Immunochemistry of Purified Polysaccharide Type Antigens of Group B Streptococcal Types Ia, Ib, and Ic

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The HCl-extracted purified polysaccharide type antigens of group B Streptococcus types Ia and Ib were composed of galactose and N-acetylglucosamine in a molar ratio of 3:1 for the Ia antigen and 2:1 for the Ib antigen. Immunochemical data were the same for the Ia antigens of type Ia, purified in this study, and type Ic, purified earlier. Glucosamine inhibited the Ib quantitative precipitin reactions more effectively than did N-acetylglucosamine, whereas the reverse was true of the Ia reactions. Ouchterlony studies were consistent with these observations and also revealed two type-specific precipitin bands with the HCl-extracted Ia antigens. All saline-extracted type antigens, however, formed single Ouchterlony bands that were only partially identical to the corresponding HCl antigens. Purification of the saline antigens was accomplished by treatment with cold trichloroacetic acid and by fractional ethanol precipitation. Immunelectrophoresis experiments showed that the saline antigens were more negatively charged than the HCl antigens. The presence of sialic acid in the saline antigens probably accounted for their net negative charge and the fact that they were partially degraded by mild acid hydrolysis. The same serological specificities were observed with saline- and with HCl-extracted antigens.

Approximately 99% of the group B streptococci isolated from human clinical material may be divided into types by allowing Lancefield HCl extracts of the organisms to react in a precipitin test with specific antisera (5, 6, 18, 19). The same antisera afford passive protection in mice against challenge by the homologous mouse virulent strain (5, 6; R. C. Lancefield, personal communication). Protection presumably depends on opsonic antibodies specific for surface polysaccharide and protein antigens that occur in characteristic combinations in the five types: (i) type Ia, Ia carbohydrate (CHO) antigen; (ii) type Ib, Ib CHO and Ibc protein (formerly, Ic protein [18]); (iii) type Ic, Ia CHO and Ibc protein; (iv) type II, II CHO and occasionally, Ibc protein; and (v) type III, III CHO and rarely, Ibc protein.

Ia CHO antibodies, whether elicited by type Ia or type Ic cells, often cross-react with Ib CHO in precipitin and protection studies (6). The reverse situation also occurs: some Ib CHO antibodies cross-react with the Ia CHO of type Ia and Ic. This phenomenon, presumably due to a chemical similarity of the Ia and Ib CHO and not to a separate cross-reactive antigen (6), is called the Ia-Ib-Ic cross, for convenience. The Ibc protein seems to elicit antibodies protective against type Ib and type Ic strains. This observation holds only if no new antigens specific for type Ic are found (R. C. Lancefield, personal communication).

In addition, R proteins frequently occur in type III strains (17). These antigens are specific for neither group nor type, nor are they protective (9).

The protective property of some of the B type antigens in mice (and by analogy, perhaps in humans) and their usefulness in epidemiological investigations have prompted several immunochemical studies on the purified polysaccharide (3, 8, 14, 18) and partially purified protein antigens (18). Lancefield and Freimer (8) found D-galactose, D-glucose, and N-acetyl-D-glucosamine in the HCl-extracted type II CHO, the specificity of which was due to β-galactose residues. In the antigen extracted with trichloroacetic acid, they found an additional component (later identified as sialic acid [7]) which elicited mouse-protective and precipitating antibodies in rabbits and gave a net negative charge to the antigen. Russell and Norcross (14) found the same three monosaccharides in their HCl-extracted III CHO, but the basis for type III specificity seemed to reside in a minor component, glucuronic acid. Whether type III antibodies are protective is not known since numerous mouse passages have not caused a
selection of cells that were mouse virulent enough to use in protection studies (R. C. Lancefield, personal communication).

A search for the Ia CHO immunodominant group of type Ic was disappointing (18). This HCl-extracted antigen was composed of 70% galactose and 30% N-acetylgalcosamine, but neither hapten inhibited the quantitative precipitin reaction of antigen and type Ia antiserum. The purpose of the present study was to study this antigen further, to purify Ia CHO from type Ia and Ia CHO from type Ib, and to compare the immunochemistry of the three antigens. Experiments designed to more completely characterize the Ibc protein were performed concurrently with this study but will be described in a later report.

