USE OF TOTAL LOOSE CONNECTIVE TISSUE PREPARATIONS IN IMMUNOFLUORESCENCE EXPERIMENTS

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In immunomorphologic research in which labeled antibodies are used, sections prepared from frozen tissue, paraffin sections, smears, impressions, or monolayer cell cultures are generally preferred [1]. Total tissue preparations, for example, axial fibers of diaphragm muscle, are more rarely used [8]. Antibodies can react with antigen other than those at the surface of unbroken (total) cell structures; for instance, they can react with components of intracell structures. An example is the reaction of anti-nuclear antibodies with antigens of the nuclei of blood smear components [3]. In this connection we propose that in experiments with antigens of surface or intracell structures of connective-tissue components, thin-film preparations of loose connective tissue might be successfully used. The advantages of total films over other kinds lie in the simplicity of preparation of the former, in which no special equipment is required, and in which effects which lead to rapid denaturation of tissue components during treatment can be excluded. Another advantage of this method lies in the conservation of the natural juxtaposition of various types of cell components, which allows simultaneous comparative research.

It is known that immunoglobulins (Ig) can react with tissue antigen as well as with active centers of the antibody. They can bind with components of surface cell structures at nonactive areas of the molecule, in particular, with Fc-receptors through the Fc-section of the Ig molecule [4]. For instance, human and animal Ig can actively bind with components on the surface of mast cells [9].

Here we present an investigation of methods for producing immunofluorescence reactions of anti-tissue antibodies with antigen of tissue structures, and of certain features in the binding of Ig with mast cells when total preparations of loose connective tissue of animals are used.

The method of preparing the films is described in [5] and elsewhere. It includes the following: A small pincer-clamped piece of loose connective tissue is detached with scissors and placed on a slide. The tissue is stretched with dissecting needles over the surface of the slide in the form of a thin film. The needles must touch only the edges of the film, leaving the middle undisturbed. After room-temperature air desiccation for 20-30 min the preparation can be used in either a nonfixed or fixed (with alcohol, acetone, or other fixatives) form. Here the experimental serum was introduced in the required ratios after 45 min. After rinsing (for 10 min) in a 0.85% NaCl solution with a phosphate buffer (pH 7.0), it was treated with luminescence antibodies for 30 min; after immediate rinsing (for 10 min) it was placed under a cover glass in 60% glycerol (pH 7.0). It was necessary to preserve in the nonfixed preparation the structure of the mast cells, which can be damaged by the serum; thus, when using the indirect method of immunofluorescence the preparations were preliminarily treated for 10 min with a 0.01% solution of EDTA.

We used thin-film preparations of the loose connective tissue of mouse, rat, guinea pig, and hamster. We tested four sequences of fluorescein-isothiocyanate antibodies to human IgG and three sequences of antibodies to rabbit Ig (prepared from donkey antiserum to human or rabbit Ig). Preparations of rabbit $F(ab')_2$ and Fab'Ig fragments supplied by I. A. Tarkhanova were used.

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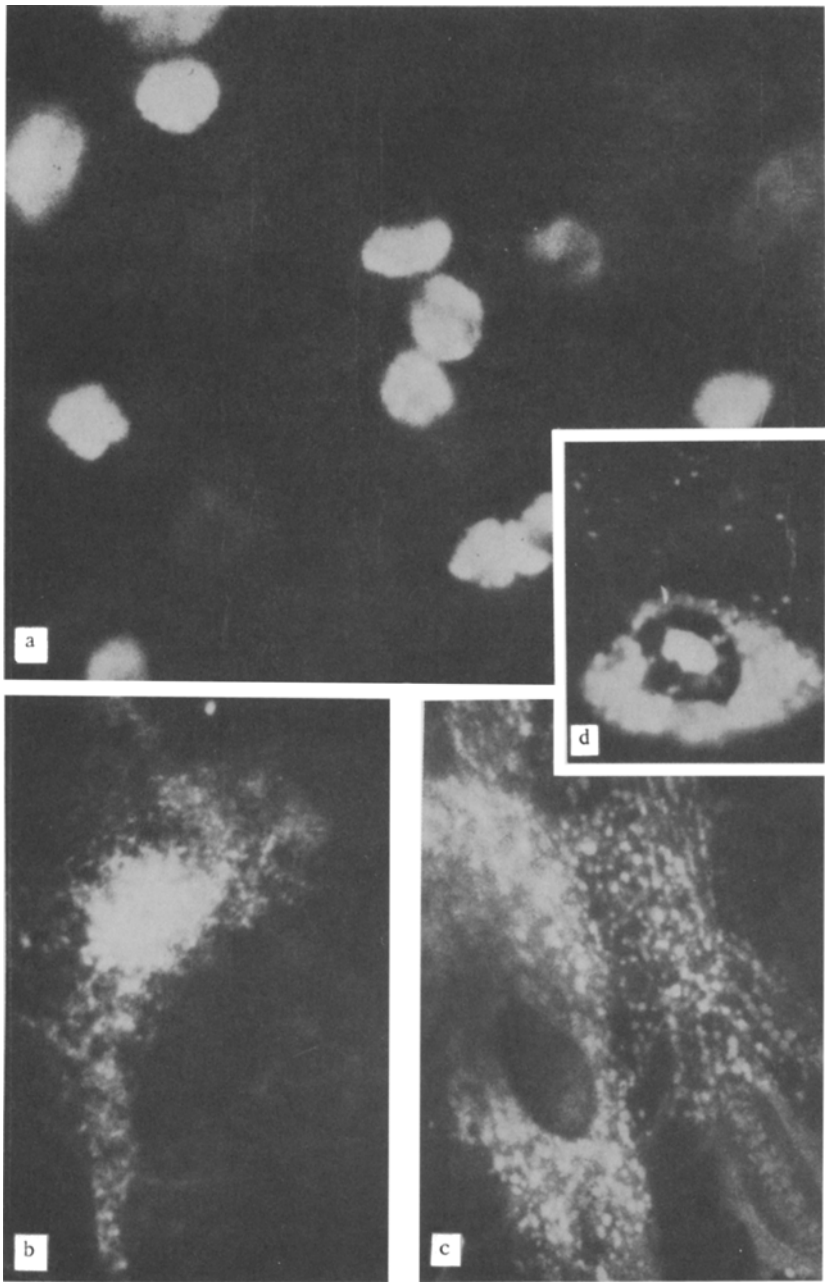

