Seroepidemiology of group B streptococcus type II antibody specificity

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SUMMARY

The specificity of human antibodies for the two major sidechain determinants of the type II group B streptococcal (GBS) polysaccharide was examined in 90 pairs of maternal and cord sera. Using an ELISA system, total antibody was measured against the complete (sialylated) type II antigen and the proportion of antibody against the galactose determinant was estimated by inhibition with free β -methylgalactopyranoside. Mothers colonized by type II or by other GBS types had higher levels of total specific antibody (means, 3.3 and 4.7 $\mu\text{g/ml}$, respectively) than those not colonized (mean, 2.2 $\mu\text{g/ml}$). Cord sera averaged 1-2 $\mu\text{g/ml}$ lower than maternal sera. Colonization with GBS was also associated with higher levels against the galactose determinant (mean, 1.5 $\mu\text{g/ml}$, compared to 0.7 $\mu\text{g/ml}$ for those not colonized). The distribution of specificities favoured antibodies against the sialic acid determinant in maternal but not cord sera. Specificity as well as antibody level may play a role in the epidemiology of GBS type II.

INTRODUCTION

The group B streptococcus (GBS) type II is an important cause of perinatal infections in both mothers and infants (Wilkinson, 1978; Gray & Dillon, 1985). Antibody against the type-specific capsular polysaccharide is thought to play a role in host defence, but few data are available on type II antibody levels in humans, and there is no information regarding the specificity of human antibodies against the known antigenic determinants of this complex capsular antigen. The type II polysaccharide is unique among the group B streptococci in having two different single sugar determinants, a galactose residue and a sialic acid (*N*-acetylneuraminic acid, NANA) linked directly to the backbone of the polymer (Jennings *et al.* 1983). The presence of these two major determinants was first suggested by Lancefield and Freimer, who showed that rabbits immunized with type II made two populations of antibodies: one precipitated only with the complete (sialylated) antigen, while the other precipitated with either the complete antigen or an incomplete (desialylated) antigen prepared by hot acid

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extraction (Lancefield & Freimer, 1966). The importance of the NANA sidechain in the complete antigen was not recognized until later years (Lancefield, 1972), but the chemical basis for the serological specificity of the incomplete antigen was surmised from the observation that precipitation by certain antisera could be inhibited by galactose and various β -galactopyranosides (Freimer, 1967).

Of course, it is simplistic to speak of a 'galactose determinant', inasmuch as the actual determinant probably consists of several sugars in a precise conformation in the tertiary structure of the polymer. Nevertheless, the identification of distinct specificities for GBS antigens has proved to be especially useful. Antibodies against the complete, but not the incomplete, type III GBS polysaccharide antigens have been shown to be protective in mice (Egan *et al.* 1983; Pritchard *et al.* 1988). With type II, however, rabbit antibodies against the two determinants were equally effective in protecting mice from an otherwise fatal challenge with live type II organisms (Lancefield & Freimer, 1966). Antibodies against either may also be protective in humans, but direct evidence is lacking. We have previously reported seroepidemiological studies of IgG antibodies against type II GBS in maternal and cord sera (Gray, Pritchard & Dillon, 1985). In this investigation we examined the specificity of the type II antibodies in a similar population.

MATERIALS AND METHODS

Ninety maternal and cord serum pairs were obtained at delivery from patients in the obstetric and newborn services at Cooper Green Hospital, located within the University of Alabama Medical Center at Birmingham, Alabama. The patient population was similar to that described in our earlier report (Gray, Pritchard & Dillon, 1985) but drawn from years 1982-3 of the 6-year prospective study (Dillon, Khare & Gray, 1987). Cultures for GBS colonization were taken from infants within an hour of birth and processed using selective media (Gray, Pass & Dillon, 1979; Dillon *et al.* 1982). The patients were divided into three groups on the basis of colonization with GBS: 29 were colonized by GBS type II, 31 were colonized by other GBS serotypes, and 30 were not colonized.

