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Group B Streptococcal Ibc Protein Antigen: Distribution of Two Determinants in Wild-Type Strains of Common Serotypes

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Studies were carried out on the distribution of the Ibc protein antigenic marker in wild-type strains of group B streptococci of diverse serotypes isolated from epidemiological studies. Rabbits were immunized with group B streptococcal strain H36B, a prototype Ib strain, to produce antibody to the Ibc protein antigens. One antiserum (no. 970) contained antibody only against the trypsin-sensitive (TS) portion of the Ibc antigen. A second antiserum (no. 973), however, contained antibody to both the TS and the trypsin-resistant (TR) determinants or components of the antigen. A total of 785 wild-type strains of group B streptococci were serotyped by using antiserum 973 as well as antisera to the polysaccharide types Ia, Ib, II, III, and IV. Remarkably, 59% of all the strains tested (462 of 785) reacted positively with the Ibc antiserum, although not all carried both components of the Ibc antigen. Of the 99 Ib strains examined, 84% carried both TS and TR components. In contrast, 96% of the 202 Ic strains carried only the TR component of the Ibc antigen. Antiserum 970 failed to identify these strains. Routine typing with an antiserum which contains antibodies to only one portion of the Ibc antigen could result in significant serotype misidentification. Differentiation of group B streptococcal strains by the presence or absence of individual TS or TR components of the Ibc antigen could prove to be a useful additional epidemiological and serological marker. It is noteworthy that wild-type Ic strains carry, ordinarily, only the TR component, in contrast to the prototype Ic strain, which possesses the complete Ibc protein antigen. The possible contribution of the Ibc antigen to group B streptococcal virulence is of interest and requires further study.

Increased awareness of group B streptococci as a primary cause of serious human neonatal infections has emphasized the need to understand better the antigenic composition of these organisms and the relationship of strain differences to the infectious process. Crucial to this understanding was the development of a typing system for group B streptococci. Lancefield (14, 15) established the basic classification system, which consists of types Ia, Ib, II, and III based on differences in capsular type-specific polysaccharide antigen. Wilkinson and Moody (24) later added the type now known as Ic. Numerous epidemiological studies of population surveys have been conducted in an effort to establish whether specific serological types of group B streptococci have an increased association with disease in humans. Published information from various laboratories (1, 2, 4, 8, 10, 20, 21, 23) about distribution of serotypes reveals interesting differences as well as similarities. Some of these differences may be due simply to geographical separation or to sampling populations at different times since shifts in the prevalence of serotypes may occur over prolonged periods of time (10, 21).

The Ibc protein antigen was first described by Wilkinson and Eagon (22) in 1971 and was shown to consist of two active determinants or components. Lancefield et al. later reported (16) that this antigen was associated with virulence of group B streptococci in mice and that antibody to the Ibc protein provided partial protection in mice injected intraperitoneally with group B streptococci possessing this antigen. The association of Ibc antigen with virulence in humans has not been clearly defined, but it has been reported that serotypes with this antigen are found in association with adult meningitis (21). In addition, strains of serotypes Ib and Ic, both possessing the Ibc protein antigen but different type polysaccharide antigens, constitute approximately one-third of the types prevalent in colonized mothers, colonized asymptomatic newborn infants, and newborn infants with early onset of bacteremia or sepsis (1–4, 8). The studies presented here make use of antisera specific for the Ibc antigen and its individual components. Application of these antisera to the serotype identification of group B streptococci from a variety of clinical sources revealed that this antigen in its complete or incomplete form may be more prevalent than has previously been considered.

MATERIALS AND METHODS

Bacterial strains. Group B streptococcal type reference strains used as controls in serological typing were obtained from the late R. C. Lancefield of The Rockefeller University, New York, N.Y. They were type Ia (Lancefield collection 090, University of Minnesota [U. of M.] 71-732), type Ib (Lancefield collection H36B, U. of M. 71-733), type Ic (Lancefield collection A909, U. of M. 71-736), type II (Lancefield collection 18RS21/19/2, U. of M. 71-734) and type III (Lancefield collection D136C, U. of M. 71-735). The prototype Ic strain A909 of Lancefield was also used in these studies as the source of reference Ibc antigen. The type IV reference strain 3139 (U. of M. 81-305) was obtained from S. Bergner-Rabinowitz, National Streptococcal Reference Center, Jerusalem, Israel. The wild-type strains of group B streptococci described were primarily collected during epidemiological studies of maternal-infant colonization with group B streptococci at the University of Minnesota, Minneapolis, Minn.

