Detection of Group B Streptococcal Antibodies in Human Sera by Radioimmunoassay: Concentrations of Type-Specific Antibodies in Sera of Adults and Infants Infected with Group B Streptococci

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Received for publication 10 November 1977

Current interest in determining the possible protective role of antibodies against group B streptococcal disease prompted this study of the feasibility of using a radioimmunoassay to measure type-specific immunity in humans. The radioimmunoassay was standardized as a quantitative test for antibodies against the carbohydrate (CHO) antigens of all five group B types. The data showed that the CHO antigens extracted by a cold trichloroacetic acid-sonification method measure more antibodies than do the corresponding CHO antigens extracted by hot hydrochloric acid; that the Ia CHOs extracted from two different types, Ia and Ic, measure the same quantity of Ia antibodies; and that human sera contain antibodies reactive with all five type-specific CHOs. No evidence of "protective" antibody was found in the serum samples studied, although there was evidence of an antibody response in adults to prolonged colonization by group B streptococci. The wide ranges of antibody concentration in a serum bank collection, the broad reactivity of all human sera tested, and the mixed populations of antibodies in human sera that react with different determinants on the same type-specific CHO antigen (type III) indicate that further studies must be done to better define normal and susceptible populations and to determine antigenic components important in protection.

The fulminant nature of group B streptococcal infections in neonates has led current researchers to explore the feasibility of using antibiotic prophylaxis or passive immunization as preventive measures (3, 8). The former method relies on identifying and treating infants at risk (or their colonized mothers, during pregnancy) because of the otherwise large numbers of expected adverse reactions to indiscriminate antibiotic prophylaxis (1). Problems with this approach, however, include the absence of identifiable risk factors until an overwhelming infection is already present (8, 11) and the incomplete eradication of group B streptococci in small-scale efficacy trials to date (9, 18). Passive immunization, on the other hand, depends on using maternal vaccines to immunize fetuses transplacentally (3) or on administering gamma globulin preparations to neonates at risk (19a). The efficacy of either method can be determined only by identifying protective antibodies in human sera.

Results of several studies suggest that immunity to group B streptococcal disease may be

type specific. Lancefield showed that homologous type-specific rabbit antisera protected mice against challenge by virulent strains of types Ia, Ib, Ic (14), and II (13) and also showed that antibodies against one or several antigenic determinants within the same type strain were multiply protective. More recently, Stewardson-Krieger et al. found that certain human sera or pooled human gamma globulin preparations protected mice against challenge by type Ia organisms but nevertheless concluded that the presence of such antibodies in maternal sera did not provide reliable evidence of protection of their neonates (19a). Several *in vitro* studies have suggested that opsonization of group B streptococci is type specific with rabbit antisera (2, 6, 10, 19; B. F. Anthony, N. F. Concepcion, D. Okada, and C. J. Hobel, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 14th, San Francisco, Calif., Abstr. No. 253, 1974) and with human sera (10, 19, 19a) and that the opsonic activity of the latter is sometimes enhanced by complement (10, 19a). Perhaps pertinent to the latter finding is the demonstration

of complement activation in the sera of infants with group B streptococcal septicemia (7a). Baker et al. have published data obtained from testing human sera in a radioimmunoassay (RIA) for type III antibody (5). They found higher type III antibody concentrations in convalescent than in acute sera of four adults with puerperal sepsis and two infants with bone and joint disease and a higher median III antibody level in 43 mothers of healthy infants than in 29 mothers of infants with type III disease. As they pointed out, however, there were wide ranges of antibody concentrations within each age group studied, and the antigen used in the RIA was not type specific.

Recently, we described an RIA with which type-specific antibodies in rabbit antisera can be measured (22). In it, carbohydrates (CHOs) extracted from all five types by sonification of cells suspended in cold 2.5% trichloroacetic acid (TCA) and purified by fractional ethanol precipitations (TCA CHOs) are used. The present study was done to: (i) standardize the RIA to measure group B antibodies against all five types quantitatively; (ii) explore the feasibility of substituting HCl for TCA CHOs because of the greater yield obtained by HCl extraction; (iii) determine whether it is necessary to use Ia CHOs extracted from both type Ia and type Ic strains to measure Ia antibodies; and (iv) investigate the potential use of RIA for predicting the presence or absence of immunity to group B infections in humans.