Antibodies against complete GBS type II antigen were measured by an ELISA method similar to that previously described (Gray, Pritchard & Dillon, 1985), except that the assays were done entirely in plastic microtiter wells (Dynatech Laboratories, Alexandria, VA) and an anti-F(ab')₂ conjugate was used to detect antibodies of all classes (Gray, Springfield & Dillon, 1987). The GBS type II polysaccharide was prepared from Lancefield strain 18RS21 by mutanolysin extraction essentially as described by DeCuenink *et al.* (1982). Antigen was coupled to poly-lysine (Gray, 1979) and dispensed in 300 μ l volumes into the wells of microtiter plates, allowed to adsorb overnight, rinsed with water, and kept filled until use with 0.1% (w/v) bovine serum albumin in phosphate buffered saline (PBS, 0.05 M phosphate, 0.1 M-NaCl, pH 7.4).

The amount of antibody specific for the galactose sidechain was estimated by performing assays with and without preincubation of serum samples for 30 min with 1-O-methyl- β -D-galactopyranoside (β -MeGal, Sigma Chemical Co., St Louis, MO) in PBS containing Brij-35 (0.05% (w/v) Aldrich Chemical Co., Atlanta, GA). The amount of β -MeGal required for inhibition of galactose-specific antibodies was

Fig. 1. Serum from two patients was measured for antibody against the complete GBS type II antigen in the ELISA in the presence of increasing concentrations of β -methylgalactopyranoside (β -MeGal). Serum from patient A was not inhibited; serum from patient B was strongly inhibited, indicating that about 75% of antibodies were against the galactose determinant of the type II polysaccharide. A β -MeGal concentration of 10 mg/ml was selected for routine use in this study.

determined for six sera, two of which are illustrated in Fig. 1. The greatest inhibition of antigen-antibody binding was accomplished at a β -MeGal concentration of 10 mg/ml. The proportion of antibodies against the galactose determinant was calculated as the percent reduction of binding in the presence of β -MeGal, relative to that in plain buffer. For example, in Figure 1 the absorbance value for serum B was reduced from 0.33 to 0.08, a 76% decrease in binding.

To determine the optimal incubation time for the assay, selected sera were incubated in buffer with 10 mg/ml β -MeGal for 1–3 h prior to adding the second antibody. This is illustrated in Fig. 2. As the incubation time was increased, there was less apparent inhibition by β -MeGal, presumably because of greater avidity for the complete antigen compared to that for the single sugar inhibitor. For the purposes of this study, an antibody incubation time of 1.5 h was used. The relative proportion of antibody bound in the presence of β -MeGal was assumed to approximate the proportion directed against the NANA determinant of the type II antigen. The proportion of antibody inhibited by β -MeGal approximated to that directed against the galactose determinant. Two dilutions of serum, usually 8 and 4 μ l per 300 μ l well, were added to antigen coated microwells, after preincubation for 1.5 h, unbound antibody was washed out, and the wells were filled with 300 μ l of β -galactosidase-conjugated goat anti-human F(ab')₂ (Southern Biotechnology Associates, Inc., Birmingham, AL). This conjugate detected IgG, IgM, and IgA equally well on a weight basis. After 2 h the wells were again washed, and 300 μ l of *o*-nitrophenyl- β -D-galactopyranoside substrate (Sigma) was added at a concentration of 1 mg/ml in 0.01 M Tris buffer, pH 7.5, containing 0.01 M concentrations of NaCl, MgCl₂, and 2-mercaptoethanol. The enzyme conjugate did not interact with galactose determinants of the antigen. The change in absorbance was measured after 15 min at 414 nm with an eight channel

Fig. 2. Inhibition of human antibodies against GBS type II by 10 mg/ml β -methylgalactopyranoside (β -MeGal) was examined as a function of incubation time in the ELISA. As incubation time increased the proportion of antibody inhibited by the free β -MeGal decreased, presumably because of greater avidity for the complete type II antigen on the microwells. An incubation time of 1.5 h was selected for routine use in this study.

photometer (Titertek Multiskan; Flow Laboratories, McLean, VA). Results, expressed as μ g of antibody protein per ml, were obtained from a standard curve constructed for IgG antibody independently measured against a pneumococcus type 19 antigen by quantitative precipitation (Gray, Pritchard & Dillon, 1985). In the present assay, values for the two dilutions were averaged. Sera were further diluted as necessary to bring both values within the limits of the standard curve, but were considered to be zero if less than 0.2 μ g/ml at the 8 μ g/ml dilution.

