IMMUNOCHEMICAL ANALYSIS OF THE RAT LEPROMA

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Examination of a saline extract of rat leproma by the precipitation test in agar with rabbit antisera revealed four antigenic components absent in the serum and tissues of healthy rats. Two of these components (L-1 and L-2) were present not only in extract of rat leproma, but also in the liver and spleen of rats and mice with Stefanskii's murine leprosy. Component L-1 was also found in water-soluble extract of BCG, in tuberculin, and also in a saline extract from human lepromas.

Infection with leprosy is characterized by the involvement of many organs and tissues in the pathological process, and by the presence of numerous mycobacteria in them [8, 11]. It is therefore interesting to study tissue antigens in order to investigate the antigenic spectrum of the diseased tissues, as well as to look for antigen-identifying proteins in them. Murine leprosy, described by V. K. Stefanskii in 1902 [3], was used to simulate human leprosy. The similarity between these two mycobacterioses has frequently been confirmed [4, 9].

In the investigation described below, an attempt was made to find specific proteins in soluble extracts of rat lepromas.

EXPERIMENTAL METHOD

The internal organs of 80 rats and 60 mice infected intraperitoneally with a suspension of rat leproma were used in the experiment. Lepromas were obtained from 15 rats infected subcutaneously. The degree of saturation of the organs with mycobacteria was determined with the aid of the bacterioscopic index, calculated from smears taken from the organs of the animals [5]. Microprecipitation in agar [1] and immunoelectrophoresis [6] were used for the immunological analysis.

Antigens. A weighed sample of tissue was ground in a glass homogenizer with physiological saline, and centrifuged at 10,000-12,000 rpm for 40 min in the cold. The supernatant was used as the antigen.

Antiserum. Six rabbits were immunized subcutaneously with rat leproma homogenate by the following scheme: first injection with Freund's incomplete adjuvant, second and third injections without adjuvant, interval between injections 14 and 7 days. Between 3 and 5 reimmunizations were given after 30-40 days. The antiserum was exhausted with serum and tissue extracts from healthy rats and mice, and concentrated by salting out the globulin fraction with ammonium sulfate. Two antisera which continued to react with leproma extract after absorption were used in the work.

EXPERIMENTAL RESULTS

When the exhausted "leproma" antisera were tested in the precipitation reaction, one of them (No. 72) revealed four antigenic components in the leproma extract which were absent from the serum and tissues

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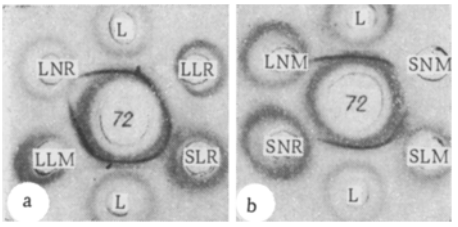

Fig. 1

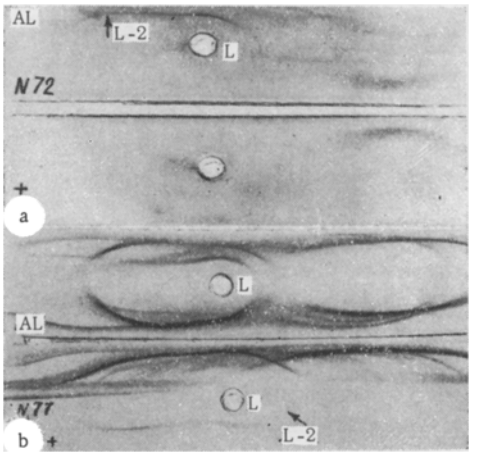

Fig. 2

Fig. 1. Precipitation test in agar using standard test system (antiserum No. 72 against rat leproma, antigen – extract from rat leproma in dilution 1 : 8). a) L – test antigen; LNR – liver of normal rats; LLR – liver of leprous rats; LLM – liver of leprous mice; SLR – spleen of leprous rats; b) LNM – liver of normal mice; SNM – spleen of normal mice; SNR – spleen of normal rats; SLM – spleen of leprous mice.

Fig. 2. Immunoelectrophoresis of leproma antigens. a) Component L-1; b) component L-2; L – leproma extract; Nos. 72 and 77 – exhausted "leproma" antisera; AL – unexhausted antiserum No. 77.

of healthy animals. In subsequent analyses, one test system was used to detect the antigenic component, which was called L-1. As Fig. 1 shows, this antigen was present not only in the leproma, but also in extracts from the liver and spleen of infected mice and rats. Microscopic examination of smears from these animals showed that the bacterioscopic index was highest in these organs. In its electrophoretic mobility, the L-1 antigen belongs to the γ-globulin group (Fig. 2a).

The other exhausted antiserum (No. 77) formed only one precipitation arc with leproma extract, in the zone of α_1- and α_2-globulins (Fig. 2b). The antigen of this test system was called component L-2. Titration showed that component L-2 is also present in extracts of the liver and spleen of the diseased animals, i.e., in the tissues containing the largest number of mycobacteria.

Antigens L-1 and L-2 are evidently not of tissue origin, but are mycobacterial in origin, for they are found in the tissues of animals of different species and they correlate with the degree of saturation of the organs with mycobacteria. Staining after immunoelectrophoresis showed that component L-1 interacted both with the protein stain and with Schiff's reagent, thus demonstrating its protein-polysaccharide nature. Component L-2 stained only with amido black.

Many workers have stated that the mycobacteria of leprosy and tuberculosis are antigenically related [2, 7, 10]. The test system used in the present experiments was also tested in the precipitation reaction against water-soluble extract from BCG vaccine and against tuberculin. For comparison, a saline extract from human lepromas also was used. During the comparative immunochemical analysis component L-1 formed a common precipitation line with BCG and tuberculin, which also distinctly curved under the location of the saline extract from human lepromas. This confirms the mycobacterial origin of antigen L-1 and is evidence of the presence of a common antigenic determinant in the mycobacteria of murine and human leprosy and of tuberculosis. Component L-1 discovered in the present experiments is evidently one of the "mother" antigens of Mycobacterium tuberculosis described by Kulagin [2]. Component L-2 was not detected in any specimen of antigens taken for comparative analysis. It can accordingly be concluded that it is specific for the mycobacterium of murine leprosy only.

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