MATERIALS AND METHODS

Antigens. Extraction and purification of the five group B type CHO antigens by a TCA-sonification method was described previously (22). After extraction with TCA, the type III cell sediment was resuspended in 10 ml of 0.2 N HCl and placed in a boiling-water bath for 15 min to extract any residual III CHO (HCl CHO). The HCl CHO was then purified by methods already described (20). The group B CHO used as the heterologous antigen in co-precipitation tests (described below) was extracted from type Ib cells (strain H36B) with hot HCl and purified by cold TCA and ethanol precipitations (20). After removal of the type CHO with 1.5 volumes of ethanol, the B CHO was precipitated with an additional 2.5 volumes of ethanol (final concentration, 4 volumes), collected by centrifugation, resuspended in water, and lyophilized. This antigen preparation contained no detectable Ib CHO.

Sera. Type-specific rabbit antisera that contained negligible amounts of non-precipitating antibodies were kindly supplied by the Center for Disease Control (CDC) Biological Products Division or by Rebecca C. Lancefield. These sera were used to standardize the RIA. Types Ia, Ib, and II antisera contained antibodies reactive with both TCA and HCl CHO antigens of the

homologous type strain, whereas the type III antisera contained either TCA antibodies (used to standardize the RIA with the III TCA CHO) or HCl antibodies (used to standardize the RIA with the III HCl CHO), but not both.

Human sera were obtained from the CDC Serum Bank collection or were sent by private investigators directly to the CDC Streptococcus Laboratory and stored at -70°C until tested. Group B streptococcal strains sent with the latter sera were typed by established methods (21).

Quantitative precipitin tests. Each antiserum used as a standard in the RIA was tested by quantitative precipitation analysis as follows. Increasing concentrations of homologous type CHO (from 2.5 to 100 μg in 0.9 ml of saline) were added in Kahn tubes to 0.1 ml of type-specific antiserum. Contents were mixed thoroughly, and the tubes were initially incubated for 1 h at 35°C and then for 10 days at 4°C . Immune precipitates were collected by centrifugation, washed twice in 1 ml of saline, and dissolved in 0.15 ml of 0.1 N NaOH, and then appropriate dilutions of the solubilized precipitate were analyzed for antibody protein by the method of Lowry et al. (15). Control tubes that had been set up with antigen alone or antiserum alone contained no detectable protein.

To determine whether the standard sera contained enough co-precipitating anti-immunoglobulin G to give elevated antibody values in quantitative precipitin tests, each serum was tested by a method based on that of Bokisch et al. (7). That is, 0.1 ml of each type-specific antiserum or of normal rabbit serum (control) was allowed to react in the quantitative precipitin test with 25 μg of group B CHO and 0.1 ml of group B antiserum. A group B antiserum prepared with type III vaccine (strain D136C) was used in co-precipitation tests of the type Ia or Ib antisera. Similarly, a group B antiserum prepared with type Ia vaccine (strain 090R) was used in co-precipitation tests of type II or III antisera. Although probably not a necessary precaution, the two different group B antiserum preparations were used to avoid any type-specific antigen-antibody precipitation that might occur with group B antiserum containing type antibodies. The difference between the protein value obtained with normal rabbit serum and that obtained with type-specific antiserum represents the amount of co-precipitating antibody in the latter.

RIA. CHO antigens were labeled with ^{125}I and used in the RIA as described previously (22). The test was modified by diluting each test serum in fetal calf serum, which in turn had been diluted 1:10 in phosphate-buffered saline. The latter dilution was made to avoid nonspecific binding of antigens and undiluted fetal calf serum noted in prior studies. The test was quantitated by plotting the percentage of bound antigen values versus the logarithm of decreasing concentration of type-specific antibody.

