Diversity of surface protein expression in group B streptococcal colonizing & invasive isolates

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Background & objectives: The classification of group B streptococcal (GBS) isolates is based on the capsular polysaccharides (Ia-VIII), and antigenic characterization of clinical isolates is augmented by the detection of various surface-localized protein antigens. In our laboratory, all GBS isolates are routinely analysed for the alpha trypsin-resistant and the beta trypsin-sensitive c protein antigens, as well as other trypsin-resistant proteins R1, R3, and R4, as well as BPS. The purpose of this work was to study diversity of protein expression in colonizing isolates (vaginal and rectal sites) from non-pregnant women and from invasive isolates (blood or CSF) from mothers and their less than seven day old newborn infants.

Methods: A total of 289 invasive isolates and 2660 colonizing isolates were collected between 1993-2002. All isolates were tested for polysaccharide serotype and cell surface-expressed protein profile by double immunoprecipitation in agarose using monospecific antisera.

Results: Among the 289 invasive isolates, 89.6 per cent expressed one or more trypsin-resistant proteins; 93 per cent of the colonizing isolates expressed one or more of these proteins. Overall, the most common surface protein expression profile by GBS serotype was: alpha in type Ia; alpha plus beta in type Ib; alpha and R4 in type II; R4 in type III; and co-expression of R1 plus R4 in isolates of type V. BPS was found in five (1.7%) invasive isolates, alone in two isolates and with other proteins in three isolates. Among 2660 colonizing isolates, BPS was found alone in 15 (0.6%) and in 57 additional isolates with other proteins. Among the total isolates, BPS was found predominantly in serotype Ia isolates, also expressing R1. Uncommon protein profiles of known serotypes included 11 type III isolates expressing alpha plus beta. Among 72 nontypable colonizing isolates, expression of R1 plus R4 was the commonest (33.3%) profile.

Interpretation & conclusion: The GBS surface proteins and the common serotypes were distributed comparably in colonizing and invasive isolates. Trypsin-resistant, alpha and alpha-like proteins, R1 and R4 were the most prevalent. The phenotypic diversity of the surface-localized protein antigens of GBS is intriguing, and genotypic analysis will permit consensus in nomenclature from laboratory to laboratory.

Key words Alpha protein - GBS serotypes - R proteins

The importance of group B streptococci (GBS) as a major perinatal pathogen for invasive disease has been well documented1-3. There are now nine distinct capsular polysaccharide (CPS) types of GBS, designated Ia, Ib, II, III, IV, V, VI, VII, VIII1,4,6. In addition to the CPS types, we have investigated basic immunologic, and phenotypic characterization of surface localized protein antigens of GBS, such as the c protein, comprising the alpha (trypsin-resistant) and beta (IgA binding and trypsin susceptible) antigens that are commonly found in GBS types Ia, Ib, and II12. Other trypsin-resistant proteins, known as R proteins10,11,13,14 have been described by
us originally in types II and III GBS. The R proteins have been found to occur, with some exceptions, independently of the c protein. We have also demonstrated that some molecular species of the R proteins occur in one of the more newly described CPS types, i.e., type V.

Characterization of the surface protein markers of the GBS clinical isolates is important since there is evidence that the c protein, particularly the alpha protein, as well as other trypsin-resistant proteins that resemble the alpha protein, may contribute to strain differences in virulence, are immunogenic, and antibodies to them may be protective. The designation of other alpha-like repetitive molecules, such as Alp2 and Alp3, is under study, but at this time we shall refer to them as the R proteins, of which R1 and R4 are the major ones found in the clinical GBS isolates. The Rib protein described by Wästfelt et al. has been thought by us and Norwegian investigators to be the same as R4 protein, based on epidemiological and antigenic studies, but recently by means of gene sequencing we demonstrated that the R4 protein is identical to the sequence of Rib. The gene sequence of R5 surface protein has been reported, as well as, data indicating that antibodies to this surface protein are protective, and this protein has been renamed group B streptococcal protective surface protein (BPS).

