INFECTION AND IMMUNITY, Mar. 1978, p. 983-991
0019-9567/78/0019-0983$02.00/0

Vol. 19, No. 3
Printed in U.S.A.

Existence of Multiple Immunodeterminants in the Type-Specific Capsular Substance of Group B Type Ia Streptococci

JUDITH A. KANE* AND WALTER W. KARAKAWA

Department of Biochemistry and Biophysics, The Pennsylvania State University, Paul M. Althouse Laboratory, University Park, Pennsylvania 16802

Received for publication 15 September 1977

Rabbits immunized with group B, type Ia streptococci produce two distinct populations of protective antibodies. Evidence is presented indicating that these antibodies are directed against two major immunodeterminants which coexist in the specific type Ia antigen. Immunochemical data, using purified antibody preparations, indicate that the type substance, a sialic acid polymer consisting of galactose, glucose, glucosamine, and sialic acid, possesses two distinct immunodominant determinants, terminal sialic acid residues and a galactosyl oligosaccharide. Antibodies directed against either of these determinants were shown to possess in vivo and in vitro opsonic capabilities.

The immunology of group B streptococci, type Ia, has been the subject of many laboratories, and detailed immunochemical analysis of the antiphagocytic capsular antigens has indicated that this type-specific substance consists of galactose and *N*-acetylglucosamine (8, 22). In continued immunochemical studies on the type Ia antigen, a picture has emerged suggesting that the previously recognized pH 2.0-extracted type-specific Ia antigen may, in fact, represent a partially degraded antigen (3). Recent studies have indicated that extraction of type Ia streptococci with pH 7.0 buffer yielded an acidic (sialo) polysaccharide antigen containing galactose, glucosamine, glucose, and sialic acid (3). The major difference observed between the pH 7.0-extracted sialo antigen and the conventional pH 2.0-extracted antigen appears to be the presence of sialic acid moieties in the former substance. Since sialic acid residues are extremely labile in pH 2.0 buffer at 100°C, it is possible that the conventional pH 2.0 antigen may represent a partially degraded type Ia sialo antigen. In an extension of this observation, immunochemical evidence has indicated that the sialo antigen of type Ia may possess two major immunodeterminants that coexist on the same antigen, an acid-labile sialic acid moiety and galactosyl moieties (3). This possibility is not unlikely, for Lancefield and Freimer have clearly demonstrated that the type-specific antigen of group B streptococci, type II, consists of two immunodeterminants, a sialic acid and β-linked galactosyl determinants (2, 8, 9).

This report substantiates the view that the type Ia antigen possesses two major immunodeterminants, terminal sialic acid residues and galactosyl oligosaccharide determinants. Evidence is presented indicating that rabbits immunized with type Ia organisms produce two distinct populations of anticapsular antibodies and that these antibodies are directed against terminal sialo determinants and galactosyl oligosaccharide determinants. Purified specific preparations of both populations of antibodies are shown to possess opsonic properties.

MATERIALS AND METHODS

Strains of streptococci. Types Ia (090/14/4), Ib (H36B/60/1), and Ic (A909/14) were kindly supplied by R. C. Lancefield, The Rockefeller University.

Antisera. Reference type-specific group B streptococcal antisera were obtained from R. C. Lancefield. Unabsorbed anti-type Ia, Ib, and Ic sera were obtained from rabbits immunized according to the method of McCarty and Lancefield (13).

Immunological methods. Procedures for the qualitative and quantitative precipitin analyses of the carbohydrate antigens have been previously described (13). Total protein was determined by the method of Lowry et al. (11). Specific absorption of antisera with whole bacterial cells was performed according to the method described by Lancefield et al. (10). Passive protection tests in mice were carried out according to the method described by Lancefield et al. (10). In vitro opsonic test was performed by the method of Roberts (15). Purified antibody preparations were obtained from antibody-antigen complexes by the method described by Braun et al. (1). Immunoelectrophoresis was carried out by the micromethod of Scheidegger (17).

Isolation and purification of antigens. Isolation and purification of antigens from strain O90/14/4, type Ia, have been previously described (3).

Chromatography. Paper chromatography and identification of individual monosaccharides were per-

formed as previously described (4, 14).

Analytical methods. Quantitative analysis of amino sugars was performed according to the modified method of Spackman et al. (18) and also by the method of Weber et al., which used a Technicon autoanalyzer (21). Hexoses and deoxy sugars were detected and quantitated with an autoanalyzer according to the method of Kesler (5). Sialic acid was detected and quantitated by the method described by Warren (20). Terminal galactose was quantitated enzymatically by a modification of the method described by Roth et al. (16).

RESULTS

Immunochemical specificity of the type Ia neutral and sialo polysaccharides. Two antigen preparations were obtained from the cell extracts of group B streptococci, type Ia, by procedures previously described (3). The acidic or sialo polysaccharide obtained from the pH 7.0 buffer extract was shown to contain glucosamine, galactose, glucose, and sialic acid in the mole ratio of 1.0:2.1:0.5:0.7; the neutral polysaccharide obtained from the hot pH 2.0 extract contained glucosamine, galactose, and glucose in the mole ratio of 1.0:2.1:0.4. As previously shown, the sialic acid residues can readily be removed from the sialo antigen with 0.05 N H_2SO_4 at 80°C for 1 h (19). The resulting asialo polymer, when subjected to immunoelectrophoretic analysis, was shown to possess an electrophoretic mobility similar to that of the neutral polymer and not the native sialo polymer (3). Double-diffusion analysis in agar also indicated that the asialo polymer was identical to the neutral pH 2.0-extracted polysaccharide (3).

