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Production of *Vibrio cholerae* O1 and Non-O1 Typing Sera in Rabbits Immunized with Polysaccharide-Protein Carrier Conjugates

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Two systems are currently used to serologically type *Vibrio cholerae* O1 and non-O1 isolates. Anti-serovar-serotype serum in the Smith system is produced in rabbits immunized with live whole-cell vaccines, and that in the Sakazaki system is produced in rabbits immunized with heat-killed vaccines. In neither system is the serovar-serotype-specific antigen clearly defined. During the course of a serological survey, ca. 10% of more than 2,500 *V. cholerae* isolates examined agglutinated in the optimal dilutions of two, three, or four different anti-serovar sera prepared by the methods of Sakazaki. An occasional isolate agglutinated in both anti-O1 and non-O1 sera. Lipopolysaccharide was extracted from eight of these possible multiple serovars, coated onto rabbit erythrocytes, and retested in these same antisera by passive hemagglutination. With one exception the lipopolysaccharide-rabbit erythrocytes were now agglutinated in a single antiserum. Antipolysaccharide sera were produced in rabbits immunized with the polysaccharide moiety extracted from eight non-O1 and two O1 vaccine strains conjugated to bovine gamma globulin protein carrier. The antipolysaccharide sera showed passive hemagglutination titers versus lipopolysaccharide-rabbit erythrocytes comparable to those achieved in antisera from rabbits immunized with heat-killed whole-cell vaccines. In the slide agglutination test antipolysaccharide sera serologically discriminated between two O1 isolates that were previously agglutinated in both anti-O1 and anti-non-O1 whole-cell sera. It is recommended that serological types or varieties of *V. cholerae* non-O1 be based upon serologically recognizable differences in lipopolysaccharide-associated antigens as are antigens A, B, and C in the O1 group.

Presently, two systems are used to serologically discriminate *Vibrio cholerae* non-O1 serotypes. Sakazaki et al. (8–10) extended the number of serologically detectable *V. cholerae* O-antigen groups from the 7 reported by Gardner and Venkatraman (2) to 60. The immunological procedures and serogroup numerical designations suggested by Gardner and Venkatraman were continued by Sakazaki, except that Sakazaki identifies O-antigen groups in *V. cholerae* non-O1 as serological varieties (serovars) rather than O groups. A second typing system, developed by Smith (11, 12), presently recognizes 72 serotypes, and the numerical designations assigned to non-O1 serotypes in this system were arrived at independently. The lack of correlation in serological nomenclature has caused considerable confusion when attempts are made for epidemiological reasons to translate a Sakazaki serovar into a Smith serotype or vice versa. Brenner et al. (1) attempted to correlate serovar and serotype designations in each system by testing the non-O1 vaccine strains from each collection in the typing sera of the opposite collection. Unfortunately, only 20 of the Sakazaki vaccine strains were found to be serologically identical to 20 in the Smith system, whereas 15 serovars and 13 serotypes, respectively, appeared to be unique to each system. The remaining vaccine strains in each system were agglutinated by two or more antisera in the opposite system, which made consistent correlations difficult.

Two reasons for the apparent lack of correlation between these serological nomenclatures originate with the disparities in the methods used by each investigator to prepare vaccines and immunization protocols. First, typing sera produced in rabbits immunized with live vaccines in the Smith system will potentially react with heat-labile antigens that antisera produced in rabbits immunized with heat-killed vaccines in the Sakazaki system will not. Second, Sakazaki uses a series of injections of the killed vibrios, in contrast to a single immunization which Smith uses, thereby increasing the chances of the production of antibodies in response to minor antigens. At the moment it appears that typing sera in each system may detect a serovar-serotype-specific antigen (yet to be defined); but these sera also contain serological activity directed against additional non-serovar-serotype-specific antigens, which accounts for the cross-reactions reported by Brenner et al. (1). To reconcile the two systems, it is imperative that the serovar-serotype-specific antigen be defined.

The Kauffmann-White (4) scheme used to serologically group salmonellae is predicated on the serological detection of polysaccharide (PS)-associated O-antigens in the smooth core of lipopolysaccharide (LPS) in the outer membrane envelope. Similarly, serovar-serotype-specific antigens A, B, and C in *V. cholerae* O1 have been located serologically in the PS portion of the smooth core in LPS (5, 6). Because of these precedents and for the sake of uniformity, it makes sense to serologically group *V. cholerae* non-O1 organisms based upon the detection of serovar-serotype-specific LPS-associated O-antigens.

