INFECTION AND IMMUNITY, May 1985, p. 569–572
0019-9567/85/050569-04$02.00/0

Vol. 48, No. 2

Antigenic Properties of *Chlamydia trachomatis* Lipopolysaccharide

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Received 1 October 1984/Accepted 4 February 1985

The antigenic properties of the lipopolysaccharide (LPS) of *Chlamydia trachomatis* L2 were investigated. By means of passive hemolysis, passive hemolysis inhibition, and absorption experiments, it was shown that antiserum raised against chlamydial elementary bodies contained at least two different antibody specificities which reacted with different antigenic determinants of chlamydial LPS. One of these antibodies cross-reacted with enterobacterial Re LPS, recognizing a structure which is shared by both LPSs, whereas the reactivity of the second antibody was restricted to chlamydial LPS. The former antibody could be absorbed with *Salmonella minnesota* Re LPS, whereas the latter was not affected by this absorption. Therefore, chlamydial LPS possesses two distinct antigenic determinants, one of which is *C. trachomatis* specific, the other of which is responsible for the cross-reactivity with enterobacterial Re-type LPS. Both antigenic determinants were destroyed during mild acid-catalyzed hydrolysis. It was further shown that free chlamydial lipid A exhibits antigenicity that cross-reacts with free enterobacterial lipid A. This antigenicity, however, as in enterobacterial LPS, is present in a cryptic form, i.e., it is unmasked only after acid hydrolysis of LPS.

Chlamydiae are important pathogens causing a number of diseases in animals and humans (13, 20, 22). The typical chlamydial infection is characterized by a long-term persistence of the infectious agent, leading to recurrent clinical symptoms, i.e., chronic infections of the genitourinary tract (15). The mechanisms by which these organisms cause infections and escape the immune defense system are presently not understood. Since surface structures are most likely involved in the pathogenicity of chlamydiae, we became interested in the antigenic and immunogenic properties of the surface antigens of chlamydial cells.

One of the major antigens of the chlamydial cell wall is a specific glycolipid which has been shown to contain a 2-keto-3-deoxycarbohydrate as the immunodominant sugar (8, 9). This antigen has been reported by several authors to cross-react with certain strains of *Acinetobacter calcoaceticus*, a member of the *Neisseriaceae* (1, 2, 17, 19, 21, 23). In addition, we have found by the immunoblotting technique that the chlamydial antigen cross-reacts with the lipopolysaccharide (LPS) of *A. calcoaceticus* and *Salmonella* Re mutants (19). Moreover, Caldwell and Hitchcock have suggested that there are at least two different epitopes on chlamydial LPS, one of which cross-reacts with enterobacterial Re-type LPS (7).

These data suggested that chlamydiae possess an LPS which in its saccharide portion is similar to enterobacterial Re-type LPS (16). That this is actually the case was shown only recently by chemical analysis, which revealed the presence of typical LPS constituents in the chlamydial antigen, e.g., D-glucosamine, long-chain hydroxy fatty acids, 2-keto-3-deoxyoctonic acid (KDO), and phosphate (18).

In the present paper, the antigenic properties of chlamydial LPS are further studied by applying the passive hemolysis and passive hemolysis inhibition test as well as absorption experiments. It will be shown that a *Chlamydia trachomatis*-specific and a cross-reacting antigen can be clearly differentiated from each other. In addition, it will be demonstrated that after acid hydrolysis the chlamydial LPS develops the typical enterobacterial lipid A antigenicity (12).

MATERIALS AND METHODS

LPS and lipid A. LPS of *C. trachomatis* was prepared as described elsewhere (18), and the LPS of *A. calcoaceticus* was that described in an earlier paper (3). LPS of the *S. minnesota* Re mutant strain R595 was obtained by the phenol-chloroform-petroleum ether method (11), purified, and converted to the uniform triethylammonium salt form as reported earlier (10). Free lipid A was obtained after hydrolysis of the LPS in 0.1 M acetate buffer, pH 4.4, at 100°C for 1 h. Deesterified LPS (LPS-OH) and free lipid A (lipid A-OH) were prepared by treatment with sodium methylate as described previously (24).

Antisera. Rabbit antiserum against *C. trachomatis* was that described previously (19). Rabbit antiserum against Re-type LPS was prepared by immunizing New Zealand white rabbits with phenol-killed *S. minnesota* R595 (Re mutant) bacteria as described previously (16). Preimmune sera were tested against all antigens used throughout this study, and only seronegative animals were chosen for immunization. Normal mouse serum was obtained from NMRI mice from the Lippische Versuchstieranstalt (Hannover, Federal Republic of Germany). All sera were absorbed with sheep erythrocytes (SRBC) and stored at −20°C.