MATERIALS AND METHODS

Strains, antisera, and serological tests. Formalinized whole-cell vaccines of group B type Ia (strain 090), type Ib (strain H36B), type Ic (strain A909), type II (strain 18RS21), and type III (strain D136C) were used to produce group B typing antiserum as described previously (18, 20). Capillary precipitin tests, Ouchterlony slides, immunoelectrophoresis, and qualitative precipitin tests were also described previously (18). Antiserum were absorbed with purified antigens by placing a mixture of 200 μg of lyophilized antigen and 0.2 ml of antiserum at 35 C for 30 min and then by centrifuging the mixture to obtain the clear supernatant. The strains listed above were used for production of all type-specific antiserum, for extraction of all antigens, and for performance of all tests. Therefore, strain numbers will not be repeated unless necessary for clarity.

Purification of antigens extracted by HCl. Proteins and nucleic acids were precipitated from HCl extracts of whole cells of types Ia and Ib with cold 10% trichloroacetic acid (18). Polysaccharide antigens were then precipitated from the trichloroacetic acid supernatant fluid with 4 volumes of cold ethanol. Antigens were suspended in saline, neutralized, and clarified by centrifugation, and since no significant absorption occurred at 260 or 280 nm, they were immediately subjected to cold ethanol fractionation cycles to separate type CHO antigens from group B CHO. Most of the type antigens precipitated in 1.5 volumes of ethanol. The purified antigens were dissolved in water and then lyophilized. This purification procedure was modified from the one described for type Ic (18) only by omitting the treatment with nucleases and trypsin, after which dialysis and additional trichloroacetic acid precipitation were necessary to eliminate the added materials.

Search for Ibc protein in type Ia cells. The Ibc protein occurs in types Ib and Ic, respectively, but has not been found in type Ia (18). The possibility that it might be present in minute quantity was tested in Ouchterlony and capillary precipitin tests with Ibc-specific antiserum and a neutralized solution (protein solution) of the trichloroacetic acid precipitate of the type Ia HCl extract.

Enzyme susceptibility tests. The ability of β-galactosidase (Worthington) to inhibit the precipitin reactions of the Ia and Ib CHO antigens with specific antiserum was tested by adding a solution of the enzyme to crude HCl extracts of types Ia and Ib in a final concentration of 1.5 mg of enzyme per ml of extract. Capillary precipitin tests were done after incubation of the extracts at 35 C for 30 min; 1, 2, 3, 5, and 23 h; and 1 week. The ability of lysozyme (Sigma) to bind to and possibly cleave N-acetylgalcosamine from the Ia and Ib antigens of types Ia, Ib, and Ic was tested by incubating 1.0 mg of enzyme with 0.2 ml of HCl extract for 1, 4, and 18 h. Capillary precipitin tests were done at these times.

Analytical methods. Thin-layer chromatographic and quantitative chemical analyses were described previously (18). The resorcinol method (16) was used to measure sialic acid.

Extraction of saline antigens. Approximately 5 g of wet, packed whole cells of types Ia, Ib, and Ic were each stirred in 20 ml of saline for 18 h at 4 C. The supernatant fluids were used for capillary precipitin tests with specific antiserum. Provided the supernatant fluids were separated from the sediments adequately by centrifugation, further saline extractions were unnecessary. However, hot HCl extractions of the packed cells yielded a large amount of polysaccharide type antigens and, in addition, the group B and Ic antigens. Since the release of type antigens by saline from type Ib and Ic cells was less than that from type Ia cells, the saline suspensions of the former strains were exposed to sonification (Bronwill Biosonic IV) for 8 min to obtain better yields of antigens.

RESULTS

Tests for Ibc protein in type Ia cells. The 2-ml protein solution of type Ia was tested with Ibc-specific antiserum in capillary precipitin and Ouchterlony tests and then was concentrated in vacuo to 0.4 ml. Negative tests of even this highly concentrated extract indicated that 13 g of wet, packed whole cells of type Ia contained no detectable Ibc protein antigen. This is consistent with previous serological observations (18, 20).

Absorption of antiserum with purified antigens. Previous observations on the serological specificities of the polysaccharide type I antigens tested with antiserum absorbed with whole cells were confirmed by the experiments summarized in Table 1. Absorptions of type Ia or type Ic antiserum with the purified Ia antigens of either type Ia or type Ic removed precipitating antibodies for type Ia extract and a type Ic extract digested by pepsin to remove the Ibc protein (18). Similarly, type Ib antiserum absorbed with Ib CHO no longer reacted with the type Ib pepsin-digested extract. Positive reac-
tions of this antiserum with type Ic and Ib untreated extracts were due to the Ibc antigen.