To determine the immunochemical relationship between the sialo and the neutral pH 2.0-extracted antigens, the following experiments were performed. Illustrated in Fig. 1 are the results of the chromatographic analyses of the various timed hydrolysates of the sialo antigen, the asialo antigen, and the conventional pH 2.0-extracted neutral antigen. An acidic-like compound, which had an R_f value identical to that of acid-treated neuraminic acid, was initially released from the sialo antigen after only 5 min of hydrolysis with 0.1 N HCl at 100°C (Fig. 1, left frame), while galactose was initially released

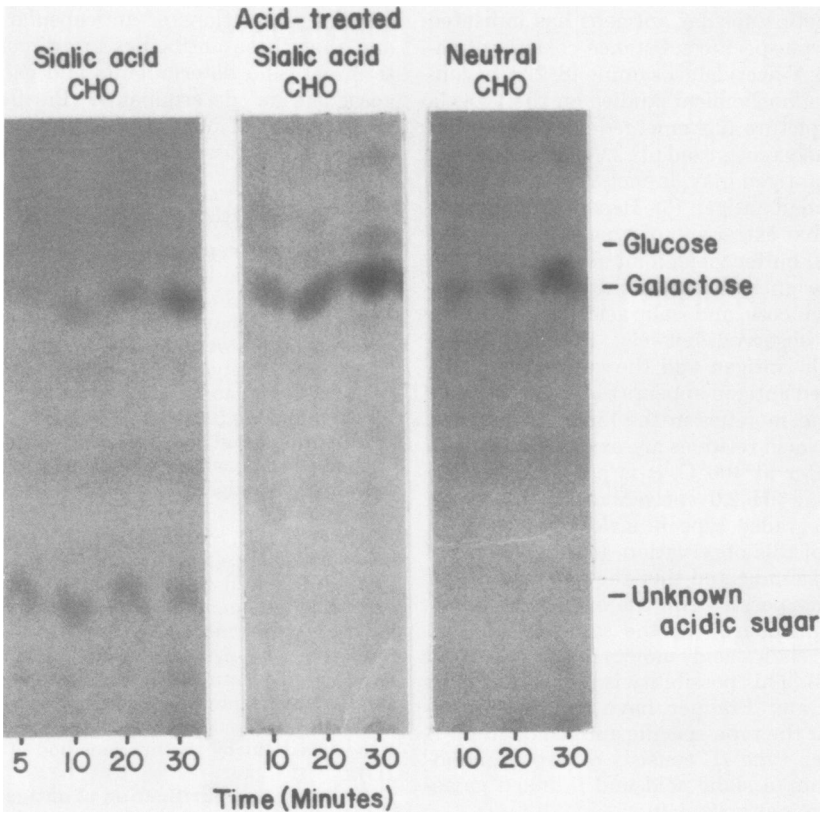

FIG. 1. *Paper chromatographic analyses of the acid hydrolysates of the sialic acid (sialo) polysaccharide (left), the acid-treated sialic acid (asialo) polysaccharide (middle), and the neutral polysaccharide (right). Hydrolysis was carried out with 0.1 N HCl at 100°C for 10, 20, and 30 min.*

after 10 min of hydrolysis. Analysis of the timed hydrolysates of both the asialo and neutral antigens (Fig. 1, middle and right frames, respectively) indicated similar hydrolytic profiles for both polymers. In both instances, galactose was the initial sugar released after 10 min of hydrolysis, whereas no acidic-like compounds were released. Reaction of the sialo and asialo antigens with galactose oxidase indicated that the sialo antigen possessed galactosyl residues that were masked by sialic acid residues. For example, the sialo antigen gave a negative galactose oxidase test before the mild acid treatment, but after removal of the acidic groups, the asialo antigen was shown to be galactose oxidase positive, suggesting the presence of terminal galactose residues.

In an extension of this study, sialic acid residues were sequentially released from the sialo antigen with 0.05 N H_2SO_4 at 80°C, and the released sialic acid residues were monitored by the thiobarbituric acid method of Warren (20). In addition, the resulting asialo antigens obtained at the various timed intervals were subjected to a galactose oxidase test. Illustrated in Fig. 2 are the results of the correlation between the release of sialic acid residues from the sialo antigen and the concomitant appearance of terminal galactose residues in the asialo antigens. The sequential release of sialic acid from the sialo antigen was directly related to the availability of terminal D-galactose. Under these test conditions, approximately all of the available sialic acid residues were released from the sialo antigen after about 45 min of hydrolysis. Concomitantly, the maximum level of available terminal D-galactose was observed after about 45 min of hydrolysis. Significantly, the detected level of D-galactose in the asialo antigen obtained after 45 min of hydrolysis was compatible with the level detected in the neutral pH 2.0-extracted antigen. These results indicate that the sialo antigen may possess terminal sialic acid residues with adjacent D-galactose residues.

