Studies on *Treponema pallidum* haemagglutination antibodies

I. TPHA antibodies in experimental syphilitic rabbits

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The antilipoidal antibodies produced in the early period of syphilitic infection are found in the 19S fraction, and thereafter a gradual shift takes place to the 7S fraction (Tsunoda, 1966; Aho, 1967). In primary and secondary syphilis, fluorescent treponemal antibody absorption (FTA-ABS) antibodies have already been proved to be both IgG and IgM (Atwood and Miller, 1969). The experiments of Tomizawa and Kasamatsu (1966) in which erythrocytes sensitized with *Treponema pallidum* were used in haemagglutination assays attracted our interest.

The present paper deals with the development of *Treponema pallidum* haemagglutination (TPHA) antibodies in sera from rabbits with experimental *T. pallidum* infection of various durations, employing density gradient ultracentrifugation and gel-filtration methods to separate the antibody-containing fractions.

Material and methods

Syphilitic rabbit sera

Sera were obtained at various intervals from five rabbits inoculated intratesticularly with 0.2 ml. of a suspension containing 60 *T. pallida* (Nichols strain) per field for each testis. In the first group (Rabbits 1, 2, and 3), the sample sera were collected before inoculation, and 11, 22, 52, 62, and 82 days thereafter. In the second group (Rabbits 4 and 5), the samples were taken before and 11, 22, 32, and 52 days after inoculation.

Procedure of fractionation

(1) DENSITY GRADIENT ULTRACENTRIFUGATION

Continuous density gradients were prepared in 2 ml. each of 10 and 40 per cent. sucrose solution in polyvinyl tubes. 0.2 ml. of the sera were added to the top of these gradients. The RPS 40A swing rotor and Hitachi Model RP 55 ultracentrifuge were employed in a 20-hour run at 40,000 rpm. Twenty successive 15-drop fractions were collected by puncturing the tubes through the bottom by a fractionating apparatus.

(2) GEL-FILTRATIONS

These were performed on 40 × 2 cm. column of Sephadex G-200 superfine (Pharmacia AB, Sweden), equilibrated with borate buffered saline, pH 8.4; 2 ml. of the sample

serum were added and column effluents were collected usually in 2.5 ml. fractions, and protein concentrations were determined at 280 mμ. with a Hitachi spectrophotometer.

TPHA test

This was performed according to the instructions supplied by the Fuji Zohki Pharmaceutical Co., Tokyo, Japan.

(1) REAGENTS

Reagent A – absorbent (lyophilized), Reagent B – test diluent, and Reagent C – sensitized sheep cells (lyophilized) (Tomizawa and Kasamatsu, 1966; Cox, Logan, and Norins, 1969).

(2) PROCEDURE

(a) Rabbit whole sera

The unheated whole serum was absorbed by mixing 0.95 ml. reagent A and 0.05 ml. rabbit serum, followed by incubation at 2 to 6°C. for at least 30 min. and centrifugation at 2,500 r.p.m. for 5 min. The supernatant fluid was a 1 : 20 dilution of the absorbed serum. A 1 : 80 dilution of the absorbed serum was made by mixing 0.2 ml. of this 1 : 20 dilution and 0.6 ml. of Reagent B, and a further nine 4-fold serial dilutions were made. 0.25 ml. of each of these dilutions were pipetted into plastic trays, and 0.025 ml. of Reagent C (2.5 per cent. *T. pallidum* sensitized sheep blood cells) were added. These mixtures were incubated for 18 hrs at room temperature.

(b) Fractionated samples

0.05 ml. of the fractions from ultracentrifugation or gel-filtration were absorbed with 0.35 ml. of Reagent A, incubated at 2 to 6°C. for at least 30 min. and centrifuged at 2,500 r.p.m. for 5 min. A 1 : 80 dilution of the absorbed fractions was made by mixing 0.05 ml. of the supernatant and 0.45 ml. of Reagent B, and from this six 4-fold dilutions were prepared. 0.25 ml. of each of these dilutions and 0.025 ml. of Reagent C were transferred to plastic trays, and incubated for 18 hrs at room temperature. Titres of these fractions were taken to be the last dilution giving weakly positive degrees of haemagglutination.

Other serological tests

Quantitative measurement was performed by VDRL slide tests.

Immunoelectrophoresis

Agar double diffusion immunoelectrophoresis was carried out with antirabbit serum (Behringwerke AG.).

Results

Range of TPHA antibody titres in sera from the rabbits inoculated with Treponema pallidum (Nichols strain)

The five rabbits became reactive in the TPHA assay on the 11th day after inoculation. The median and range of the TPHA antibody titres detected at the intervals in the course of infection with *T. pallidum* are shown in Fig. 1. The TPHA antibody titres ranged from 1:80 to 1:327,680. The median increased rapidly until the 22nd day, and then slowly till the 32nd day; thereafter titres did not increase.