CIE. Each human serum was tested for soluble group B and type CHO antigens by CIE (12). Veronal buffer, pH 8.6, 0.05 μ , was used in preparing agarose slides (1%) and in the electrophoresis chamber. Purified TCA antigens were used as controls. Electrophoresis was allowed to proceed for 30 min with a constant

current of 20 mA/glass slide (8.3 by 10.2 cm). Slides were examined immediately after electrophoresis, after 2 and 20 h at room temperature and again after 3 days at 4°C. All reactions, however, were visible immediately after slides were removed from the electrophoresis chamber.

RESULTS

Figure 1 shows representative standard curves for each of the six radiolabeled antigens used in the RIA: TCA CHO's from all five types and the HCl CHO from type III. The highest value obtained in co-precipitation tests for anti-immunoglobulin G in any of the standard sera was 0.2 mg/ml, a value that had no influence on subsequent calculations. The RIA was linear between 1 and 10 µg of antibody per ml when tests were done with 3 µg of CHO per ml and between 10 and 100 µg of antibody per ml when tests were

FIG. 1. RIA binding curves with ^{125}I -labeled type CHO antigens and homologous type-specific rabbit antisera. Antigens were extracted with hot HCl ($\text{III}_{(\text{h})}\text{CHO}$) or cold TCA sonification (all others) and used in a concentration of 30 µg/ml (III CHO) or 3 µg/ml (all others). Ia CHO's were extracted from two different strains, type Ia ($\text{Ia}_{(\text{a})}\text{CHO}$) and type Ic ($\text{Ia}_{(\text{c})}\text{CHO}$).

done with 30 µg of CHO per ml. Similar RIA slopes were obtained in preliminary studies of multiple dilutions of selected human sera, but to assure that subsequent results would fall within the linear range of the test, the mean antibody concentration obtained with at least two different dilutions of each test serum had to be within 1 µg/ml before it was considered valid. Reproducibility was determined by repeating tests of selected human sera after 1 week and after 10 months. Repeat test results agreed with those obtained in the original tests within 1 µg/ml. Tests performed 10 months apart were done with two different lots of radiolabeled antigens.

A total of 40 sera from individuals of various ages were tested in the RIA for antibody reactive with each of the six CHO antigens (Table 1). The mean antibody concentration for each type antigen was higher in sera from the adult group than in those from the infant group, as might be expected, with type III antibody present in the highest concentrations and type Ib antibody in the lowest. Although there were wide ranges of antibody concentrations for each type, those measured by the type III TCA CHO varied most with concentrations ranging from 4.1 to 242.1 µg of antibody per ml. The differences in mean values obtained with the Ia CHO's extracted from types Ia (6.7 µg/ml, overall mean) and Ic (5.7 µg/ml) were not significant at the 0.05 level. Antibody concentrations measured by the III HCl CHO were consistently lower than those measured by the III TCA CHO, but a value obtained with one could not be used to predict the value obtained with the other. Although values obtained with the III HCl CHO are included in the tables, further mention of type III antibodies in the test is based on tests with the III TCA CHO.

A total of 17 sera were from infants who (or from mothers of infants who) carried or had symptoms of group B type III disease (Table 2). There was no appreciable difference in the mean type III antibody concentration in the sera of infants carrying type III (11.0 µg/ml) and those

TABLE 1. Mean type-specific antibody concentrations in sera from individuals of different ages

Age (yr)	No. of sera tested	Mean type-specific antibody concn (µg/ml) of:					
		Ia(Ia) ^a	Ia(Ic) ^a	Ib	II	III	III (H) ^b
<1	17	3.5	3.6	1.0	9.0	22.1	4.0
1-11	5	3.3	1.5	0.6	6.8	87.3	4.9
12-17	3	4.4	2.2	0.9	7.2	24.9	16.3
>17	15	11.7	10.0	5.4	16.1	35.0	13.5
Total (Range)	40	6.7 (0.6-33.0)	5.7 (0.9-27.4)	2.6 (0.0-32.5)	11.3 (2.5-44.2)	36.2 (4.1-242.1)	8.6 (1.2-65.3)

^a Group B serotype strain from which Ia CHO was extracted: type Ia(a) or type Ic(c).

^b CHO antigen extracted with hot HCl rather than cold TCA.