Antisera. Group B antiserum was prepared in our laboratory by immunization of rabbits (14) using Lancefield strain 090R, which possesses group B antigen but little or no type-

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specific antigen. Antisera for routine identification of types Ia, Ib, II, and III were obtained from the Centers for Disease Control, Atlanta, Ga., or were prepared in our laboratory. Antiserum to newly described type IV (11, 17) was supplied by S. Bergner-Rabinowitz and by J. Jelinkova of the Institute of Hygiene, Prague, Czechoslovakia. Antiserum to the Ibc protein antigen was prepared in our laboratory with formalinized vaccines prepared with either the Ib or Ic strain of Lancefield by standard immunization techniques (14). A reference Ibc antiserum was also supplied to us by Lancefield.

Grouping and typing methods. Antigens were extracted by the hot HCl method (19). Grouping of streptococci was done by using the classic capillary precipitin reaction (19). Typing was performed by the Ouchterlony double immunodiffusion method (18). Slides were prepared with 0.8% agarose in 0.01 M phosphate buffer with 1% polyethylene glycol 6000 added to enhance the sensitivity of the system (9) and were stained with Coomassie blue R-250 (Colab Laboratories, Inc., Glenwood, Ill.). All extracts not reacting in the double diffusion system were rechecked by the capillary precipitin method (19) with a final reading being done after overnight incubation at room temperature (22). However, no results were considered final that could not be verified by the double diffusion method. Extracts that reacted only in capillary tubes, or that did not react with any of the type-specific antisera, or reacted only with Ibc antiserum, or gave equivocal reactions with any of these antisera were retested in the standard double diffusion system after 10× concentration with polyethylene glycol, compound 20-M (Carbowax, Union Carbide Corp., New York, N.Y.).

Proteolytic digestion of acid-extracted antigens. Sensitivity of the Ibc antigen to the proteolytic activity of trypsin (ICN Nutritional Biochemicals, Cleveland, Ohio) and pepsin (Sigma Chemical Co., St. Louis, Mo.) was tested as described by Wilkinson and Eagon (22).

RESULTS

Evaluation of rabbit antisera to Ibc protein antigen. Antiserum to the Ibc protein antigen was produced by immunizing three rabbits with Ib strain H36B. Two of the rabbits, numbers 970 and 973, developed satisfactory titers of Ibc antibody as measured by reactions of these sera with acid extracts of Ic strain A909 in capillary precipitin tests. The minor cross-reacting antibodies present were easily removed by standard absorption techniques. Antibodies to the Ib polysaccharide were present only at very low titers and did not interfere with the Ibc reaction, for which the final interpretation was based solely on the immunodiffusion test.

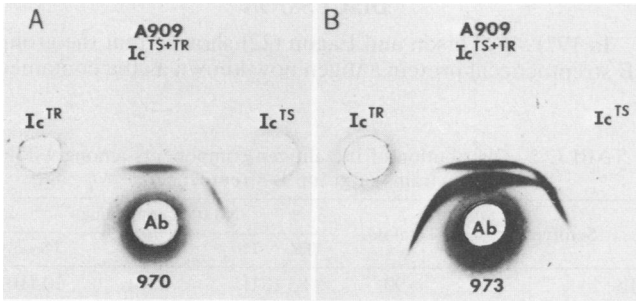

FIG. 1. Identification of Ibc antigen components in two wild-type Ic strains and prototype Ic strain A909 by the Ouchterlony double diffusion method. (A) Center well contained antiserum 970; peripheral wells contained acid extracts of wild-type Ic strains containing TR or TS components (to the left and right of prototype Ic extract A909). (B) Center well contained antiserum 973; peripheral wells were the same as in (A).

