OCCASIONAL REVIEW

GROUP-B STREPTOCOCCUS—PROFILE OF AN ORGANISM*

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Introduction and historical review

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Nocard and Mollereau (1887) were the first to describe the isolation of streptococci from bovine mastitis and these organisms were likely to have been the group-B streptococci (GBS). Many years elapsed, however, before these streptococci featured again in the literature. In 1933, Rebecca Lancefield divided streptococci into groups on the basis of carbohydrate antigens in the cell wall; her classification scheme was based on precipitation reactions of hot-hydrochloric-acid extracts of whole organisms (Lancefield extracts) with antisera raised in rabbits against whole-cell formalinised streptococci. These classical studies clarified the taxonomy of streptococci to a considerable degree because the cultural and biochemical patterns that had been used before 1933 had added little to streptococcal identification.

The streptococci that Lancefield described as group B were isolated from bovine and dairy sources. In 1934 she divided GBS into types I, II and III and in 1938 subdivided type I into Ia and Ib. All the antigens were polysaccharides. Pattison, Matthews and Maxted (1955) added to the serological profile of GBS by describing two protein antigens, R and X. They also stated that GBS from human and bovine sources were not similar and this was corroborated by Butter and de Moor (1967). Wilkinson (1975) discovered a third protein antigen and called it Icp. It is generally referred to as the Ic protein and is usually found in strains with the Ia or Ib polysaccharide. Strains possessing the Ia and Ic antigens are said to belong to type Ic. The Icp antigen may be found in strains with the II or the III polysaccharide or without a detectable polysaccharide antigen. With serological tests that identify carbohydrate and protein antigens, many combinations of antigens are found—Ia, Ib, Ic, Icp, II, II/R, II/X, II/Icp, III, III/R, III/X, III/Icp, Ia/Rp, Icp/Xp, Rp, Xp.

After Lancefield's recognition of GBS, sporadic reports of their clinical significance for man began to appear in the literature. Lancefield and Hare (1935) reported that GBS were often present in the postpartum period and that they were associated occasionally with mild fever. A little later, Colebrook and Purdie (1937) first

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described the isolation of GBS from the blood of a woman with puerperal septicaemia and Fry (1938) isolated these bacteria from three fatal cases of postpartum infection in a maternity hospital in London.

During the next 14 years, interest in GBS appeared to wane and only scanty information exists for that period anywhere in the world. Between 1938 and 1943 only 42 GBS infections were reported in the UK; the large majority were associated with puerperal or post-abortion sepsis and only a very few with the neonatal period.

From 1958 the scene began to change and GBS infections were documented with increasing frequency, particularly in neonates and infants, and during the following years systemic neonatal infection due to GBS was reported in the UK, USA, Holland, Germany and Scandinavia. Infection took one of two forms, either septicaemia that occurred within hours after birth, or meningitis that appeared several days after birth, and by 1973 several workers in the USA, including Franciosi, Knostman and Zimmerman (1973) and Quirante, Ceballos and Cassady (1974) had established that the natural history of these two forms of GBS neonatal infections was quite different.

During the last decade GBS have been the focus of intensive world-wide research and a massive bibliography has accumulated. However, despite the increased awareness of GBS and improvements in laboratory technology, it is generally agreed that this alone would not account for the dramatic increase in GBS infections, and that the increase is real and significant, for reasons not readily apparent at present.

Importance of GBS as human pathogens

GBS cause infections in adults but it is their potential for causing serious disease in neonates, particularly septicaemia and meningitis, that has given cause for alarm in recent years. In the neonate, GBS infections are termed "early" or "late" but these terms are not uniformly defined. Whereas the "early-onset" term for septicaemic infection is correct, because this occurs within several hours of birth, the timing of "late-onset" infection has proved highly arbitrary. It is generally agreed, however, that GBS infection occurring beyond the immediate neonatal period falls into the "late-onset" category. With increasing age and maturity of the infant, late-onset infections are expressed more as localised infection of target sites, such the meninges, bones and joints.

Early-onset infection is an acute, undifferentiated, fulminating sepsis during the first 2 days, and generally within 24 h of birth. As with meningococcaemia, it causes severe shock. The lung is the site of initial and major involvement and in those dying within a few hours of birth the inflammatory response is minimal or absent. In the majority of cases the neonate appears to become infected by aspirating infected vaginal secretions, but GBS can occur in babies delivered by caesarian section, implying that GBS can enter the amniotic fluid through intact membranes. Predisposing factors to this form of GBS infection are low birth weight, prematurity, prolonged rupture of the membranes, a maternal vagina colonised by GBS and the degree of vaginal colonisation.