It is the intent of this communication to show that LPS-associated antigens unique to *V. cholerae* non-O1 vaccine strains are serologically detectable and that antisera specific for these LPS-associated antigens can be produced in rabbits immunized with PS-protein carrier conjugates.

MATERIALS AND METHODS

Vaccine strains. Table 1 lists the *V. cholerae* non-O1 strains used to prepare vaccines. The Sakazaki serovar and the Smith serotype designations are provided. *V. cholerae* el tor Inaba (La 5875) and *V. cholerae* Ogawa (CA 411) were the O1 serovars used.

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TABLE 1. *V. cholerae* non-O1 vaccine strains used to produce serological typing sera

V. cholerae non-O1 vaccine strain source[a]	Designation		
	LSU serovar[b]	Sakazaki serovar[c]	Smith serotype[d]
Crab hemolymph	G	23	19
Sewer, Lake Arthur, La.	O	42	999[e]
Human, Louisiana	Q	2	17
Human, Louisiana	X	57	999
Sewer, Lake Charles, La.	Y	X64	350
Sewer, Welsh, La.	FF	X82	999
Human, Dacca, Bangladesh	UU	Unknown[f]	21
Water, Florida	XX	Unknown	Unknown

[a] N. C. Roberts (Department of Health and Human Resources, State of Louisiana).
[b] Vaccine strains were arbitrarily assigned an alphabetical Louisiana State University (LSU) serovar designation (Siebeling et al., in press).
[c] Isolates serologically typed by T. Donovan (Public Health Laboratory, Preston Hall Hospital, Maidstone, Kent, England).
[d] Isolates serologically typed by H. L. Smith, Jr. (Jefferson Medical College, Philadelphia, Pa.).
[e] Not typable.
[f] Serovar-serotype unknown.

Typing sera. Antisera to *V. cholerae* O1 and non-O1 strains (Table 1) were produced in rabbits immunized with live vaccines prepared by the methods of Smith (11), heat-killed vaccines prepared by the methods of Sakazaki et al. (9), and PS-protein carrier conjugates prepared from detoxified LPS conjugated to bovine gamma globulin (BGG).

Preparation of PS-BGG immunogen. LPS was extracted from 4 to 10 g of acetone-dried cells prepared from each of the *V. cholerae* vaccine strains by the method of Westphal and Jann (14). Each LPS preparation (1 mg/ml) was detoxified in 0.25 N NaOH at 37°C for 4 h, followed by 1 h at 56°C. Detoxified LPS was dialyzed against water overnight, and then the lipid was sedimented by centrifugation at 105,000 × *g* for 3 h. The supernatant fluid, PS O-antigen, was lyophilized. To conjugate PS to BGG, 10 mg of PS was dissolved in 2 ml of 0.15 M H_3BO_3–0.5 M NaCl, pH 8.6. Next, 7 mg of CNBr crystals was added to the PS, and the solution was stirred for 15 min while the pH was maintained at 8.6. Activated PS was added to 10 ml of a 0.1% solution of BGG (Sigma Chemical Co., St. Louis, Mo.) in borate-sodium chloride buffer, and the coupling reaction was allowed to proceed, with stirring, overnight at 4°C. The PS-BGG conjugate was dialyzed against 0.067 M phosphate-buffered saline (PBS, pH 7.3) for 6 h and then against saline overnight. The PS-BGG preparation was concentrated to 5 ml, and 2.5 ml (ca. 5 mg of the conjugate) was emulsified in an equal volume of Freund adjuvant (Difco, Laboratories, Detroit, Mich.). Rabbits were immunized by injecting 0.5 ml of the PS-BGG adjuvant intramuscularly into each hindquarter and in 7 to 10 sites subcutaneously along the spine and the nuchal region. The animals received a second immunization 30 days later of 2.5 ml of PS-BGG incorporated into incomplete adjuvant.