Hydrolysis of the neutral antigen in 0.1 N HCl for 10 min resulted in the selective removal of essentially only galactose residues (Fig. 1). Illustrated in Fig. 3 are the results of the quantitative precipitin analyses of the neutral antigen before and after removal of galactosyl residues. The reactivity of the acid-treated neutral antigen with anti-type Ia serum was noticeably diminished. A similar decrease in antigenicity was also observed between the asialo antigen devoid of significant levels of galactosyl residues and anti-type Ia serum. These results support the thesis that both the neutral and asialo antigens possess terminal galactosyl residues that may play a role in the antigenicity of these polymers. Results of quantitative precipitin analyses, using the neutral antigen treated with galactose oxidase, indicated that conversion of the D-galactosyl residues to D-galactohexodialdose by galactose oxi-

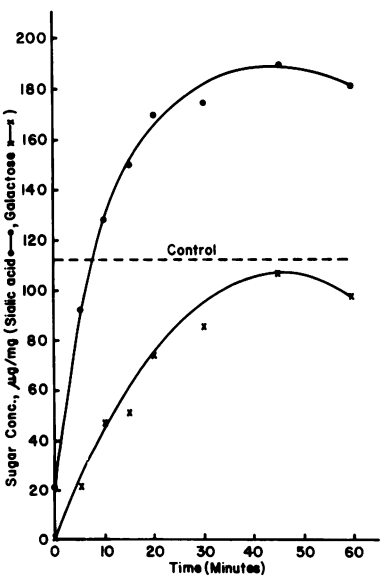

FIG. 2. *Correlation of the release of sialic acid residues from the sialic acid polymer with the appearance of terminal galactose residues. Control depicts the maximum amount of terminal nonreducing galactose residues available in the neutral polymer.*

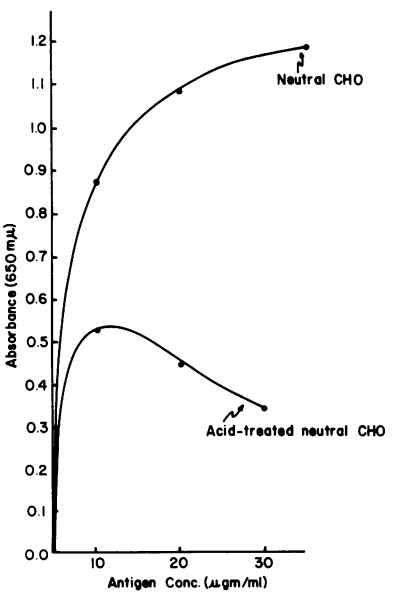

FIG. 3. *Quantitative precipitin reactions between the neutral polysaccharide, the neutral polysaccharide treated with 0.1 N HCl for 10 min, and anti-type Ia serum.*

dase had little effect on the antigenicity of the treated antigen. In addition, quantitative precipitin inhibition studies, which used various galactosyl derivatives as inhibitors, suggested that the galactosyl residues do not function alone as the immunodominant determinant. For example, D-galactose was shown to be a weak inhibitor of the precipitin reaction between the neutral antigen and homologous antiserum. Lactose, a 4-*o*-*β*-D-galactosyl-D-glucose, and *p*-nitrophenyl-*β*-D-galactopyranoside were moderate inhibitors, whereas melibiose, a 6-*o*-*α*-D-galactosyl-D-glucose, and *p*-nitrophenyl-*α*-D-galactopyranoside were poor inhibitors of the precipitin reaction. These results suggest that the immunodominant determinant of the neutral and asialo antigens may represent an oligosaccharide comprised of galactosyl residues *β*-linked to adjacent monosaccharides. In view of these observations, it could be concluded that the sialo antigen is the type-specific antigen of type Ia, which consists of terminal sialic acid residues with an adjacent *β*-linked galactosyl oligosaccharide.

Immune response in rabbits to the type Ia capsule. In view of the observed antigenicity of the galactosyl-linked oligosaccharide of the neutral antigen and of the sialic acid residues of the sialo antigen, the possibility exists that both immunodeterminants may function simultaneously in the immune response of animals to the type Ia-specific polysaccharide. Illustrated in Fig. 4 are the results of the quantitative precipitin analyses between antisera derived from a representative rabbit taken over a period of 210 days and the neutral and sialo antigens. At 14 days after the initial injection of the whole-cell vaccine, a representative rabbit consistently developed significant levels of anti-sialo-antigen antibodies but only very low levels of anti-neutral-antigen antibodies. In antiserum taken at 20 days, both types of antibodies were detected. The neutral-antigen antibody concentration peaked at about 20 days, whereas the sialo-antigen antibody concentration peaked at about 30 days. Seven months after the initial injection, the sialo-antigen antibodies still persisted, whereas the level of neutral-antigen antibodies was greatly diminished. These results indicated that rabbits immunized with the Ia vaccine elicited the production of two major populations of antibodies, namely, antibodies that were directed toward both the sialic acid residues and the galactosyl-linked residues of the type Ia antigen.