Serological methods. Antibodies were determined in microtiter plates by the passive hemolysis test with SRBC sensitized with LPS-OH and guinea pig complement which had been absorbed with SRBC. Inhibition studies were performed by the passive hemolysis inhibition test with three hemolytic units of antibody (12). The following antigen/antibody systems were employed: Re LPS-OH/Re antiserum, chlamydial LPS-OH/anti-*C. trachomatis* antiserum, and lipid A-OH/anti-lipid A antiserum to determine Re-type, chlamydial, and lipid A specificity, respectively. In addition, *A. calcoaceticus* lipid A-OH/normal mouse serum was used to measure the common LPS specificity. Details of the latter test system have been reported elsewhere (4, 5).

Absorption experiments were carried out with antigen-

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TABLE 1. Reactivity of anti-*C. trachomatis* and anti-Re antisera with SRBC coated with chlamydial or Re LPS-OH

Sensitizing antigen	amt (μg/0.2 ml of SRBC)	Hemolytic titer with antiserum against:	
		C. trachomatis	*S. minnesota* Re
C. trachomatis LPS-OH	4	320	<10
	8	5,120	80
	40	20,480	640
	80	20,480	640
	200	20,480	1,280
	400	20,480	2,560
S. minnesota Re LPS-OH	4	1,280	80
	8	1,280	160
	40	1,280	640
	80	2,560	2,560
	200	2,560	2,560
	400	2,560	2,560

coated SRBC (4°C, 30 min) as outlined in detail elsewhere (5).

Hydrolysis kinetics were done as described previously (4), except that 0.1 M instead of 20 mM acetate buffer (pH 4.4) was used.

RESULTS

Hemolytic activity of anti-chlamydia antiserum with homologous and Re-type LPSs. Rabbit antiserum obtained after immunization with outer membranes of *C. trachomatis* elementary bodies was tested by the passive hemolysis test against SRBC coated with various amounts of chlamydial LPS-OH and *S. minnesota* R595 LPS-OH (Re type). The reactivity was compared with that of an Re (*S. minnesota*) antiserum (Table 1). The chlamydial antiserum yielded a significantly higher titer with the homologous antigen (20,480) than with Re LPS-OH (2,560), whereas the Re antiserum gave the same titer, 2,560, with both antigens. Both antisera gave maximal titers at a sensitizing dose of 40 to 80 μg per 0.2 ml of packed SRBC except when Re antiserum was tested with chlamydial LPS-OH, when a plateau value was not attained even at 400 μg per 0.2 ml of SRBC. Doses higher than 400 μg could not be tested since nonspecific lysis occurred. In all further experiments, both antigens were used at a concentration of 80 μg per 0.2 ml of SRBC.

Absorption of anti-chlamydia antiserum with Re LPS-OH. The chlamydial antiserum was absorbed with Re LPS-OH coated onto SRBC. The titer against chlamydial and Re LPS-OH was tested both before and after absorption. As a control, Re antiserum was absorbed in the same way. The reactivity of the chlamydial antiserum with Re LPS-OH could be completely absorbed, whereas the hemolytic titer with the homologous antigen was not affected (Table 2). The reactivity of the Re antiserum was abolished after absorption, independent of whether the homologous or heterologous test antigen was employed.

TABLE 2. Hemolytic activity of anti-*C. trachomatis* and anti-Re antisera before and after absorption with Re LPS-OH coated on SRBC[a]

Antiserum	Hemolytic titer against SRBC coated with:	
	Chlamydial LPS-OH	Re LPS-OH
C. trachomatis		
Before absorption	20,480	2,560
After absorption	20,480	<20
S. minnesota Re		
Before absorption	640	2,560
After absorption	<20	<20

[a] SRBC were sensitized with 80 μg of the respective LPS-OH per 0.2 ml of packed cells; 0.5 ml of diluted (1:5) antiserum was absorbed with 50 μl of SRBC sensitized with Re LPS-OH.

Inhibition tests. The inhibiting capacity of chlamydial LPS-OH for the homologous test system (chlamydial LPS-OH/chlamydial antiserum) was determined by the passive hemolysis inhibition test; Re LPS-OH was tested in parallel. The amount of chlamydial LPS-OH causing 50% inhibition of lysis in the homologous and heterologous (Re LPS-OH/Re antiserum) test systems was 1 and 32 ng, respectively. The Re LPS-OH inhibited the homologous system with 16 ng, whereas the *C. trachomatis*-specific system was not inhibited by amounts up to 1,000 ng.

