IMMUNODETERMINANT SPECIFICITY OF HUMAN IMMUNITY TO TYPE III GROUP B *STREPTOCOCCUS**

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Early studies by Lancefield on the immunochemical character of the type-specific antigens of group B *Streptococcus* utilized hot hydrochloric acid (HCl) treatment of whole bacteria for extraction of these polysaccharides (1, 2). The HCl-extracted antigens have been shown to be of low molecular size, immunologically incomplete, and to consist of galactose, glucosamine, and glucose in a molar ratio of 2:1:1, respectively, (3, 4).[1] Extraction of cells by more gentle techniques such as washing with neutral buffer solutions allows isolation of more complete, high molecular weight native polysaccharides which contain in addition to these three monosaccharides a terminal acid-labile determinant, sialic acid (5, 6).[1] The molar ratio of sugars in the repeating unit of this native polysaccharide is 2:1:1:1, galactose, glucose, glucosamine, and sialic acid, respectively. Acid treatment of these large molecular size antigens results in degradation to core fragments which are immunochemically identical to the HCl-extracted polysaccharides and contain no sialic acid (6).

It has been our hypothesis that investigation of human immunity to the group B *Streptococcus* should employ purified polysaccharides from the bacterial cell surface or capsule which exist in native form (6). These native antigens would be desirable for study since, theoretically, they most closely resemble those which the infected host recognizes immunologically.

Our studies have demonstrated a significant association between low concentrations of maternal antibody directed against the native type III polysaccharide and risk for infant disease due to type III, group B streptococci (7). Furthermore, development of anti-native III antibody results during recovery from natural infection or after immunization of adults with purified native type III polysaccharide (8, 9).

Although it appeared that antibody to native type III polysaccharide was of great importance in our understanding of human immunity to type III strains of group B *Streptococcus*, the relationship between this antibody and that to the core or HCl antigens has not been studied. Recently, Wilkinson has reported that high levels of antibody to acid-extracted antigens (hot hydrochloric acid or cold trichloracetic acid) exist in acute sera of infants infected with group B streptococci (10). These data made clear the necessity for experiments to assess the relative importance of antibody to undegraded native sialic acid-containing polysaccharide and antibody to the core antigens which lack these acid labile determinants.

Unfortunately, experiments aimed at directly determining the relative importance

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[1] Jennings, H. J., et al. Structural studies of the polysaccharide antigen of type III group B Streptococcus. Manuscript in preparation.

in human immunity of antibody to these terminal acid-labile constituents or to the core structure have not been feasible because of the very small molecular size of the hot HCl-extracted antigen and its likely nonimmunogenicity in humans. However, the findings of Fischer et al. (11) that the type XIV capsular polysaccharide of *Streptococcus pneumoniae* cross-reacted immunologically with the core HCl antigen of type III group B *Streptococcus*, in conjunction with our own analysis of the type III core antigen showing chemical similarity to Lindberg's et al. (12) description of the pneumococcal XIV capsule[1] suggested that antibody to core type III antigen might be induced by immunization of adults with pneumococcal type XIV polysaccharide.

Studies were performed to determine the biological relevance of antibody directed against native and core type III antigen in human infection as well as in adult volunteers immunized with native type III group B streptococcal and multivalent pneumococcal polysaccharide vaccines (9).

Materials and Methods

Bacterial Strains. Prototype strains of group B *Streptococcus* representing each of the five serotypes and the group B variant strain (devoid of type-specific antigen) were kindly supplied by Dr. Rebecca Lancefield, The Rockefeller University. These strains are designated 090 (type Ia), H36B (type Ib), A909 (type Ic), 18RS21 (type II), D136c (type III), and 090R (group B); in addition, strains M732 and M735 (type III) were isolated from infants with meningitis. Lyophilized strains were rehydrated with Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) and incubated overnight at 37°C in 1-ml portions of Todd-Hewitt broth.

Preparation of Rabbit Antisera. New Zealand white rabbits were immunized with formalin-killed whole cell vaccines of the prototype strains of group B *Streptococcus* according to the method of McCarty and Lancefield (13). For type Ic-specific antiserum to strain A909, the method of Wilkinson and Eagon was used (14). All rabbit sera had 1:10,000 merthiolate added, and were stored at 4°C. Hyperimmune rabbit antisera to type XIV *S. pneumoniae* was kindly supplied by Dr. Michael Grizzard, Channing Laboratory, Boston, Mass. and was prepared by the New York State Department of Health.

