deposition of bound immunoglobulins in these tissues in rheumatic fever [6]. Despite the discovery of Fc-receptors on fibroblasts of human heart valves, no specific reactions could be found when patients' sera were tested on these cells [2]. In the present investigation, cultures of stromal and embryonic human fibroblasts were used to determine antibodies against connective-tissue antigens in sera from patients with rheumatic fever. In parallel experiments, the sera were studied on cultures of stromal guinea pig fibroblasts. Most of the experiments were conducted by the indirect immunofluorescence method. The complement-dependent cytotoxicity test was used to determine the presence of cytotoxic antibodies in the sera.

EXPERIMENTAL METHOD

Sera from patients with rheumatic fever in the active phase were studied: 32 sera from young patients and 12 from middle-aged and old patients. Ten sera from patients with rheumatoid arthritis (RA) and 12 sera from healthy blood donors were tested as the control. The sera were kept at −20°C and studied in dilutions of 1:4-1:16. IgG were isolated from the sera by ion-exchange chromatography on DEAE-cellulose.

Human (bone marrow) and guinea pig (thymus and bone marrow) stromal fibroblasts were cultured in medium 199 with the addition of 15% embryonic calf serum; human embryonic fibroblasts were cultured in Eagle's medium with 10% embryonic serum. To obtain a monolayer on a coverslip, $(3\text{-}5) \times 10^4$ cells were introduced into a flask containing 2 ml medium and cultured for 4 days at 37°C. The coverslip was washed with medium, then with 0.85% NaCl solution made up in 0.015 M phosphate buffer, pH 7.0 (BSS) and dried. The cell monolayer was fixed with acetone at 4°C for 20 min, and with 3% formaldehyde in BSS at room temperature for 15 min. Some of the cells were dried for 30 min at 37°C and used unfixed. The preparations were kept at −20°C.

To increase the permeability of the cell membrane the monolayer was treated with 0.1% Triton solution in BSS for 3 min at room temperature and then washed with BSS for 30 min. The cells were treated with neuraminidase (*Vibrio cholerae*, from Serva, West Germany) with the enzyme present in concentrations of 1, 0.1, and 0.01 mg/ml in 0.05 M acetate buffer with or without the addition of 0.005 M $CaCl_2$. The neuraminidase solution was applied to the cells for 30 min at 37°C. After freezing and thawing the cell suspension was applied to a slide, dried, and fixed with acetone.

To determine antibodies by the direct immunofluorescence method pure antibodies against human IgG labeled with fluorescein isothiocyanate, as described previously [1], were used. The serum was applied to the wet monolayer, incubated for 1 h at 37°C, washed for 10 min with BSS, and then treated with labeled antibodies for 30 min at 37°C. After rinsing the preparations were mounted in glycerol, pH 7.0, and examined in the ML-2 luminescence microscope in blue-violet

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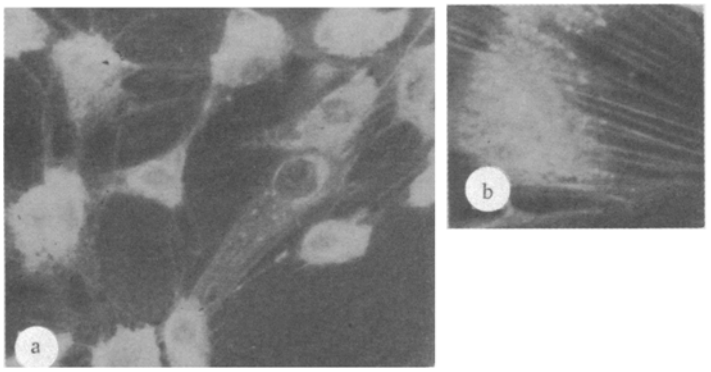

Fig. 1

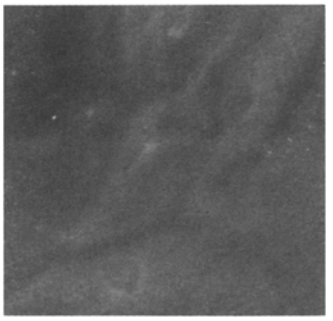

Fig. 2

Fig. 1. Reaction of sera from rheumatic fever patients with culture of guinea pig fibroblasts: a) fluorescence of fine network in cytoplasm; brighter fluorescence in region of endoplasm; b) fluorescence of fibrils.

