NOTES

Hemagglutination Is a Novel Biological Function of Lipopolysaccharide (LPS), as Seen with the Vibrio cholerae O139 LPS

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It has been generally thought that the polysaccharide moiety of lipopolysaccharide (LPS) maintains only serological specificity, while the lipid A portion determines various biological functions. However, we found that hemagglutination was a common function of the polysaccharide moiety of LPSs from important human enteropathogenic bacteria. Of the LPSs examined, Vibrio cholerae O139 LPS showed the highest hemagglutinating activity. Glycoproteins, such as mucin and fetuin, showed efficient inhibition of the hemagglutinating ability. Since cell-mediated hemagglutination is known to be correlated with bacterial adherence, hemagglutination induced by the polysaccharide moiety is interpreted to indicate that cell-surface LPS is a potential adhesin.

Human enteropathogens, like Vibrio cholerae or Escherichia coli, colonize the host intestinal mucosa to cause a diarrheal disease. In the course of a crisis of a diarrheal disease, cell surface components including lipopolysaccharide (LPS) interact directly with the host intestinal mucosa, which results in induction of antibody production (10). The production of antibodies against enterotoxins is evident; however, antibacterial immunity confers more effective protection than the antitoxic immunity (8). During studies with human volunteers, the attenuated live vaccine administered parenterally has protected the population against cholera (12, 13).

The bacterial adherence to the intestinal mucosa is a prerequisite in colonization, and therefore, considerable attention has been given to the factor(s) related to adherence. The adhesive ability of an enteropathogen is usually assessed by determining the hemagglutinating ability, because the erythrocyte membrane is believed to possess the homolog(s) of the mucosal substance(s) involved in bacterial adherence (14, 23, 25). Actually, in many human pathogens, including Vibrio mimicus, the hemagglutinating ability of bacterial cells has been shown to be closely correlated with the bacterial ability to adhere to the host intestine (2, 5, 14, 25). We recently isolated two cell-associated hemagglutinins (HAs) from V. mimicus E-33 (1), which adheres strongly to the small intestine (2). Additionally, the HAs were found to correspond to a major outer membrane protein and the polysaccharide moiety of LPS (1).

In the present study, we report hemagglutinating ability as a common property of the polysaccharide moiety of LPS isolated from enteric pathogens including V. cholerae O139 Bengal, a newly recognized pathogen of the prevailing eighth pandemic of cholera (3, 20). We also report that V. cholerae O139 LPS has the highest hemagglutinating activity, suggesting involvement of LPS in the increased pathogenicity of this vibrio.

We (1) recently reported that one of the cell-associated HAs of V. mimicus E-33 was LPS and that only the polysaccharide moiety mediated hemagglutination. In order to examine the hemagglutinating ability of other vibrio LPSs, LPS was isolated from several strains including two isolates of V. cholerae O139. The vibrio was grown in tryptic soy broth at 37°C overnight with shaking, and the cells were harvested by centrifugation at 6,000 × g for 30 min and killed by acetone treatment. The acetone-killed bacterial cells were freeze-dried, and LPS was purified by hot phenol-water extraction followed by repeated ultracentrifugation (24). The purity of each LPS preparation obtained was confirmed by sodium dodecyl sulfate–12.5% polyacrylamide gel electrophoresis followed by silver staining (1). From 2.5 g (dry weight) of the bacterial cells, 5 to 10 mg of LPS was isolated from each vibrio strain.

The hemagglutinating activity of vibrio LPS (500 µg/ml) was assayed by the method of Jones et al. (7). A serial twofold dilution of 50 µl of the sample in 20 mM Tris-HCl buffer containing 0.9% NaCl (pH 7.5) was mixed with 50 µl of 1.5% rabbit erythrocytes in the wells of a 96-well polystyrene V-bottom microtiter plate (Greiner, Nurtingen, Germany). The plate was incubated at room temperature for 45 min, and the hemagglutination was monitored visually. The HA unit was defined as the reciprocal of the highest dilution of the sample causing visible agglutination of the erythrocytes. As shown in Table 1, all vibrio LPS preparations had hemagglutinating activity against rabbit erythrocytes. However, it should be noted that LPS from either of the V. cholerae O139 strains showed activity four times greater than others (Table 1).

By using V. mimicus E-33 LPS, we previously showed that hemagglutination was elicited by the polysaccharide fraction but not by the lipid A fraction (1). Therefore, in the present study, each of the vibrio LPS preparations was also hydrolyzed with 1% acetic acid at 100°C for 90 min, and the lipid A fraction was spun down (16). Thereafter, the supernatant containing the polysaccharide region was collected and lyophilized, and the hemagglutinating activity was then determined. For V. mimicus E-33 LPS, 100% of the hemagglutinating activity was recovered in the polysaccharide fraction (data not
It was confirmed that, although LPS from V. cholerae O139 Bengal conferred the highest HA activity, hemagglutination was a common biological property of LPS from gram-negative bacteria.

Each vibrio LPS was mixed with an appropriate amount of each vibrio LPS preparations, but some LPSs were found to be considerably resistant to fetuin.

In order to test the LPS-inactivating potential of the polypeptide portion of the glycoprotein, exhaustive pronase digestion of the glycoprotein was carried out as described by Saha and Banerjee (17). Mucin, fetuin, or asialofetuin was mixed with pronase at a ratio of 50:1 in 50 mM Tris-HCl buffer supplemented with 1 mM CaCl₂ (pH 8.0), and the mixture was incubated at 37°C for 2 hours. Thereafter, pronase was inactivated by heating at 80°C for 20 min, and the digestion was monitored. The HA unit (HAU) was defined as the reciprocal of the highest dilution of the sample causing visible hemagglutination. Erythrocytes from guinea pigs, sheep, cows, horses, and chickens showed no hemagglutination (i.e., activity of <20 HAU units).

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V. cholerae O139 Bengal endangers human lives and is currently the subject of extensive global studies because of its extraordinary ability to cause cholera. Studies of its pathogenicity suggest a resemblance between O139 Bengal and O1 biotype El Tor (3, 20). The most notable finding in the present study may be the high HA activity of V. cholerae O139 LPS. Oral immunization against V. cholerae O139 has implied that LPS conferred the most efficient protection (3, 4). However, a study on biological activities of O139 LPS suggested no contribution of the lipid A moiety in the high pathogenic potential of this vibrio (21). It is therefore plausible that, as cholera researchers worldwide search clues to what makes O139 Ben-
gal so efficient in its pathogenicity, the high hemagglutinating ability of the polysaccharide moiety may be of interest.

The correlation between cell-mediated hemagglutination and intestinal adherence in several enteropathogenic vibrios has been established (2, 14, 23, 25). *V. cholerae* has been reported to express rigid pili controlled by a transmembrane protein, ToxR, which also controls expression of cholera toxin (11). This toxin-coregulated pilus is known to have a role in bacterial adherence (6), but this pilus is restricted to *V. cholerae* serogroups, O1 (6) and O139 (19). The hemagglutinating function of outer membrane proteins has also been documented (1, 18, 22). However, considering the cell surface architecture, direct interaction of the outer membrane protein with the host mucosa seems to be impossible. In contrast, the extended polysaccharide moiety of LPS may be the first to come into contact with the mucosal membrane. On balance, it seems likely that, although the toxin-coregulated pilus may be essential for the cholera vibrios, the polysaccharide moiety with the strong hemagglutinating ability may function as the ubiquitous adhesin in the enteropathogenic vibrios.

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REFERENCES


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