Human Sera. Acute and convalescent sera from 11 patients with type III group B streptococcal infection were selected from our collection of patient sera and were chosen because of the availability of serum from the mothers of these same infants at the time of infant infection. These women, like the great majority of mothers of infected infants, had low antibody levels to native type III antigen (8).

The sera of 19 randomly selected normal women collected at delivery and cord sera from their healthy infants were obtained for antibody studies. None of these women were type III group B streptococcal vaginal carriers.

In another group of 12 adult volunteers selected for low levels of serum antibody to native type III antigens, preimmunization serum specimens were collected, and written consent was given before their immunization with multivalent pneumonococcal polysaccharide vaccine (Pneumovax, Merck, Sharp, & Dohme Research Laboratories, West Point, Pa., Lot no. 2871W) containing pneumococcal XIV polysaccharide antigen. Postimmunization sera were obtained from these subjects 2 wk after immunization. Subjects were matched according to preimmunization antibody level as determined by a radioactive antigen-binding assay (7, 8), age and sex, with volunteers receiving type III group B streptococcal vaccines who have been previously reported (9). Adults with low levels of antibody to the native polysaccharide were chosen for study because this has been shown to be a risk factor for development of neonatal disease (7, 8). Sera from selected volunteers who were immunized with type III group B streptococcal polysaccharides extracted by neutral buffer ethylenediamine tetra-acetic acid (EDTA)[2] solution or cold trichloroacetic acid (TCA) methods were also studied (9, 15). These sera were selected for study because they contained opsonophagocytic antibodies as determined by the method of Baltimore et al. (16) 2 wk after immunization.

[2] *Abbreviations used in this paper:* cfu, colony-forming units; EDTA, ethylenediamine tetraacetic acid; RABA, radioactive antigen-binding assay; TCA, trichloroacetic acid.

Preparation of Antigens. Native (EDTA-extracted) and TCA-extracted type III, group B streptococcal polysaccharide antigens were isolated from strain M732 by methods described previously (6, 15). Type XIV pneumococcal polysaccharide was obtained from Dr. Grizzard and came from the Bureau of Biologics, Food, and Drug Administration (Eli Lilly and Co., Indianapolis, Ind., Lot no. 812430, Kd = 0.21 Sepharose 4B, information kindly supplied by Dr. John B. Robbins, Bureau of Biologies, Food and Drug Administration, Bethesda, Md.).

The core type III specific polysaccharide of group B *Streptococcus* was extracted from strain M732. This strain was grown in Todd-Hewitt broth and the organisms pelleted by centrifugation. The pelleted bacteria were suspended in 0.2 N HCl and boiled for 10 min according to the method of Lancefield (1). Debris was removed by centrifugation at 10,000 *g* for 15 min at 4°C. The supernate was then neutralized with NaOH to pH 7.0, fractionated with 30% alcohol, and the precipitate discarded. A crude polysaccharide extract was then precipitated from the supernatant with 1.5 vol of alcohol, and after centrifugation, this precipitate was saved. The supernate was then adjusted to 80% alcohol, centrifuged, and the precipitate was dissolved in 0.05 M Tris (Trishydroxymethylaminomethane) buffer, pH 7.4, and this solution was again fractionated with 1.5 vol alcohol. The 1.5 vol alcohol precipitates were combined, and extraction with cold 8% TCA for 4 h at 4°C was performed to further remove proteins and nucleic acids (17). After centrifugation at 10,000 *g* for 10 min, the supernate was neutralized with 1 N NaOH. At this stage the supernate, which had both type III and group B serologic reactivity in capillary precipitin tests, was chromatographed on 2.6 × 85 cm column of Sephacryl S-200 (Pharmacia, Uppsala, Sweden) in 0.05 M Tris, pH 7.4. The serologically active fractions containing type III or group B reactivity (13), excluding UV absorbable material, which was of small molecular size, were combined, concentrated on a PM-30 membrane (Amicon Corp., Lexington, Mass.). precipitated with 4 vol of alcohol, and suspended in 0.05 M Tris, pH 8.4. Final purification of that type III core antigen devoid of group B antigen was achieved on a column of DEAE-Sephacel (Pharmacia) equilibrated in 0.05 M Tris pH 8.4. The K_{av} of the type III core antigen was 0.435 (G-100) corresponding to a molecular size of 46,000 daltons. Immunologically, this antigen formed precipitates only with type III-specific antiserum, and not with other type-specific or with group B-specific streptococcal antisera (13).