TABLE 2. *Type-specific antibody concentration in sera from infants or mothers of infants with group B type III carriage or disease*

Serum no.	Infant diagnosis	Type-specific antibody concn (μg/ml) of:					
		Ia(Ia) ^a	Ia(Ic) ^a	Ib	II	III	III (H) ^b
Infant							
1	Meningitis (D) ^c	3.7	2.6	1.1	20.2	16.8	13.4
2	Meningitis (S) ^c	4.1	5.1	0.7	14.2	9.3	5.7
7	Septicemia (S)	3.0	2.2	1.2	3.5	ND ^d	6.7
9	Septicemia (S)	4.4	2.2	0.8	28.6	9.3	2.6
11	Meningitis (S)	0.6	1.0	0.4	4.5	7.5	6.2
{44} ^{e, f}	Septicemia, pneumonia (D)	2.8	1.9	1.4	4.0	7.4	2.2
{45}		1.5	3.0	0.6	2.7	8.0	2.3
51 ^f	Septicemia, pneumonia (S)	7.1	7.8	2.5	31.6	5.4	1.2
40	Colonized (cord, gastric)	3.1	3.3	1.2	6.6	16.4	3.9
{46} ^{e, f}	Colonized (ear)	2.1	2.9	0.7	3.8	8.6	2.3
{47}		1.7	1.7	0.0	3.1	8.7	2.2
50 ^f	Colonized (ear)	4.0	ND	ND	ND	8.0	2.3
Maternal							
3 ^h	Septicemia (D)	3.8	2.3	1.6	8.6	71.0	65.3
4	Meningitis (S)	4.7	2.5	0.4	15.8	35.1	26.9
10	See no. 11	3.2	1.7	2.0	8.6	10.4	2.6
41	See no. 40	3.8	5.5	1.7	9.5	14.0	4.0
52	See twins, Nos. 50 & 51	7.1	7.2	3.3	36.0	10.2	2.7

^a Group B serotype strain from which Ia CHO was extracted: type Ia(a) or type Ic(c).

^b CHO antigen extracted with hot HCl rather than cold TCA.

^c D, Patient died or S, patient survived.

^d ND, Not done.

^e First number indicates value obtained with cord serum, second number indicates value obtained with serum drawn several hours later.

^f Sets of twins: 44 (45) and 46 (47); 50 and 51.

^g Group B and type III soluble CHOs detected in serum by CIE from 45, only.

^h Mother had postpartum septicemia, stillborn infant.

with type III meningitis or septicemia (9.3 $\mu\text{g/ml}$). The mean type III antibody concentration in the sera of mothers of infants with type III disease was higher than in those whose infants were colonized with type III but not ill (31.7 $\mu\text{g/ml}$ and 12.1 $\mu\text{g/ml}$, respectively). Serum no. 3, with 71.0 μg of III antibody per ml, was from a woman who had postpartum fever after giving birth to a stillborn infant from whom type III was cultured at autopsy.

Sera from two sets of twins, who were recently described by Fenton and Strunk (7a), were available for analysis. The mother (serum no. 52) of the first set (serum no. 50 and 51) had type III isolated from her vagina and throat and a III antibody concentration of 10.2 $\mu\text{g/ml}$. The twin who survived type III sepsis and pneumonia (no. 51) had 5.4 μg of III antibody per ml of serum. The twin colonized with type III but not ill (no. 50) had 8.0 μg of III antibody per ml of serum. Of the second set of twins, represented by serum no. 44 through 47, one baby (no. 44 and 45) had a fatal type III septicemia and pneumonia with III antibody concentrations of 7.4 $\mu\text{g/ml}$ at birth and 8.0 $\mu\text{g/ml}$ at several hours of age. The second baby (no. 46 and 47) was colonized with type III

and had III antibody concentrations of 8.6 $\mu\text{g/ml}$ at birth and 8.7 $\mu\text{g/ml}$ at several hours of age. This baby had mild, transient, respiratory distress. Serum from the mother of these twins was not available. Soluble group B and type III CHOs were detected by CIE in the serum of the baby who died at 41 h of age (no. 45) but not in his cord serum (no. 44) and not in either serum from his colonized twin (no. 46 and 47).

