JOURNAL OF CLINICAL MICROBIOLOGY, July 1980, p. 7-9
0095-1137/80/07-0007/03$02.00/0

Vol. 12, No. 1

Comparative Studies on Two Antigens (F4 and TM) Extracted from Leptospires

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F4 and TM antigens extracted from *Leptospira interrogans* serovars *pomona*, *icterohaemorrhagiae*, and *copenhageni* were compared by immunodiffusion, by hemagglutination inhibition, and by selective absorption of antisera. These data, together with previous information on the serological specificities of the two antigen preparations, suggest that F4 and TM are different antigens.

Since 1972 there have been several studies on two leptospiral antigen preparations, the TM antigen of Shinagawa and Yanagawa (10) and the F4 antigen of Faine et al. (6). There is now considerable information available on both these antigens and on the human antibody response to one of them (see Discussion for references). In addition, there are some similarities between their serological activities. It is therefore important to establish whether their reactions represent different manifestations of similar determinants or activities of independent antigenic systems. Thus the objectives of this paper were to examine the serological activities of F4 and TM antigens from two very closely related *Leptospira interrogans* serovars (*copenhageni* and *icterohaemorrhagiae*) and from an unrelated serovar (*pomona*).

MATERIALS AND METHODS

Antigens and serological methods. TM antigens were prepared by one of the authors (R.Y.) from *L. interrogans* serovars *pomona* (Pomona), *copenhageni* (Shibaura), and *icterohaemorrhagiae* (RGA) at Hokkaido University, Sapporo, Japan, as described previously (11). They were sent in lyophilized form to the other authors at Monash University, Melbourne, Australia. F4 antigens were extracted in Melbourne from serovars *pomona* (Pomona), *copenhageni* (M20), and *icterohaemorrhagiae* (RGA) as described previously (6).

Hemagglutination (HA) tests with antigen preparations were performed in V-bottom microtiter trays as described previously (6), using sheep erythrocytes that were glutaraldehyde fixed (5) before sensitizing with antigen.

Antiserum for use in HA inhibition (HI) tests was first titrated against sensitized erythrocytes, and the highest hemagglutinating dilution was taken to contain 1 HA unit of antiserum. For HI tests, the antigen was serially diluted, and 1 volume of antiserum containing 2 HA units was added, followed by 1 volume of sensitized erythrocytes. The lowest concentration of antigen to show HI was taken as the minimal HI dose.

Immunodiffusions (6) and absorption of sera with F4-sensitized erythrocytes (3) were performed as described.

Antisera. Antileptospiral antisera were produced in Melbourne in New Zealand White rabbits by intravenous injection of antigen as described by Adler and Faine (3) (Melbourne [Mel] antiserum) or in Sapporo as described by Yanagawa et al. (11) (Sapporo [Sap] antiserum).

RESULTS

Reactions of Sap and Mel antisera with F4 and TM antigens. Both Sap and Mel antisera reacted with homologous F4 antigens optimally sensitized to sheep erythrocytes. The difference between the two antisera in titers for each serovar was only one doubling dilution (Table 1).

All antisera reacted by gel diffusion with homologous TM antigen at concentrations of 200 μg/ml to give a single line. Lines of identity between TM antigens from *copenhageni* and *icterohaemorrhagiae* were observed with both Sap and Mel antisera prepared against either serovar.

HI of F4-sensitized erythrocytes. Both F4 and TM antigens inhibited, to varying extents, the agglutination of erythrocytes sensitized with homologous F4 (Table 2). However, it was necessary to sonicate TM antigen to disperse it in aqueous solution before it became active in HI tests. Unsonicated TM had no HI activity at concentrations up to 200 μg/ml.

When tested for specificity with Mel sera and homologous F4-sensitized erythrocytes, both the F4 and TM antigens of *copenhageni* and *icterohaemorrhagiae* cross-reacted, but very little or no reaction was seen between the corresponding preparations from *pomona* and the other two serovars (Table 3).