**Thin-layer chromatography.** Chromatographic analyses of the purified Ia CHO of type Ia cells and Ib CHO of type Ib suggested that they were composed of galactose and N-acetylgalactosamine. The latter substance was detected by spraying the chromatograms with p-dimethylaminobenzaldehyde (Ehrlich reagent; Elson-Morgan test) as described by Partridge (13). Both the Ia and Ib hydrolyzed antigens gave positive color tests without prior condensation of the antigens with acetylace tone; therefore at least some N-acetyl groups were not destroyed by mild acid hydrolysis. In addition, the unhydrolyzed Ia antigen was Elson-Morgan positive though the unhydrolyzed Ib antigen was negative. Acetyl groups may be inaccessible to Ehrlich reagent in the native Ib CHO.

**Chemical composition.** The close serological and protective relationships of the CHO antigens of types Ia, Ib, and Ic also extended to their chemical composition (Table 2). More than two-thirds of the dry weight of these antigens consisted of galactose; N-acetylgalactosamine was the other constituent. Molar ratios of galactose to N-acetylgalactosamine were 3:1 for the Ia antigen of types Ia and Ic and 2:1 for the Ib antigen.

**Inhibition studies.** Efforts were made to find the immunodominant groups of the three CHO antigens. Several commercially obtained monosaccharides and oligosaccharides effected little or no inhibition of the quantitative precipitin reactions of purified Ia antigens with homologous antisera (Table 3). A similar lack of

<table>
<thead>
<tr>
<th>Designation</th>
<th>Immunizing strain no.*</th>
<th>Absorbed by purified HCl antigens</th>
<th>Precipitin reactions with HCl extract of strain:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>090</td>
<td>None</td>
<td>090  A909  A909*  H36B  H36B*</td>
</tr>
<tr>
<td>Ia</td>
<td>090</td>
<td>Ia(090)</td>
<td>4+  4+  4+  0c  0</td>
</tr>
<tr>
<td>Ia</td>
<td>090</td>
<td>Ia(A909)</td>
<td>0  0  0  0  0</td>
</tr>
<tr>
<td>Ic</td>
<td>A909</td>
<td>None</td>
<td>4+  4+  4+  4+  0</td>
</tr>
<tr>
<td>Ic</td>
<td>A909</td>
<td>Ia(090)</td>
<td>0  4+  0  3+  0</td>
</tr>
<tr>
<td>Ic</td>
<td>A909</td>
<td>Ia(A909)</td>
<td>0  4+  0  3+  0</td>
</tr>
<tr>
<td>Ib</td>
<td>H36B</td>
<td>None</td>
<td>0   4+  0  4+  0</td>
</tr>
<tr>
<td>Ib</td>
<td>H36B</td>
<td>Ib(H36B)</td>
<td>0   4+  0  4+  0</td>
</tr>
</tbody>
</table>

* Vaccines of formalinized whole cells.
* Extract digested with pepsin.
* No precipitin reaction observed.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Antigen (% dry wt)</th>
<th>Type Ia (090)</th>
<th>Type Ib (H36B)</th>
<th>Type Ic (A909)</th>
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</thead>
<tbody>
<tr>
<td>Galactose</td>
<td>71.0</td>
<td>60.0</td>
<td>69.0</td>
<td></td>
</tr>
<tr>
<td>Glucosamine</td>
<td>25.4</td>
<td>24.0</td>
<td>24.8</td>
<td></td>
</tr>
<tr>
<td>Nitrogen</td>
<td>1.5</td>
<td>2.0</td>
<td>1.7</td>
<td></td>
</tr>
</tbody>
</table>

* No phosphorus, galactosamine, glucose, methyl pentose, uronic acid, ketose, pentose, heptose, amino acid, nucleic acid, or sialic acid detected.
* Strain numbers.
* See reference 18.

hapten inhibition with other lots of antisera was reported previously for the Ia antigen from type Ic cells (18). More than 50% of the Ib-specific precipitate was inhibited, however, by glucosamine or by γ-D-galactonolactone. β-Linked galactose derivatives were more effective inhibitors than those that were α-linked. The significance of these observations was further investigated by plotting a series of hapten inhibition curves.

