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Normal Human Sera Contain Bactericidal IgG That Binds to the Oligosaccharide Epitope Expressed within Lipooligosaccharides of *Neisseria gonorrhoeae*

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Although more than several investigators reported the presence of antibodies in normal human sera (NHS) that bind to lipooligosaccharide (LOS) of *Neisseria gonorrhoeae*, the specificities of those antibodies were not fully characterized. To identify anti-LOS antibodies in NHS, we used LOS from a serum-sensitive strain, JW31R, as an affinity ligand and purified IgG from NHS that bound to JW31R LOS. The affinity purified IgG (AP-IgG) binds to the oligosaccharide (OS) moiety of both the ligand LOS and its truncated form, 15253 LOS. Lipid A could be essential for maximum expression of the carbohydrate epitope that resides on 15253 OS. We also found that AP-IgG is capable of killing a serum-sensitive strain JW31R. The present work provided direct evidence that NHS contain bactericidal antibodies specific for a site close to the inner core OS expressed on gonococcal LOS. The present results not only show that anti-LOS antibodies specific for the inner core OS could play a major role in our defense against gram-negative bacteria. But also they demonstrated that such core OS or a nearby site could be utilized as possible targets for vaccine development against microbial infections.

Key words: human antibodies, lipooligosaccharide, lipopolysaccharide, *Neisseria gonorrhoeae*, oligosaccharide, vaccine.

Abbreviations: GlcNAc, *N*-acetyl glucosamine; Hep, heptose; LOS, lipooligosaccharide; LPS, lipopolysaccharide; MAb, monoclonal antibody, NHS, normal human sera; OS, oligosaccharide; PBS, phosphate buffered saline.

Lipooligosaccharide (LOS) produced by *Neisseria gonorrhoeae* is one of the major antigenic and immunogenic components (*1–7*). LOS consists of oligosaccahride (OS) and lipid A, and the OS moiety of LOS consists of a structurally variable region and a conserved inner core linked to the lipid A moiety. Recent immunochemical and structural analyses (*8–12*) showed that gonococci elongate a conserved trisaccharide consisting of *N*-acetylglucosamine (GlcNAc) and two heptose (Hep) residues, GlcNAc-Hep[II]-Hep[I]. Glycoses are sequentially added on Hep[I] only or on both Hep[I] and Hep[II] to produce the variable region of two different OS elongation patterns (Fig. 1).

In a recent publication (*12*), we reported that gonococcal LOS having OS on both Hep[I] and Hep[II] contain an epitope that could potentially be utilized as a vaccine target against *N. gonorrhoeae* (*12–14*). Although this carbohydrate epitope is recognized by a murine monoclonal antibody (MAb) 2C7, it remains an important question to be answered whether such carbohydrate epitopes within gonococcal LOS are truly immunogenic in humans.

To date, more than several publications have reported the presence of antibodies in normal human sera (NHS) that are directed against gonococcal LOS (*1–4*, *6*, *15*, *16*). However, those human anti-LOS antibodies in NHS or in patients' sera have not yet been fully characterized. It is still unclear whether they are specific for the lipid A or OS moiety of the LOS, and to our knowledge, direct evidence of human antibodies binding to the core OS expressed on LOS has not yet been provided.

To isolate and characterize antibodies that bind to the OS of gonococcal LOS, we selected LOS from strain JW31R (*17*) as an affinity ligand. This serum-sensitive strain is easily killed by NHS, and we reasoned that the killing of this strain would partially take place *via* antibody-dependent activation of the classical pathway and therefore anticipated the isolation of bactericidal antibodies that bind to JW31R LOS by using it as an affinity ligand. We also anticipated that such antibodies may recognize the OS moiety of the LOS, especially the OS epitope similar to MAb 2C7, because this JW31R LOS is an elongated form of 15253 LOS containing the 2C7 epitope.

By sequential affinity chromatography, we purified the IgG bound to the ligand LOS and determined that it recognizes the OS but not the lipid A. This affinity-purified IgG (AP-IgG) also showed a bactericidal activity against strain JW31R. For the first time, we obtained direct evidence for the presence of a bactericidal IgG that recognizes the OS moiety of gonococcal LOS.