Erythrocyte-sensitizing properties of TM antigen. TM antigens from the three serovars

TABLE 1. *HA titers of rabbit antisera against erythrocytes optimally sensitized with homologous F_4*

Antiserum against serovar:	Titer of antiserum[a]	
	Mel	Sap
pomona	1,024	512
copenhageni	2,048	1,024
icterohaemorrhagiae	1,024	512

[a] Mel prepared at Monash University, Melbourne (3); Sap prepared at Hokkaido University, Sapporo (11).

TABLE 2. *HI activity of F_4 and TM antigens against erythrocytes optimally sensitized with homologous F_4*

Antigens extracted from serovar:	MHID[a] (μg/ml) of antigen			
	Mel antisera		Sap antisera	
	F4	TM	F4	TM
pomona	1.2	3.1	2.4	3.1
copenhageni	1.1	3.1	2.1	6.2
icterohaemorrhagiae	0.3	3.1	0.6	12.6

[a] MHID, Minimal HI dose.

TABLE 3. *Specificity of HI of F4-sensitized erythrocytes with F4 and TM antigens*

Serovar[a]	MHID[b] (μg/ml) with antigen:					
	pomona		*copenhageni*		*icterohaemorrhagiae*	
	F4	TM	F4	TM	F4	TM
pomona	0.6	3.1	—	100	—	—
copenhageni	—	—	1.1	3.1	1.3	12.5
icterohaemorrhagiae	—	—	0.2	3.1	0.3	6.3

[a] Test used homologous Mel antiserum and erythrocytes sensitized with F4 from indicated serovar.

[b] MHID, Minimal HI dose. —, MHID > 200 μg/ml.

were tested, at concentrations of 10, 50, 100, and 200 μg/ml, for their ability to sensitize erythrocytes to agglutination by homologous antiserum. All F4 preparations sensitized erythrocytes at 100 or 200 μg/ml. TM antigens from serovars *copenhageni* and *icterohaemorrhagiae* were unable to sensitize at any of the concentrations, but *pomona* TM antigen sensitized erythrocytes optimally at a concentration of 100 μg of antigen per ml. Mel and Sap *pomona* antisera had HA titers of 256 and 64, respectively, against these erythrocytes, and serovar *pomona* F4 inhibited at a minimal HI dose of 1.1 μg/ml.

Absorption of antisera with F4. Because the above results could be explained either by identity of TM and F4 antigens or by the presence of one antigen in preparations of the other, we sought to clarify this problem by absorbing serum with F4. Mel antisera were absorbed with F4-sensitized erythrocytes, and the absorbed sera were compared with a control antiserum absorbed with unsensitized erythrocytes in precipitin reactions against TM antigen. Removal of F4 antibodies as detected by HA did not remove the capacity of either *pomona* or *icterohaemorrhagiae* antisera to react with homologous TM antigen by immunodiffusion. There was insufficient material to perform a similar experiment with *copenhageni*.

Comparison of antigens by immunodiffusion. Because purified F4 antigen does not precipitate readily in immunodiffusion tests (6), it was not possible to compare the two antigens directly by gel diffusion. However, since F4 forms a line of identity with the antiserum-adjacent line of leptospiral phenol extract (6), TM and phenol extract from serovar *copenhageni* were compared by immunodiffusion. The results showed that the TM line was different from the F4 line. Although TM always gave only a single line in such tests, occasionally a slight bending of the F4 line was observed, further suggesting the presence of F4 in the TM antigen preparation.

In addition, antiserum prepared against purified *copenhageni* F4 did not give rise to a precipitin line when reacted with homologous TM. The antiserum had an HA titer of 128 against F4.