**Inhibition of Ib precipitate.** Sugars that inhibited the precipitin reaction of Ib CHO and type Ib antiserum greater than 30% at a concentration of 40 mg/ml are shown in Fig. 1. As observed in the previous experiments, γ-D-galactonolactone and D-glucosamine were the most effective inhibitors. The other hapten shown may have been inhibited by virtue of their stereochemical likeness to glucosamine or to γ-galactonolactone.
TABLE 3. Hapten inhibition of quantitative precipitin reactions obtained with antisera produced with whole-cell vaccines and purified polysaccharide antigens extracted by hot HCl from the vaccine strains

<table>
<thead>
<tr>
<th>Potential inhibitor*</th>
<th>Inhibition (%) of:</th>
<th>Type Ia reactions</th>
<th>Type Ic reactions</th>
<th>Type Ib reactions</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>(900)*</td>
<td>(A909)*</td>
<td>(H36B)*</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>ND</td>
<td>9</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>D-Glucose</td>
<td>0</td>
<td>1</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>D-Galactose</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>D-Glucosamine</td>
<td>10</td>
<td>5</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>N-acetyl-D-glucosamine</td>
<td>15</td>
<td>0</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>D-Galactosamine</td>
<td>5</td>
<td>0</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>N-acetyl-D-galactosamine</td>
<td>4</td>
<td>0</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Phenyl-β-D-galactoside</td>
<td>16</td>
<td>0</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>α-Methyl-D-galactoside</td>
<td>3</td>
<td>12</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>λ-d-Galactonolactone</td>
<td>28</td>
<td>0</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>2-Deoxy-D-galactose</td>
<td>7</td>
<td>16</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>β-Lactose</td>
<td>10</td>
<td>18</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>α-D-Melibiose</td>
<td>2</td>
<td>17</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>D-Raffinose</td>
<td>5</td>
<td>8</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Stachyose</td>
<td>1</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>D-Galactose + N-acetyl-D-glucosamine</td>
<td>8</td>
<td>0</td>
<td>45</td>
<td></td>
</tr>
</tbody>
</table>

*a Reaction mixture consisted of 20 mg of inhibitor, 0.1 ml of antiserum produced with the same strain used for antigen extraction, and the amount of purified polysaccharide antigen that gave maximum precipitation with 0.1 ml of antiserum (15 μg of Ia CHO from strain 909, 10 μg of Ia CHO from strain A909, and 35 μg of Ib CHO from strain H36B) in a total of 1 ml.

*b Strain numbers.

*c ND, Not done.

The inhibition of the Ib quantitative precipitin reaction by γ-galactonolactone, not a constituent of the purified antigen, was investigated in a different way. D-Glucosamine (10 mg) inhibited the quantitative curve through 90 μg of purified antigen and, therefore, may compete more effectively for antibody binding sites than does the same quantity of γ-galactonolactone (Fig. 2).

**Inhibition of Ia precipitates.** Numerous inhibition curves were obtained by using both type Ia and Ic antisera with each of the purified CHO antigens extracted from type Ia and Ic cells. No hapten that was tested inhibited type Ic antiserum from combining with the antigens. Type Ia antiserum, on the other hand, was partially inhibited from combining with Ia CHO of either strain by γ-galactonolactone and by N-acetylgalactosamine (Fig. 3 and 4), an interesting finding since the Ib reaction was inhibited to a greater extent by the unacetylated hexosamine. Again, the inhibition by N-acetylgalactosamine may reflect a stereochemical relationship to the immunodominant group of the antigen.

**Ouchterlony studies of HCl antigens.** Figure 5 shows the reactions of each type-specific antiserum with the three purified CHO antigens described above. Further investigation of the double band between type Ia antiserum and Ia CHO of type Ic (center pattern) showed that a double band also occurs occasionally with the Ia CHO of type Ia and with certain lots of type Ic antiserum. The spur seen in the right pattern...
occurred because of Ia-Ib-Ic cross-reactive antibodies in this lot of type Ic antiserum.

The results (not shown) of incorporating various haptens (1% concentration) into the agar gel were consistent with the quantitative studies in that N-acetylgalactosamine was the more effective inhibitor of the Ia reactions while unacetylated glucosamine was the more effective inhibitor of the Ib reaction.