**Material & Methods**

**GBS clinical study isolates:** A total of 2949 GBS isolates collected between 1993 and 2002 were examined. The 289 invasive isolates were collected in Houston, Minneapolis-St. Paul, Pittsburgh and Seattle from blood or cerebrospinal fluid (CSF) of mothers or newborn infants with early-onset disease while the 2660 colonizing isolates were collected in Pittsburgh from rectal (R) or vaginal (V) cultures of non-pregnant women. Isolates were sent on blood agar slants to the GBS Molecular Reference Laboratory at the University of Minnesota where they were stored frozen at -30°C in Todd-Hewitt blood broth until they were serotyped.

**Prototype strains and typing antisera:** The prototype strains, with their original source designations, used as controls were from the University of Minnesota collection and are as follows: group B: O90R (Lancefield); Ia: S3-3630P (U of M); Ib: H36B (Lancefield); II: 18RS21/19/2 (Lancefield); III: M781 (Houston); IV: Wilkinson 3139 (Rabinowitz, Israel); V: SS-1169 (Prague); VI: SS-1214 (Prague); VII: Perch “7271” (Prague); VIII: JM9-130013 (Boston/Prague); c protein (alpha and beta components): A909 (Lancefield); alpha only: 80-426 (U of M); beta only: 81-418 (U of M); R1: D136C (Lancefield); R3, R4, and BPS (group B protective surface protein): Compton 25/60 (Prague). The typing antisera, prepared as we have described before by immunizing rabbits with formalin-killed whole bacterial cells, were made monospecific by cross-absorption.

**Antigen extraction and serotyping by immunoprecipitation in agarose:** Testing for the polysaccharide serotype and cell surface-expressed protein profile of each isolate was done, using a hot HCl extract prepared from the cell pellet of overnight growth in Todd-Hewitt broth. Extracts were tested by double immunoprecipitation in agarose using the monospecific antisera to GBS capsular polysaccharide types Ia, Ib – VIII and to the c (alpha and beta components) and R (R1, R3, R4 species) proteins. The extracts also were tested with antiserum to the group B surface protective protein (BPS, formerly known as R5). Extracts of control prototype strains were included in all tests and positive identification was based on observing a precipitin band with a reaction of identity between the extracts of the clinical and the control isolates.

**Results**

Serotypes Ia, III, and V were the three most common serotypes recovered from nonpregnant women, followed by serotype II. There were a few isolates of serotypes IV, VII, and VIII identified. A low number of the isolates, 2.2 per cent, were nontypable (Fig.1). Among invasive isolates, serotypes Ia, III, and V also were the most prevalent. There was a single isolate of serotype VII and three isolates (1.0%) were nontypable (Fig.1).

The protein expression profiles of these 2660 GBS colonizing isolates (Fig.2) revealed that approximately 92.6 per cent expressed one or more of the trypsin-
resistant proteins. The strains expressing BPS with another surface protein, usually the alpha protein were also included. The most common pattern was expression of alpha alone for 40.5 per cent or R4 alone for 27.3 per cent. Only 6.6 per cent of the isolates expressed none of the above described surface proteins. Among the 289 invasive isolates a similar distribution of surface protein expression was seen when compared to that of the colonizing isolates, and 88.9 per cent expressed one or more trypsin-resistant proteins. Among these isolates 10.4 per cent did not express the proteins that were examined phenotypically. For both colonizing and invasive isolates, BPS expression was uncommon. The IgA binding beta protein was expressed in only a minority of invasive and colonizing isolates, 8.6 and 10.6 per cent, respectively.

The predominant surface protein expressions among the colonizing and invasive isolates, respectively, were alpha alone for 92 and 97 per cent in serotype Ia isolates; alpha plus beta proteins for 86 and 81 per cent for serotype Ib; alpha only in 64 and 57 per cent of

Fig.1. Comparison of CPS serotype distribution between invasive and colonizing isolates of group B streptococci. NT, nontypable.

Fig.2. Cell surface proteins expressed by invasive and colonizing isolates of group B streptococci. BPS, group B streptococcal protective surface protein.
serotype II; R4 for 95.6 and 96 per cent in serotype III; and R1 plus R4 for 71.4 and 53.8 per cent of serotype V.

The surface-localized protein antigens were also studied among 72 nontypable isolates and 79.2 per cent expressed one or more of the trypsin-resistant proteins, with a predominance of R1 and R4 proteins in one-third of these isolates.