Several sera were available from infants with type Ia or type Ic septicemia (Table 3). The infant who died of type Ia septicemia (no. 48 and 49; reference 7a) had a Ia antibody concentration at birth of 7.1 $\mu\text{g/ml}$ (10.9 $\mu\text{g/ml}$ when measured by Ia CHO from type Ic) and at several hours of age of 4.4 $\mu\text{g/ml}$ (5.1 $\mu\text{g/ml}$ by Ia CHO from type Ic). Soluble group B, type Ia, and type III CHOs were detected by CIE in the latter serum sample, whereas none was detected in the cord serum of the baby. Similar Ia antibody concentrations were found in the serum of another infant (no. 6) who survived a type Ia septicemia.

The preceding data obtained with infant sera show no relationship between group B type-specific antibody serum levels and the presence or absence of group B streptococcal disease.

Whether differences in antibody concentrations occur in adult sera was investigated in the next RIAs (Table 4). Five adults colonized with type III streptococci had a mean III antibody concentration of 34.5 $\mu\text{g/ml}$, a value lower than the mean value of 42.6 $\mu\text{g/ml}$ obtained with sera from individuals who were culture negative for group B type III at the time blood was collected (rectal and pharyngeal cultures; serum no. 27 and 39, Table 4, and no. 22, 23, and 26, Table 5) and lower than the overall mean value of 36.2 $\mu\text{g/ml}$ shown in Table 1. However, the wide range of 8.3 to 69.0 $\mu\text{g/ml}$ suggests that there may be individual variations in response.

Sera from two individuals colonized with types other than III had antibody concentrations against the colonizing type that were higher than the corresponding overall mean values. Serum no. 42 from a patient colonized vaginally with type II/Ic had 43.2 μg of II antibody per ml, a value considerably higher than the mean II antibody concentration of 9.0 $\mu\text{g/ml}$ in the sera of individuals culture negative for types II or II/Ic (no. 22, 23, 26, 27, and 39) or the overall mean II

antibody value of 11.3 $\mu\text{g/ml}$ (Table 1). Similarly, serum no. 27, 38, and 39 were from an individual who had group B type Ib isolated from his throat three times in three years. During this time, his Ib antibody concentrations ranged from 7.2 to 32.5 $\mu\text{g/ml}$, high values in comparison to the overall mean Ib value of 2.6 $\mu\text{g/ml}$ (Table 1, including no. 27, 38, and 39) or 1.2 $\mu\text{g/ml}$ (excluding no. 27, 38, and 39). The Ia antibody concentrations in these sera (27.9 to 29.3 $\mu\text{g/ml}$, 25.0 to 27.4 $\mu\text{g/ml}$) were higher than the overall mean Ia value of 6.7 (5.7) $\mu\text{g/ml}$. However, the Ib antibody level varied with time, whereas the Ia antibody level was relatively constant throughout the study period.

Although the preceding observations suggest that a relationship between type-specific antibody concentration and susceptibility to group B streptococcal infection is lacking in infants and that some adults may produce type-specific antibodies in response to group B carriage or infection, these data must be interpreted in light of the wide range of antibody levels found among serum bank sera (Table 5). Type III antibody

TABLE 3. Type-specific antibody concentration in sera from infants with group B type Ia or Ic septicemia

Serum no.	Infecting type	Diagnosis	Type-specific antibody concn ($\mu\text{g/ml}$) of:					
			Ia(Ia) ^a	Ia(Ic) ^a	Ib	II	III	III (H) ^b
6	Ic	Septicemia (S) ^c	4.7	6.0	0.8	2.7	4.4	2.9
48 ^d	Ia	Septicemia (D) ^c	7.1	10.9	2.1	5.0	12.2	4.9
49 ^d			4.4	5.1	1.0	2.5	9.6	2.8

^a Group B serotype strain from which Ia CHO was extracted: type Ia(a) or type Ic(c).

^b CHO antigen extracted with hot HCl rather than cold TCA.

^c Patient survived (S) or died (D).