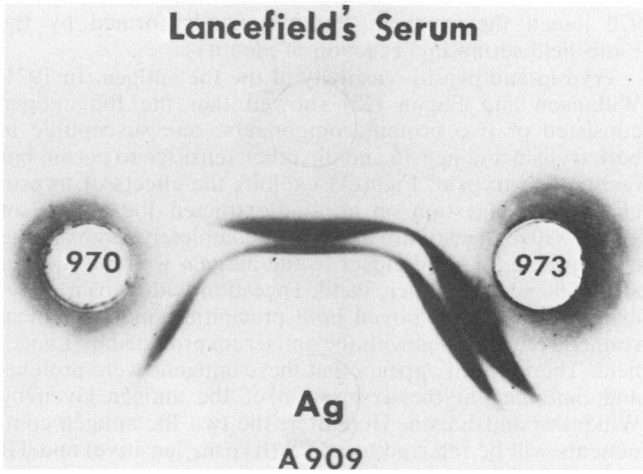

FIG. 2. Comparison of antisera 970 and 973 with the Lancefield anti-Ibc serum in reaction with an acid extract of Ic strain A909 (center well).

When absorbed antiserum 970 was put into trial use for identification of the Ibc antigen in routine typing of group B streptococci, the first strains to be tested were those strains previously identified in our laboratory as type Ic. A large proportion of these strains, however, showed no reaction with serum 970 by the capillary precipitin method or by double immunodiffusion in agar. When the typing was repeated with antiserum 973 instead of 970, all of the previously identified Ic strains gave good precipitin reactions. These findings led to the subsequent experiments described below.

Figure 1A shows the reactions obtained in an immunodiffusion-in-agar system between serum 970 and acid extracts of the reference Ic strain A909 and of two strains identified previously as type Ic. A reaction of identity took place between the A909 extract and the extract of the wild-type Ic strain on the right. No reaction took place with the extract on the left. Figure 1B reveals an immunodiffusion reaction of serum 973 with extract A909 and acid extracts of the two wild-type Ic strains shown in Fig. 1A. The extract of strain Ic on the right formed an outer precipitin band which joined the A909 band in a reaction of identity, a finding analogous to that seen with serum 970. However, serum 973 also formed a precipitin band with the extract of another Ic strain on the left. This band joined a second separate and distinct A909 precipitin band in a reaction of identity. Therefore, antiserum 973 contains antibodies with two distinct specificities. Reference Ic strain A909 has two antigens or antigenic components which react with this serum, whereas the wild-type strains tested varied in their antigenic composition (see below).

To evaluate these antisera and strains further, antiserum specific for the Ibc antigen, prepared by Rebecca Lancefield in her laboratory, was used. Figure 2 compares the reaction of this antiserum and antisera 970 and 973 with an acid extract of prototype strain A909. Two distinct precipitin bands were formed by both the Lancefield serum and serum 973, and these were reactions of identity, whereas serum 970 formed only one band. The precipitin band seen with serum

970 joined the inner of the two bands formed by the Lancefield serum in a reaction of identity.

Trypsin and pepsin sensitivity of the Ibc antigen. In 1971, Wilkinson and Eagon (22) showed that the Ibc antigen consisted of two protein components, one susceptible to both trypsin and pepsin and the other sensitive to pepsin but resistant to trypsin. Figure 3 exhibits the effects of trypsin and pepsin digestion on an acid-extracted Ibc antigen of strain A909. Digestion with trypsin completely removed the outer precipitin band closer to the antigen well but did not affect the second, inner, band. Digestion with pepsin, however, completely removed both precipitin bands. Identical results were obtained with the antiserum provided by Lancefield. Therefore, it appears that these antigens were proteins and that they fit the description of Ibc antigen given by Wilkinson and Eagon. Hereafter, the two Ibc antigen components will be referred to as TS (trypsin sensitive) and TR (trypsin resistant).

Detection of Ibc antigen among wild-type strains of group B streptococci. Antiserum 973, which contained antibody to both the TS and TR components of the Ibc antigen, was included in the typing of 785 wild-type strains of group B streptococci collected from 1978 to 1980. Table 1 shows the distribution of serotypes among these strains. The predominant serotypes in this population were Ic, II, and III. Serotypes II and III possessed their type-specific polysaccharide antigen, either alone or in combination with the Ibc protein antigen. These three types were present in nearly equal proportion and together accounted for nearly 80% of all strains examined. Type Ib accounted for about 13% and type Ia for only 2% of the strains examined. Approximately 2% of these 785 strains reacted only with serum 973 and are thus classified as Ibc protein only. A small number of strains of the provisional type IV possessed the Ibc protein antigen.