Immunospecificity of the two populations of antibodies. Quantitative precipitin tests between the sialo antigen, the neutral antigen, and anti-type Ia sera were performed. The results (Fig. 5, left frame) indicated that the

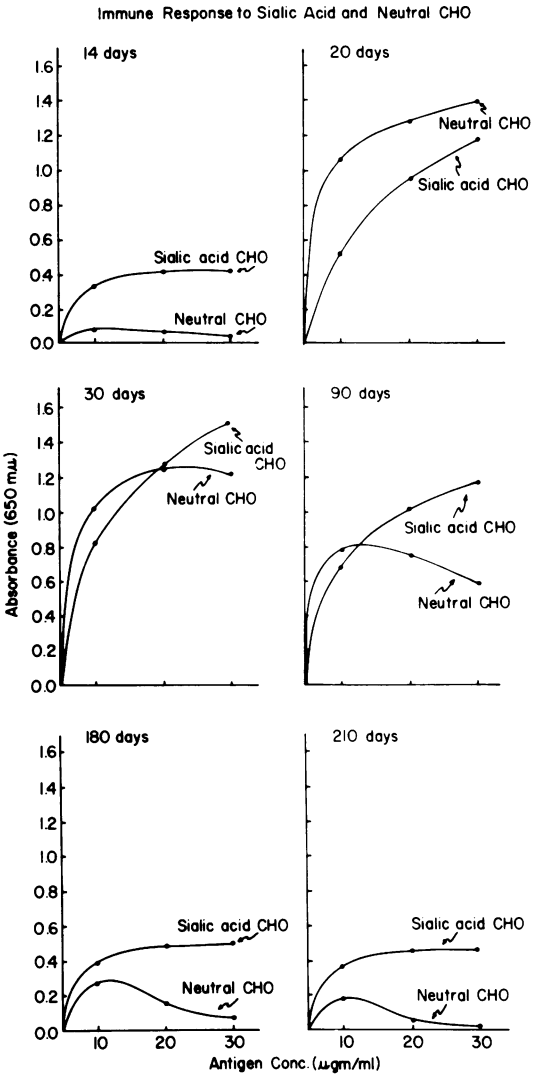

FIG. 4. *Precipitin analyses of antisera derived from a representative rabbit at 14, 20, 30, 90, 180, and 210 days after immunization with group B, type Ia organisms.*

equivalence point of the sialo antigen reaction was approximately 60 μg, whereas the equivalence point of the neutral antigen reaction was 40 μg. Samples of anti-type Ia sera were absorbed with either the sialo antigen or the neutral antigen at equivalence, and each absorbed antiserum was used in the quantitative precipitin test (Fig. 5, right frame). It should be noted that the serum absorbed with the neutral polymer still gave significant reaction with the sialo antigen but not with the neutral antigen. However, serum absorbed with the sialo antigen failed to react with either the neutral antigen or the sialo antigen. Since the sialo antigen pos-

sesses both immunodeterminants, it is capable of absorbing out both populations of antibodies, whereas the neutral polymer can absorb out only the antibodies with galactose specificity. This notion is supported by additional absorption studies, depicted in Fig. 6. In this experiment, antiserum was allowed to react with fresh type Ia whole cells for 24 h at 4°C. After washing, the agglutinated cells were treated with propionic acid (pH 2.7), and the eluted antibodies were precipitated with 50% cold $(NH_4)_2SO_4$, dialyzed, and concentrated by vacuum evaporation to a final concentration of approximately 10 mg/ml. The eluted antibody preparation consisted of antibodies directed against both the sialo and neutral antigens (Fig. 6, left frame), indicating that the capsular substance of fresh type Ia cells possesses immunologically functional sialic acid and galactosyl-linked immunodeterminants. Results of the quantitative precipitin analyses, us-

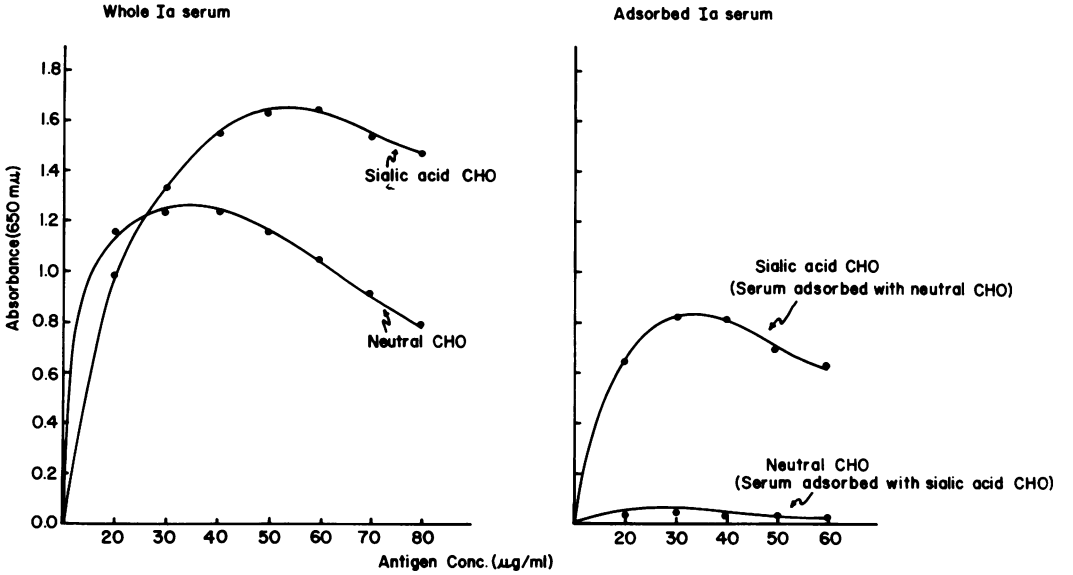

FIG. 5. *(Left) Quantitative precipitin reactions between the sialic acid (sialo) antigen, the neutral antigen, and anti-type Ia serum. (Right) Quantitative precipitin reactions between the sialic acid (sialo) antigen and antiserum absorbed with the neutral antigen and between the neutral antigen and antiserum absorbed with the sialo antigen.*