RESULTS

Fig. 1 illustrates that the binding of antibodies to type II GBS could be effectively blocked by preincubation with β -MeGal in some sera but not in others. This was interpreted to indicate that patient A made essentially no antibody against the galactose determinant, whereas 76 percent of antibodies in the sera of patient B could be inhibited by β -MeGal. The distributions of antibody specificities in maternal and cord serum pairs with detectable antibody levels are summarized in Table 1, according to whether or not they were colonized with GBS. Few mothers had more than 75% of the antibodies to type II GBS inhibited by β -MeGal, and the majority had less than half of their antibodies directed against the galactose determinant. The distribution departed considerably from that expected by the χ^2 test for goodness of fit. The specificities of cord sera were fairly evenly distributed, except for a larger than expected proportion of samples among non-colonized infants that could be inhibited more than 75% by β -MeGal.

Antibody levels in the 90 maternal and cord sera pairs are summarized in Table 2. Mean antibody concentrations against the complete type II antigen were 3.3 μ g/ml for mothers colonized by type II and 4.7 μ g/ml for those colonized by other

Table 1. *Distribution of antibody specificities against GBS type II in maternal and cord sera*

Colonization	No. of sera with detectable antibody	No. of patients with given percentages of antibodies inhibited by β -MeGal†				<i>P</i> -value‡
		< 25 %	25-50 %	50-75 %	> 75 %	
By type II:	29 Maternal	5	11	11	2	< 0.02
	29 Cord	6	6	7	10	NS
By other types:	30 Maternal	13	11	4	2	< 0.01
	28 Cord	9	8	4	7	NS
Not colonized:	30 Maternal	15	8	5	3	< 0.01
	25 Cord	8	4	2	11	< 0.02

† Estimate of antibodies directed against the galactose determinant (see text).

‡ χ^2 for goodness of fit.Table 2. *Levels of antibodies against GBS type II in maternal and cord sera*

Colonization	No.	Antibody to the complete antigen μ g/ml			Antibody inhibited by β -MeGal			Antibody inhibited (%)
		Mean	1 s.d.	(highest)	Mean	1 s.d.	(highest)	
By type II:	29 Maternal	3.3†	2.2	(8.2)	1.5‡	1.2	(3.8)	45
	29 Cord	2.0†	1.9	(7.9)	0.9	1.0	(3.0)	45
By other types:	31 Maternal	4.7†	5.7	(30.7)	1.5	2.3	(11.1)	32
	31 Cord	1.8†	1.7	(7.9)	0.5	0.5	(2.1)	28
Not colonized:	30 Maternal	2.2	1.5	(6.6)	0.7	0.7	(2.8)	32
	30 Cord	0.9	1.0	(4.4)	0.3	0.3	(1.2)	33

† Higher than for those not colonized (Student's *t* test, *P* < 0.05).

‡ Estimate of antibody directed against the galactose determinant (see text).

GBS types. Levels for non-colonized mothers were significantly lower (2.2 μ g/ml; *P* < 0.05). Antibody in cord sera averaged 1-2 μ g/ml lower than the maternal levels, presumably because cord sera contained predominantly IgG, whereas maternal sera may also have IgM and IgA antibodies. Mothers colonized by type II and those colonized by other types averaged 1.5 μ g/ml against the galactose determinant, about twice the level of those not colonized.

