CROSS-REACTING ANTIGENS OF MYOID CELLS OF THE HUMAN THYMUS AND STABLE L-FORMS OF GROUP A STREPTOCOCCUS

L. V. Beletskaya, Yu. V. Vul'fovich
É. V. Gnezditskaya, and S. A. Goncharova

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Antigens of several microorganisms are known to contain common antigenic determinants with tissue antigens and to belong to what are known as cross-reacting antigens (CRA). It has been shown that autoantibodies may appear against certain streptococcal CRA, but the role of these antigens in the genesis of an autoimmune process has not been fully studied. Meanwhile under certain conditions CRA can evidently promote the long persistence of a pathogenic agent in the body in man and animals, thus giving rise to the phenomenon of microbial mimicry [7]. CRA of mammalian tissues and the human pathogenic group A streptococcus have been described by several workers. CRA common with components of the myocardium [7, 9, 13, 15] and antigens of the renal glomeruli [14] have been found. A cross reaction of the group polysaccharide of group A streptococcus with the components of some mammalian epithelial tissues and, in particular, the epithelial reticulum of the thymus [2, 8], has been recorded. Antigens of microorganisms common with the components of thymus tissues may be of special importance from the standpoint of representation of heteroorganic antigens in this central lymphoid organ [1, 8, 12], for the function of the thymus evidently determines the development of natural immunologic tolerance to antigens of the body's own tissues [11]. Disturbance of tolerance is known to lead to the development of an autoimmune response. Some infectious processes are accompanied by a marked autoimmune reaction, especially in rheumatic fever, a disease caused by a group A streptococcus [6]. Besides the intact streptococcus, in rheumatic fever its L-form, capable of long persistence in mammalian tissues [4] and of causing a chronic progressive disease with immunopathologic disturbances in cases of experimental infection [4, 5], can also be isolated. The presence of L-forms in patients with rheumatic fever has been confirmed serologically [10]. However, the pathogenetic role of L-forms of streptococcus in this disease has not yet been fully explained, and for that reason the search for CRA in L-forms of streptococcus and in human tissues is worthwhile. In a previous investigation the presence of CRA was established in stable L-forms of group A streptococcus and in the plasma membrane of muscle fibers from the human myocardium.

In the investigation now described cross-reactions between antigenic components of stable L-forms of group A streptococcus and myoid cells of the human thymus were studied by an immunofluorescence method.

EXPERIMENTAL METHOD

Experiments were carried out on tissues of the human thymus obtained at thymectomy on patients with myasthenia and also the thymus of persons dying from acute trauma and with blood groups 0, II, and III. Liver tissue was used as the control. Sections of the organs 5 μ thick were cut in a cryostat from tissue frozen to -76°C and fixed in cold acetone (2 min at 4°C). In some cases the tissues were treated by Zabriskie's method [15].

The animals were immunized with a culture of L-forms (strain L-406), isolated in 1963 from the blood of a patient with rheumatic carditis, and reverted initially into a group A streptococcus, disintegrated by heating and thawing. This strain was cultured for 15 years in nutrient medium, and at the present time reversion to its original form cannot be obtained. The culture of L-forms was grown on medium based on a digest of bovine heart muscle or casein hydrolysate with the addition of horse serum (10%), penicillin (100 i.u./ml), and an osmotic stabilizer [10].

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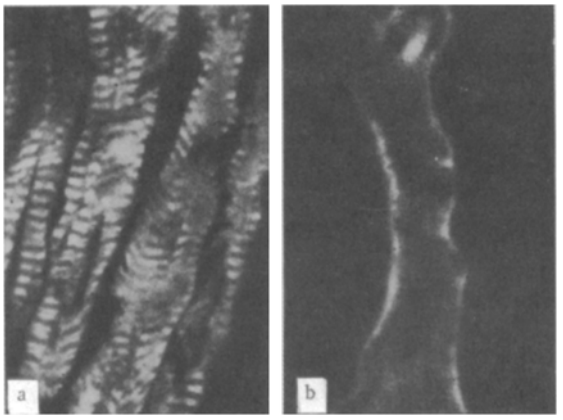

Fig. 1. Sections through human myocardium: a) treatment with serum of myasthenia patient containing antibodies against antigens of striated muscle fibers; reaction in zone of A disks of muscle fibers; b) treatment with rabbit serum against antigens of L-forms of group A streptococcus; reaction in zone of sarcolemma of muscle fiber. Here and in Fig. 2 – indirect immunofluorescence method. Magnification: objective 40 (water immersion), ocular, homal 3.