MATERIALS AND METHODS

Strains and Lipooligosaccharide (LOS)—Dr. Robert E. Mandrell (USDA/ARS, Albany, CA) kindly provided the two strains used (JW31R and 15253). The gonococci were cultured on GC agar base (Difco Laboratories, Detroit,

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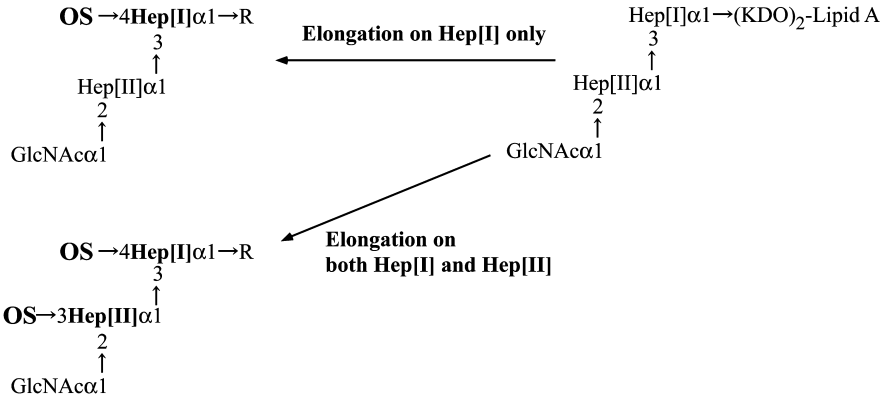

Fig. 1. **OS elongation patterns in *Neisseria gonorrhoeae*.** To distinguish the two heptoses (Hep) in the core OS, we defined the Hep linked to KDO as Hep[I] and the other Hep linked to Hep[I] as Hep[II] (*12*). R = $(KDO)_2$-Lipid A.

MI) containing 1% defined supplement (*18*) in a CO_2 incubator at 37°C. We used the following LOS samples whose structures had been characterized: JW31R (*12*, *19*), 15253 (*11*), 15253 lgtE mutant (*12*, *20*), WG (*12*), F62 (*8*), 24-1 (*12*, *21*), and MS11mk (variant A) (*10*). We modified LOS samples (WG and 15253 LOS) by chemical and enzymatic methods as described previously (*12*). *Salmonella minesota* mutant Re LPS was purchased from Sigma Chemical Co. (St Louis, MO, USA).

Human Sera, Monoclonal Antibodies (MAb), and Secondary Antibodies—Ten ml of blood were collected by staff nurses at a Health Care Center in Tottori University from healthy volunteers (six males and two females whose ages range from 21 to 38) with no history of gonococcal or meningococcal infection. After clotting and centrifugation, each serum was aliquoted, and equal amounts of each serum were pooled. Both the individual and pooled sera were stored at –70°C. The following secondary antibodies (alkaline phosphatase conjugate) were purchased from Sigma Chemical: mouse anti–human IgG MAb (γ-chain specific), mouse anti–human IgA MAb (α-chain specific), and mouse anti–human IgM Ab (μ-chain specific).

Immunochemical Analyses—Antibodies were characterized by enzyme-linked immunosorbant assay (ELISA), PAGE/blot and TLC immunostaining. Dilutions of LOS for PAGE and ELISA analyses were done with phosphate buffered saline (PBS) containing 10 mM magnesium chloride. Typical procedures for ELISA (*12*), PAGE/blot and TLC immunostaining (*12*, *22*, *23*) have been described previously.

Purification of Anti-LOS Human Antibodies—Protein contents were estimated by using a BCA Protein Assay Kit (Pierce, Rockford, IL, USA). Anti-LOS antibodies were dialyzed using a MEGA System Microdialyzer (Health Products Int., Rockford, IL) or a microdialyzer system 500 (Pierce), and concentrated using a CENTRIPREP-10 column (Amicon, Bevery, MA, USA) and a Speedvac concentrator A160 (Yamato, Tokyo, Japan).

For the isolation of antibodies binding to JW31R LOS, we prepared a column by coupling JW31R LOS hydrazide with CNBr-activated Sepharose 4B (Amersham Pharmacia, Uppsala, Sweden) according to the manufacturer's protocol. The coupling of JW31R LOS to the Sepharose was confirmed by using a soy bean lectin (*Glycine max*, Sigma Chemical Co.) that binds to JW31R LOS (*24*); the lectin was conjugated with alkaline phosphatase using a conventional glutaraldehyde method (*25*), and the coupled gel was stained with the conjugated lectin and Western Blue™ stabilized substrate (Promega Co., Madison, NJ, USA). Human sera (8 ml) diluted with PBS were loaded on the LOS affinity column (0.7 × 6 cm), and then the column was washed with 50 mM Tris/HCl in 0.1 M NaCl (pH 7.4, 400 ml, 50 ml per fraction) and then with 200 mM glycine in 0.5 M NaCl (pH 2.8, 40 ml, 2 ml per fraction). The acidic eluate was neutralized with 0.5 M Tris/HCl (pH 8.0, 0.6 ml per fraction), pooled and then concentrated. The presence of Igs in the fractionated eluate was monitored by ELISA using a goat anti–human polyvalent Igs (alkaline phosphatase conjugate, Sigma Chemical Co.) as a probe.