Serologic Methods. Capillary precipitin tests were performed by the method of Lancefield (1) and immunodiffusion tests were done in agar gel by the method of Ouchterlony (18).

The radioactive antigen-binding assay (RABA) was performed as previously described by using [^{3}H]labeled native type III polysaccharide as the antigen (7, 8). A similar assay employing intrinsically [^{3}H]labeled core type III polysaccharide antigen was designed.

For preparation of the intrinsically labeled core antigen, strain M732 was grown in Todd-Hewitt broth supplemented with 5 mCi of [^{3}H]labeled sodium acetate per liter. The specific activity of the purified polysaccharide was equal to 1,200 counts per minute per microgram. The RABA reported by Farr (19) and modified for the detection of antibody to the group B streptococcal capsular polysaccharides was employed (7, 8). The percentage of antigen bound was related linearly to the logarithm of the antibody concentration (μg/ml) as determined by quantitative precipitin tests on five selected human sera. Using the method of least squares, the concentration of antibody could be determined from percentage of binding (20) (r = 0.97, slope = 0.0147, intercept = 0.101).

The opsonophagocytic assay described by Baltimore et al. was used (16). In this test bacteria, human polymorphonuclear leukocytes, antibody-free human complement, and patient serum are mixed by end over end rotation. Greater than 90% reduction of colony-forming units (cfu) in 1 h is considered significant opsonization. The titer of serum is the highest dilution of antiserum which causes >90% reduction of cfu in 1 h.

Antibody to pneumococcal polysaccharide types I, III, VIA, VII, XIV, XVIII c (Danish nomenclature), were measured by using the radioimmunoassay method of Schiffman and Austrian (21) and are expressed as nanograms of antibody nitrogen/ml.

Statistical Methods. Differences in antibody levels between groups were compared using the Mann-Whitney U test (22) because of the nonparametric distribution of the data. The method of least squares was used to relate the rise in opsonic titer (expressed in $\log_2$) and rise in antibody concentration to both the native and core polysaccharides. The relative significance of the correlation coefficients between antibody to these two antigens and opsonins was tested by Z transformation (23). The significance of differences in antibody levels within paired sera of groups was estimated with the paired t test (24) which is employed for analysis of these

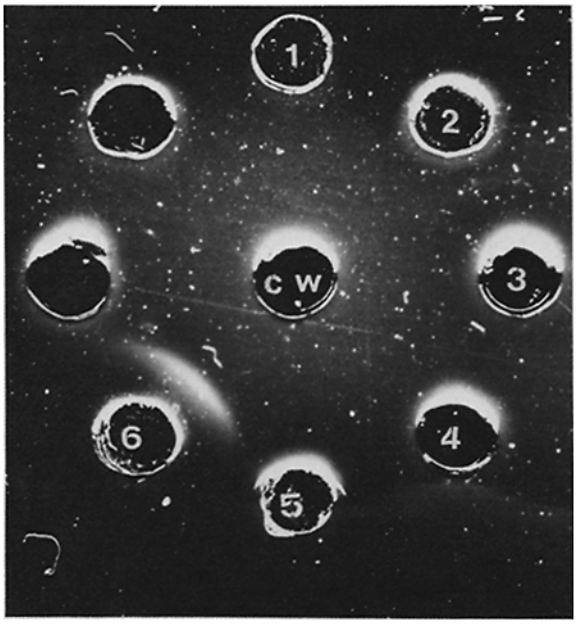

FIG. 1. Immunodiffusion in agar of type XIV polysaccharide of *S. pneumoniae* (cw) with rabbit antisera prepared to group B variant strain (090R) (1), type Ia strain 090 (2), type Ib strain H36B (3), type Ic strain A909 (4), type II strain 18RS21 (5), and type III strain D136C (6).

nonparametrically distributed data. For pneumococcal antibody studies the rise in geometric mean titer is also given so that comparison to previously published data could be made (21).