Serology. Antibody activity in each preparation of anti-*V. cholerae* serum was tested by both slide agglutination and passive hemagglutination (PHA). *V. cholerae* cells harvested in PBS or 0.3% formolized PBS from a 24-h slant were mixed with various dilutions of each antiserum on a glass slide and examined over a 1-min period for evidence of agglutination. The PHA test was used to test each O1 and non-O1 antiserum for anti-PS activity by the addition of LPS-sensitized rabbit erythrocytes (RRBC) to dilutions of each antiserum. LPS was extracted from environmental and vaccine strains. Before RRBC were sensitized, LPS was treated with 0.02 N NaOH (1 mg/ml) for 5 h at 37°C and then neutralized in 19 volumes of PBS. To a 5% suspension of RRBC washed three times (in PBS) was added an equal volume of alkaline-treated LPS (50 μg/ml), and the suspension was incubated for 2 h at 37°C. LPS-sensitized RRBC were washed three times in PBS and resuspended to 0.5% (vol/vol) in PBS. Serial dilutions of heat-inactivated antiserum were made in PBS, and an equal volume of LPS-sensitized RRBC was added to each tube. Agglutination patterns were examined after 90 min at 37°C, and titers were determined after overnight incubation at 4°C.

RESULTS

Over a 3-year period, more than 2,500 environmental *V. cholerae* isolates were examined serologically by slide agglutination in antisera raised in rabbits immunized with heat-killed vaccines prepared from 46 different *V. cholerae* non-O1 serovars (7). Three hundred of the environmental isolates were agglutinated in the optimal dilutions of two or more of the anti-serovar sera. Table 2 lists eight representative isolates recovered in Louisiana which agglutinated in two, three, and four different antisera. Two isolates, La 8584 and La 9214, agglutinated in optimal dilutions of anti-O1 sera (Inaba, 1:80) and in an optimal dilution of anti-non-O1 serum XX. This serological dilemma occurred frequently, making it difficult to serologically discriminate between O1 and non-O1 serogroups. Kaper et al. (3) detected the gene for cholera

TABLE 2. Examples of *V. cholerae* isolates which agglutinate in two or more anti-serovar sera

Live *V. cholerae* suspensions of:	Slide agglutination reaction[a] in serum produced against heat-killed vaccine strains of:									
									O1	
	G[b]	O	Q	X	Y	FF	UU	XX	La 5875 Inaba	CA 411 Ogawa
La 8584								++	++	++
La 6480						++				+
La 9214								++	++	++
La 9296		+		+		++		++		
La 6169			++		++					
La 6195	++						++			
La 9313		+		+		++		++		
La 9302		+		+		++		++		

[a] ++, Agglutination in optimal dilutions of antiserum within 1 min; +, agglutination in low dilutions of antiserum within 1 min.
[b] See Table 1 for Sakazaki serovar and Smith serotype designations.

TABLE 3. Serological examination of LPS extracted from *V. cholerae* isolates for evidence of existence of multiple serotypes

LPS extracted from isolates listed and coated onto RRBC	PHA titer vs LPS-sensitized RRBC in serum produced against heat-killed vaccines of:									
									O1	
	G[a]	O	Q	X	Y	FF	UU	XX	AC Inaba	AB Ogawa
La 8584								0	40,000	20
La 6480						160				0
La 9214								0	40,000	20
La 9296		0		320		0		40,000		
La 6169			0		0					
La 6195	2,560						2,560			
La 9313		0		320		0		10,000		
La 9302		0		640		0		40,000		

[a] See Table 1, footnotes *b*, *c*, and *d*.

toxin in La 8584 which made it important to ascertain whether this isolate was O1 or non-O1. A second group of *V. cholerae* isolates, La 9296, La 9313, and La 9302, were agglutinated in optimal dilutions of anti-XX but not in O1 serum (Table 2). These three isolates were also agglutinated in optimal dilutions of anti-FF and in low dilutions of anti-O and anti-X sera. Do these findings imply that O1 and non-O1 serovar-serotype-specific antigens are coexpressed in *V. cholerae* isolates, or are different classes of O-antigens being detected? If La 8584 is an O1 serovar, then anti-XX serum is reacting with either group-specific O1 LPS-associated antigen A, B, or C or with a non-LPS-associated antigen.