Behavior of the different antigenic specificities after mild acid hydrolysis. Chlamydial LPS was subjected to mild acid-catalyzed hydrolysis (0.1 M acetate buffer, pH 4.4) at 100°C for various lengths of time (Fig. 1). After neutralization, the samples were tested for their ability to inhibit the Re-specific and *C. trachomatis*-specific hemolytic test systems and to inhibit the lipid A-specific system of lipid A-OH/anti-lipid A antiserum. Untreated LPS (time zero) did not exhibit lipid A antigenicity. However, after acid hydrolysis of the LPS, the lipid A antigenicity was exposed, reaching a maximum after 30 min of hydrolysis (inhibition value of 16 ng).

The *C. trachomatis*-specific and Re-specific antigens were destroyed during hydrolysis, as indicated by inhibition values greater than 1,000 ng after 60 min. Whereas the disappearance of Re specificity and the appearance of lipid A antigenicity followed similar kinetics, the *C. trachomatis*-specific antigen was destroyed at a slower rate.

A test system specific for the recently described common LPS specificity (*A. calcoaceticus* lipid A-OH/normal mouse

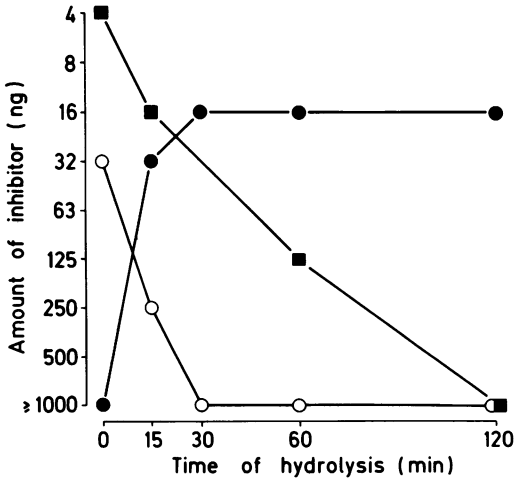

FIG. 1. Behavior of different antigenic specificities in chlamydial LPS after mild acid hydrolysis. LPS was hydrolyzed in 0.1 M acetate buffer, pH 4.4, at 100°C for the time intervals indicated on the abscissa and tested by the passive hemolysis inhibition test for reactivity in the antigen/antibody systems of chlamydial LPS-OH/anti-*C. trachomatis* antiserum (■), Re LPS-OH/anti-Re antiserum (○), and lipid A-OH/anti-lipid A antiserum (●). Amounts of inhibitor causing 50% inhibition of lysis are indicated on the ordinate.

serum [4, 5]) was not inhibited by chlamydial LPS either before or after hydrolysis (data not shown).

DISCUSSION

Passive hemolysis and passive hemolysis inhibition test systems were used for further characterization of the antigenic cross-reaction between chlamydial and enterobacterial Re LPSs.

Antiserum against chlamydial outer membranes was found to react with chlamydial and Re-type LPSs in the passive hemolysis test; however, the homologous titer was significantly higher (20,480) than the titer against Re LPS (2,560). To determine whether this difference was due to two different epitopes, the chlamydial antiserum was absorbed with Re LPS, after which reactivity with Re LPS disappeared while the homologous titer remained unchanged. This indicates that the chlamydial antiserum contained at least two different antibody specificities. One of these recognizes a structure which is expressed by chlamydial and Re LPS, and the other reacts with a *C. trachomatis*-specific determinant not present in Re LPS. This is further supported by the results of inhibition experiments, in which the chlamydial LPS was demonstrated to inhibit both the *C. trachomatis*- and Re-specific hemolytic test systems, whereas the Re LPS was only active in the homologous test system. The failure of Re LPS to inhibit the chlamydial test system is explained by the fact that in the chlamydial antiserum, the titer against the *C. trachomatis*-restricted determinant is about 10 times higher than that against Re specificity (Table 1). Therefore, in the inhibition test, the Re-specific antibodies are diluted out at the dilution used to give three hemolytic units (antiserum dilution of 1:6,000). However, when the hemolytic system of Re LPS-OH/chlamydial antiserum was employed, chlamydial and Re LPSs exhibited similar inhibition values (data not shown). It is stressed that in this system, the Re-specific antibodies are determining the dilution of the chlamydial antiserum to give three hemolytic units, which in this case was achieved at a dilution of 1:600. This confirms the existence of two different antibodies in the chlamydial antiserum and shows that the chlamydial LPS expresses two distinct antigenic determinants.