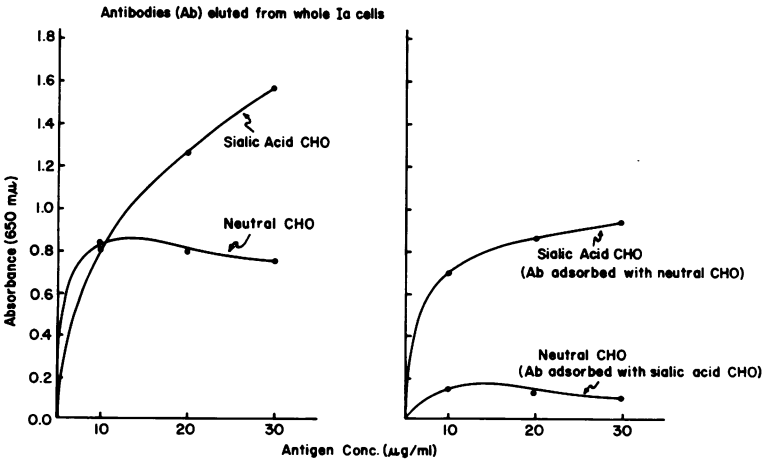

FIG. 6. *(Left) Quantitative precipitin reactions between the sialic acid (sialo) antigen, the neutral antigen, and antibodies eluted from whole type Ia cells. (Right) Quantitative precipitin reactions between the sialic acid (sialo) antigen and whole-cell-eluted antibodies absorbed with neutral antigen and between the neutral antigen and whole-cell-eluted antibodies absorbed with the sialo antigen.*

ing eluted antibody preparations that were selectively absorbed with either the sialo antigen or the neutral antigen, are depicted in Fig. 6 (right frame). Note that the sialo antigen was able to absorb out both types of eluted antibodies, whereas the neutral antigen was only about 50% effective. These studies underscore the view that the sialo antigen consists of two major immunodeterminants that coexist on the same antigen and are both immunogenic in rabbits.

Opsonic properties of the neutral and sialo antibodies present in rabbit anti-type Ia serum. Antibodies with specificity toward the neutral and sialo carbohydrates of type Ia have been shown to possess opsonic properties in mice (3). Illustrated in Fig. 7 are the results of the in vitro interaction between type Ia organisms and human polymorphonuclear leukocytes (PMN) in the presence of preimmune serum, immune serum containing antibodies against the two type-specific determinants as well as against the group B antigen, and absorbed-immune serum. The preimmune serum had no appreciable opsonic capacity, whereas the homologous immune serum possessed very effective opsonins. In the case of the absorbed-immune serum, it should be noted that serum absorbed with the neutral carbohydrate still contained opsonins, whereas immune serum absorbed with the sialo carbohydrate contained minimal levels of opsonins. The almost complete removal of the serum opsonins by the sialo antigen could be attributed to the fact that the sialo antigen contains two immunodeterminants and thus is able to remove both populations of specific opsonins. In contrast, the neutral carbohydrate contains only one major immunodeterminant and therefore is capable of removing only a portion of the existing serum opsonins. Group-specific antibodies were suggested to be ineffective opsonins, for immune serum absorbed with a nontypable group B streptococci was still very effective in promoting phagocytosis (Fig. 7). These results support the view that the sialo antigen consists of two major immunodeterminants and that both determinants are significant in eliciting the production of opsonins in rabbits.

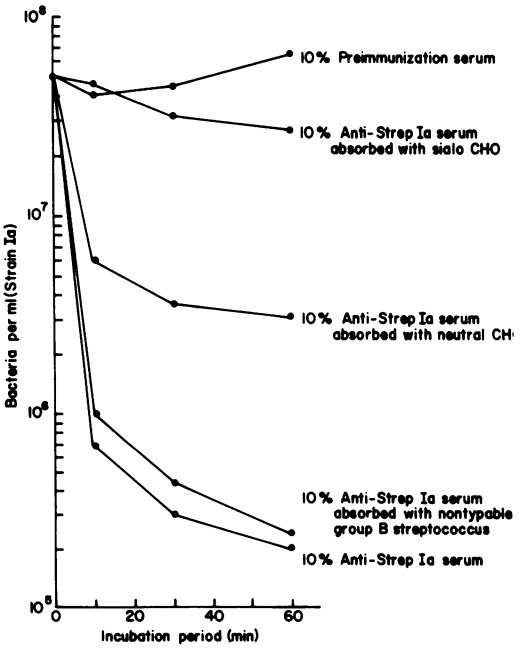

FIG. 7. *Interaction between type Ia streptococci and human leukocytes in the presence of normal preimmune rabbit serum, anti-type Ia serum, and anti-type Ia serum absorbed with either the sialo antigen, the neutral antigen, or a nontypable group B streptococcus.*