Since serovar-specific O1 antigens A, B, and C are structural components of LPS (5, 6), it would be logical to serologically group *V. cholerae* non-O1 isolates through serologically unique LPS-associated antigens. To determine whether isolate La 8584 exhibits O1 antigens A, B, and C and non-O1 antigen XX in LPS, this cell wall component was extracted from each of the eight environmental isolates, coated onto RRBC, and retested by PHA in the same 10 antisera. Table 3 shows the PHA titers of antisera produced against heat-killed whole-cell vaccines and tested against LPS-sensitized RRBC. Anti-XX serum did not react with La 8584 LPS-coated RRBC, whereas anti-Inaba serum produced a PHA titer of 40,000. This indicates that anti-XX serum, when tested by slide agglutination (Table 2) against isolate La 8584, reacted with a non-LPS-associated antigen. The implication is that La 8584 is a *V. cholerae* O1 serovar. La 9214 LPS-sensitized RRBC were also agglutinated in anti-O1 serum only.

Isolates La 9296, La 9313, and La 9302 appear to be *V. cholerae* non-O1 serovar XX since LPS extracted from these organisms exhibited elevated PHA titers in anti-XX serum, with no reaction in anti-O or anti-FF serum, but showed low-level activity in anti-X serum (Table 3). Isolate La 6169 does not appear to be serotype Q or Y but some yet to be identified serovar; however, the validity of this interpretation should be questioned based upon the extremely low yield of LPS extracted from this isolate. It is possible that RRBC were not optimally sensitized with La 6169 LPS, if at all. It appears that typing sera raised in rabbits immunized with heat-killed vaccines possess serovar-specific anti-LPS activity if the serovar-specific antigen is defined as LPS; and some typing sera also possess high levels of antibody activity to a yet to be defined heat-stable, non-LPS antigen shared among serogroups.

When a *V. cholerae* isolate is agglutinated in two or more anti-serovar-sera, it is not expedient to extract LPS to ascertain by PHA the serological type or variety. Rather, serovar-specific (anti-LPS) typing sera are required. With this objective in mind, rabbits were immunized with PS-BGG conjugates to produce LPS-specific typing sera. PS-BGG conjugates were prepared from LPS extracted from the eight non-O1 vaccine strains (Table 1) and *V. cholerae* O1 (La 5875 and CA 411). Figure 1 compares the anti-LPS antibody responses in representative rabbits immunized with a live vaccine, a heat-killed whole-cell vaccine, or PS-BGG conjugate prepared from *V. cholerae* O1 Inaba (La 5875) and Ogawa (CA 411). Fifty percent of the rabbits immunized with live O1 or non-O1 vaccines died by days 3 to 4 postimmunization. Those that survived showed low levels of anti-PS (1:80) at the time of exsanguination on day 14. Smith (11) collects typing sera from rabbits 10 to 14 days after the

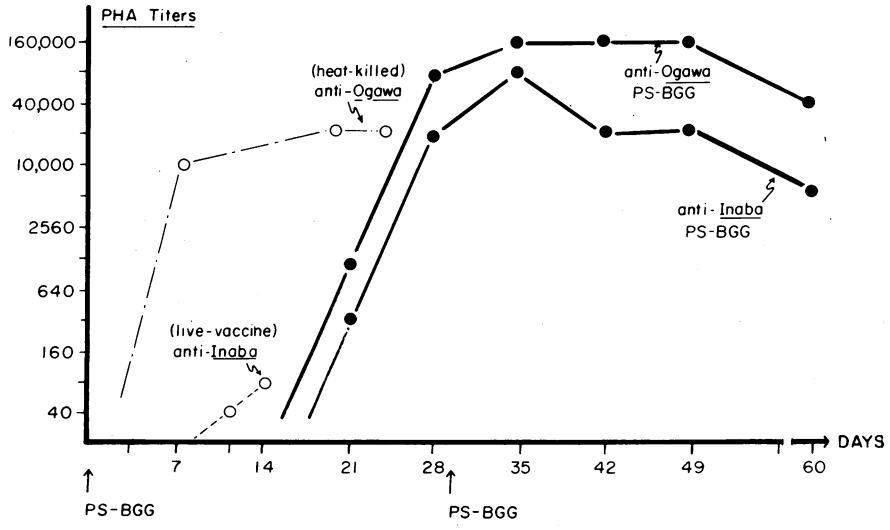

FIG. 1. Anti-LPS PHA titers in antisera produced in rabbits immunized with three different vaccine preparations of *V. cholerae* O1. Antisera produced in rabbits immunized with live whole-cell vaccine, heat-killed whole-cell vaccine, and PS-BGG carrier conjugates were tested against homologous LPS coated onto RRBC.