Fig. 1. Total (film) preparations of animal loose connective tissue. a) Guinea pig connective tissue, treated with serum of SLE sufferers (1:100), reacting with antigen of cell-structure nuclei; b) rat connective tissue, treated with serum of RA sufferers (1:64), reacting in endo- and ectoplasmic zone of the fibroblast; c) hamster connective tissue, treated with serum to fibronectin — the surface protein of fibroblasts (1:64); the reaction is localized in point zones of the cell cytoplasm; d) rat connective tissue, treated with nonimmunized rabbit serum (1:10), reacting with mast-cell components. The indirect method of immunofluorescence was used. Magnification 270 ×.

As a source of antibodies to the tissue antigens we used: serum of individuals suffering from systemic lupus erythematosus (SLE); this serum contained antibodies to antigens of cell nuclei [10]; serum of individuals suffering from rheumatoid arthritis (RA); this serum contained antibodies that react with cytoplasmic antigens of connective-tissue cells [2]; rabbit serum, containing antibodies to the surface antigen of fibroblasts, fibronectin (supplied by A. A. Ivanov-Smolensk). As a control we used serum of donors and nonimmunized animals. An ML-2 microscope with 40 × (water-immersion) and 90 × (oil-immersion) objectives was used.

Upon introducing serum of SLE sufferers (in 1:100 and higher ratios) to the nonfixed films of animal loose connective tissue, with subsequent application of labeled antibodies to human IgG, a reaction in the form of bright green luminescence in the neighborhood of the nuclei of the majority of the loose connective tissue cells was observed (Fig. 1a). Donor serum introduced in identical ratios did not react with components of the nuclei of cell structures. Introduction of the serum of individuals with RA (in 1:64 and higher ratios), containing antibodies to cytoplasmic components of connective-tissue cells, leads to a reaction in the zone of pulverized structures of ecto- and endoplasmic fibroblasts (Fig. 1b). Sera of RA sufferers react only weakly with components of histiocytes (tissue macrophages); here the reaction diffusively appears in the cytoplasm and its islands. In such cases, in which sera of RA sufferers contains anti-nuclear antibodies belonging to the class IgG, one also observes a reaction in the neighborhood of the cell nuclei of the loose connective tissue; this reaction was established with the aid of labeled IgG antigens. Donor sera introduced in the same ratios (1:64 and higher) did not react with components of cell structures. When donor serum was used in small ratios (1:16), we observed in a number of cases (3 out of 10) a reaction with components of cytoplasmic cell structures in the same neighborhood as the reaction with serum of individuals suffering from RA. In the control experiments, upon introduction of labeled antibodies to human IgG, no reaction with nuclear components of cytoplasmic cell structures was seen on the film. Upon application of fibronectin to nonfixed preparations of rabbit serum containing antibodies to fibronectin (in 1:64 and 1:128 ratios), an intensive reaction in the cytoplasmic zone of all types of cell structures from loose connective tissue was observed. There was practically no reaction with surface glycocalyx fibrous structures. After application of cold acetone (4°C) to the preparations (in order to fix the fibronectin) and the subsequent introduction of serum, the intensity of the reaction sharply decreased; however, the luminescence was clearly localized in point or hatched cytoplasmic zones on the fibroblasts (Fig. 1b) and on the surface of the loose connective tissue fibers. Serum of nonimmunized rabbits, introduced in identical ratios, did not react with components of loose connective tissue of total preparations either before or after fixation.

All the sera used in either direct or indirect immunofluorescence — including sera of immunized and nonimmunized animals, sera of donors and of individuals with SLE and RA, and preparations of pure antibodies to human and rabbit IgG taken in low ratios (1:4-1:16) — reacted with mast cells of nonfixed preparations of loose connective tissue. The most intensive reaction was observed on preparations of rat connective tissue, which contains, as is well known, a large number of mast cells. In cases in which the preparation was subsequently treated with intermediate serum and labeled antibodies without the preliminary application of the EDTA solution to the tissue, a rather rapid (after 15-30 min) destruction of mast-cell structures and a weakening of the immunofluorescence reaction were observed. EDTA was found to stabilize the mast cells, and in this case the immunofluorescence reaction continues for a protracted period (5-7 days). Preparations of Fab' and $F(ab')_2$ rabbit IgG fragments did not react with the mast cells; this supports the hypothesis that Ig is bound with mast-cell components along the Fc-section of the molecule [6, 7].

Thus, the results of this investigation indicate the possibility of using total preparations of loose connective tissue (requiring no special preparation equipment) to study the reaction of anti-tissue antibodies with corresponding tissue components and certain parameters in the binding of Ig with mast-cell components.

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