DETECTION OF ANTIGEN OF L-FORMS OF HEMOLYTIC STREPTOCOCCI IN TISSUES OF EXPERIMENTALLY INFECTED MICE BY THE IMMUNOFLUORESCENCE TEST

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Antigen of L-forms of hemolytic streptococci were found by the indirect immunofluorescence test in the tissues of organs of experimentally infected mice from 1-3 h to 10 weeks after their infection. The dynamics of distribution of this antigen was determined in different organs of the mice. For instance, antigen of L-forms was detected in liver tissue 1 h after injection and persisted in some animals until 10 weeks. In the spleen it appeared after 24 h and was present throughout the rest of the period of observation. In the kidneys, antigen was detected mainly 2-3 weeks after infection. It was found in the heart tissues first after 2 weeks, and remained during the next 8 weeks.

The problem of whether bacteria can exist in a latent state as L-forms in the infected organisms and in cell cultures has not yet been solved. Various biological, chemical, and physical methods have been used for its investigation: feeding on artificial nutrient media suitable for growth of L-forms, vital staining of microorganisms, electron-microscopy of ultrathin sections, and the immunofluorescence method [3-6]. Direct seeding of test material on artificial nutrient media is the simplest and most reliable method of detection of microorganisms, but in the case of L-forms of bacteria seeding often gives negative results. If the results of seeding are positive, difficulties arise in the species identification of isolated stable L-forms, the biological characteristic of which is that they have lost the power of reversion to bacteria of the original species.

Electron microscopy can give nonspecific results because of the impossibility of morphological differentiation of the species of L-forms, and vital staining of L-forms can be used only under restricted

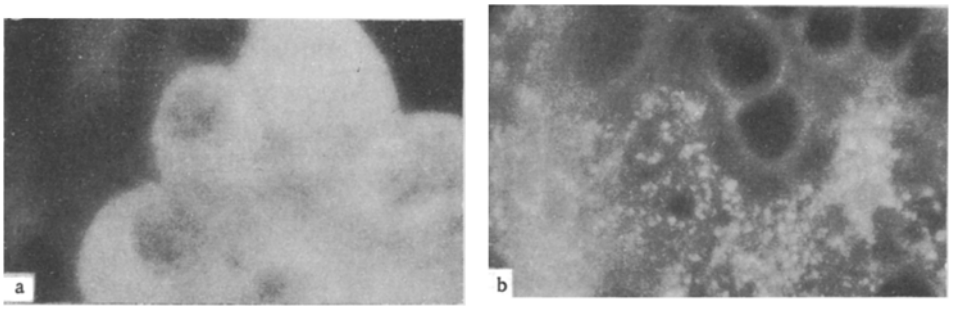

Fig. 1. Immunofluorescence reaction in sections of tissues from infected mice: a) liver cells 3 h after infection; b) spleen cells 10 weeks after infection, 500 ×.

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TABLE 1. Dynamics of Distribution of L-Forms of Hemolytic Streptococcus in Organs of Mice at Various Times after Infection

Organ	Time after infection					
	1–3 h	24 h	1 week	2 weeks	3 weeks	10 weeks
Spleen	1/10	4/12	9/12	10/12	11/12	6/6
Liver	9/10	8/12	6/12	7/12	6/12	3/6
Kidney	0/10	2/12	2/12	9/12	8/12	0/6
Heart	0/10	0/12	0/12	3/12	4/12	4/6

Note: Numerator shows number of mice with positive reaction, denominator shows total number of mice infected.

experimental conditions. Little information is available on the use of the immunofluorescence method for the detection of L-forms [7]. Nevertheless, this method is highly sensitive and also specific.

In the present investigation the indirect immunofluorescence test was used to detect antigen of L-forms of hemolytic streptococci in organs of experimentally infected mice.

EXPERIMENTAL METHOD

Strain L-406 of Streptococcus haemolyticus used in the investigation was isolated by Kagan and Mikhailova from the blood of a patient with rheumatic carditis in 1959 [1]. The strain was grown in broth based on a tryptic digest of bovine heart muscle with the addition of 10% horse serum, 1,000 units/ml penicillin, and an osmotic stabilizer (NaCl) in a final concentration of 2.6%. The broth culture, washed off and concentrated 100 times, was resuspended in 1.5% NaCl solution up to a titer of 5×10^6 colony-forming units (c.f.u.) per ml.

The suspension of L-forms was injected in a volume of 0.5 ml intraperitoneally into albino mice weighing 16-18 g. The mice were decapitated 1, 3, and 24 h and 1, 2, 3, 5, and 10 weeks after infection and impression preparations were made of the liver, spleen, kidneys, and heart. The immune serum used was obtained by immunizing a rabbit with the same strain, grown on the medium described above but with the addition of 10% rabbit serum [2]. This serum agglutinated a culture of L-forms of L-406 in a dilution of 1:640. In Ouchterlong's gel diffusion test this serum gave two clear precipitation bands in a dilution of 1:10 with the soluble fraction of the homologous antigen. Ass antirabbit globulin, labeled with fluorescein isothiocyanate (FITC) and bovine albumin labeled with rhodamine were obtained from the Laboratory of Luminescent Sera, N. F. Gamaleya Institute of Epidemiology and Microbiology.

The specificity of staining was verified by the use of normal rabbit serum, by blocking the antibodies of the immune serum by preliminary contact with homologous antigen of the L-forms, and by using impressions of organs of uninfected animals and of animals which had received injections of the culture medium and a suspension of L-forms heated for 30 min at 80°C. The ML-2 luminescent microscope was used to examine the preparations.

EXPERIMENTAL RESULTS

The results given in Table 1 and Fig. 1 show that in the early stages (1-3 h) the antigen was found in highest titer in the liver tissue. After 24 h and throughout the test of the period of observation (10 weeks) the antigen was found in the liver in 50% of the infected animals.

Antigen was found in the spleen after 24 h in 30% of the experimental mice, and during the next 1-10 weeks in the overwhelming majority of animals. In the kidneys it was found most commonly after 2-3 weeks, but toward the end of the period of observation as a rule the antigen was not found in the kidneys. The antigen appeared latest of all in the heart tissues, where it was detected 2 weeks after infection and remained during the next 8 weeks of observation. In all the control preparations the reaction was negative, except in the control with heated antigen, when it was found in the early stages (1-24 h) in the liver tissue, probably because of its selective absorption by this tissue.

These results demonstrate that antigen of the L-forms of hemolytic streptococcus is found in the tissues of organs of experimentally infected animals after 1-3 and until 10 weeks after infection.

LITERATURE CITED

1. G. Ya. Kagan and V. S. Mikhailova, Zh. Mikrobiol., No. 7, 327 (1963).
2. V. N. Petrosova, I. V. Rakovskaya, T. M. Raskova, et al., Zh. Mikrobiol., No. 10, 11 (1969).
3. N. D. Berliner, R. B. Kundsin, and E. N. Allred, J. Bact., 99, 1 (1969).
4. G. Drach and J. Schmitt-Slomska, C. R. Acad. Sci. (Paris), 269, 2467 (1969).
5. H. A. Clasener, H. L. Ensering, and W. Hijmans, J. Gen. Microbiol., 62, 195 (1970).
6. Pham Hun Trung, J. Schmitt-Slomska, and A. Bone, Path. et. Biol., 16, 431 (1968).
7. G. Tallarida, L. Perroni, V. Velli, et al., Boll. Soc. Ital. Biol. Sper., 44, 356 (1968).