TABLE 4. Specificity of anti-PS serum: PHA titers of anti-PS sera tested against homologous and heterologous LPS prepared from *V. cholerae* O1 and non-O1 vaccine strains

RRBC coated with LPS from:	PHA titer of antiserum produced against:									
	G[a]	O	Q	X	Y	FF	UU	XX	O1	
									Inaba	Ogawa
G	10,240									
O		160,000	2,560							
Q		2,560	160,000							
X				80,000				640		
Y	640				1,280					
FF						160,000				
UU							2,560			
XX								160,000		
Inaba (La 5875)									1,280	160,000
Ogawa (CA 411)									640	160,000

[a] See Table 1, footnotes *b*, *c*, and *d*.

single immunization. Rabbits immunized with heat-killed vaccines showed a rapid and elevated anti-PS response which persisted through day 28. The anti-PS responses in rabbits immunized with PS-BGG conjugates were delayed in comparison; however, high PHA titers to PS were produced in these animals by day 30, and the antibody activity persisted through day 50. PS-BGG conjugate-immunized animals were exsanguinated 50 to 60 days postimmunization.

Polysaccharide was used as an immunogen to produce a high-titer antiserum to the PS O-antigen in the *Vibrio* cell wall which would be serologically reactive with only those vibrios which express that O-antigen determinant. To assess the serological specificity of each anti-PS serum produced against the eight non-O1 serovars and two P1 strains, the titers of these sera were determined by PHA in a checkerboard fashion (Table 4).

The PHA titers suggest that each of the eight non-O1 vaccine strains possesses a unique (serovar-specific) LPS-associated antigen. In addition, either vaccine strains O and Q share a second minor LPS antigen, or each vaccine strain expresses the Q- and O-antigen, respectively, at a low concentration. Vaccine strain XX probably expresses antigen X, whereas strain X does not express detectable levels of antigen XX. When a similar serological checkerboard titration was done by testing the anti-PS sera with live cell suspensions of the vaccine strains by slide agglutination, an identical result pattern was seen.

The eight environmental isolates were retested by slide agglutination in each of the 10 anti-PS sera (Table 5). Isolate La 8584 had agglutinated in both anti-non-O1 XX serum and anti-O1 serum produced in rabbits immunized with heat-killed whole-cell vaccines (Table 2), whereas LPS extracted from this isolate was agglutinated by PHA in anti-O1 serum only (Table 3). When anti-PS serum was used to retest this isolate by slide agglutination, a live suspension of La 8584 cells was agglutinated in anti-O1 serum only. The slide agglutination patterns of the eight environmental isolates in the anti-PS serum (Table 5) parallel the PHA results when LPS of each isolate was tested in antisera raised in rabbits immunized with heat-killed whole-cell vaccines (Table 3). The single exception was La 6169, which agglutinated in anti-PS serovar Q. The anti-LPS PHA titers (Table 4) imply that vaccine strains X and XX share at least one LPS antigen, a finding substantiated in environmental isolates. The anti-PS sera provide a level of specificity in slide agglutination comparable to that of the PHA test, which improves upon the presently used typing sera produced in rabbits immunized with whole-cell vaccines.

DISCUSSION

It was not our intention to add a third serological typing scheme and its inherent nomenclature to the two systems presently in use (9, 11). The typing serum we have used to serologically examine thousands of environmental *V. cholerae* isolates (7) was prepared by using the methods of Sakazaki (9). It became apparent that typing sera raised in rabbits immunized with heat-killed whole-cell vaccines did not possess the specificity necessary to serologically discriminate between non-O1 serotypes and serovars (Table 2).