Purulent meningitis is the prototype of late-onset infection and this can occur in otherwise healthy full-term infants, born to mothers who are not GBS carriers. The organisms are thought to be acquired from other persons in contact with the baby who are GBS carriers, e.g., nursing staff in hospital and, perhaps, colonised babies sharing

the same ward. The time range for these infections can be from several days to months after birth and the mortality rate of 10–20% is considerably lower than the 50–60% associated with early-onset infection. Other later forms of GBS infection in infants can involve almost any area of the body.

Although newborn and young infants are the serious casualties of GBS infections, these also occur in adults, and have a bimodal distribution: a young, healthy female population in which GBS infection is a complication of pregnancy, abortion or of the postpartum period, and the elderly and “compromised host”—the person with some underlying disease or deficiency whether hormonal, nutritional, immunological or metabolic. Eickhoff *et al.* (1964) isolated GBS from gangrenous lesions of eight diabetics and Bayer *et al.* (1976) described a wide spectrum of clinical illnesses in compromised persons, the most common of which were pneumonias, bacteraemic pyelonephritis, endometritis, meningitis and arthritis; despite the severity of many of these infections the mortality rate was only 8%.

Sampling and isolation of GBS

If the neonate is colonised by GBS, the area most likely to yield growth of these organisms is the external ear, therefore this must always be swabbed (Ferrieri, Cleary and Seeds, 1977). GBS may also be isolated from the anterior nares, the umbilical stump and the rectum.

In adults the gastrointestinal tract is the major site of carriage of GBS and the organism can be isolated from the female cervix, urethra, perineum and anorectal areas. It is now accepted that GBS carriage in the female genital tract is a sequel to perineal contamination from the gastrointestinal tract (Badri *et al.*, 1977; Dillon *et al.*, 1982). Critical factors in the isolation of GBS from the adult female are types of swabs used, numbers of areas swabbed and frequency of swabbing; these variables may partly account for the different carriage rates of GBS described in the literature. It has been reported that the use of swabs held in transport media does not favour prolonged survival of GBS and that the yield of organisms is much greater from swabs not held in such media. Plain cotton swabs, albumen-coated and dacron swabs all give satisfactory results (Ross and Cumming, 1979).

The isolation rate of GBS is proportional to the number of areas swabbed. In studies on women attending an antenatal clinic in Edinburgh the isolation rate was 16%. Had the cervix only been swabbed, only 65% of the women would have been identified as carriers, with an isolation rate of 10·3% and if the urethra only had been swabbed the isolation rate would have been 14·4% (Ross and Neilson, 1982). In these antenatal studies it was not possible to obtain rectal swabs, but in a study of GBS carriage in female patients attending a clinic for sexually transmitted diseases, cervical, urethral and rectal swabs were obtained. The rectal swab produced the highest GBS isolation rate (80% of positive patients). Although positive rectal swabs were usually accompanied by a positive cervical or urethral swab, nevertheless 30% of GBS isolations were from the rectal swab alone (Ross and Cumming, 1982).

Laboratory identification of GBS

GBS grow rapidly on media enriched with blood, serum or glucose. After incubation for 24 h, colonies measure c. 1 mm in diameter and appear pearly grey. In

general, colonies are larger than those of group A streptococci and are mucoid and soft. Haemolysis is variable; most human strains produce a narrow zone of β -haemolysis, although this may appear hazy, whereas many bovine strains are either α - or non-haemolytic.

Although GBS grow well on blood agar, the use of a selective medium is usually necessary, particularly for epidemiological purposes. The addition of nalidixic acid 15 mg/L and gentamicin sulphate 6 mg/L to blood agar or a broth medium selects GBS satisfactorily. Recent studies have shown that 30–40% of positive GBS cultures can be missed if selective media are not used (Ross and Cumming, 1982). For a busy service laboratory the most feasible approach is to inoculate solid selective media so that results are obtained on the following day.

Several tests are available for the presumptive diagnosis of GBS. These are sodium hippurate hydrolysis (Ayers and Rupp, 1922; Hwang and Ederer, 1975), the CAMP test (Christie, Atkins and Munch-Petersen, 1942), and pigment production (Fallon, 1974; Islam, 1977). Other tests are less satisfactory; only 5% of GBS are sensitive to bacitracin; 80% are tolerant of a 10% concentration of bile but only 8% of a 40% concentration; none ferments pyruvate. Human strains ferment salicin but not lactose, whereas bovine strains ferment lactose but not salicin.

