A CROSS-REACTING ANTIGEN OF GROUP A STREPTOCOCCUS AND STROMAL FIBROBLASTS OF LYMPHOID ORGANS

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UDC 612.41+612.438].017.1:[579.862.1:612.017.1

KEY WORDS: streptococcus; fibroblasts; cross-reacting antigens.

Among cell wall proteins of the group A streptococcus a nontype-specific (NTS) antigen, common with the interstitial connective tissue (ICT) antigen of the mammalian myocardium, has been found. It has been shown that the cross-reacting antigen (CRA) in sections through human heart tissue is located in ICT cells morphologically similar to fibroblasts [4, 7]. A solution to the problem of whether cells containing CRA are in fact fibroblasts is of great importance to our understanding of the role of the CRA found in the streptococcus in the development of autoimmune responses (AIR) to connective tissue of the heart in rheumatic fever. It is also important to determine whether CRA is present or not in the connective-tissue stroma of lymphoid organs and, in particular, in the stroma of the thymus. According to one hypothesis, streptococcal antigens common with thymus antigens can induce the development of AIR to thymus antigens and, as a result of this, an autoimmune thymitis, a "triggering mechanism" of the autoimmune process in rheumatic fever [5].

To examine these problems, in the investigation described below CRA was determined in pure cultures of fibroblasts from lymphoid organs.

EXPERIMENTAL METHOD

Explanation of thymus, spleen, and bone marrow cells from adult noninbred guinea pigs and also thymus and bone marrow cells from 18-week human fetuses were explanted into monolayer cultures by the method in [8]. Medium 199 with the addition of 20% calf serum (for guinea pig cells) or healthy human serum (for human cells) was used for explanation. The gaseous phase consisted of air containing 5% CO_2. The first passage of the cultures was carried out 12-16 days after explanation and subsequent passages as the cultures reached the confluent state. Pure cultures of fibroblasts (after 3-5 passages), not contaminated with macrophages, were used for the experiment. Into flat-bottomed tubes with a coverslip on the bottom 3×10^5 cells were introduced. After culture for different periods the coverslips with fibroblasts were removed from the tubes, washed with Hanks' solution followed by phosphate-buffered physiological saline (BPS), and fixed for 10 min in cold acetone. Experiments also were carried out on suspensions of living stromal fibroblasts. CRA was detected by the indirect immunofluorescence method (IIFM) with pure antibodies against rabbit IgG labeled with fluorescein isothiocyanate. The technique of isolation and labeling of antibodies against rabbit IgG and schemes for processing the fixed preparations and cells in suspension were described previously [2, 3]. The specimens were examined in the ML-2 luminescence microscope.

Rabbit sera obtained after 4 cycles of immunization with fractions containing NTS antigens of group A streptococcus [7] were used as test sera for detection of CRA. The most active sera, reacting in the IIFM with ICT cells of human myocardium to a dilution of 1:64-1:28 were selected. Fractions of NTS antigens used for immunization were isolated from HCl extracts of types 1 and 29 streptococcus by the method of preparative electrophoresis [6]. To inhibit the reaction of immune sera with fibroblasts, fractions of NTS antigens isolated from HCl extracts of group A streptococcus of heterologous type (i.e., not of the type which was used for immunization) were used. Homogenate of guinea pig bone marrow fibroblasts, ob-

N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 98, No. 12, pp. 708-710, December, 1984. Original article submitted November 26, 1983.

0007-4888/84/9812-1693$08.50

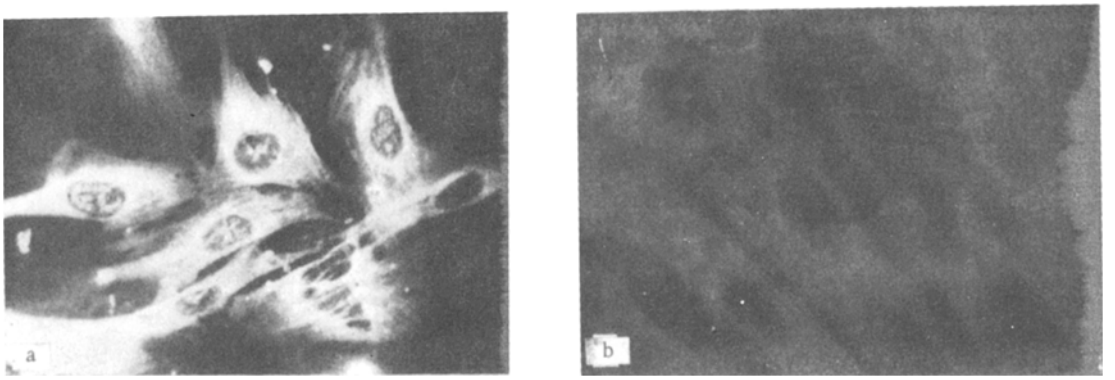

Fig. 1. Culture of guinea pig bone marrow fibroblasts. Reaction of $F(ab')_2$-fragments of IgG isolated from antiserum against streptococcal NTS antigens with cytoplasm of fibroblasts (a). Inhibition of reaction after absorption with fraction containing streptococcal NTS antigens (b). Indirect immunofluorescence method. Magnification 120.