For further chromatographic purification of the anti-LOS antibodies, we used an ÄKTA explorer 10S (Amersham Pharmacia, Uppsala, Sweden). To remove non-specific antibodies and proteins binding to the gel matrix, the concentrated eluate described above was passed through a column (0.7 × 6 cm) of inactivated Sepharose prepared by treating CNBr-activated Sepharose 4B with an excess of triethanolamine. The same buffers as described above were used for this chromatography. Purification was also attempted by gel chromatography using a Superose 12HR column (Amersham Pharmacia, Uppsala, Sweden). IgG was purified by affinity chromatography using a HiTrap Protein G column (Amersham Pharmacia) [washing buffer: 0.1 M sodium phosphate (pH 8.0); elution buffer: 0.1 M glycine/HCl (pH 2.7); buffer for neutralization; 2 M Tris/HCl (pH 9.0)], and we used a monoclonal anti–human IgG antibody as a probe for this affinity purification. The neutralized fractions were pooled, dialyzed and then concentrated. The subclasses of the purified IgG were determined using a Human Ig Subclass Typing Kit (The Binding Site, Birmingham, U.K).

Bactericidal Activity—To measure the activity of the IgG against two strains, JW31R and 15253, we used the methods reported by McQuillen *et al*. (*26*) and Schneider and Griffiss (*4*). In brief, the complement source serum was prepared by pre-absorbing the serum with either organism, JW31R or 15253. Each bacterial suspension was prepared by diluting a mid-log-phase concentration (OD_{650} ~ 0.2) with 0.5% bovine serum albumin (BSA)/Gey's balanced salt solution. After incubating a mixture of IgG (50 μl), the source complement (50 μl) , and each bacterial suspension (25 μl) for 45 min at 37°C, a choco-

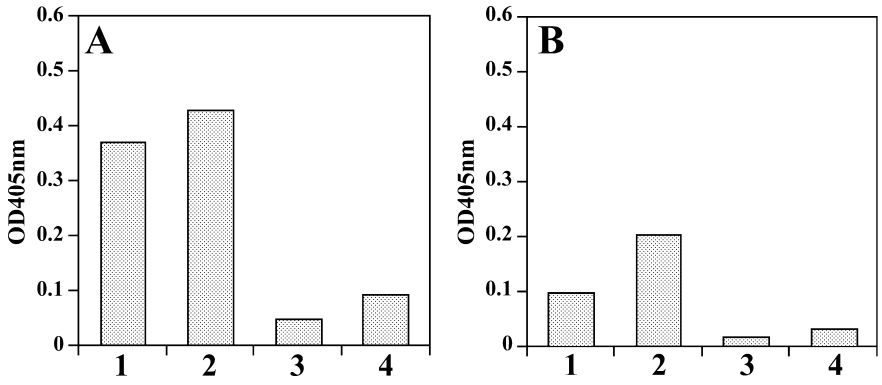

Fig. 2. **ELISA analysis of normal human sera.** After coating with each antigen (JW31R LOS, F62 LOS, and Re LPS), wells were sequentially treated with 1% BSA for blocking, sera (75 μl), and anti–human polyvalent antibodies (alkaline phosphatase conjugate, 1:1,000 dilution). *p*-Nitrophenylphosphate was used as a substrate. A: 1:10 dilution; B: 1:100 dilution. The results are expressed as mean values of duplicate analyses. 1: JW31R LOS (200 ng); 2: JW31R LOS (500 ng); 3: F62 LOS (200 ng); 4: Re LPS (200 ng).

late agar plate was inoculated with the mixture (50 μl). Colonies were counted after incubating the plates in a CO_2 incubator overnight at 37°C.