Results

Immunological Definition of the Specific Relationship between the Type XIV Pneumococcal Capsule and Type III Group B Streptococcal Antigens. The immunological relationship between the pneumococcal type XIV antigen and various type III group B streptococcal antigens was studied in agar gel diffusion. The pneumococcal XIV antigen formed an immunoprecipitin with antiserum prepared to Type III group B streptococci, but not with that prepared to group B serotype Ia, Ib, Ic, or II strains (Fig. 1).

When pneumococcal XIV antigen and type III core polysaccharide (0% sialic acid) were reacted with type III group B streptocccal rabbit antiserum, a line of identity was observed (Fig. 2). However the immunoprecipitins formed with either of these two immunologically identical antigens formed partial identities with the immunoprecipitin resulting from the reaction of native type III (23% sialic acid) or TCA-extracted (9.6% sialic acid) type III antigens and this same antiserum. Identical precipitins were formed by the reactions of the native type III antigen and the TCA antigen with type III group B streptococcal antiserum (Fig. 2).

When pneumococcal type XIV and the core type III group B streptococcal antigens were reacted with pneumococcal type XIV antiserum, a line of identity was seen (Fig. 3). The TCA extracted type III group B streptococcal antigen also reacted with the pneumococcal XIV antiserum to give a line of identity with the previous two precipitins (Fig. 3). However, the native type III group B streptococcal antigen did not form immunoprecipitins with the pneumococcal XIV antiserum.

Antibody Levels to the Core and Native Antigens in Natural Infection. The acute and

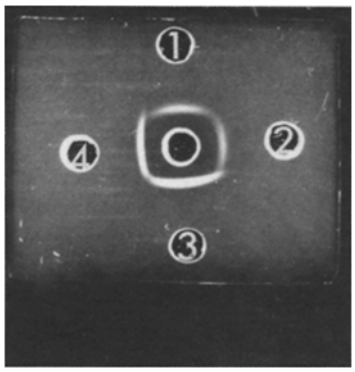

FIG. 2. Immunodiffusion in agar of type XIV capsular polysaccharide of *S. pneumoniae* (1), core capsular antigen of type III group B *Streptococcus* (M732) (2), TCA extracted capsular antigen of type III group B *Streptococcus* (M732) (3), and native capsular antigen of type III group B *Streptococcus* (M732) (4) with rabbit antiserum prepared to type III strain, M735, of group B *Streptococcus*. (Center well).

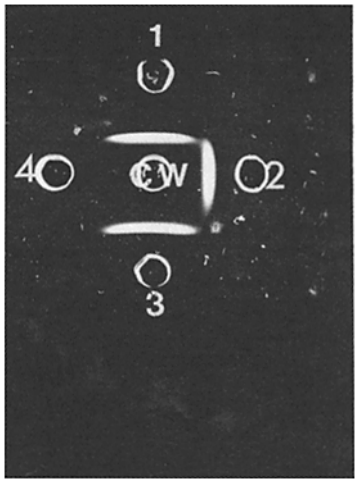

FIG. 3. Immunodiffusion in agar of type XIV capsular antigen of *S. pneumoniae* (1), core capsular antigen of type III group B *Streptococcus* (M732) (2), TCA-capsular antigen of type III group B *Streptococcus* (M732) (3), and native capsular antigen of type III group B *Streptococcus* (M732) (4) with rabbit antiserum prepared to type XIV *S. pneumoniae*. (center well).

convalescent sera of 11 infants with invasive type III group B streptococcal infection were studied to determine whether natural infection was more likely to result in a rise in antibody concentration to the native or to the core type III group B streptococcal polysaccharide antigens (Table I). Quantitative antibody concentrations in acute and convalescent sera from the patients were determined by radioactive antigen-binding assay. Only 2 of these 11 infants had a detectable rise in antibody to the core antigen during convalescence ($P > 0.5$). Similarly, no significant rise in antibody concentration was detected to the pneumococcal XIV ($P > 0.5$) or pneumococcal III ($P > 0.05$) polysaccharides. In contrast 9 of 11 infants developed detectable rises in serum antibody to the native type III group B *Streptococcus* polysaccharide antigen ($P < 0.02$, paired t test). Therefore, in natural type III group B streptococcal infection, the young host is more likely to develop serum antibody directed against native than against

TABLE I

Antibody Concentrations in Sera from Infants with Invasive, Type III, Group B Streptococcal Infection