The behavior of these two determinants after mild acid hydrolysis was studied by performing hydrolysis kinetics. It was found that both determinants are destroyed; however, the *Chlamydia*-specific seemed to be more resistant to acid than the Re-specific determinant.

KDO has been reported to act as the immunodominant sugar for the enterobacterial Re specificity (16). Since KDO has been found to be a constituent of chlamydial LPS, it is likely that it is also involved in the Re specificity of chlamydial LPS. Because of the extremely acid-labile ketosidic linkage of this sugar, the Re specificity is easily destroyed by mild acid hydrolysis.

It is not known at present what role KDO may play in the *C. trachomatis*-specific antigen. Dhir et al. have claimed the importance of a 2-keto-3-deoxycarbohydrate for the antigenicity of the group-specific antigen of chlamydiae which could be destroyed by periodate oxidation or by mild acid hydrolysis (8). However, these authors reported that the compound released after hydrolysis was not identical with KDO as determined by paper chromatography. Very recently, we have identified KDO in chlamydial LPS which was indistinguishable from authentic KDO by using combined gas-liquid chromatography and mass spectrometry of reduced and permethylated derivatives (18). This discrepancy may be explained by the different hydrolysis conditions employed by us and by Dhir et al. or by substituents which were removed during the derivatization procedure (Hakomori methylation [14]).

A hypothetical substituent could also change the stability of the ketosidic linkage (6), leading to different hydrolysis kinetics after mild acid hydrolysis, but other possibilities cannot be excluded at this time. Thus, the heterogeneity of LPS and the possibility that the *C. trachomatis*-specific determinant is not attached to KDO but to other LPS regions have to be considered.

In addition to the aforementioned two antigenic determinants, the chlamydial LPS also harbors lipid A antigenicity. As in enterobacterial LPS, the lipid A antigenicity of chlamydial LPS is cryptic and only exposed after acid hydrolysis (12). The rate of exposure of lipid A antigenicity is similar to that observed for enterobacterial LPS, in which the appearance of lipid A antigenicity is paralleled by the loss of polysaccharide (O and R specificities). Finally, there is yet another similarity between chlamydial and enterobacterial Re LPS: they both lack the common LPS specificity which is found in all LPSs except those belonging to the Re chemotype (4, 5).

Caldwell and Hitchcock recently described a monoclonal antibody against the *C. trachomatis*-specific antigen. In addition, they reported a polyclonal anti-lipid A antibody which was able to bind to chlamydial and enterobacterial Re LPSs (7). This finding is not in agreement with our data and those of other investigators (12) and could be the result of partial cleavage of KDO residues during preparation of the LPS. The low KDO value (8.8%) given by these authors for the chlamydial LPS, compared with 20% KDO in enterobacterial Re LPS, suggests this possibility. However, the different immunochemical technique (immunoblotting versus a complement-dependent hemolytic test system) could also explain these differing results, and further studies are indicated to resolve this discrepancy.

ACKNOWLEDGMENTS

We are indebted to E. T. Rietschel for fruitful criticism. The expert technical assistance of U. Albert is gratefully acknowledged.

This work was supported by a grant of the Deutsche Forschungsgemeinschaft to H.B. (Br 731/3).

LITERATURE CITED

1. **Brade, H., and H. Brunner.** 1979. Serological cross-reactions between *Acinetobacter calcoaceticus* and chlamydiae. J. Clin. Microbiol. **10**:819–822.
2. **Brade, H., and H. Brunner.** 1980. Detection of chlamydial inclusion bodies with antisera to *Acinetobacter calcoaceticus* subspecies *anitratus* by indirect immunofluorescence. Infection **8**:215–216.
3. **Brade, H., and C. Galanos.** 1982. Isolation, purification, and chemical analysis of the lipopolysaccharide and lipid A of *Acinetobacter calcoaceticus* NCTC 10305. Eur. J. Biochem. **122**:233–237.
4. **Brade, H., and C. Galanos.** 1983. Common lipopolysaccharide specificity: new type of antigen residing in the inner core region of S- and R-form lipopolysaccharides from different families of gram-negative bacteria. Infect. Immun. **42**:250–256.
5. **Brade, H., and C. Galanos.** 1983. A new lipopolysaccharide antigen identified in *Acinetobacter calcoaceticus*: occurrence of widespread natural antibody. J. Med. Microbiol. **16**:203–210.
6. **Brade, H., C. Galanos, and O. Lüderitz.** 1983. Isolation of a 3-deoxy-D-*manno*-octulosonic acid disaccharide from *Salmonella minnesota* rough-form lipopolysaccharide. Eur. J. Biochem. **131**:195–200.
7. **Caldwell, H. D., and P. J. Hitchcock.** 1984. Monoclonal antibody

against a genus-specific antigen of *Chlamydia* species: location of the epitope on chlamydial lipopolysaccharide. Infect. Immun. **44**:306–314.