Concentration of cell extracts (see above) with polyethylene glycol followed by retesting by immunodiffusion permitted assignment of a type designation for 25 strains, 15 of which gave a weak precipitin reaction before concentration and 10 of which had been negative. Fifteen of these strains were finally classified as type Ic, four as type Ia, two as type II, and four as type III.

As was shown in Fig. 1B, not all strains of group B streptococci carrying the Ibc protein antigen possessed both the TS and TR components. Table 2 shows the distribution

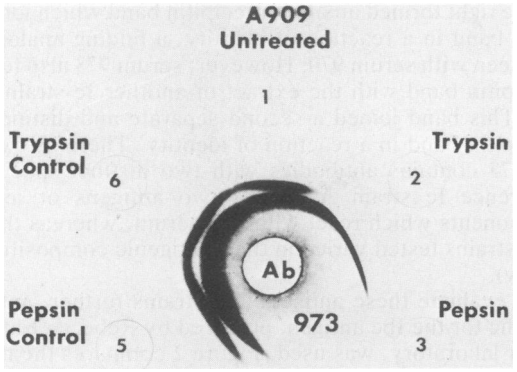

FIG. 3. Effect of trypsin and pepsin digestion on an HCl extract of prototype Ic strain A909. Well 1, Untreated extract of Ic strain A909; well 2, trypsin-treated A909 extract; well 3, pepsin-treated A909 extract; wells 5 and 6, A909 extracts exposed to the same conditions as extracts in wells 2 and 3, but without enzyme added.

TABLE 1. Distribution of serotypes among 785 wild-type strains of group B streptococci collected from 1978 to 1980

Serotype	No.	%
Ia	16	2.0
Ib	99	12.6
Ic	202	25.7
II	90	11.5
II-Ibc[a]	130	16.6
III	199	25.4
III-Ibc[a]	3	0.4
IV-Ibc[a]	10	1.3
Ibc protein only	18	2.3
Nontypable	18	2.3

[a] React with antiserum to Ibc protein also.

of the TS and TR components of Ibc antigen among the 462 strains which carried the Ibc antigen. These 462 strains of various serotypes, carrying the Ibc marker antigen, represented 59% of the 785 strains during this period. Of the type Ib strains examined, 84% were found to carry both TS and TR components. In sharp contrast, the remaining serotypes were composed primarily of strains carrying only the TR portion of the Ibc antigen. Ninety-six percent of type Ic and 81% of type II-Ibc strains carried only the TR component. Although the numbers were small for III-Ibc, IV-Ibc, and Ibc protein only strains, it appeared that strains carrying only the TR component were predominant. Strains carrying only the TS component of the antigen (in addition to type polysaccharide antigen) were found but were relatively uncommon, accounting for 14 of 462 or 2.8% of all strains with Ibc antigen.

Comparison of Ibc protein antigens among group B streptococcal serotypes. Figure 4 compares the serological reactivity of the Ibc antigen components from HCl extracts of wild-type strains representing some of the more commonly encountered types of group B streptococci. The TS components from wild-type strains of types Ib and Ic formed a reaction of identity (outer precipitin band) with each other and with the TS band of Ic strain A909. Similarly, the precipitin bands reflecting TR antigen reactivity from these strains, from an additional Ic strain, and from a II-Ibc strain all joined in reactions of identity (inner band). Numerous additional HCl extracts of strains representing all serotypes possessing the Ibc antigen were tested in this manner, and in all cases the resulting precipitin bands expressed reactions of identity.