One plausible explanation for the apparent lack of correlation between the two currently used serological typing schemes originates with the disparity in the methods used to

TABLE 5. Reexamination of *V. cholerae* isolates by slide agglutination in anti-PS serovar-specific sera

Live *V. cholerae* suspensions of:	Slide agglutination reaction[a] in antiserum to PS-BGG conjugates of:								
	G[b]	O	Q	X	Y	FF	UU	XX	O1
La 8584								0	++
La 6480						++			0
La 9214								0	++
La 9296		0		++		0		++	
La 6169			++		0				
La 6195	+						+		
La 9313		0		++		0		++	
La 9302		0		++		0		++	

[a] ++, Agglutination in optimal dilutions of antiserum within 1 min; +, agglutination in low dilutions of antiserum within 1 min.
[b] See Table 1, footnotes *b*, *c*, and *d*.

prepare vaccines. The diverse methodology is further clouded by the fact that neither the Sakazaki nor the Smith system attempts to define or identify the serovar-serotype-specific antigen(s) that each system purports to detect. It is probable that each system may be detecting different classes of antigens, a conclusion one might reach when examining the efforts of Brenner et al. (1) to correlate the two systems. From our vantage point it appears that the serological nomenclatures, serovar or serotype notations, of the two systems are irreconcilable. Whole-cell vaccines, living or heat killed, present a diverse offering of serovar-specific and non-serovar-specific antigens to the antibody-producing facilities of rabbits, resulting in the production of a spectrum of antibodies possessing an array of specificities. This appears to be the case when the two environmental *V. cholerae* isolates, La 8584 and La 9214, were serologically tested with serum produced in rabbits immunized with heat-killed whole-cell vaccines. These isolates agglutinated in optimal dilutions of anti-O1 serum and anti-non-O1 serum XX. The public health implications are significantly greater if *V. cholerae* O1 organisms are recovered from recreational waters, seafood, or sewerage than if non-O1 isolates are detected. If the typing serum fails to discriminate among the O-antigens, then a breakdown in the follow-up toxigenic studies and resulting interpretations ensues.

To us it makes good sense to dedicate or commit the serological typing of *V. cholerae* to the detection of LPS-associated antigens for reasons previously presented. This approach could lead to a standardized serological nomenclature which defines the cell wall component which carries the serovar-specific antigen. Antisera which exhibit specificity for LPS-associated antigens of eight non-O1 and two O1 serovars were produced in rabbits immunized with the PS moiety of LPS prepared from each serovar and conjugated to a protein carrier. A high-titer antibody was produced after two immunizations, with slide agglutination titers equal to and often four times the level seen in sera produced in rabbits immunized with whole-cell vaccines; and in each case anti-PS sera showed specificity for homologous LPS. In three instances it appears that either a second LPS-associated antigen may be shared by two vaccine strains (i.e., O and Q [Table 4]) or each strain expresses at a low concentration the reciprocal serovar-specific antigen. The question could be resolved by absorption studies, and it should not be surprising for non-O1 strains to express multiple LPS determinants since O1 Inaba and Ogawa each express antigen A. Both Smith (12) and Shimada and Sakazaki (10) detected cross-reactions in their respective antisera and conducted absorption studies which implied the existence of multiple O-antigen determinants on *V. cholerae* non-O1. When isolates La 8584 and La 9214 were tested by slide agglutination in anti-PS sera, they each agglutinated in anti-O1 only (Table 5), which was not the case when these isolates were tested in antisera raised in rabbits immunized with whole-cell vaccines (Table 2). The slide agglutination patterns of the eight environmental *V. cholerae* isolates in anti-PS sera paralleled the PHA patterns obtained when anti-whole-cell sera were tested against LPS extracted from these same eight isolates. These findings suggest that anti-whole-cell sera, when tested against live or Formalin-killed cell suspensions by slide agglutination, react with a heat-stable non-LPS antigen(s) shared across serovar boundaries.

At this time neither the efficiency of the PS conjugation process nor the molecular size of the PS moiety conjugated to the protein carrier is known, nor have the constitutive sugars that comprise the O-determinants in the non-O1 strains been identified. However, in the absence of this information, preliminary findings suggest that the PS-BGG conjugate as an immunogen is superior to native LPS. Rabbits immunized with O1 and non-O1 LPS by intraperitoneal or subcutaneous injection yielded low and transitory PHA and slide agglutination titers (R. J. Siebeling, L. B. Adams, Z. Yusof, and A. D. Larson, *Proceedings of the Conference on Vibrios in the Environment*, in press). Staub (13) prepared LPS-RBC stromata immunogens with *Salmonella* LPS and successfully produced anti-LPS activity in rabbits, an approach which was not consistently reproducible when we used *V. cholerae* non-O1 LPS-stromata or LPS-whole RBC as immunogens (Siebeling et al., in press). It is conceivable that the PS-BGG immunogen described and the immunization regimen used can be improved upon by determining the optimum PS-to-BGG coupling ratio, identifying the O-determinant in crude PS, identifying a more efficient carrier, and evaluating different immunogen doses and immunization schedules.