**Discussion**

Prior to our studies over the past decade, isolates from neonates with early-onset infections were found to be evenly divided among serotypes I, II, and III and the majority of those with early-onset sepsis/meningitis and the vast majority of those from late-onset infection were caused by serotype III1-5. In our prospective surveillance studies, we have shown that there has been a shift with the current CPS types causing early-onset maternal and infant infection to be serotypes Ia, III, and V1-5. Serotype V has emerged in the United States and Europe not only as a cause of GBS genital colonization in nonpregnant women, but it is a prominent cause of early-onset invasive disease in newborn infants and is the major serotype recovered from invasive infections in nonpregnant adults1-5,15. The reason for the emergence of serotype V over the past decade is unclear, but this is a reminder that more newly described GBS polysaccharide serotypes, *i.e.*, VIII, may emerge and replace the currently defined serotypes. For example, serotype VIII is a predominant cause of GBS colonization in women, as well as a pathogen for newborn infants in Japan6.

Our laboratory was the first to describe the ladder-like appearance of the alpha protein by Western immunoblotting (WB)4,11. We also described the ladder-like effect of the R1 and R4 proteins by WB4,11. The genetic basis for this observation was studied by Michel *et al*28, documenting the presence of tandem repeating units in the alpha antigen gene. The alpha protein is the prototype for comparison among these trypsin-resistant proteins, which also include Alp2 and Alp3 that may be highly related to R1 and R4 proteins20. Antigenic diversity, as well as similarity, within this family of proteins is startling, and it may be due to genetic recombination.

The great similarities in cell surface protein expression profiles between the colonizing isolates and the invasive GBS isolates suggest that there may be no single protein that enhances for virulence of the organism. Similarly, although the women were not the mothers of the infected newborn infants, the CPS types were similar among the colonizing and invasive isolates. The higher prevalence of nontypable isolates among the colonized women compared to rare nontypable invasive isolates, may reflect loss of capsule in the genital tract of women colonized for long periods.

The dual presence of R1 and R4 proteins in GBS isolates, predominantly in type V was first described in our laboratory4,15. The R4 protein has been studied extensively in our laboratory at the phenotypic, as well as molecular level10,11,23. We have demonstrated its immunogenicity in rabbits and humans and the transplacental passage of anti-R4 antibody from mothers to infants16. In the present study, R4 was seen alone primarily in serotype III (>95%), and in serotype II (20-22%) and in serotype V (54-75%) either alone or in combination with R1. More recently, Smith *et al*23 sequenced the R4 gene from a clinical isolate of serotype III and showed that the nucleotide sequence was identical to the *rib* gene.

Over the past few years we have discovered uncommon protein profiles among GBS of known CPS type with patterns never described before. For example, we have found the presence of the R1 and R4 proteins in CPS type Ib, and the alpha and beta proteins, expressed without R4, in serotype III (Ferrieri P, and Flores AE, unpublished observations). It is possible, that significant genetic exchange is occurring within the genital tract of women, particularly in the presence of simultaneous colonization with more than one serotype, and lengthy periods of colonization, which may enhance for these potential genetic events.

The importance of characterizing, at an antigenic level, these other epidemiological protein markers of GBS is that they may play a future role in development of capsular polysaccharide protein conjugate GBS vaccines2. The alpha protein alone17 or alpha protein in combination with Rib protein18 has shown immunogenicity and protection in infant mice. Examples of other highly conserved surface-localized proteins
(expressed by 100% of strains tested) include the C3 binding protein\(^2\) and surface immunogenic protein Sip\(^3\), which may hold promise as future vaccine candidates.

In summary, the GBS surface proteins and the common serotypes were distributed comparably in large numbers of invasive and colonizing isolates studied. Trypsin-resistant proteins, alpha and the alpha-like proteins, R1 and R4, were the most prevalent. Nontypable GBS isolates frequently express these proteins, which can serve as molecular epidemiological markers in the absence of a defined capsular polysaccharide antigen. Ultimately, resolution of phenotypic similarities among these surface proteins will be determined by DNA sequencing. Combinations of these protein, with or without polysaccharides, may prove valuable as GBS vaccines.

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**References**


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