RESULTS

Confirmation of the Presence of Anti–JW31R LOS Antibodies by ELISA—Prior to affinity purification, we examined antibodies against gonococcal JW31R LOS in pooled NHS obtained from eight individuals with no history of gonococcal infection. For this ELISA analysis, we examined BSA and casein as a blocking reagent and found that the use of 1% BSA gave lower non-specific binding. The pooled NHS was diluted to 1:10 and 1:100 with PBS and then examined for binding to JW31R LOS using anti–human Igs as a probe. In addition to JW31R LOS, we used a gonococcal LOS produced by strain F62 and *Salmonella minesota* mutant Re LPS as a control, and Fig. 2 represents partial results of the experiment. The NHS showed higher binding to JW31R LOS than to F62 LOS and Re LPS, and the binding to F62 LOS is the lowest although this strain is also serum-sensitive (*27*). The binding to JW31R LOS was found to be proportional to the concentration of the sera. As Fig. 3 shows, each individual's serum was also examined for binding to JW31R and Re LPS. Similar to the pooled NHS, each individual's serum showed higher binding to JW31R LOS (Fig. 3), although the antibody titer of each serum was different as expected. This analysis showed that the presence of antibodies binding to JW31R LOS in the pooled NHS is also reflected in each serum and not due to specific individuals possessing higher titers of the antibodies. After confirming the binding of NHS to JW31R LOS, we characterized the antibodies that truly bind to the LOS as will be described below.

Affinity Purification of Anti–JW31R LOS Ab—Because affinity purification of each individual's serum did not provide enough antibodies for characterization of their specificities and biological functions, the pooled NHS described above was used. We chromatographed the pooled sera on an affinity column prepared by treating a hydrazide of JW31R LOS with CNBr-activated Sepharose. Prior to the chromatography, the coupling of the LOS was confirmed by examining the prepared gel matrix with a soybean lectin that binds to the LOS (*24*). As Fig. 4 shows, screening by ELISA showed the presence of immunoglobulins in the fractions eluted with the 0.2 M glycine buffer (pH 2.8), showing that the immunoglobulins bound to the LOS affinity column. The acidic fractions were neutralized, pooled, concentrated, and then applied to an inactivated Sepharose column to remove

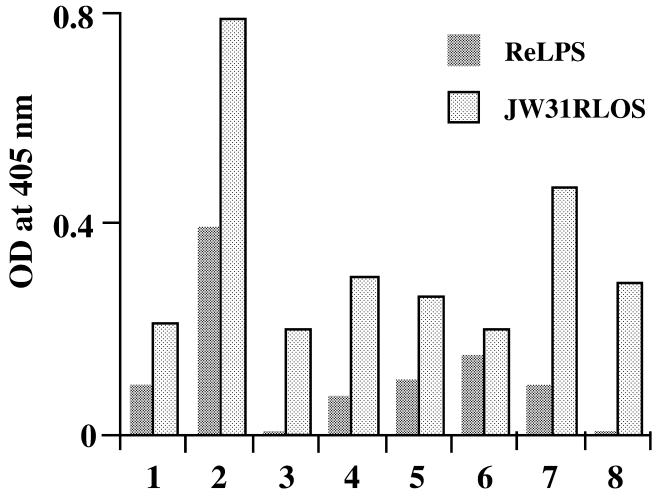

Fig. 3. **ELISA analysis of each individual's serum.** After coating with LOS (JW31R LOS and Re LPS, 200 ng each), wells were treated with each serum (1:25 dilution) in a similar manner as described as in Fig. 2. The results are expressed as mean values of duplicate analyses.

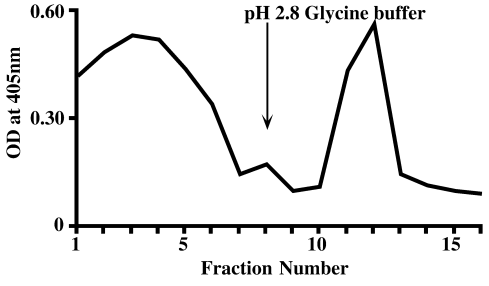

Fig. 4. **Affinity purification of anti-LOS human antibodies.** After applying the pooled human sera, the column (0.7 × 6 cm) was washed with 50 mM Tris/HCl and 0.1 M NaCl (pH 7.4), and then with 200 mM glycine (pH 2.8) containing 0.5 M NaCl. The glycine solution was neutralized with 0.5 M Tris/HCl (pH 8.0). Each fraction (50 ml and 2 ml for the wash and the elution fractions, respectively) was analyzed by ELISA using the same anti–human Igs as in Fig. 2.

Table 1. **ELISA analysis of the non-absorbed fraction obtained by affinity chromatography using an inactivated Sepharose column[a].**

	IgG	IgM	IgA
OD at 405 nm	0.65	0.04	<0.01

[a]Wells were coated with JW31R LOS (200 ng), and the binding of anti-LOS antibodies was examined by using anti–human IgA, IgG and IgM (alkaline phosphatase conjugates), and the same substrate in Fig. 2. The results are expressed as mean values of duplicate analyses.

both non-specific proteins and antibodies that bind to the gel matrix (data not shown). The non-absorbed fractions obtained with this second affinity chromatography were pooled, concentrated and then examined by ELISA using anti–human IgA, IgG, and IgM Abs as probes. As Table 1 shows, the IgG in this fraction bound to JW31R LOS. Residual IgM binding was also detected, whereas hardly any IgA binding was detected under the conditions used. Although this ELISA analysis indicated that minute amounts of IgM may bind to JW31R LOS, we focused our efforts on characterization of the IgG that binds to the LOS.