**Lack of enzyme inhibition.** Incubation of the purified antigens with either β-galactosidase or lysozyme did not noticeably affect the type Ia or Ib precipitin reactions. Ehrlich reagent, used as described above, detected no differences after incubation of the antigens with lysozyme.

**Lack of serological cross-reactions with antigens of glucosamine immunodominance.** Neither type Ia nor type Ib antisera formed a visible precipitate with HCl extracts of streptococcal groups A(10), L(4), or O(11) in the capillary precipitin test. Group A, L, and O antisera did not react with the group B extracts.

**Partial serological identity of B-I HCl and saline antigens.** The experiments of Lancefield and Freimer (8) with the II CHO suggested that a partial hydrolysis of B type antigens occurred in the HCl extraction process. The labile component (sialic acid [7]) imparted a net negative charge to the antigen and formed a reaction of partial identity with the HCl-extracted CHO on Ouchterlony analysis. The same observations were made on the type I antigens in the present study. Saline-extracted antigens formed spurs with HCl-extracted antigens when homologous whole-cell antisera were used (Fig. 6). The incorporation of 1% sialic acid in the agar had no effect on the occurrence of spurs (not shown), although each type-specific saline antigen contained sialic acid in addition to galactose, glucose, and N-acetylgalactosamine. The double band seen in other experiments when type Ia antiserum and Ia (HCl) CHO of type Ic were allowed to react appears in the center pattern. The extra band between type Ib antiserum and Ib saline antigen, seen in the right pattern, is due to the presence of Ibc protein in the Ib sonic material and anti-Ibc in this antiserum.

The difference in charge of the HCl and saline antigens is seen in Fig. 7. The purified HCl antigens migrated slightly toward the cathode by endosmosis during electrophoresis at alkaline pH. The more negatively charged saline antigens, however, moved toward the anode as shown in the bottom pattern when the Ia antigen of type Ia cells was used. The saline-extracted CHO of types Ib and Ic had the same electrophoretic characteristics as that of type Ia.

**Characteristics of B-I saline antigens.** Whether the saline and HCl antigens of the various types have similar serological specifi-

![Fig. 3. Hapten inhibition curves of type Ia antiserum and purified Ia (HCl) CHO of type Ia strain. Reaction mixture are the same as that for Table 3 except for varying concentrations of haptons. Abbreviations of haptons are listed under Fig. 1; also, N-acetyl-D-galactosamine, N-ac-Gal.](image)

![Fig. 4. Hapten inhibition curves of type Ia antiserum and purified Ia (HCl) CHO of type Ic strain. Reaction mixture consisted of 0.1 ml of antiserum, 20 μg of antigen, and the varying amounts of hapten indicated, in a total volume of 1.0 ml. See Fig. 1 and 3 for abbreviations.](image)

![Fig. 5. Ouchterlony slide showing precipitin reactions of purified type (HCl) CHO antigens and whole-cell rabbit antisera. Antigens were: A, Ia CHO of type Ia strain 090; B, Ib CHO of type Ia strain H36B; C, Ia CHO of type Ic strain A909. Antisera were: A-S, type Ia; B-S, type Ib; C-S, type Ic.](image)
ties was determined by performing capillary precipitin tests with saline extracts and antisera of types Ia, Ib, Ic, II, and III. Reactions were as type specific with the saline as with the HCl extracts. Furthermore, the Ia saline extracts of types Ia and Ic formed a reaction of identity on Ouchterlony slides with type Ia antiserum. This indicated that the homology observed with the HCl Ia antigens was also noted with the saline antigens of types Ia and Ic.

To determine whether the saline antigens were labile to heat alone, the Ia saline antigen of type Ia was tested with antiserum specific for the labile portion of the antigen. Absorption of the antiserum with living or with heat-killed (60 C, 1 h) type Ia cells removed precipitating antibodies to the labile antigen. Furthermore, heating the antigen at 60 C for 1 h had no detectable effect on its reaction with the specific antiserum. Subjecting the antigen to 100 C at pH 2 for 10 min (Lancefield extract), however, made it unreactive with the labile-specific antiserum. These data suggested that the antigen was heat stable but that part of it had been hydrolyzed during HCl extraction.

