USE OF THE METHOD OF LUMINESCENT ANTIBODIES IN IMMUNOLOGIC INVESTIGATIONS IN TUBERCULOSIS

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There is no information in the literature on the use of the luminescent antibody technique for the detection of tuberculosis. It has been shown [1] that intact cells of Mycobacterium tuberculosis do not give specific luminescence, but if the cells are broken up and the antigens liberated, luminescence of this type is possible.

It was accordingly decided to study the possibility of using the Coons' method for special immunologic investigation in tuberculosis.

EXPERIMENTAL METHOD AND RESULTS

The indirect Coons' method was used to study the possibility of detecting intact cells of various strains of M. tuberculosis (H_{37} Rv, H_{37} Rv Czechoslovakia, bovinus-8, academia) and BCG (Moscow production substrain, N. F. Gamaleya Institute of Microbiology and Epidemiology), and also BCG antigens in films (native, adsorbed on erythrocytes, agglutinated), and H_{37} Rv antigens in tissue sections from infected animals and the refected lungs of patients with tuberculosis.

Antigens were prepared from a 2- to 3-week culture of BCG by destroying the bacterial cells in a ball mill, followed by lyophilization for keeping. The antigens were tested for their protein content by the micro-Kjeldahl method. The broken-up mass of the BCG culture with the antigens thus obtained had a protein content of 7.1 mg/ml, and in the gel-precipitation reaction gave a reaction of complete identity with destroyed H_{37} Rv cells. In the complement fixation reaction (CFR) the titer of the antigens was 1:1024, and in Boyden's passive hemagglutination inhibition reaction (PHIR) the titer was 1:2560.

The tuberculosis antiserum was obtained by repeated (5-6 times at intervals of 4-5 days) immunization of rabbits with antigens prepared by the authors from BCG vaccines of Moscow and Japanese substrains. The titers of the prepared sera in the CFR were 1:1280-1:2560, in the PHIR, 1:10240-1:20480, while their precipitating titer was 1:512-1:1024. The sera gave 8-10 precipitation lines on immunoelectrophoresis.

A fluorescent serum against rabbit globulins was obtained from the N. F. Gamaleya Institute of Epidemiology and Microbiology (batch 356, working dilution 1:32). Sections, 3-4 μ in thickness, from the fresh tissues of the organs of the infected animals and the refected lungs from patients with tuberculosis were obtained on a cryostat.

The sections and films were treated by the usual method.

The specificity of the immunologic reactions in the films and sections was determined with the aid of the following controls.

1. Staining the preparations with a fluorescent anti-rabbit immune serum only.

2. Treating the preparations with a fluorescent immune serum preliminarily adsorbed with rabbit globulins.

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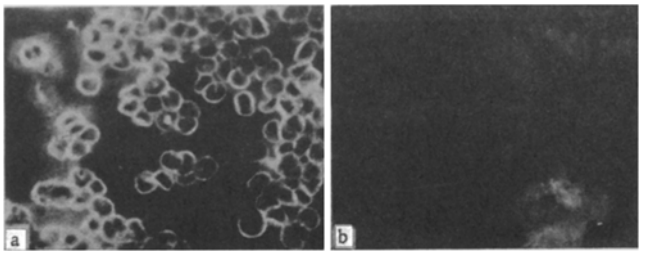

Fig. 1. Luminescence of antigens adsorbed on erythrocytes. a) Experiment; b) control.

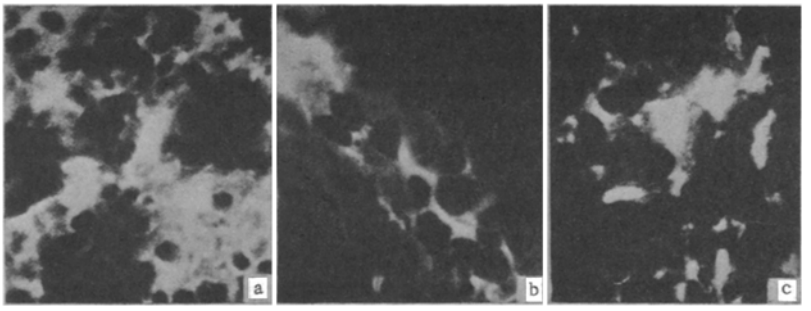

Fig. 2. Luminescence of antigens in tissues of the organs of a guinea pig infected with tuberculosis. a) Follicle of the spleen; b) interalveolar septum of the lung; c) tubercle of epithelioid and lymphoid cells in the lung.