The IgG in the non-absorbed fractions obtained with the second affinity chromatography was further purified using a HiTrap Protein G column. The chromatographic fractions obtained using the three affinity columns were monitored by PAGE/blot, and Fig. 5 shows the partial results. Lanes 1, 2 and 3 show the results of the pooled NHS, the unbound and bound fractions obtained with Protein G affinity chromatography, respectively. The NHS bound to the JW31R LOS column (lane 1 of Fig. 5) contained significant amounts of non-IgG proteins. Of these proteins, the major protein of ~60 kDa, serum albumin, was not removed completely by single chromatography using the inactivated Sepharose column or a Superose 12 HR column (data not shown). But its removal was accomplished by Protein G affinity chromatography (lane 3 in Fig. 5). Also, we found that the non-IgG proteins (Fig. 5, lane 2) obtained by the protein G affinity chromatography showed no inhibitory activity on the AP-IgG binding to JW31R LOS (data not shown), confirming that they non-specifically bound to the gel matrix. Double immunodiffusion analysis (Fig. 6) showed that the affinity-purified IgG (AP-IgG) (lane 3 in Fig. 5) is mostly IgG2, with residual amounts of IgG1 and IgG4. The concentration of AP-IgG in the pooled NHS was estimated to be ~2 μg ml^{-1} based on the results using the BCA protein assay.

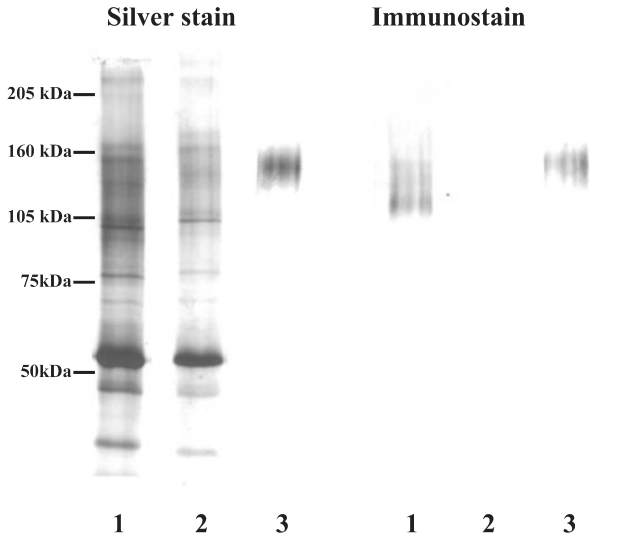

Fig. 5. **PAGE (6% acrylamide)/blot analysis of fractions obtained with chromatography on three different affinity columns.** One gel was silver stained and the other was used for Western blotting. The same anti–human IgG MAb as in Table 1 and Western Blue (as a substrate) were used for immuno-staining. 1: The fraction eluted from the JW31R LOS affinity column (Fig. 4); 2: the non-absorbed fraction obtained by affinity chromatography on a protein G column; 3: the fraction bound to the protein G column.

The Specificity and Bactericidal Activity of the AP-IgG—Then, we analyzed the specificity of the AP-IgG by PAGE/blot, ELISA, and TLC immunostaining using various gonococcal LOS samples. Fig. 7 summarizes the results of PAGE/blot analysis, and its partial results are shown in Fig. 8. As expected, the AP-IgG bound to the JW31R LOS but not to any of the Hep[I]-elongated LOS as represented by the results for MS11mkA , F62, and 24-1 LOS (Fig. 7 and lanes 4–6 in Fig. 8), confirming that the epitope of the AP-IgG does not reside on gonococcal LOS containing OS only on Hep[I]. In addition to the ligand LOS, the AP-IgG bound to its truncated form, 15253 LOS (lane 1 in Fig. 8), showing that it also recognizes the endo portion of JW31R LOS (Fig. 7). However, the AP-IgG did not bind to WG LOS. Although this LOS is an elongated form of 15253 LOS, like JW31R LOS, their OS structures are different; the lactose on Hep[I] of 15253 LOS is elongated to produce WG LOS, whereas the other lactose on Hep[II] is elongated in the case of JW31R LOS. The inert binding of the AP-IgG to WG LOS showed that the AP-IgG is distinct from MAb 2C7 because MAb 2C7 binds to both 15253 LOS and WG LOS.