DISCUSSION

We considered it important to examine the relationship between F4 and TM antigens because detailed information is available on the composition, serological specificity, and role in agglutination of these antigens (7, 8, 10). In addition, the immune response to F4 antigen in humans after leptospiral infection has been examined (3). Thus it was of interest to determine whether the two groups of workers were detecting identical, similar, or completely different antigens. Our results indicate that different antigens are involved in the reactions of sera with F4 by passive HA and with TM by immunodiffusion. TM preparations inhibited HA tests with F4-sensitized erythrocytes, although there was a quantitative difference in HI activity between TM and F4. However, results of the absorption experiments indicated that TM antigen detected by immunodiffusion was not itself active in HI tests, but the TM preparations used apparently contained some F4 which was responsible for the HI activity exhibited by TM. Absorption of all F4 activity from antisera did not affect the reaction with TM by immunodiffusion.

The findings that F4 cross-reacts widely

among different serogroups (4), whereas TM is serovar specific (Shimono et al., Jpn. J. Vet. Sci, in press), further support the contention that the two antigens are different.

TM antigen did not sensitize erythrocytes, nor did the F4 that was presumed to be in TM preparations, except for serovar *pomona*, in which case the erythrocytes behaved as described for F4-sensitized erythrocytes. The lack of sensitizing activity by the other two serovars may be due to the state of aggregation of the F4, which is affected by the age of the preparation. The importance of aggregation state in determining the activity of bacterial lipopolysaccharide is well known (9), and similar observations have been made with F4 antigen (Adler, unpublished data). Although F4 and TM antigens were different, the specificities of the two preparations in HI tests were similar, further supporting the contention that HI detected similar haptens in both antigen preparations. This was supported by the finding that the TM precipitin line in immunodiffusions was different from the F4 line visualized in phenol extracts. The F4 line bent slightly when influenced by the TM preparation, consistent with the presence of F4 hapten in the TM preparation.

We conclude that the TM and F4 antigens, as detected by immunodiffusion and HA, respectively, and as reported by the two groups of workers (6, 10), are different leptospiral antigens.

ACKNOWLEDGMENT

This work was supported by a grant from the National Health and Medical Research Council, Canberra, Australia.

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. **Adler, B., and S. Faine.** 1978. Serological specificities of erythrocyte-sensitizing antigens of leptospires. FEMS Microbiol. Lett. **4**:35-36.
3. **Adler, B., and S. Faine.** 1978. The antibodies involved in the human immune response to leptospiral infection. J. Med. Microbiol. **11**:387-400.
4. **Adler, B., and S. Faine.** 1979. Serological cross-reactions of leptospiral lipopolysaccharide (F4) antigen. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **244**:291-301.
5. **Baker, L. A., and C. D. Cox.** 1973. Quantitative assay for genus-specific leptospiral antigen and its antibody. Appl. Microbiol. **25**:697-698.
6. **Faine, S., B. Adler, and A. Palit.** 1974. Chemical, serological and biological properties of a serotype-specific polysaccharide antigen in *Leptospira*. Aust. J. Exp. Biol. Med. Sci. **52**:311-319.
7. **Kasai, N., and R. Yanagawa.** 1974. Studies on the antigenic determinant group of the type specific antigen of *Leptospira canicola*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **228**:533-541.
8. **Kawaoka, Y., M. Naiki, and R. Yanagawa.** 1979. Radioimmunoassay system using a serovar-specific lipopolysaccharide antigen of *Leptospira*. J. Clin. Microbiol. **10**:313-316.
9. **Ribi, E., R. L. Anacker, R. Brown, W. T. Haskins, B. Malmgren, K. C. Milner, and J. A. Rudbach.** 1966. Reaction of endotoxin and surfactants. I. Physical and biological properties of endotoxin treated with sodium deoxycholate. J. Bacteriol. **92**:1493-1509.
10. **Shinagawa, M., and R. Yanagawa.** 1972. Isolation and characterization of a leptospiral type-specific antigen. Infect. Immun. **5**:12-19.
11. **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.