	Antibody to the type III core antigen		Antibody to the type III native antigen	
	Acute	Convalescent	Acute	Convalescent
	μg/ml		*μg/ml*	
1	10.98	5.04	0.44	0.37
2	2.4	2.3	0.34	0.37
3	10.98	10.98	0.44	7.88
4	3.48	4.12	0.34	13.0
5	3.72	2.94	0.6	5.0
6	14.88	14.39	0.0	36.0
7	18.85	23.89	0.5	9.2
8	4.04	4.04	0.38	6.7
9	4.96	4.18	0.49	36.12
10	2.48	3.54	0.37	3.9
11	4.05	4.05	0.42	49.42

Mean difference in antibody concentration between paired sera ± standard error 0.1 ± 0.76 ($P > 0.5$). 16.01 ± 5.53 ($P < 0.02$).

TABLE II

Antibody Concentrations in Sera from Mothers of Infants with Invasive, Type III Group B Streptococcal Infection

	Antibody to the type III core antigen	Antibody to the type III native antigen
	μg/ml	*μg/ml*
1	13.2	0.85
2	8.2	2.52
3	10.8	2.0
4	5.4	0.58
5	5.7	0.46
6	4.0	0.42
7	8.2	0.68
8	8.2	0.55
9	6.72	1.06
10	5.86	1.00
11	7.96	0.77

core antigenic determinants. Notably, sera from these infants and their mothers (Table II) had significantly higher levels of antibody to the core than to the native antigen ($P < 0.001$ Mann-Whitney U test).

The maternal and cord sera of 19 women and their healthy offspring were compared to the maternal and acute sera from women whose infants had invasive type III group B streptococcal infection for antibody levels to the type XIV and III pneumococcal polysaccharides. No significant differences were found (Mann-Whitney U test). These studies support our earlier observations that very low antibody levels to the native type III group B streptococcal antigen are found in sera from infected infants and their mothers (7, 8) and suggests the unlikelihood that antibody to the core antigen

is protective, when considering the concentrations of this antibody which are present in the acute sera of infected infants and their mothers and in normal healthy controls.

Antibody Response to Group B Streptococcus Type III Antigens in Recipients of Multivalent Pneumococcal Polysaccharide Vaccine. 12 volunteers who had low levels of antibody to the native type III group B streptococcal antigen were given a single injection of multivalent pneumococcal polysaccharide vaccine. Antibody to types I, III, VIa, VII, XIV, and XVIIIc pneumococcal polysaccharides were measured in the paired sera of these volunteers. Data were analyzed by three methods. Using the criterion of Fikrig et al. (25) that a >40% increase between paired serum samples is significant, 9/12 volunteers responded to pneumococcal types I and III, 11/12 responded to type VIa, 12/12 responded to type VII, 8/12 responded to type XIV and 11/12 responded to type XVIIc. The rise in geometric mean titer for each polysaccharide was 3.83 (type 1), 11.95 (type III), 8.0 (type VIa), 16.9 (type VII), 2.55 (type XIV), and 6.69 (type XVIIIc). Utilizing the paired t test, the mean differences between pre- and post-immunization sera for each pneumococcal type (± standard error) were 855.26 ± 363.9 (type I), 1,321.2 ± 352.87 (type III), 211.2 ± 54.16 (type VIa), 563.37 ± 133.47 (type VII), 295 ± 238.43 (type XIV), and 1,463.06 ± 495.80 (type XVIIIc). As analyzed by this latter method, the antibody response to the pneumococcal vaccine recipients was significant for type I ($P < 0.05$), and types III, VIa, VII, or XVIIIc, ($P < 0.01$). This group of vaccine recipients did not have a significant rise to the type XIV pneumococcal polysaccharide by using the paired t test for data analysis.

Despite the fact that when analyzed as a group by the paired t test the overall change in antibody concentration to pneumococcus XIV antigen was not significant, 8 of the 12 individual recipients responded with over a twofold increase in antibody concentration, and these responders had a rise in geometric mean titer of 3.92 fold. Therefore, there were enough high-level responders to type XIV pneumococcal polysaccharide upon which to base further analysis of the immunodeterminant specificity of group B streptococcal antibodies.