Furthermore, we examined expression of the AP-IgG epitope by using 15253 LOS derivatives. As lanes 7 and 8 (Fig. 8) show, it did not bind to the truncated 15253 LOS derivatives: one lacking the Gal residue of the lactose linked to Hep[I] and the other lacking both Gal residues of the two lactoses present at the non-reducing ends,

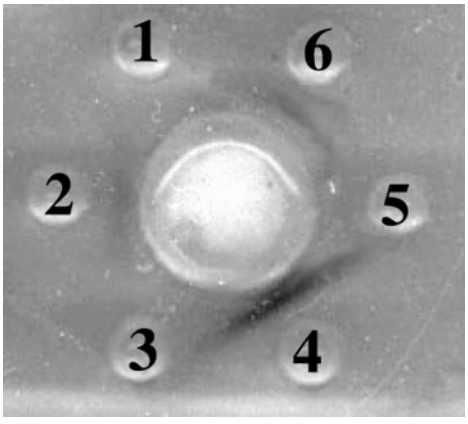

Fig. 6. **Double immunodiffusion analysis of the purified IgG.** The IgG was analyzed using a MAb IgG/IgA subclass typing kit. 1: IgA1; 2: IgG1; 3: IgA2; 4: IgG2; 5: IgG3; 6: IgG4.

IgG binding	Strain	R_1	R_2
-	F62	GalNAcβ1-3Galβ1-4GlcNAcβ1-3Gal β1-4Glcβ1-4	
-	F62, PID-2	Galβ1-4GlcNAcβ1-3Gal β1-4Glcβ1-4	
-		GlcNAcβ1-3Gal β1-4Glcβ1-4	
-		Gal β1-4Glcβ1-4	
-	MS11mkA[a]	Glcβ1-4	
-	WG	GalNAcβ1-3Galβ1-4GlcNAcβ1-3Gal β1-4Glcβ1-4	Gal β1-4Glcα1-3
-	WG	Galβ1-4GlcNAcβ1-3Gal β1-4Glcβ1-4	Gal β1-4Glcα1-3
+	JW31R	Gal β1-4Glcβ1-4	GalNAc–Gal-Galβ1-4Glcα1-3
+	15253	Gal β1-4Glcβ1-4	Gal β1-4Glcα1-3
-	15253[b]	Glcβ1-4	Gal β1-4Glcα1-3
-	15253lgtE	Glcβ1-4	Glcα1-3

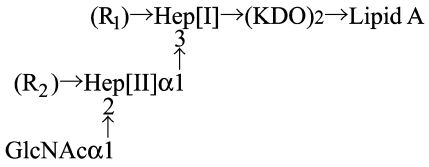

Fig. 7. **PAGE/blot and ELISA results of AP-IgG binding to gonococcal LOS.** [a]Prepared as described previously (*10*); [b]prepared as described previously (*12*).

showing that either or both β-Gal residues could be essential for expression of the epitope. However, the β-Gal residues that could be involved in the epitope are not identical to those of the Galβ1-4Glc or Galβ1-4GlcNAc structures expressed at the non-reducing ends of the Hep[I]-elongated LOS such as MS11mk, F62 and 24-1, because the AP-IgG binds to none of these LOS as described above.

As exemplified by the above results, in particular, the inactive binding of the AP-IgG to the truncated 15253 LOS lacking Gal at either or both non-reducing ends (Fig. 7), the IgG was found to bind to the OS moiety but not to the lipid A moiety. If it is directed to the lipid A moiety, it should bind to not only these truncated LOS but also all gonococcal LOS examined. TLC immunoblot analysis also showed that the AP-IgG did not bind to the lipid A prepared from JW31R LOS (data not shown). Taken together, we excluded the possibility that the Ab bound to the lipid A of a specific structure only expressed on both 15253 LOS and JW31R LOS.