^d Cord serum (no. 48) and serum drawn at several hours of age (no. 49) from same patient. Group B, Ia, and III soluble CHOs detected in serum no. 49 by CIE.

TABLE 4. Type-specific antibody concentration in sera from adults colonized with group B streptococci

Serum no.	Type isolated	Culture source	Sex	Type-specific antibody concn ($\mu\text{g/ml}$) of:					
				Ia(Ia) ^a	Ia(Ic) ^a	Ib	II	III	III (H) ^b
4	III	Cervix	F	4.7	2.5	0.4	15.8	35.1	26.9
5	III	Urethra	M	4.3	2.1	0.5	14.9	49.8	26.4
24	III	Rectum	M	4.4	1.7	1.1	8.0	8.3	4.3
25	III	Rectum	F	10.4	12.6	3.9	44.2	69.0	13.9
52	III	Vagina, throat	F	7.1	7.2	3.3	36.0	10.2	2.7
42 ^c	II/Ic ^d	Vagina	F	4.6	7.9	2.4	43.2	28.7	14.0
38 ^e	Ib	Throat	M	27.9	25.5	7.2	7.2	16.1	3.1
27 ^e				29.3	25.0	32.5	7.6	46.5	2.9
39 ^e				28.1	27.4	18.5	7.0	14.0	4.6

^a Group B serotype strain from which Ia CHO was extracted: type Ia(a) or type Ic(c).

^b CHO antigen extracted with hot HCl rather than cold TCA.

^c Patient had vaginitis.

^d Type II/Ic contains II CHO and Ibc protein antigens.

^e Same individual: serum no. 38 drawn March 1973, culture obtained December 1972; serum no. 27 drawn and culture obtained August 1976; serum no. 39 drawn and culture obtained November 1976.

TABLE 5. *Type-specific antibody concentration in sera from CDC serum bank*

Serum no.	Age (yr)	Sex	Type-specific antibody concn ($\mu\text{g/ml}$) of:					
			Ia(Ia) ^a	Ia(Ic) ^a	Ib	II	III	III (H) ^b
28	Infant	NS ^c	2.6	0.9	0.7	3.5	93.0	2.1
29	Infant	NS ^c	2.9	0.9	1.1	7.0	151.8	4.5
30	1	M	2.4	1.0	0.3	2.6	4.1	1.4
31	2	F	2.6	1.7	0.2	16.6	52.7	2.3
32	7	F	2.8	1.2	0.2	3.0	129.0	4.3
33	8	M	4.2	1.6	1.6	5.0	242.1	11.4
34	11	F	4.6	2.2	0.6	6.7	8.4	4.9
35	12	M	3.8	1.6	0.8	6.6	33.6	30.7
36	15	M	3.7	1.4	0.4	8.5	20.1	9.0
37	17	F	5.7	3.5	1.5	6.4	21.0	9.1
23 ^d	23	M	33.0	25.0	4.1	15.2	93.8	19.8
22 ^d	35	F	6.0	2.1	1.4	7.5	12.3	3.5
26 ^d	38	F	4.8	2.1	0.8	7.7	46.3	7.8

^a Group B serotype strain from which Ia CHO was extracted: type Ia(a) or type Ic(c).

^b CHO antigen extracted with hot HCl rather than cold TCA.

^c NS, Sex of infant not specified.

^d Culture negative for group B streptococci at the time blood was drawn.

concentrations, for example, ranged from 4.1 to 242.1 $\mu\text{g/ml}$. Furthermore, sera from individuals who were culture-negative for group B streptococci at the time blood was drawn (no. 22, 23, 26; rectal and pharyngeal cultures) ranged widely in antibody concentrations against CHOs of all types.