DISCUSSION

In 1971, Wilkinson and Eagon (22) showed that the group B streptococcal protein antigen now known as Ibc contained

TABLE 2. Distribution of Ibc antigen components among wild-type strains of group B streptococci[a]

Serotype	Total no.	No. (%) containing:		
		TR + TS	TR only	TS only
Ib	99	83 (84)	6 (6)	10 (10)
Ic	202	9 (4)	193 (96)	0
II-Ibc	130	21 (16)	105 (81)	4 (3)
III-Ibc	3	0	3 (100)	0
IV-Ibc	10	2 (20)	8 (80)	0
Ibc protein only	18	5 (28)	13 (72)	0

[a] Components were extracted by the hot HCl method of Lancefield.

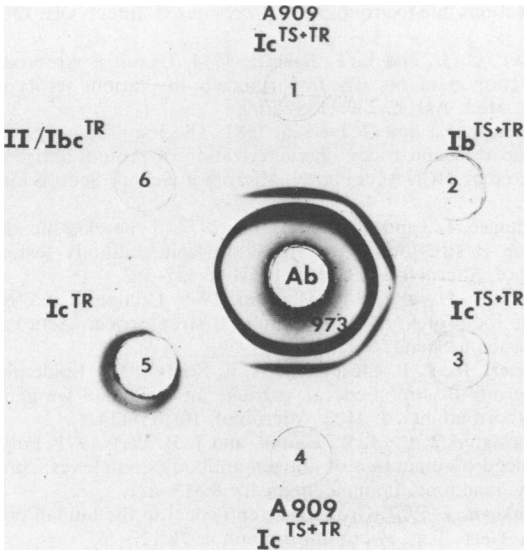

FIG. 4. Comparison of Ibc antigen components among wild-type strains of common serotypes. Wells 1 and 4, Extract of Ic strain A909; well 2, type Ib extract with TS and TR components; well 3, type Ic extract with TS and TR components; well 5, type Ic extract with TR component only; well 6, type II extract with TR component only.

two determinants or components: one sensitive to trypsin and pepsin and the other sensitive to pepsin but resistant to trypsin. Jensen (12) and Bevanger and Maeland (6, 7) have subsequently referred to the presence of these two Ibc antigen components and the development of corresponding antibodies in antisera prepared for identification of group B streptococci. Immunization of rabbits in our laboratory with either prototype Ib strain H36B or Ic strain A909 also resulted in production of antibodies to both the TS and TR components of Ibc. This was verified both in tests with extracts digested with the appropriate proteolytic enzymes and by comparison of these antisera with one produced by Lancefield and verified by her as having antibodies to both components of Ibc antigen.

A potential problem of importance to laboratories involved in the production of antisera for serotype identification of group B streptococci is the possibility that not all animals immunized with a vaccine strain possessing complete Ibc antigen will necessarily produce antibodies to both components of the antigen. Rabbit number 970, immunized with the same vaccine on the same schedule as rabbit number 973, never produced satisfactory antibody to the TR portion of the Ibc antigen, although the reaction to the TS component was adequate. Use of such an antiserum for routine identification of Ibc antigen would result in misidentification of a majority of these strains since 328 of 462 (71%) wild-type strains examined carried only the TR portion of the antigen. Since 96% of the wild-type Ic strains possessed only the TR component, these strains would not be identified by a serum like 970, and the strains would be misidentified as type Ia. Further, since the prototype strain A909 possesses both TR and TS antigens, it would give a positive capillary precipitin reaction with such a serum, indicating that the positive control was identified correctly and that the serum was valid for use. We have authenticated this potential serotyping problem in a number of strains sent to our laboratory from another institution (unpublished data). An unusual increase in Ia strains in a laboratory where such strains were previously not prevalent should prompt very careful study of the strains to avoid incorrect identification of true Ic strains. Immunodiffusion in agar has the advantage of identifying both the TS and TR precipitin reactions. It is not greatly appreciated that wild-type Ic strains uncommonly possess both components of the Ibc protein antigen.

Another potential source of error lies in use of the capillary precipitin test for the identification of Ibc protein antigen. This reaction, even with potent antisera and a complete antigen, occurs somewhat more slowly and is generally weaker than those obtained with the polysaccharide type antigens. When the Ibc antigen was incomplete, as was found in 74% (342 of 462) of the wild-type strains carrying Ibc protein, the capillary precipitin reaction occurred even more slowly and with less total precipitate. This could result in a true Ibc reaction being incorrectly interpreted as negative.