We further examined the structural requirements for expression of the AP-IgG–defined epitope by ELISA and TLC immunostaining (*10–12*, *22*) of the 15153 LOS derivatives and 15253 OS (Table 2) which had been characterized in the previous study (*12*). Phosphates are not necessary for expression of this epitope since dephosphorylation did not alter expression of the epitope. As expected from our previous studies on other MAbs (*10*, *12*, *22*, *23*) that bind to the OS moiety of LOS, the AP-IgG did not bind to the 15253 OS, showing that the OS itself is not enough for the epitope to be expressed. The AP-IgG bound after de-*O*-acylation of the lipid A moiety, although the binding was weak compared to that of the intact LOS or the dephosphorylated LOS, and subsequent de-*N*-acylation of the de-*O*-acylated LOS abolished the antigenicity. The loss of expression of the epitope after de-*N*-acylation demonstrated the importance of the *N*-linked fatty acids in the lipoidal moiety and/or the GlcNAc residue. Although we were not be able to determine that the GlcNAc residue linked to Hep[II] is also essential for expression of the epitope, we may be able to investigate its importance by carrying out inhibition studies using a partial 15253 OS structure, Lacα1-3Hep(2-1αGlcNAc) (*28*). In the present study, however, both the inactivity of 15253 OS and the decreased antigenicity of the de-*O*-acylated LOS showed that the AP-IgG carbohydrate epitope requires the lipid A moiety for its maximum expression.

We also investigated whether the AP-IgG exhibits the bactericidal activity against the two strains, JW31R (serum-sensitive) (*17*) and 15253 (serum-resistant) (*29*). As Fig. 9 shows, both the AP-IgG and heat-inactivated NHS were capable of killing the serum-sensitive strain JW31R in the presence of a complement source, whereas neither of them showed the activity when the source was absent. However, the AP-IgG was not able to kill the15253 organism under the conditions used (data not shown). The current results showed that the IgG was capable of killing a serum-sensitive JW31R strain but not the serum-resistant 15253 strain, although it binds to 15253 LOS.

Table 2. **AP-IgG2 binding to chemically modified 15253 LOS and 15253 OS.**

LOS and OS tested	AP-IgG2 binding
Intact LOS[a]	++
dephospholylated LOS[a]	++
De-*O*-acylated LOS[a]	+
De-*N*- and -*O*-acylated LOS[b]	–
15253 OS[b]	–

[a]Analyzed by TLC immunostaining; [b]analyzed by PAGE/blot as described in Fig. 8.

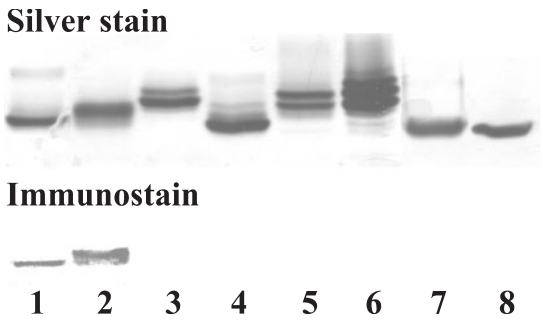

Fig. 8. **PAGE (14% acrylamide)/blot analysis of the AP-IgG defined-epitope.** Each LOS (200 ng) was separated on two separate gels and then analyzed as described in Fig. 5. 1: 15253 LOS; 2: JW31R LOS; 3: WG LOS; 4: MS11mkA; 5: F62; 6: 24-1; 7: 15253 LOS treated with β-galactosidase; 8: 15253 lgtE LOS.

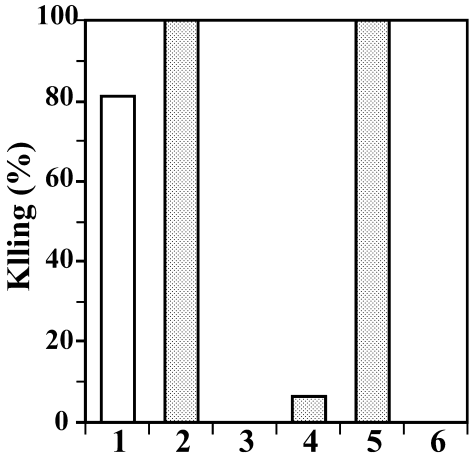

Fig. 9. **Bactericidal activity of the AP-IgG against the JW31R strain of *N. gonorrhoeae*.** The killing activity is expressed as (1 – [CFU in test sample with intact complement/CFU in the buffer control]) × 100, CFU: the average of duplicate analyses). 1: AP-IgG + complement source; 2: heat-inactivated NHS + complement source; 3: AP-IgG + heat-inactivated complement source; 4: buffer (0.5% BSA/GBSS) + complement source; 5: NHS; 6: heat-inactivated NHS. The results are expressed in reference to the activity of NHS as mean values of duplicate analyses.