Type Ia specificity of the rabbit serum opsonins. To confirm the view that the type Ia carbohydrate used in these studies is, in fact, present in the prototype Ia isolated by Dr. Lancefield, immunological tests were performed that used standard reference type Ia, Ib, and Ic antisera, kindly supplied by Dr. Lancefield. Illustrated in Fig. 8 are the results of the quantitative precipitin analyses between the sialo and neutral antigens and Lancefield's reference type Ia, Ib, and Ic antisera. Reference type Ia serum contained antibodies with both sialo-antigen and neutral-antigen specificities (Fig. 8, left frame). On the other hand, reference type Ib antiserum had only low levels of antibodies directed against each of these antigens. Anti-type Ic serum (right frame) contained high levels of both sialo-antigen and neutral-antigen antibodies, which is consistent with the findings of Wilkinson (23). To determine the immunochemical relationship between types Ia and Ic, double-diffusion analysis in agar between the neutral pH 2.0-extracted antigen, the asialo antigen, and anti-type Ia and type Ic sera was performed. The precipitin band formed between the asialo antigen and anti-type Ia serum merged to form lines of identity with the precipitin line formed between the asialo antigen and anti-type Ic serum (Fig. 9). Further, the precipitin line formed between the neutral type Ia antigen and anti-type Ic serum merged to form lines of identity with the precipitin line formed between the asialo antigen and anti-type Ic serum. The results of these precipitin reactions indicate that type Ic possesses an antigen that is immunochemically identical to the type Ia neutral antigen, thus accounting for the observed cross-reactivity between these serotypes.

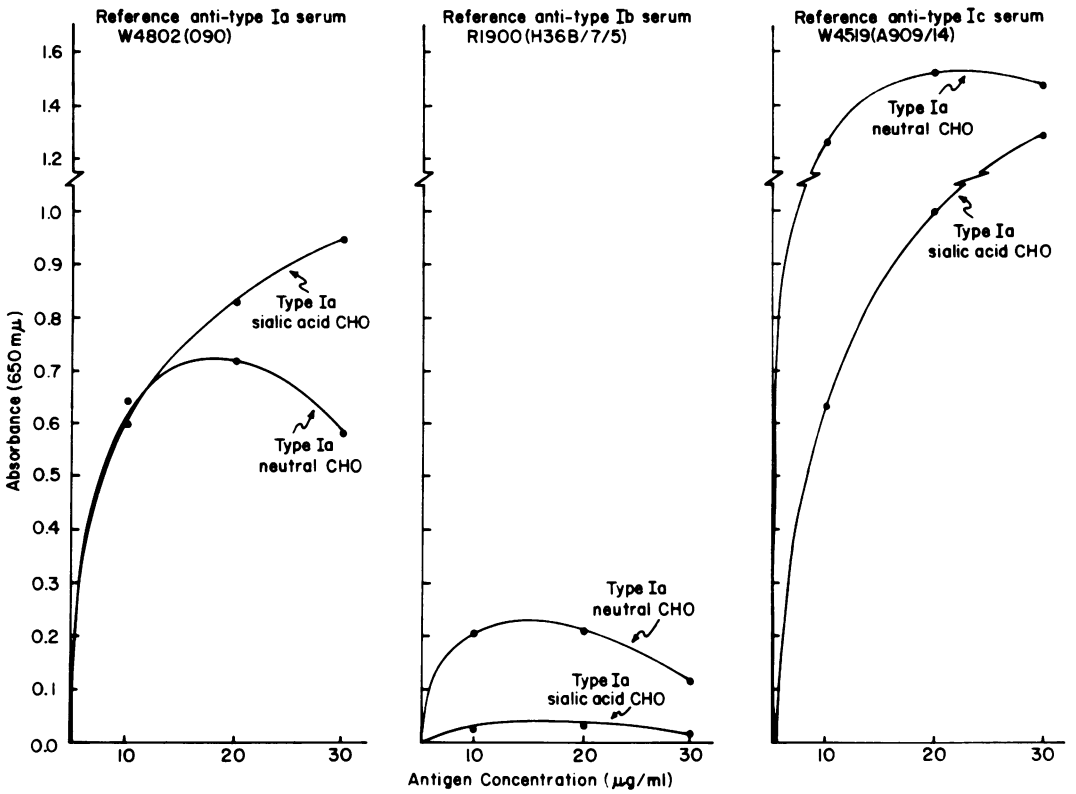

FIG. 8. *Quantitative precipitin reactions between type Ia sialic acid (sialo) antigen, type Ia neutral antigen, and reference anti-type Ia serum (left), reference anti-type Ib serum (middle), or reference anti-type Ic serum (right).*

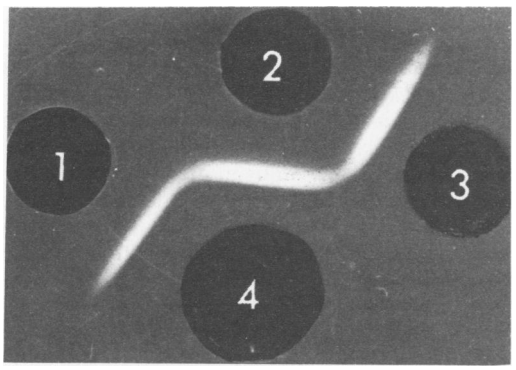

FIG. 9. *Immunodiffusion studies in agar gel between the type Ia asialo antigen, the type Ia neutral antigen, anti-type Ia serum, and anti-type Ic serum. (Well 1) Neutral antigen; (well 2) asialo antigen; (well 3) anti-type Ia serum; and (well 4) anti-type Ic serum.*

The presence of the type Ia neutral antigen in type Ic organisms explains the cross-protection observed in the mouse neutralization studies depicted in Table 1. Type Ia and Ic group B streptococci, both of which carry the type Ia-specific antigen, were each mixed with either type Ia neutral-antigen antibodies or sialo-antigen antibodies and subsequently were injected into mice according to the method described by Lancefield et al. (10). All control animals were killed by both Ia and Ic organisms, whereas all animals injected with either Ia or Ic organisms treated with purified neutral-antigen antibodies or sialo-antigen antibodies were protected against a challenge dose of virulent organisms (Table 1).