DISCUSSION

These data show that humans make antibodies to both major determinants of the GBS type II polysaccharide. In most people, somewhat more than half of their total antibody against the type II antigen was directed against determinants that were not inhibited by the presence of β -MeGal in the assay system. These antibodies were presumably against the NANA determinants. Using mouse monoclonal antibodies against the NANA and galactose determinant, we have shown that the binding of antibodies of one specificity precludes the binding of antibodies of the other specificity, apparently by competing for distinct binding sites within a combining area that includes both determinants (Gray, Egan &

Pritchard, 1988; Pritchard *et al.* 1988). We also found that oligosaccharide and mucin components of human milk and saliva inhibited the binding of antibodies to the galactose determinant (Gray *et al.* 1987). This raises the possibility that some antibodies in human secretions may bind to oligosaccharide components of the secretions and may not be available for binding to bacteria. It is also conceivable that such antibodies have a beneficial role in binding simultaneously to both bacteria and mucin, thus fixing organisms to the mucus layer and preventing invasion of the epithelia. The mouse monoclonal antibodies against the NANA sidechain were not inhibited with physiologic concentrates of secretions. Human antibodies to this determinant may be especially critical in defending against GBS type II. Although antibody levels in human saliva and vaginal secretions do not correlate exactly with serum levels, or with colonization status, we have detected antibodies against GBS type II in both saliva and vaginal secretions of healthy young women (Gray *et al.* 1987).

We used β -MeGal to inhibit antibodies to the galactose determinant rather than attempting to measure these antibodies directly with desialylated antigen in the ELISA. The type II polysaccharide is not susceptible to common bacterial neuraminidases (Jennings *et al.* 1983), and acid hydrolysis does not result in a uniformly desialylated antigen without some concomitant damage to the polymer. By using complete antigen in the solid phase for both determinants we avoided the problem of structural or conformational differences inadvertently introduced by desialylating the antigen. The use of a β -galactopyranoside inhibitor was similar in concept to methods employed by Freimer to identify the galactose determinant in precipitation experiments with rabbit antisera (Freimer, 1967). We could thus be fairly sure of the specificity of antibodies inhibited by β -MeGal, although there might remain some question about antibodies not inhibited. In more recent studies of rabbit antisera raised against GBS type II, we found that certain rabbits made responses that included antibodies which bound to both the complete and the non-sialylated antigen but were not inhibited by β -MeGal (Gray *et al.* 1988). We suspect that these antibodies may be directed against determinants on the backbone of the polymer. We have not yet examined human sera for such antibodies, but should they exist, their role in protection would require further study.

This investigation differs from our previous seroepidemiologic study of GBS type II (Gray, Pritchard & Dillon, 1985) in that here we measured total antibody, rather than IgG alone. Levels were somewhat higher in maternal but not cord sera, probably because IgM and IgA antibodies were also being detected. In a study of naturally occurring antibodies against GBS type III, IgM was found to be the major isotype of antibodies in maternal sera (Anthony *et al.* 1984). We have yet to examine these sera to see if the response to GBS type II is similar, and if there is any isotypic preference for either of the two major specificities. Antibodies against the NANA determinant were predominant in more maternal than cord sera, but the similarity in the mean proportion of galactose-inhibitable antibodies in maternal and cord sera would argue against a strict isotype restriction.

Other host differences, including IgG subclass distribution and the carbohydrate composition of secretions, may need to be examined in light of the epidemiologic characteristics of GBS type II. In the United States this type predominates in

adult infection, including meningitis and perinatal disease; it is important in neonatal bacteraemia but seldom causes meningitis or 'late-onset' disease in infants (Wilkinson, 1978). For reasons we do not completely understand, disease rates in Europe and the United Kingdom are considerably lower for GBS of all serotypes (Mayon-White, 1985). In our earlier seroepidemiologic study of GBS type II (Gray, Pritchard & Dillon, 1985) we found that the prevalence of IgG antibody was quite low, only about 6% of mothers were estimated to have more than 2 µg/ml IgG against type II. We also found that 5 of 23 patients with systemic type II infections had IgG antibody levels more than 2 µg/ml. Antibody 'deficiency' was widespread and did not adequately account for the risk of infection. The specificity of a patient's antibodies, along with the presence of structurally similar substances in secretions, are factors that require further investigation. Antigenic specificity as well as antibody level, may well play an important role in GBS type II colonization and in the development of disease.

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