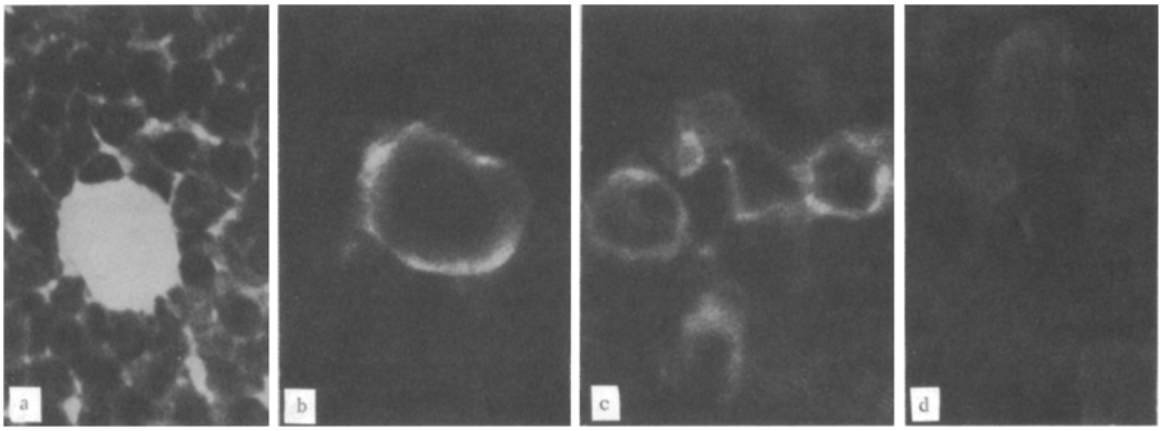

Fig. 2. Sections through human thymus: a) treatment with serum of myasthenia patient containing antibodies against antigens of striated muscle fibers, reaction extends over all areas of cytoplasm of thymus myoid cells; b, c) treatment with serum against antigens of streptococcal L-forms; b) reaction localized in outer zone of cytoplasm of myoid cell; c) group of myoid cells in medullary zone of thymus; reaction at periphery of cells; d) treatment with serum against streptococcal L-forms absorbed beforehand with culture of L-forms; no reaction.

Hyperimmune serum to L-forms was obtained by immunizing rabbits by the combined scheme described previously [3]. This serum reacted with antigens of L-forms in the immunofluorescence test in a dilution of 1:20-1:80, in the gel precipitation test in dilutions of 1:8-1:16, and in the passive hemagglutination test in a dilution of 1:100,000. In parallel tests a preparation of antibodies (900 μg protein/ml) obtained by absorption of antiserum with a suspension of culture of L-forms, followed by elution of the antibodies with glycine-HCl buffer, pH 2.6, was used. Normal rabbit serum (1:10, 1:20) and serum from patients with myasthenia (1:100) were used as the control. Luminescent antibodies against human and rabbit immunoglobulins were prepared by the method described in [8]. Details of the conduct of the immunofluorescence test were described previously [3].

EXPERIMENTAL RESULTS

On treatment of sections through the human thymus with serum of myasthenia patients containing antibodies against antigens of A disks of striated muscle fibers (Fig. 1a), followed by application of antibodies against human IgG labeled with fluorescein isothiocyanate, muscle antigens could be demonstrated in the medullary zone of the lobules in large round cells (myoid cells), the intensity of luminescence of which was assessed at 3+ on a 4+ system (Fig. 2a). Preliminary absorption of the serum with homogenate of cardiac or skeletal muscles (2:1) prevented the reaction both with muscle fibers and with thymus myoid cells. Absorption with liver homogenate did not affect the character of the reaction. On treatment of sections of human myocardium with rabbit antiserum against streptococcal L-forms, followed by application of fluorescent antibodies against rabbit IgG, specific luminescence was observed in the zone of the sarcolemma of the muscle fibers, with an intensity estimated at 3+ (Fig. 1b). On treatment of thymus sections with serum against streptococcal L-forms or with eluate of antibodies against antigens of L-forms obtained from this serum, a reaction of fluorescence was found (intensity 3+) in the peripheral region of the cytoplasm of the myoid cells (Fig. 2b). Absorption of antiserum against L-forms of streptococcus by heart tissue homogenate prevented the reaction both with components of myocardial muscle fibers and with antigens from the peripheral zone of the cytoplasm of thymus myoid cells. The same result was obtained by absorption of the serum with a suspension of a culture of L-forms grown either on meat or on casein medium (Fig. 2). Adsorption of the serum with liver tissue homogenate and with nutrient medium did not affect the result of the reaction.

The investigation thus showed that sera of rabbits immunized with a culture of stable L-forms of streptococcus contain antibodies which react not only with antigen of the L-forms themselves, but also with antigenic components of the peripheral zone of cytoplasm of myoid cells of the human thymus and muscle fiber cells of the human myocardium. Since it was shown previously [3] by an immunomorphologic method that an antigen common to streptococcal L-forms and myocardial muscle fibers is localized in the cell membrane of muscle fibers, it can be tentatively suggested that the CRA in myoid cells is also located in the cytoplasmic membrane. As already stated, the presence of a CRA in microorganisms is linked not only with the appearance of autoimmune reactions, but also with the condition of immunologic unresponsiveness, leading to microbial mimicry [7]. In this connection the common nature of the antigenic determinants of the cytoplasmic membrane of streptococcal L-forms and human somatic cell membranes in some cases may be the cause of mimicry and the large persistence of these microorganisms in the body, and in others the cause of development of an autoimmune reaction. In the case examined above both muscle cells and thymus myoid cells may act as target for the autoantibodies, with the consequent disturbance of thymus function connected with the formation of natural immunologic tolerance to muscle antigens [1].

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