tained by fragmenting living cells by rapid freezing and thawing 3 times, also was used for inhibition. The fraction containing NTS antigens was used for inhibition in a dose equivalent to 10 mg protein, whereas the fibroblast homogenate was used in a dose equivalent to 5×10^6 fragmented cells to 1 ml of antiserum diluted 1:8. Inhibition continued for 2 h at 37°C and 18 h at 4°C. The immunologic specificity of the reaction of the antisera with fibroblasts was monitored by testing pepsin $F(ab')_2$-fragments of IgG isolated from sera against NTS antigens. To obtain $F(ab')_2$-fragments, immunoglobulins were subjected to hydrolysis by pepsin and fractionation on a column with Sephadex G-150 [12].

EXPERIMENTAL RESULTS

On treatment of a suspension of living stromal fibroblasts from guinea pig spleen with antisera against streptococcal NTS antigens, followed by luminescent antibodies against rabbit IgG, no fluorescence of the cells was found. Investigation of cells cultured on coverslips showed absence of fluroescence also in preparations fixed during the first 18 h of culture. Toward the end of the 1st day of culture, fluorescence appeared in most preparations in small areas of cytoplasm adjacent to the cell nucleus. On the following days the intensity of fluroescence increased in the perinuclear zone of the cytoplasm. The clearest reactions were found in preparations fixed toward the end of the 3rd day of culture (Fig. 1). Intensive fluorescence of the perinuclear zone of the cytoplasm could be seen in these preparations. Weaker fluorescence of the peripheral zone of cytoplasm and the cell membranes also was observed. Similar results were obtained in a study of thymus and bone marrow fibroblast cultures derived from guinea pigs and human fetuses.

Fluorescence of the cytoplasm and cell membranes in fixed monolayer fibroblast cultures from human and guinea pig lymphoid organs also was observed during testing of preparations of pepsin $F(ab')_2$-fragments of IgG isolated from antisera against NTS antigens. Sera of unimmunized rabbits and $F(ab')_2$-fragments of normal rabbit IgG reacted with none of the fibroblast cultures used in the work.

Considerable or complete inhibition of the reaction of antisera against NTS antigens with fibroblasts was induced by fractions containing NTS antigens of group A streptococcus. Complete suppression of these reactions also was observed after exhaustion of the antisera with homogenate of guinea pig bone marrow fibroblasts. Fractions containing NTS antigens and homogenate of cultured fibroblasts also inhibited the reaction with fibroblasts observed during testing of $F(ab')_2$-fragments of IgG isolated from antisera against NTS antigens (Fig. 1b). Absorption with guinea pig or human (blood groups I-IV) erythrocytes did not affect the reaction with fibroblasts.

A CRA common with NTS antigen of group A streptococcus was thus found in pure cultures of stromal fibroblasts from the thymus and other lymphoid organs of man and guinea pigs. Cross reactions were demonstrated not only with antisera against NTS antigens, but also with $F(ab')_2$-fragments of IgG isolated from these antisera. The use of $F(ab')_2$-fragments of IgG rules out the possibility of "imitation" of cross reactions. According to some workers [11], these may arise on account of the reaction of immunoglobulins with tissue Fc-receptors when whole

antistreptococcal sera are tested by the IIFM, and on account of nonspecific inhibition of these reactions by absorption of the sera with preparations containing streptococcal Fc-receptors.

CRA was found in all fibroblast cultures tested irrespective of the organ and species of their origin. These data confirm the previous hypothesis [4] that this CRA belongs to the category of tissue-specific antigens present in connective tissue of different species of mammals. It must also be noted that this CRA is a so-called hetero-organic antigen of the thymus. A number of such thymus antigens, common with antigens of certain highly specialized tissues, were described previously [1].

Investigation of fixed monolayer cultures of fibroblasts enabled the intracellular location of CRA to be studied. This antigen was found both in cytoplasm and on the membrane of fixed fibroblasts. At the earliest stages of cell culture the CRA was found in the perinuclear zone of cytoplasm. CRA was found later in the peripheral zone of cytoplasm and on the membrane. CRA was not found on the membranes of living cells, evidently because of their destruction during removal of the cell monolayer with trypsin.

The presence of CRA in stromal fibroblasts of the thymus and other lymphoid organs indicates that should autoantibodies appear during immunization with the corresponding streptococcal CRA, these organs may be injured. The appearance of AIR against CRA of stromal thymus cells may evidently have an important role in the disturbance of immunoregulation during the autoimmune process. This view is based on data on the role of thymus cells, in whose microenvironment T lymphocytes undergo maturation [8, 9]. The opinion is held that injury to the thymus in rheumatic fever is autoimmune in nature and is a "triggering mechanism" for further generalization of the autoimmune process [5, 10]. It has also been suggested that group A streptococcal CRA may be a cause of the development of AIR to thymus antigens [5]. In this connection it will be interesting to determine in future investigations the ability of this CRA to induce the formation of autoantibodies and of cellular AIR against the corresponding thymus antigen in animals on immunization with streptococcal NTS antigens and in human patients with rheumatic fever.

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