These collective data show the following: (i) the AP-IgG obtained using JW31R LOS as an affinity ligand recognizes the truncated form of LOS, 15253 LOS; (ii) the epitope resides on the OS of the JW31R OS and is probably the structure of 15253 OS, and the β-Gal residue(s) at the non-reducing end(s) of the OS are essential for expression of the epitope; (iii) in addition, the lipid A is essential for maximum expression of the carbohydrate epitope; (iv) although both the AP-IgG and MAb 2C7 recognizes 15253 LOS, the epitope of the human IgG is distinct from that of MAb 2C7 because the IgG does not bind to WG LOS (Fig. 7) that is recognized by 2C7; (v) the AP-IgG is bactericidal.

DISCUSSION

As described earlier, we predicted that the killing of a serum-sensitive strain, JW31R, would be partially due to the antibody-dependent activation of the classical pathway. As we anticipated, we were able to isolate IgG from NHS by using JW31R LOS as an affinity ligand, and showed that the AP-IgG bound to the OS of both JW31R LOS and 15253 LOS. Although several investigators had both suggested and reported the presence of anti-gonococcal LOS antibodies in NHS (*1–4*, *6*, *16*), they had not determined whether the antibodies are specific for the OS or the lipid A moiety. Human immunoglobulins have been purified by affinity chromatography using *E. coli* J5 LPS (*6*) or gonococcal LOS (*15*) as a ligand. The former study reported that the affinity-purified antibodies (IgM and IgA) showed bactericidal activities against gonococci. However, both the OS pattern and OS structure of the J5 LPS are different from those of gonococcal LOS, and it had not been determined whether the anti-J5 LPS antibodies (*6*) truly bind to the gonococcal strains examined in the bactericidal assays or the LOS produced by those strains. Although the latter study showed IgG and IgM from both NHS and convalescent sera bound to some gonococcal LOS, the specificities of those antibodies were not determined. In this study, we obtained direct evidence for the presence of a human bactericidal IgG that binds to the OS structure of gonococcal LOS. Because affinity purification of each individual's serum did not provide enough Ab for characterization of anti-LOS antibodies, we used pooled NHS for isolation of the IgG. As mentioned earlier (Fig. 3), each individual's serum contained antibodies that bound to JW31R LOS, indicating that the IgG could be a common Ab present in NHS.

In a previous study, we characterized the specificity of a murine MAb 2C7 (*12*). Because MAb 2C7 is bactericidal and does not cross-react with human carbohydrate epitopes, the 2C7 epitope is regarded as one of the potential vaccine targets (*12–14*). However, it was not yet known whether the OS structure defined by MAb 2C7 is truly immunogenic in humans. The isolation of IgG that binds to the OS moiety of 15253 LOS constituted important evidence that a 2C7-like OS epitope is immunogenic. Also, Plested *et al.* (*30*, *31*) recently indicated the presence of human Abs against the inner core of meningococcal LOS based on inhibition studies using a murine MAb. These results show that anti-LOS antibodies that recognize the inner core OS expressed in LOS could play a major role in our defense against Gram-negative bacteria. They also show that such a core OS could be utilized for the development of vaccines against microbial infections.

The presence of antibodies that bind to gonococcal LOS in NHS with no history of gonococcal infection raises a question on how this immunity has been acquired. We are currently investigating the binding capability of this Ab to LOS produced by other Gram-negative bacteria to gain some insight regarding the above question. In addition, we are not only investigating a better purification method that would lower the loss of the Ab and its activity that we experienced during each purification procedure including dialysis and concentration, but also are developing a sensitive blot assay for determination of the specificities of anti-LOS antibodies that are present in minute amounts. Because the titer of the AP-IgG is much lower than those of murine MAbs, larger amounts of the pooled sera (~100 ml) were necessary for us to obtain

enough antibodies for characterization of the epitope and bactericidal activity. After developing a sensitive assay, we will investigate other anti-LOS antibodies, and the results will be published elsewhere.

In summary, we isolated IgG from NHS using LOS from a serum-sensitive strain, JW31R, as an affinity ligand. By immunochemical analyses using a series of LOS and modified LOS samples, we determined that the AP-IgG recognizes the OS expressed on both JW31R and its truncated form, 15253 LOS, and found that the lipid A moiety could be essential for the OS epitope to be expressed, as had been observed with other MAbs that bind to the OS of gonococcal LOS (*10*, *12*, *22*, *23*). In addition, we found that the AP-IgG is bactericidal. For the first time, our study provided conclusive evidence that normal human sera contain bactericidal antibodies that bind to the OS expressed on LOS produced by *N. gonorrhoeae*. This demonstrates not only that anti-LOS antibodies binding to the inner core OS expressed in LOS could play a major role in our defense against gram-negative bacteria, but also that the inner core OS or its nearby site could be utilized as possible targets for vaccine development against microbial infections.

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