Antibodies to the type III group B streptococcal core and native polysaccharide antigens were quantitated in these vaccine recipients before and 2 wk after immunization by means of the RABA (Table III). In the preimmunization sera of these volunteers, as in the mothers of infected infants, significantly higher concentrations of antibody were detected to the core antigen than to the native antigens ($P < 0.001$ Mann-Whitney U test). This indicates that different populations of antibody are reacting with the core and the native antigens. The geometric mean rise in antibody concentration to the core antigen in postimmunization sera from these 12 subjects was 3.2 μg/ml and 9 had >1 μg/ml increase in antibody concentration. As was demonstrated in the pneumococcal XIV antibody assay, the overall rise in antibody concentration to the core antigen of this group was not significant using the paired t test ($P < 0.1$), however, there was a significant antibody response to the core antigen in nine of these pneumococcal vaccine recipients ($P < 0.05$ paired t test). The geometric mean increase in antibody concentration to the native antigen in postimmunization sera from these same individuals was 0.99 μg/ml, and only three demonstrated >1 μg/ml increases ($P > 0.1$, paired t test). Pneumococcal vaccine induced significantly greater responses to the core than to the native type III antigen ($P < 0.01$ Mann-Whitney U test) and was significantly less effective in inducing antibody to the native antigen than was the native type III group B streptococcal polysaccha-

TABLE III

Antibody Levels in Sera of Adult Volunteers Immunized with Multivalent Pneumococcal Vaccine

Antibody to type III group B streptococcal core antigen		Antibody to type III group B streptococcal native antigen		Type III group B *Streptococcus* opsonic titer	
Weeks postimmunization					
0	2	0	2	0	2
23.5	47.95	1.63	13.58	<1:2	<1:2
9.43	10.44	0.47	0.52	<1:2	<1:2
11.17	13.88	2.26	3.68	<1:2	<1:2
26.09	58.75	8.8	38.4	<1:2	1:5
14.15	10.44	3.9	3.9	<1:2	<1:2
7.97	9.76	0.49	0.49	<1:2	<1:2
8.81	5.88	0.42	0.52	<1:2	<1:2
11.55	11.17	0.42	0.49	<1:2	<1:2
15.14	26.00	0.58	0.55	<1:2	<1:2
19.18	30.9	0.68	1.12	<1:2	<1:2
11.95	13.68	0.52	0.47	<1:2	<1:2
9.43	11.17	0.49	0.49	<1:2	<1:2

Mean difference in antibody concentration between paired sera ± standard error 6.80 ± 3.26 ($P < 0.1$). 3.63 ± 2.55 ($P > 0.1$).

ride. The geometric mean rise in 11 recipients of native type III polysaccharide was 9.86 μg/ml, and 8 demonstrated >1 μg/ml increase in antibody in postimmunization sera ($P < 0.01$) (9).

Antigenic Specificity of Human Opsonic Antibodies to Type III Group B Streptococcus. To determine whether antibody directed against the core or the native type III polysaccharide antigens was more important in opsonic immunity to type III group B *Streptococcus,* the pre- and postimmunization sera of nine subjects developing opsonins who received the native (EDTA) or TCA-extracted type III group B streptococcal vaccine and the one multivalent pneumococcal vaccine recipient who developed opsonins were studied. The antibody levels to the native antigen of the individuals of this group who received EDTA or TCA vaccines have been reported elsewhere (9). Opsonic titer and quantitative antibody concentrations to the core and native antigens in sera from these volunteers are summarized in Table IV. All subjects were selected because they had low levels of antibody to the native antigen and lacked type III opsonic activity in their preimmunization sera. These sera as well as those from mothers of infants with invasive infection contained much higher levels of antibody to the core than to the native antigen ($P < 0.001$ Mann-Whitney U test). Immunization with the sialic acid containing antigens elicited a significant rise in antibody concentration to both the core antigen ($P < 0.05$) and the native antigen ($P < 0.02$, paired t test). However, the rise in opsonic antibody titer ($\log_2$) correlated much better ($P < 0.001$, Z transformation) with the rise in antibody concentration to the native antigen ($r = 0.94$, $r^2 = 0.88$) than with the rise in antibody concentration to the core antigen ($r = 0.51$, $r^2 = 0.26$). These opsonic titers were similar in tests with two strains of type III group B *Streptococcus.* Notably, the one pneumococcal vaccine recipient who developed a rise in opsonic antibody after immunization had high concentration of antibody to the native antigen as well as the core antigen. These findings indicate that opsonic antibodies to type III group B *Streptococcus* are usually

TABLE IV

Antibody Levels in Sera from Adult Volunteers Immunized with Type III Group B Streptococcal Vaccine