DISCUSSION

The group B streptococcal RIA, which measures anti-CHO type-specific antibodies (22), was standardized in this study as a quantitative test for assaying human sera. Because the latter often contain non-precipitating antibodies (16), as standards they could cause subsequent test results to be deceptively low. Hyperimmune rabbit sera, on the other hand, often contain co-precipitating anti-immunoglobulin G antibodies (7) that could falsely elevate values in tests on unknown sera. Therefore, type-specific rabbit antisera with negligible non-precipitating or co-precipitating antibodies were used as standards in the RIA in this study. Plots of logarithm antibody concentration versus purified ¹²⁵I-labeled CHOs showed that a linear relationship existed for each type antibody test. Replicate antibody determinations on human sera, tested within the demonstrably linear range, agreed within 1 μg of antibody per ml, even in tests that were performed 10 months after the initial studies and in which different lots of radiolabeled antigens were used.

A total of 40 human sera were tested with five labeled TCA CHOs in the RIA and with a labeled III CHO extracted with hot HCl (Lancefield extract). The latter was used to explore the possibility of substituting HCl for TCA CHOs in

the RIA because of the proportionately greater yield of CHO obtained with HCl (unpublished data). Because HCl CHOs appear to be degraded forms of TCA antigens, it seemed likely that the TCA CHOs would measure more antibodies in the RIA than would HCl CHOs. Immune rabbit sera often contain mixed populations of antibodies against both the HCl and TCA determinants (13, 20; III TCA CHO not studied, although a buffer-extracted CHO appears to have similar characteristics [4]). This was indeed found to be the case in testing human serum samples. Antibody concentrations measured by the III TCA CHO were always greater than those measured by the III HCl CHO. Inconsistent ratios of TCA- to HCl-measured antibodies occurred, but such ratios apparently were not useful in predicting immunity to type III disease. HCl CHOs, therefore, will not be used in future studies.

The detection of antibodies reactive with all group B CHO-type antigens in all human sera tested suggests that humans have a broad immunological experience either with group B streptococci or with antigens cross-reactive with the group B type CHOs. Similarly, Riesen et al. (17) found group A streptococcal CHO antibodies in concentrations as high as 131 $\mu\text{g/ml}$ in "normal" human sera. It would be tempting to speculate that group B type-specific antibodies in maternal sera are responsible for the high colonization to disease ratio (100 to 1) found so far in epidemiological studies of group B infant disease (1); however, no evidence was found in this study for type-specific protective antibody in infants. To the contrary, infants with group B type III meningitis or septicemia had approximately the same mean antibody concentrations

as did those colonized with type III but not ill. There were no appreciable differences in antibody concentration in the sera of babies with group B disease and in that of their colonized twins (several sets studied). Of special interest was the fact that soluble group B and type-specific CHOs were detected by CIE in two of the sera. The possibility of deceptively low antibody values being caused by antigenemia should be considered in future studies.

In contrast to the data obtained in tests of infant sera, evidence suggested that adults produce type-specific antibodies in response to group B streptococcal colonization or infection. Serum samples from an individual with type Ib pharyngeal colonization had Ib antibody levels ranging from 3 to 12 times that of the overall mean antibody level of 36 other sera. Another individual colonized vaginally with type II/Ic had a type II antibody concentration five times the overall mean. A third serum sample from an individual from whom cultures were unavailable but who had postpartum fever after giving birth to a stillborn infant infected with type III had a type III antibody concentration twice that of the overall mean. The wide ranges found among serum bank sera, however, illustrate the inadequacies of our present understanding of normal and "susceptible" populations.

The data reported in this paper suggest the need for several precautions in future studies. Tests should be done with at least four CHOs (TCA CHOs Ia, Ib, II, and III) to give more information regarding the broad immunological reactivity of human sera. Further attempts should be made to identify antigenic components that might be important in protection. There is a need for quantitative protection assays done concomitantly with quantitative antibody determinations. Both normal and susceptible populations should be better defined, a process that will probably be possible only in prospective studies of larger samples accompanied by complete culture information. Adequate culture techniques have rarely been used in past studies. Only after such information is available will it be possible to use antibody tests as clinical tools to aid in predicting susceptibility to group B streptococcal disease.

ACKNOWLEDGMENTS

I thank the following individuals who contributed some of the serum samples analyzed in this study: Robert C. Aber, Lawrence J. Fenton, Samuel P. Gotoff, and Charles F. Peters. I am also grateful to Barry Gray for very helpful discussions and to William Fulcher for excellent technical assistance in the early phases of standardization.

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