**Effect of trichloroacetic acid on saline antigens.** Saline extraction of sonically treated cells probably releases more than surface polysaccharide antigens, and therefore isolation and purification must proceed in a manner similar to that for HCl-extracted antigens. Experiments done to see what effect trichloroacetic acid has on the antigens showed serological identity of the saline antigens and those treated with 2.5% or 5% cold trichloroacetic acid (Fig. 8). Thus proteins could be precipitated by trichloroacetic acid without destroying the "labile" antigens in the supernatant. These antigens were further purified by fractional ethanol precipitation. Figure 9 shows the reactions of partial identity between the purified HCl and saline (trichloroacetic acid) antigens of the three types and shows a reaction of complete identity between the saline (trichloroacetic acid) Ia antigens of types Ia and Ic.

**DISCUSSION**

The serological relationships of the polysaccharide type antigens of group B streptococcal types Ia, Ib, and Ic were confirmed in immunochmical studies with type Ia strain 090, type Ib strain H36B, and type Ic strain A909. The Ia CHO antigens extracted by hot HCl from types Ia and Ic were composed of galactose (70% of...
weight) and N-acetylglucosamine (30%) in a molar ratio of 3:1. Similarly, the Ib CHO of type Ib was composed of galactose (60%) and N-acetylglucosamine (30%) in a molar ratio of 2:1. Color tests with Ehrlich reagent and hapten inhibition tests suggested that the specificity of Ia CHO depends on N-acetylglucosamine and that of the Ib CHO on unacetylated glucosamine. If true, the difference could be due to cryptic acetyl groups in the Ib antigen, which, in turn, could depend on nonterminal acetyl groups or apodeterminants (15) that turn a different stereochemical face to the antibody than that of the Ia CHO. The quantity of hapten required to inhibit the quantitative precipitin reactions suggests, however, that these sugars have only a stereochemical similarity with, but not identity to, the immunodominant groups. This is also suggested by the lack of binding to lysozyme (12) and by the inhibition curves obtained with γ-galactonolactone. Furthermore, secondary linkages could be important. As mentioned previously (18), the small yield of purified antigens made sequence studies impractical.

Results of Ouchterlony tests were consistent with the quantitative precipitin data in that type Ib reactions were inhibited more by glucosamine and type Ia reactions more by N-acetylglucosamine. Two precipitin bands were occasionally seen between the Ia CHO of type Ia or Ic and certain lots of Ia antisera produced with type Ia or Ic cells; both bands were partially inhibited by N-acetylglucosamine. This observation suggests that HCl extraction cleaves the antigen into two fragments with the same serological specificity.

Hot HCl also extracted CHO antigens only partially identical to antigens extracted from the same cells by saline. Neither 60°C heat, cold trichloroacetic acid nor cold ethanol affected the saline antigens serologically, but treatment with 0.2 N HCl at 100°C for 10 min produced antigens identical to the HCl-extracted antigens on Ouchterlony analysis and immunoelectrophoresis. These observations are similar to those made on the II CHO (3, 8), in which the labile residue was composed of sialic acid. Immunelectrophoresis experiments showed that the type I saline antigens were more negatively charged than their corresponding HCl antigens and therefore might also contain sialic acid. Recent experiments detected galactose, N-acetylglucosamine, glucose, and sialic acid in approximate molar ratios of 5:2:1:1 in the purified saline Ia CHO of type Ia and Ib CHO of type Ib. However, immunoechemical differences in the saline antigens of different group B types are implied by the fact that they are type specific.

The different yields of saline antigens observed with different strains probably reflect differences in the amounts synthesized and released from the streptococcal cell envelope into a microcapsule. Strain 090 (type Ia) has a large capsule, and therefore Ia CHO washes off in large amounts (R. C. Lancefield, personal communication). Sonic treatment was necessary to obtain comparable amounts of saline-CHO from H36B and A909. Sonic treatment also released the group B CHO and Ibc protein antigens. After the extracellular antigens were removed, hot HCl extraction of the cell sediments released large amounts of "HCl antigens."

These studies are pertinent to the epidemiology of group B neonatal disease. Prevention of neonatal septicemia and meningitis could be approached in two ways, by antibiotic prophylaxis of mother and infant or by maternal vaccination. The former method is the subject of considerable controversy (1, 2); the latter has not been tried. Antibody studies and efficacy trials would depend on purified and characterized antigens. It is possible that human immunity, if present, would be type specific since this specificity has been found in passive mouse protection studies. The B type antigens could, therefore, be virulence factors analogous to the M protein antigens of group A streptococci.
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LITERATURE CITED