FIG. 1 Median and range of TPHA antibody titres of sera taken from experimentally infected rabbits at various intervals after inoculation

Ultracentrifugal analysis

23 sera obtained from the five inoculated rabbits were fractionated. The fractions were titrated as described above. As will be seen from Fig. 2, the TPHA antibodies in Rabbit 1 were detected in the 19S fraction exclusively on the 11th day after inoculation, then in equal amounts in the 19S and 7S respectively on the 22nd day, and were mainly present in the 7S fraction after the 52nd day. The distribution of the TPHA antibody activity in the five rabbits is shown in the Table. On the 11th day after inoculation there were four sera with detectable antibodies only in the 19S fraction. On the 22nd day, in one serum, TPHA antibody was detectable only in the 19S, two sera showed approximately equal titres of TPHA antibody in both the 19S and 7S, and the other two sera gave higher titres in the 7S than in the 19S fractions. All the sera showed higher titres in the 7S than in the 19S fractions after the 32nd day.

FIG. 2 TPHA antibody titres of ultracentrifugal fractions of sera from an experimentally infected rabbit (Rabbit 1)

TABLE Distribution of TPHA antibodies in experimentally infected rabbits in the 7S and 19S fractions

Days after inoculation	Number of titrated sera		
	19S exclusively	19S = 7S	19S < 7S
11	4	1	0
22	1	2	2
32	0	0	2
52	0	0	5
62	0	0	3
82	0	0	3

Gel-filtration

Gel-filtrations were carried out on the sera from Rabbit 4 (Fig. 3). The curves show protein concentration measured spectrophotometrically at 280m μ . The first peak of these curves corresponds to the 19S and the second peak to the 7S fractions. TPHA-positive fractions of the first and second peaks were confirmed by immunoelectrophoresis to be γ M and γ G respectively. The distribution of TPHA antibody titres of this rabbit by gel-filtration showed nearly the same pattern as that from ultracentrifugation.

FIG. 3 Gel-filtration of experimentally infected rabbit sera (curve) and TPHA antibody titres of each fraction. The distribution of TPHA antibodies shows approximately same pattern as in ultracentrifugation

VDRL assay

Ultracentrifugal fractions of four sera from Rabbit 4 were tested by TPHA and VDRL assay (Fig. 4). TPHA antibody titres showed almost the same pattern as VDRL antibody titres, although TPHA antibody was detected in the 19S fraction only and VDRL antibody in both the 19S and the 7S fractions on the 11th day after inoculation.

Discussion

Our ultracentrifugation experiments confirmed that, in the early stage of infection, TPHA antibody in the majority of experimentally infected rabbits except one (Rabbit 2) could be detected solely in the 19S fraction; thereafter a gradual shift to the 7S took place. The distribution of TPHA antibody titres in the sera fractionated by gel-filtration showed nearly the same pattern as in those fractionated by ultracentrifugation. The formation of TPHA and antilipoidal antibodies showed an analogous pattern, although the two antibodies had essentially different serological properties. Seronegative rabbits infected intratesticularly became reactive in the TPHA assay as early as or earlier than in FTA 1:5 and VDRL slide tests (Cox and others, 1969). According to the present results, the TPHA antibodies in rabbits in the early stage of infection are detected in the 19S

FIG. 4 Ultracentrifugal location of TPHA and VDRL antibodies in the same syphilitic rabbit. Antibody titres of TPHA show approximately same pattern as those of VDRL, while TPHA antibodies are detected in the 19S fraction exclusively and VDRL antibodies in both the 19S and the 7S fractions on the 11th day after inoculation

fraction exclusively. This is in contrast with the fact that the 19S fraction of sera in patients with syphilis is less sensitive in the TPHA assay than that of sera in experimentally infected rabbits (unpublished data).

Summary

Quantitative assays of *Treponema pallidum* haemagglutinating (TPHA) antibodies were performed on 28 sera from five experimentally infected rabbits. After intratesticular inoculation 23 sera were fractionated by ultracentrifugation. In the majority of sera collected on the 11th day after inoculation, TPHA antibodies were detected in the 19S fraction exclusively. On the 22nd day, TPHA antibody titres were found to be equal in both the 19S and the 7S fractions. After the 32nd day 7S TPHA antibodies predominated. The distribution of TPHA antibody titres in gel-filtration fractionation showed nearly the same pattern as by ultracentrifugation. Antibody reactive in the VDRL test in the experimentally infected rabbit showed a pattern of development similar to that of TPHA antibody.

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Études sur les anticorps hémagglutinants suscités par *T. pallidum*. I. Les anticorps TPHA dans la syphilis expérimentale du lapin

SOMMAIRE

Des mesures quantitatives des anticorps hémagglutinants/ *T. pallidum* ont été faites sur 28 sérums provenant de 5 lapins infectés expérimentalement. Après l'inoculation

intratesticulaire, 23 sérums furent fractionnés par ultracentrifugation. Pour la majorité des sérums recueillis le onzième jour après l'inoculation, les anticorps TPHA furent trouvés exclusivement dans la fraction 19S. Au 22ème jour, les titres de l'anticorps TPHA étaient équivalents dans la fraction 19S et dans la fraction 7S. Après le 32ème jour, les anticorps TPHA prédominaient dans la fraction 7S. La distribution des titres de l'anticorps TPHA, lors du fractionnement par filtration sur gel, ont montré à peu près la même distribution qu'après ultracentrifugation. L'anticorps réagissant au VDRL avait un modèle de développement semblable à celui de l'anticorps TPHA.