INFECTION AND IMMUNITY, Aug. 1977, p. 466-467

Vol. 17, No. 2
Printed in U.S.A.

# Inhibition of Leptospiral Agglutination by the Type-Specific Main Antigens of Leptospiras

YOSHIKAZU ADACHI AND RYO YANAGAWA*

*Department of Hygiene and Microbiology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, 060, Japan*

Received for publication 28 February 1977

Microscopic agglutination of leptospiras was inhibited by the homologous type-specific main antigens. The result may indicate that the substance participating in the agglutination of leptospiras with antiserum is the type-specific main antigen.

The taxonomy of the genus *Leptospira* is at present under consideration by the Subcommittee on *Leptospira* of the International Committee on Systematic Bacteriology (4). For many years, strains of leptospiras have been compared and distinguished serologically, especially by means of microscopic agglutination reaction and cross-agglutinin absorption studies carried out with antisera prepared in rabbits. The substance that participates in the specific agglutination of leptospira with antiserum must be located at the surface of the leptospiral cells.

The type-specific main (TM) antigen was extracted from leptospiras and purified (3). The serovar specificity of the TM antigen was proved by immunodiffusion. The nondialyzable, delipidized TM antigen contained the antigenic determinant of the TM antigen (1). We wished to know the relation of the TM antigen to the substance participating in leptospiral agglutination. We presumed that if the substance that participates in the specific agglutination of leptospiras with antiserum is the TM antigen, then sufficient amounts of purified, homologous TM antigen should compete for limiting concentrations of antibody and prevent agglutination of leptospiras. These results would suggest a surface location of the TM antigen on leptospiras.

The strains used were serovar *canicola* strain Hond Utrecht IV, serovar *copenhageni* strain Shibaura, serovar *kremastos* strain Kyoto, and serovar *hebdomadis* strain Hebdomadis (a mutant isolated by the present authors that can grow in a modified Shenberg's synthetic medium). These strains were cultivated in Shenberg's synthetic medium (2), with the exception of strain Hebdomadis (mutant), which was grown in the same medium with 0.04% of bovine serum albumin (fraction V; Sigma) added.

The extraction and purification of the TM antigen was carried out by the method described previously (3). The TM antigens, which were dissolved in 20% ethanol at a concentration of 1 mg/ml, were diluted with saline solution and used for the microscopic agglutination inhibition test. In some cases, the TM antigens were sonically treated for 10 min before use. The antisera against the four strains of leptospiras were prepared as previously reported (5).

The microscopic agglutination inhibition test

TABLE 1. *Inhibition of microscopic agglutination of leptospiras by the homologous TM antigens*

| TM antigen | Leptospira and homologous anti-serum | TM antigen added (μg/ml) | | | | |
|---|---|---|---|---|---|---|
| | | 100 | 25 | 6.3 | 1.6 | 0 |
| *canicola* | *canicola* | 0[b] | 0 | 0 | 2 | |
| | *copenhageni* | 2 | 2 | 2 | 2 | 2 |
| | *kremastos* | 2 | 2 | 2 | 2 | 2 |
| | *hebdomadis* | 2 | 2 | 2 | 2 | 2 |
| | —[a] | 0 | 0 | 0 | 0 | 0 |
| *copenhageni* | *canicola* | 2 | 2 | 2 | 2 | 2 |
| | *copenhageni* | 0 | 0 | 0 | 2 | 22 |
| | *kremastos* | 2 | 2 | 2 | 2 | 2 |
| | *hebdomadis* | 2 | 2 | 2 | 2 | 2 |
| | — | 0 | 0 | 0 | 0 | 0 |
| *kremastos* | *canicola* | 2 | 2 | 2 | 2 | 2 |
| | *copenhageni* | 2 | 2 | 2 | 2 | 2 |
| | *kremastos* | 0 | 0 | 0 | 2 | 2 |
| | *hebdomadis* | 2 | 2 | 2 | 2 | 2 |
| | — | 0 | 0 | 0 | 0 | 0 |
| *hebdomadis* | *canicola* | 2 | 2 | 2 | 2 | 2 |
| | *copenhageni* | 2 | 2 | 2 | 2 | 2 |
| | *kremastos* | 2 | 2 | 2 | 2 | 2 |
| | *hebdomadis* | 0 | 0 | 0 | 2 | 2 |
| | — | 0 | 0 | 0 | 0 | 0 |

[a] Saline solution was used instead of antiserum.
[b] 0, No agglutination; 2, 50% agglutination.

was carried out as follows. The antiserum was diluted so as to cause 50% agglutination when mixed with 2 volumes of saline. Each 1 drop (0.025 ml) of the antiserum was mixed with the same amount of the serially diluted TM antigen in the holes of a plastic tray (Tominaga Co., Tokyo, Japan). The mixture was shaken and held at 37°C for 30 min. Each 1 drop (0.025 ml) of leptospiral culture was then mixed and left at room temperature for 1 h. Necessary controls were made. The agglutination of the leptospiras was observed under the dark-field microscope. The criteria for the agglutination of leptospiras were: 2 (50% agglutination); and 0 (no agglutination).

The 50% agglutination of leptospiras was inhibited in the presence of the homologous TM antigen (Table 1). The inhibition was attained with 6.3 $\mu$g (157 ng per drop) of the TM antigens per ml. No inhibition was found when the heterologous TM antigens were used up to the amount of 100 $\mu$g/ml. For inhibition of a stronger (70%) agglutination of leptospiras, the homologous TM antigen at a concentration of more than 50 $\mu$g/ml was necessary (data not shown).

These results may indicate that the TM antigen is the substance that participates in the agglutination of leptospira with antiserum, and its is probably located at the surface of the leptospiral cells. The results may emphasize the importance of investigation of the nondialyzable, delipidized TM antigen, which contains the antigenic determinant of the TM antigen (1).

## LITERATURE CITED

1. **Adachi, Y., and R. Yanagawa.** 1975. Possible role of protein(s) as antigenic determinant of the type-specific main antigen of *Leptospira kremastos* strain Kyoto. Infect. Immun. 11:1325–1331.
2. **Shenberg, E.** 1967. Growth of pathogenic *Leptospira* in chemical defined media. J. Bacteriol. 93:1598–1606.
3. **Shinagawa, M., and R. Yanagawa.** 1972. Isolation and characterization of a leptospiral type-specific antigen. Infect. Immun. 5:12–19.
4. **Turner, L. H.** 1974. Genus V. *Leptospira* Noguchi 1917, p. 190–192. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's Manual of Determinative Bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
5. **Yanagawa, R., M. Shinagawa, and I. Takashima.** 1974. Serological studies of leptospiras by immunodiffusion. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A 228:369–377.