Vaccine given	Antibody to type III group B streptococcal core antigen		Antibody to type III group B streptococcal native antigen		Type III group B *Streptococcus opsonic titer*	
	μg/ml		*μg/ml*		*μg/ml*	
	weeks post immunization					
	0	2	0	2	0	2
EDTA	34.2	69.6	1.31	198.0	<1:2	1:80
EDTA	15.1	15.1	1.91	4.58	<1:2	1:2
TCA	11.5	11.5	0.65	2.8	<1:2	1:2
TCA	7.4	27.8	1.63	314.0	<1:2	1:80
EDTA	49.5	53.05	2.96	172.2	<1:2	1:20
EDTA	11.2	12.78	.72	33.8	<1:2	1:5
EDTA	21.96	44.8	1.31	51.5	<1:2	1:10
EDTA	34.2	69.6	1.46	167.0	<1:2	1:40
EDTA	9.1	9.1	.58	38.1	<1:2	1:2

Mean difference in antibody concentration between paired sera standard error.
13.24 ± 5.11 ($P < 0.05$). 107.71 + 35.92 ($P < 0.02$).

directed against the acid-labile determinant of the native antigen and not against the core antigen.

Discussion

Although it seemed reasonable on an intuitive basis that native polysaccharide antigens of group B *Streptococcus* containing complete immunodeterminants from the bacterial cell surface would be best suited for candidate vaccines, no proof of the hypothesis had been given (6). In this study, various antigens have been compared for their potential ability to induce protective antibodies to type III group B *Streptococcus.* Chemically, each polysaccharide contains galactose, glucose, and glucosamine (2:1:1). The type XIV pneumococcal capsule and the core of the type III, group B streptococcal capsule are identical in chemical composition and have a trisaccharide backbone of each of these monosaccharides and an additional terminal galactose, β1-4 linked to the glucosamine of the trisaccharide (3, 12).[1] Antibodies to the type XIV pneumococcal capsule and the core type III group B streptococcal antigen are identical. The native type III antigen differs by having a terminal sialic acid (23% composition) attached to each galactose end group of the core.[1] These differences in chemical structure could explain the immunologic differences noted among the various antigens. The type XIV pneumococcal capsule, core type III group B streptococcal antigen, TCA extracted type III antigen, and the native type III group B streptococcal capsule all reacted with type III group B streptococcal antiserum. However, the reaction of identity by type III antiserum with the pneumococcal XIV or core type III antigen is not complete with the precipitate between the native or TCA-extracted antigens and this serum. Because all four antigens share a chemically similar core, this structure contains the common determinant demonstrated by the reactions of these antigens with type III group B streptococcal antiserum. The presence of terminal sialic acid residues in the native type III group B streptococcal antigen (23% sialic acid) which are partially destroyed by TCA extraction (TCA antigen, 9.6% sialic acid) provide a more complete antigenic site to precipitate antibodies in

type III antiserum which are directed against this determinant. Moreover, the presence of terminal sialic acid residues in the native antigen could likely mask the core antigenic site, thus preventing precipitation of this antigen with the type XIV pneumococcal antiserum which only contains antibodies to the core determinants. The presence of fewer sialic acid residues in the TCA antigen provides an ineffective mask; therefore, core sites are exposed and a precipitin reaction occurs with type XIV pneumococcal antiserum. The core type III group B streptococcal antigen, the type XIV pneumococcal capsule, and the TCA type III group B streptococcal antigen give identity reactions with type XIV pneumococcal antiserum.

The chemical differences among the various antigens are directly relevant to their application as vaccines. Immunization of 12 healthy adults selected for low levels of antibody to the native type III Group B streptococcal polysaccharides with multivalent pneumococcal vaccine resulted in significant responses to types I, III, VIa, VII, and XVIIIc pneumococcal polysaccharides. Interestingly, the response of this group of normal adults to the pneumococcal XIV capsule was not statistically significant using the paired t test for data analysis. Nonetheless, 8 of these 12 individuals responded to the type XIV polysaccharide with over twofold rises. The overall rise in geometric mean titer of this group of 12 adults is comparable to data published elsewhere (26, 27) using the same assay for pneumococcal antibodies as is reported here. The antibody rise in those eight individuals responding to the type XIV polysaccharide is of sufficient magnitude for analyses to determine whether human antibody induced to the pneumococcal XIV polysaccharide offers a feasible alternative to immunization with native group B streptococcal polysaccharide vaccines as a means of preventing type III group B streptococcal disease.