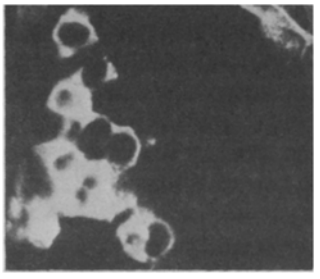

Fig. 3. Luminescence of antigens in tissues of a refected human lung. Infected interalveolar septa.

3. Treatment of the preparations with tuberculosis antiserum, exhausted with the corresponding antigen, followed by treatment with fluorescent anti-rabbit immune serum. The concentration and volume of antigen necessary for total blocking of the tuberculosis antiserum were determined from the titer in the PHIR. To produce exhaustion the antigen was mixed with the immune serum in equal volumes, incubated for 1 h at 37°, and then left overnight in the refrigerator, after which the mixture was centrifuged. The decanted supernatant was again tested in the PHIR and CFR. Usually the reactions were negative.

4. Treatment of the preparations with the tuberculosis antiserum followed by application of a heterologous fluorescent serum (against guinea pig's or human globulins).

5. Treatment of the preparations with a heterologous immune serum followed by incubation with fluorescent anti-rabbit immune serum.

The preparations were examined in the ML-2 luminescence microscope with a type AU-26 binocular adapter and the objectives were illuminated from above with blue-violet light (filters FS-1 and SES-7). Type RF-3 film and a type MFN-10 camera attachment were used for photography. The preparations were investigated simultaneously by the phase-contrast method. Parallel sections were stained with hematoxylin-eosin.

The study of film with intact cells of the various strains of M. tuberculosis and BCG revealed specific luminescence only of individual bacterial cells or of small conglomerates, whereas, during phase-contrast microscopy, large numbers of mycobacteria were observed in the same field of vision.

In films made from the specific antigens, a bright diffuse luminescence of the whole antigenic mass was observed, whereas, after the control treatment of the same films, the result was always negative.

To discover whether antigens from the cells could be detected, BCG antigens were conjugated with tanninized sheep's erythrocytes. After 40 min the erythrocytes were washed off in phosphate buffer, pH 7.2, and films were made. At the same time films were also made from clumps of erythrocytes agglutinated in Boyden's reaction.

In both cases, antigens adsorbed on the surface of the erythrocytes gave a bright specific fluorescence, surrounding the erythrocytes in the form of a halo, which was not observed in the control preparations (Fig. 1).

Having established that BCG antigens fixed to cell structures could be detected, the next step was to detect them in sections of the tissues of infected animals and refected lungs from patients with chronic fibro-cavernous tuberculosis.

Experimental tuberculosis was induced in guinea pigs by subcutaneous injection of a culture of strain H_{37} Rv in a dose of 1:10,000. The guinea pigs were sacrificed in the course of the third month after infection when severe generalized tuberculosis was present. Five healthy guinea pigs were sacrificed for control purposes. Sections of the lungs and spleen were taken for investigation.

The study of the spleen of the guinea pigs showed specific luminescence of the antigens both in the specific granulomas and caseous masses and in the cells and tissues of the organ outside the pathological foci (Fig. 2a). The antigens were located in the lymphoid follicles outside the germ centers, in the cytoplasm of the lymphoid cells, and extracellularly. Luminescence was also found in the cytoplasm of the reticular cells and macrophages. In the lung tissue, as also in the spleen, luminescence was detected in the pathological foci (caseous masses, capsules of the foci, epithelioid tubercles and foci) and also in the surrounding tissues, especially in the zone of inflammatory proliferation in the interalveolar septa (Fig. 2b, c). Antigens of the mycobacteria were found both intracellularly and in the extracellular spaces.

Examination of sections of the tissue of refected human lungs revealed specific luminescence in the same cellular and extracellular structures (Fig. 3).

After control treatment of the sections, the results were always negative. Only weak nonspecific fluorescence of the tissue was observed in the sections.

The material described thus shows clearly that the method of fluorescent antibodies can be used successfully to detect antigens of BCG and of M. tuberculosis in both films and tissue sections.

LITERATURE CITED

1. C. Shepard and D. Kirsh, Proc. Soc. exp. Biol. (New York), 106, 685 (1961).