DISCUSSION

In this report, the immunochemical relationship between the conventional type-specific or neutral polysaccharide extracted by pH 2.0 buffer and the sialic acid or sialo polysaccharide extracted by pH 7.0 buffer has been explored. The HCl-extracted polymer consists of galactose, glucose, and glucosamine, whereas the sialo polymer contains the same constituents, but in addition possesses immunodominant sialic acid residues. Evidence is also presented suggesting

TABLE 1. *Passive protection tests in mice showing the effects of antibodies directed against the sialic acid and the neutral carbohydrates of type Ia on homologous Ia and heterologous Ic organisms*

Antibody Specificity	Antibody Conc. mg/ml	Injected Organism Group B type	Organism Conc. no./ml	Death Rate no. injected/no. died
Control	Normal serum	Ia	7.0×10^2	5/5
Control	Normal serum	Ic	1.0×10^2	5/5
Sialic acid CHO	0.25	Ia	7.0×10^2	5/0
Sialic acid CHO	0.25	Ic	1.0×10^2	5/0
Neutral CHO	0.20	Ia	7.0×10^2	5/0
Neutral CHO	0.20	Ic	1.0×10^2	5/0

that the sialo antigen represents an unaltered capsular substance and possesses two major immunodominant determinants, terminal sialic acid residues and adjacent galactosyl-linked residues. In addition, the neutral pH 2.0-extracted antigen was shown to represent an asialo version of the sialo antigen. The existence of a sialic acid-galactosyl terminal in the type Ia polysaccharide appears to be reminiscent of the immunodeterminants of the capsular antigen of the type II group B streptococci (2, 8, 9). However, preliminary precipitin analysis has revealed little immunological relationship between the type Ia and type II capsular antigens. Two distinct populations of type-specific antibodies directed against each of the two different immunodeterminants of the type Ia capsule were isolated and purified from antisera derived from rabbits immunized with formalinized type Ia streptococci. In most instances, the antibodies specific for the sialic acid moiety are expressed earlier and persist longer in rabbits than do the corresponding antibodies directed against the galactosyl oligosaccharide.

The results presented in this study are thus similar to those reported by Lancefield and Freimer (2, 8, 9) in which they demonstrated that the type-specific polysaccharide of group B, type II streptococci was either acidic or neutral, depending upon the harshness of the extraction procedure used. The sialic acid residues of the type II antigen, which consisted of sialic acid, galactose, glucose, and glucosamine, were extremely labile to the conditions used in a typical pH 2.0 extraction procedure, and therefore only the neutral carbohydrate was obtained under these conditions. On the other hand, the acidic polymer, which contained sialic acid, was readily obtained by the milder cold-trichloroacetic acid method. In an extension of these observations, the existence of two types of antibodies was observed in rabbits immunized with type II organisms, namely, antibodies directed against the sialic acid residues and antibodies directed against the β-linked galactosyl residues. The complete sialic acid polymer absorbed all the protective antibodies, whereas the neutral polymer absorbed only a portion of the protective antibodies in the antiserum, namely, those antibodies with β-galactosyl specificity.

Several other antigen-antibody systems with similar dual specificities are known. These include the polyglycerophosphate antigen of group A streptococci as described by McCarty, which may exist with or without an ester-linked D-alanine immunodeterminant (12) and the group A and A-variant streptococcal carbohydrates, which possess either an *N*-acetylglucosamine or an adjacent rhamnose immunodeterminant, respectively, as their immunodeterminants (6). The coexistence of two major immunodeterminants on the type Ia antigen represents another of these unusual relationships that have long been suspected in group B streptococci.

Both populations of type-specific antibodies have been shown to be effective in protecting mice against a challenge of type Ia organisms (3, 10). These results suggest that these antibodies have the capacity to promote in vivo phagocytosis in mice. It is therefore possible that the antibodies directed against the Ia capsule may also function as opsonins in man by stimulating human polymorphonuclear leukocytes (PMN). The results of in vitro opsonic studies, using human PMN, suggested that ingestion and intracellular killing of the type Ia organisms by human PMN was strongly influenced by antibodies specific for the sialic acid moieties and for the galactosyl oligosaccharide. On the basis of these results, it is possible that a modified in vitro phagocytic method of Roberts might be useful in quantitating the level of group B streptococcal antibodies in human sera by assessing the interaction of specific group B organisms and human PMN in the presence of human sera (R. C. Lancefield, personal communication). Preliminary in vitro opsonic analyses of several human sera (heated at 56°C for 1 h) indicated that opsonic antibodies directed against type Ia streptococci were present in some of the sera

tested. Although the number of sera examined was small, the results do suggest that normal adults may possess opsonins against type Ia streptococci. For example, one serum had levels of opsonins that were comparable to those of antisera derived from rabbits hyperimmunized with killed type Ia streptococci. Also, some samples had low levels of Ia opsonins, whereas some were almost devoid of Ia opsonic properties. Further work is now in progress to determine the feasibility of using this technique to identify those individuals who may be susceptible to infection by group B streptococci.