Fig. 2. Reaction of donor's serum with guinea pig fibroblasts. Fluorescence absent.

light and the LYUMAM-I-1 microscope in UV light with 40 × and 90 × objectives. A ++ reaction with serum in a dilution of 1:4-1:8 was considered to be positive.

For the complement-dependent cytotoxicity test [3] a suspension of living human embryonic fibroblasts (5×10^6) was used. Equal volumes (0.1 ml each) of cell suspension and serum, heated for 30 min at 56°C, were incubated for 30 min at 37°C. As the source of complement, 0.1 ml of fresh rabbit or guinea pig serum was added and the mixture was allowed to stand for 30 min at 37°C. The number of viable cells was counted in a Goryaev's chamber after staining the mixture with 0.1% solutions of eosin and trypan blue.

EXPERIMENTAL RESULTS

Tests of the sera from rheumatic fever patients on cultures of human fibroblasts revealed weak and diffuse fluorescence of the cells. Only in three cases, after treatment of human embryonic fibroblasts with Triton, was brighter fluorescence (up to ++) of the cytoplasm observed. Stromal and embryonic fibroblasts gave practically identical results. When guinea pig fibroblasts were treated, positive reactions were observed in one-third of cases. As a rule, fluorescence of a fine network or of fibrils was observed in the region of the cytoplasm (Fig. 1a, b). Sometimes there was a brighter reaction in the region of the endoplasm, adjacent to the nuclear membrane (Fig. 1a). Occasionally, fluorescence of the nuclei was noted. Fluorescence of the cells was observed only with low dilutions of serum (1:4-1:8) and as a rule it disappeared with a dilution of 1:16.

Positive reactions were observed more often in patients with RA — in 50% of cases. Sera from patients with RA reacted both with human fibroblasts and with guinea pig fibroblasts, but just as with rheumatic fever patients, positive results were obtained only by the use of low dilutions of serum. Blood donors' sera virtually did not react with human or guinea pig fibroblasts (Fig. 2).

The main investigations were conducted on cells fixed with acetone. Similar results were obtained by the use of fibroblasts fixed with alcohol or formalin and of unfixed cells.

When the experiments were carried out on fibroblasts treated with neuraminidase or subjected to freezing and thawing, the intensity of fluorescence fell sharply. Lengthening the exposure of cells with sera to 18 h at 4°C led to the appearance of nonspecific reactions with normal sera. The IgG preparation isolated from sera of rheumatic fever patients reacted weakly with human and guinea pig fibroblasts; even with a protein concentration of 6 mg/ml the intensity of fluorescence of the cells did not exceed ++.

Five sera from rheumatic fever patients, two from RA patients, and two sera from healthy blood donors were studied in the complement-dependent cytotoxicity test. Sera most active in immunofluorescence were chosen for these tests, but no cytotoxic antibodies against human fibroblasts could be found in any of them.

The results indicate that sera from rheumatic fever patients in the active phase of the disease possess weak ability to react with antigens of human fibroblasts which is independent of age. The sera react more strongly with guinea pig fibroblasts, but these antibodies can evidently be classed as heterophilic. In RA antibodies against human and guinea pig fibroblasts are found more often, but in the same low titers. It was impossible to increase the intensity of the reactions either by the use of different methods of fixing the cells or by the use of neuraminidase or of Triton, which increases the permeability of the cell membrane.

These results agree with those obtained by other workers. For instance, attempts to find antibodies against synovial fibroblasts in RA by the antibody-dependent cellular cytotoxicity method likewise did not give convincing positive results [4, 5].

Despite the low level of antibodies against fibroblasts, it can be tentatively suggested that in rheumatic fever they may bind with myocardial ICT antigens. In normal heart tissue the corresponding antigenic determinants may be "latent," and for that reason it is impossible to detect antibodies against connective tissue antigens in sections of human myocardium.

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