8. **Dhir, S. P., S. Hakomori, G. E. Kenny, and J. T. Grayston.** 1972. Immunochemical studies on chlamydial group antigen (presence of a 2-keto-3-deoxycarbohydrate as immunodominant group). J. Immunol. **109**:116–122.
9. **Dhir, S. P., G. E. Kenny, and J. T. Graystone.** 1971. Characterization of the group antigen of *Chlamydia trachomatis*. Infect. Immun. **4**:725–730.
10. **Galanos, C., and O. Lüderitz.** 1975. Electrodialysis of lipopolysaccharides and their conversion to uniform salt forms. Eur. J. Biochem. **54**:603–610.
11. **Galanos, C., O. Lüderitz, and O. Westphal.** 1969. A new method for the extraction of R lipopolysaccharide. Eur. J. Biochem. **9**:245–249.
12. **Galanos, C., O. Lüderitz, and O. Westphal.** 1971. Preparation and properties of antisera against the lipid A component of bacterial lipopolysaccharides. Eur. J. Biochem. **24**:116–122.
13. **Grayston, J. T., and S.-P. Wang.** 1975. New knowledge of *Chlamydiae* and the diseases they cause. J. Infect. Dis. **132**:87–105.
14. **Hakomori, S.** 1964. A rapid methylation of glycolipid and polysaccharide catalyzed by methylsulfinyl carbanion in dimethylsulfoxide. J. Biochem. (Tokyo) **55**:205–208.
15. **Hobson, D., and K. K. Holmes (ed.).** 1977. Nongonococcal urethritis and related infections. American Society for Microbiology, Washington, D.C.
16. **Lüderitz, O., C. Galanos, H. J. Risse, E. Ruschmann, S. Schlecht, G. Schmidt, H. Schulte-Holthausen, R. Wheat, O. Westphal, and J. Schlosshardt.** 1966. Structural relationships of *Salmonella* O and R antigens. Ann. N.Y. Acad. Sci. **133**:349–374.
17. **Nurminen, M., M. Leinonen, P. Saikku, and P. H. Mäkelä.** 1983. The genus-specific antigen of *Chlamydia*: resemblance to the lipopolysaccharide of enteric bacteria. Science **220**:1279–1281.
18. **Nurminen, M., E. T. Rietschel, and H. Brade.** 1985. Chemical characterization of *Chlamydia trachomatis* lipopolysaccharide. Infect. Immun. **48**:573–575.
19. **Nurminen, M., E. Wahlström, M. Kleemola, M. Leinonen, P. Saikku, and P. H. Mäkelä.** 1984. Immunologically related ketodeoxyoctonate-containing structures in *Chlamydia trachomatis*, Re mutants of *Salmonella* species, and *Acinetobacter calcoaceticus* var. *anitratus*. Infect. Immun. **44**:609–613.
20. **Schachter, J., and H. D. Caldwell.** 1980. Chlamydiae. Annu. Rev. Microbiol. **34**:285–309.
21. **Shimizu, Y., and R. A. Bankowski.** 1963. The nature of the cross reactions between a bacterium of the genus *Herellea* and the ornithosis virus in complement fixation. Am. J. Vet. Res. **24**:1283–1290.
22. **Storz, J. (ed.).** 1971. Chlamydia and chlamydia-induced diseases. Charles C Thomas, Publisher, Springfield, Ill.
23. **Volkert, M., and M. Matthiesen.** 1956. An ornithosis related antigen from a coccoid bacterium. Acta Pathol. Microbiol. Scand. **39**:117–126.
24. **Wollenweber, H.-W., K. W. Broady, O. Lüderitz, and E. T. Rietschel.** 1982. The chemical structure of lipid A. Demonstration of amide-linked 3-acyloxyacyl residues in *Salmonella minnesota* Re lipopolysaccharide. Eur. J. Biochem. **124**:191–198.