The distribution of serotypes among the wild-type strains of group B streptococci described here differed in some respects from those reported from other geographical areas and earlier time periods (2). However, they are consonant with data from earlier epidemiological studies at the University of Minnesota (1, 8) and with other data in the American literature (2, 4). Isolates from clinical material received at the Centers for Disease Control from 1967 to 1972 contained a relatively high percentage of Ia strains (20%), whereas Ic strains accounted for only 7% (23). During the succeeding period, 1972 to 1975, the percentage of Ia strains dropped to 8%, but the Ic strains increased to a slight plurality at 11% (21). Results published from the World Health Organization reference lab in Prague (10) reveal that from 1967 to 1971 the Ia and Ic serotypes accounted for only 5 and 3%, respectively, of their group B isolates. From 1972 to 1975, however, these percentages were 6% for Ia and 21% for Ic. These percentages are similar to the 2 and 26% reported here for Ia and Ic, respectively. Differences in submission patterns, sources of isolation of strains, and reference laboratory versus research epidemiological typing laboratory can influence these results. For type II strains identified in Prague (10), there was virtually no change from the period 1967 to 1971 to the period 1972 to 1975 in the percentage carrying Ibc antigen (24 and 23%, respectively). Among strains identified at the Centers for Disease Control during approximately the same time period, however, the percentage of type II strains with Ibc antigen increased from 12 to 39% (21). A majority (59%) of the Minnesota type II strains from 1978 to 1980 carried the Ibc antigen marker. The general increases in the prevalence of Ic strains and the dramatic increase observed at the Centers for Disease Control in type II strains carrying Ibc antigen, combined with the high frequency of these two types in the Minnesota strains, suggest that the prevalence of the Ibc antigen among strains of group B streptococci is increasing. It was remarkable that 59% of the 785 strains of diverse serotypes tested in the present study possessed one or both components of the Ibc protein.

The distribution of individual components of the Ibc antigen varies dramatically from one serotype to another. Whereas 84% of type Ib strains had both TS and TR components of Ibc antigen, 89% of the remaining strains with Ibc antigen carried only the TR component. Although the usefulness of the individual Ibc components as epidemiological and biological markers would be enhanced if their presence (one or the other or both) within a serotype were more balanced, the potential application of this concept

needs further consideration. It will be interesting to see whether the composition of strains from other geographical locations and from various specific sources of infection and body sites of isolation differs from that observed in this population.

Questions concerning the stability of the Ibc antigen were raised by Jensen (13), who studied strains of group B streptococci isolated from cases of bovine mastitis in Denmark. He found that on reculturing 34 quarters known to be infected with strains carrying Ibc antigen, 10 quarters showed a change in type. Since attempts to reproduce this variation in vitro were unsuccessful, he concluded that it was an in vivo phenomenon possibly induced by type-specific antibodies. The Minnesota strains, all from human sources, seemed by contrast to be very stable. Multiple sequential vaginal and cervical cultures taken throughout pregnancy and during labor, as well as cultures from newborns at birth and upon hospital discharge, detected only one instance of change in type involving the Ibc antigen. In this case, the mother was colonized with a type Ib strain which had both the TS and TR portions of the Ibc antigen, whereas the baby carried a type Ic strain with only the TR portion of the Ibc antigen. Since this involved a change in both the protein and polysaccharide antigens, it was probably not a result of antigenic variation. More likely, there was initial colonization in the mother with more than one type, or else the strain in the baby was nosocomially acquired.

Bevanger and Maeland (7) have questioned whether strains carrying the Ia polysaccharide antigen plus an incomplete Ibc antigen should be classified as serotype Ic. The finding presented here that a large majority of strains with Ibc antigen possessed only a portion of the antigen suggests that a large proportion of group B streptococci would be affected by a change in the established system of classification. Also, further studies may reveal that the two antigenically reactive components of Ibc protein discussed here represent only a portion of a very complex antigen (5, 10, 22). An important consideration will be the role played by either or both of the individual components of Ibc protein antigen in the virulence of the organism. Although all antigens are of potential value as markers in the identification of group B streptococci, those affecting the virulence of the organism will be of special interest in devising immunological strategies for the prevention and treatment of infection and disease.

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