This report is a continued effort to better understand the immunology of the type Ia strains and to focus on the intricacies of the immunological problems. Information concerning the specific immunochemical determinants of the group B capsular antigens may shed significant light on the existing complexities of the immunological interrelationship among the five known types of group B streptococci. Elucidation of this interrelationship could lead to a better understanding of the infective properties of these organisms and thus open the way to more effective control of group B streptococcal infections in man and lower animals.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant AI-11598 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Braun, D. G., and R. M. Krause.** 1968. The individual antigenic specificity of antibodies to streptococcal carbohydrates. J. Exp. Med. **128**:969–989.
2. **Freimer, E. H.** 1967. Type-specific polysaccharide antigens of group B streptococci. II. The chemical basis for serological specificity of the type II HCl antigen. J. Exp. Med. **125**:381–392.
3. **Kane, J. A., and W. W. Karakawa.** 1977. Multiple polysaccharide antigens of group B streptococci, type Ia: emphasis on a sialic acid-containing type-specific polysaccharide. J. Immunol. **117**:2155–2160.
4. **Kane, J. A., W. W. Karakawa, and J. H. Pazur.** 1972. Glycans from streptococcal cell walls: structural features of a diheteroglycan isolated from the cell wall of *Streptococcus bovis*. J. Immunol. **108**:1218–1226.
5. **Kesler, R. B.** 1967. Rapid quantitative anion-exchange chromatography of carbohydrates. Anal. Chem. **39**:1416–1422.
6. **Krause, R. M.** 1963. Symposium on the relationship of structure of microorganisms to their immunological properties. IV. Antigenic and biochemical composition of hemolytic streptococcal cell walls. Bacteriol. Rev. **27**:369–380.
7. **Lancefield, R. C.** 1938. Two serological types of group B hemolytic streptococci with related, but not identical, type-specific substances. J. Exp. Med. **67**:25–40.
8. **Lancefield, R. C.** 1972. Cellular antigens of group B streptococci, p. 57–65. *In* L. Wanemaker and J. M. Madsem (ed.), Streptococci and streptococcal diseases: recognition, understanding, and management. Academic Press Inc., New York.
9. **Lancefield, R. C., and E. H. Freimer.** 1966. Type-specific polysaccharide antigens of group B streptococci. J. Hyg. **64**:191–203.
10. **Lancefield, R. C., M. McCarty, and W. N. Everly.** 1975. Multiple mouse-protective antibodies directed against group B streptococci. Special reference to antibodies effective against protein antigens. J. Exp. Med. **142**:165–179.
11. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
12. **McCarty, M.** 1964. The role of D-alanine in the serological specificity of group A streptococcal glycerol teichoic acid. Proc. Natl. Acad. Sci. **52**:259–265.
13. **McCarty, M., and R. C. Lancefield.** 1955. Variation in the group-specific carbohydrate of group A streptococci. I. Immunochemical studies on the carbohydrates of variant strains. J. Exp. Med. **102**:11–28.
14. **Partridge, S. M.** 1948. Filter paper partition chromatography of sugars. I. General description and application to the qualitative analysis of sugars in apple juice, egg white, and foetal blood of sheep. Biochem. J. **42**:238–248.
15. **Roberts, R. B.** 1970. The relationship between Group A and Group C meningococcal polysaccharides and serum opsonins in man. J. Exp. Med. **131**:499–513.
16. **Roth, H., S. Segal, and D. Bertoli.** 1965. The quantitative determination of galactose—an enzymic method using galactose oxidase, with applications to blood and other biological fluids. Anal. Biochem. **10**:32–39.
17. **Scheidegger, J. J.** 1955. Une micromethod de l'immunoélectrophorèse. Int. Arch. Allergy Appl. Immunol. **7**:103–110.
18. **Spackman, D. H., W. H. Stein, and S. Moore.** 1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. **30**:1190–1206.
19. **Spiro, R. G.** 1966. Analysis of sugars found in glycoproteins. Methods Enzymol. **8**:3–26.
20. **Warren, L.** 1959. The thiobarbituric acid assay of sialic acids. J. Biol. Chem. **234**:1971–1975.
21. **Weber, P., and R. H. Winzler.** 1969. Determination of hexosaminitols by ion-exchange chromatography and its application to alkali glycosidic linkages in glycoproteins. Arch. Biochem. Biophys. **129**:534–538.
22. **Wilkinson, H. W.** 1975. Immunochemistry of purified polysaccharide type antigens of group B streptococcal types Ia, Ib, and Ic. Infect. Immun. **11**:845–852.
23. **Wilkinson, H. W., and R. G. Eagon.** 1971. Type-specific antigens of group B type Ic streptococci. Infect. Immun. **4**:596–604.
24. **Wilkinson, H. W., and M. D. Moody.** 1969. Serological relationships of type I antigens of group B streptococci. J. Bacteriol. **97**:629–634.