One approach that could be followed to unify the serological nomenclature used to identify *V. cholerae* non-O1 groups (serovars-serotypes) could be done in the following manner. LPS, if it is agreed that the serovar-specific antigen resides in this cell wall constituent, could be extracted from the vaccine strains of either the Smith or the Sakazaki collection. RBC coated with LPS would be tested by PHA with the homologous and heterologous typing sera in that collection. In this manner the minimum number of unique LPS-associated antigens in the collection can be serologically identified. Determinants that may be shared among serovars, in the manner that the A-determinant is shared by the Inaba, Ogawa, and Hikojima serovars in the O1 group, may be identified. Next, the typing serum in the opposite collection would be used to serologically test the LPS-sensitized RBC in an attempt to identify its counterpart in the first collection. In addition, serogroups unique to each collection would be recognized, a finding reported by Brenner et al. (1).

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LITERATURE CITED

1. **Brenner, D. J., B. R. Davis, Y. Kudoh, M. Ohashi, R. Sakazaki, T. Shimada, and H. L. Smith, Jr.** 1982. Serological comparison of two collections of *Vibrio cholerae* non O1. J. Clin. Microbiol. **16**:319–323.
2. **Gardner, A. D., and K. V. Venkatraman.** 1935. The antigens of the cholera group of vibrios. J. Hyg. **35**:262–282.
3. **Kaper, J. B., H. B. Bradford, N. C. Roberts, and S. Falkow.** 1982. Molecular epidemiology of *Vibrio cholerae* in the U.S. Gulf Coast. J. Clin. Microbiol. **16**:129–134.
4. **LeMinor, L.** 1981. The genus *Salmonella*, p. 1148–1159. *In* M. P. Starr, H. Stols, H. G. Truper, A. Ballows, and H. G. Schlegel (ed.), The procaryotes, vol. 2. Springer-Verlag, Berlin.
5. **Redmond, J. W.** 1979. The structure of the O-antigenic side chain of the lipopolysaccharide of *Vibrio cholerae* 569B (Inaba) Biochim. Biophys. Acta **584**:346–352.
6. **Redmond, J. W., M. J. Korsch, and G. D. F. Jackson.** 1973. Immunochemical studies of the O-antigens of *Vibrio cholerae*. Partial characterization of an acid labile antigenic determinant.

Aust. J. Exp. Biol. Med. Sci. **51**:229–235.
7. **Roberts, N. C., R. J. Siebeling, J. B. Kaper, and H. B. Bradford, Jr.** 1982. Vibrios in the Louisiana Gulf coast environment. Microb. Ecol. **8**:299–312.
8. **Sakazaki, R., and T. Shimada.** 1977. Serovars of *Vibrio cholerae*. Jpn. J. Med. Sci. Biol. **30**:279–282.
9. **Sakazaki, R., K. Tamura, C. Z. Gomez, and R. Sen.** 1970. Serological studies on the cholera group of vibrios. Jpn. J. Med. Sci. Biol. **23**:13–20.
10. **Shimada, T., and R. Sakazaki.** 1977. Additional serovars and inter-O antigenic relationships of *Vibrio cholerae*. Jpn. J. Med. Sci. Biol. **30**:275–277.
11. **Smith, H. L., Jr.** 1979. Serotyping of non-cholera vibrios. J. Clin. Microbiol. **10**:85–90.
12. **Smith, H. L., Jr., and K. Goodner.** 1965. On the classification of vibrios, p. 4–8. *In* O. A. Bushnell and C. S. Brookhyser (ed.), Proceedings of the Cholera Research Symposium, Honolulu, Hawaii.
13. **Staub, A. M.** 1964. The role of the polysaccharide moiety in determining the specificity and immunological activity of the O-antigen complex in Salmonellae, p. 38–48. *In* M. Landry and W. Braun (ed.), Bacterial endotoxins. Academic Press Inc., New York.
14. **Westphal, O., and K. Jann.** 1965. Bacterial lipopolysaccharides. Extraction with phenol-water and further applications of the procedure. Methods Carbohydr. Chem. **5**:83–91.