Immunization with multivalent pneumococcal vaccine resulted in an antibody response to the core type III group B streptococcal polysaccharide in nine of the 12 recipients, yet only one developed a significant rise in opsonic titers to type III group B streptococci. Immunization with native type III antigen or TCA group B streptococcal antigen resulted in a rise in antibody to the more complete native group B streptococcal polysaccharide in addition to the core. This finding is significant because the development of opsonic antibody and natural immunity to group B *Streptococcus* is correlated with the presence of antibody to the native, rather than the core antigen.

Sera from volunteers immunized with type III group B streptococcal or pneumococcal polysaccharide vaccines who developed opsonic antibody demonstrated a very significant correlation ($r = 0.94$, $r^2 = 0.88$) between rise in serum antibody to the native antigen and opsonic titers, but a significantly poorer correlation between rise in antibody to the core antigen and opsonic titers to type III group B streptococci ($r = 0.51$, $r^2 = 0.25$) ($P = < 0.001$). All vaccine recipients were selected for immunization because their sera contained low levels of antibody to the native antigen. Interestingly, these vaccinees were found to have high levels of antibody to the core polysaccharide in their preimmunization sera. This observation may be explained by the age-related acquisition of antibody to type XIV pneumococcal polysaccharide.

In natural type III group streptococcal human infection, low levels of maternal antibody to the native antigen has been shown to be a significant risk factor for development of disease in infants (7, 8). This association is lacking for antibody to the core structure since both infants with invasive, type III group B streptococcal disease and their mothers had significantly higher levels of serum antibody to the core than

to the native type III group B streptococcal antigen. Furthermore, maternal and cord sera of infants infected with type III group B streptococci did not differ from age-matched controls with respect to anti-pneumococcal XIV capsular antibodies. The discrepancy between anti-core and anti-native antibody levels emphasizes the importance of low levels of antibody to the native type III antigen as a determinant of susceptibility in human infection, and explains the findings reported by Wilkinson (10) that high antibody levels to acid-extracted antigens are not infrequently found in acute sera of infants with group B streptococcal infection and their mothers.

The finding that immunization of adults with native type III group B streptococcal polysaccharide stimulates antibody directed against the core type III antigen might possibly be explained by degradation of the native antigen in vivo resulting in exposure of its core components. This hypothesis does not explain our observation that three adults immunized with pneumococcal XIV antigen developed antibody to the native antigen in their sera. It is conceivable that both these findings could be best explained by the hypothesis of original antigenic sin in which a secondary response to an immunogen not quite identical to the primary antigen is believed to occur (28).

These data confirm the importance of antibody directed against native type III capsular antigen in human immunity to type III group B *Streptococcus* and suggests that future investigations regarding human immunity to, or vaccine preparation for prevention of group B streptococcal disease must be based upon type-specific antigens containing native determinants.

Summary

The type III polysaccharides of group B *Streptococcus* in its native state chemically consists of glucose, galactose, glucosamine, and sialic acid. The core of this polysaccharide lacks sialic acid and precipitates with type III antiserum to give a partial identity with the precipitate between the native antigen and this serum. The core determinant is immunochemically similar to the capsular polysaccharide of type XIV *Streptococcus pneumoniae*, while the native type III group B streptococcal polysaccharide does not cross-react with type XIV pneumococcal antiserum. In human sera, it is antibody directed to the native antigen which correlates very highly with opsonic immunity ($r = 0.94$) while a poorer correlation exists between antibody to the core antigen and opsonins ($r = 0.51$ $P < 0.001$). In natural infections, an association exists between low levels of maternal antibody to the native antigen and risk of disease in the infant. This association is not true for antibody to the core structure, where both infected infants and their mothers have much higher levels of antibody to the core than the native antigens. Infected infants are also more likely to respond to infection by developing antibody to the native antigen. Immunization of 12 adults with multivalent pneumococcal polysaccharide induced significantly better antibody response to the core antigen than to the native, and this vaccine induced opsonic activity in only one recipient. Immunization of adults with type III group B streptococcal antigens induced antibody to the native determinant which correlated with opsonic activity. Therefore, it would appear that native group B streptococcal polysaccharides will provide the best candidate antigens for immunization.

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