For definitive identification of GBS, serological tests are required. These broadly fall into two categories: those that require extraction of the carbohydrate antigen, such as agglutination and precipitation tests, counterimmunoelectrophoresis and enzyme-linked immunosorbent assays and those that do not require antigen extraction. Several commercial kits incorporating the principle of latex agglutination and coagglutination are included in this category. An API system (API 20 Strep) is also available, but its use is rarely required for identifying β -haemolytic streptococci.

Epidemiology of group-B streptococcal infection

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During the past 10 years there has been a large number of surveys of GBS carriage in various sites, often with very different results. The method of processing the sample and the number of times the individual is swabbed will affect the observed GBS carriage rate. Baker and Barrett (1973) showed the effectiveness of an enrichment method using Todd-Hewitt Broth supplemented with gentamicin sulphate 8 mg/L, nalidixic acid 15 mg/L and sheep blood 5% v/v. Without blood this medium inhibits GBS. Other antibiotics such as colistin have been substituted for gentamicin (Anthony, Okada and Hobel, 1978). In a survey of GBS carriage in pregnancy that we have completed recently, the use of broth enrichment increased the detected carriage rate from 17% to 28%.

Agar media of the type described by Islam (1977), in which GBS produce a characteristic orange yellow carotenoid pigment on anaerobic incubation, are useful for epidemiological work. Small numbers of GBS can easily be distinguished amongst other colonies and solid media can also be made selective by the addition of antibiotics.

Sites and patterns of GBS carriage

GBS can be found as part of the normal flora in the upper respiratory tract, the gastro-intestinal and genital tracts and on the skin, particularly in the perineal area. Neonates can acquire GBS either from maternal or nosocomial sources within the first few days of life. In those studies in which neonates have been swabbed at birth, the external ear seems to be the most important site (Ferrieri *et al.*, 1977). The nose and umbilical stump are also colonised early, as is the rectum. During the next few days and weeks the importance of the upper respiratory tract as a site of GBS carriage declines and that of the anorectal area increases. The respiratory tract may be important as a route of entry for GBS in the neonate.

In the adult respiratory tract, GBS carriage is uncommon (Baker and Barrett, 1973) although under certain circumstances it may be significant (Easmon *et al.*, 1983). The genital tract, perineal skin and gastrointestinal tract are of far greater importance. Although the female genital tract is the main site from which the neonate acquires GBS, colonisation here probably represents contamination from the perineum and anorectal area (Kexel and Beck, 1965; Badri *et al.*, 1977). The gastrointestinal tract is the major site of GBS carriage. However, there is some debate about the extent of GBS carriage along its length (Anthony *et al.*, 1981; Easmon *et al.*, 1981; Barnham, 1983). Anorectal carriage is certainly the main determinant of patterns of GBS carriage during pregnancy (Dillon *et al.*, 1982; Easmon *et al.*, 1984). This accounts both for the resistance of genital GBS to eradication with antibiotics and for the importance of GBS as urinary-tract pathogens in pregnancy (Wood and Dillon, 1981; Hastings *et al.*, 1984). Carriage rates in pregnancy will depend upon the sites and frequency of sampling. With broth enrichment and the use of rectal and vaginal samples it is possible to predict intrapartum GBS carriage with a high degree of accuracy. Whether screening is cost effective depends upon the rate of infection.

Transmission of GBS in hospital and community

GBS carriage in the anogenital area is associated with sexual activity; high carriage rates are often found among those attending sexually transmitted disease clinics (Embil *et al.*, 1978; Jackson *et al.*, 1982). There is, however, no association between GBS and any particular sexually-transmitted disease. There is a link between GBS and balanitis which deserves further investigation (Jackson *et al.*, 1982).

Family groups appear to carry the same strain of GBS and this may provide the background for late-onset neonatal sepsis (Weindling *et al.*, 1981). Despite considerable circumstantial evidence, Jackson *et al.* (1982) could not confirm sexual transmission of GBS in a study of 92 couples that employed both serotyping and phage typing.

The neonate normally acquires GBS from its mother's genital tract during labour; the heaviness of maternal GBS colonisation and the duration of labour with ruptured membranes increases the likelihood of transmission (Ferrieri *et al.*, 1977). In some circumstances, nosocomial transmission can be an important alternative source of GBS for the neonate (Boyer *et al.*, 1980; Anthony *et al.*, 1981; Easmon *et al.*, 1983). The colonised mother-baby pair is the source of nosocomial spread. Hospital-acquired colonisation has little influence on the subsequent colonisation of the baby once it has left hospital (Easmon *et al.*, 1983).

Conclusions

Twenty to 25% of pregnant women are colonised with GBS at the time of labour; 40–70% of their babies will become colonised and a smaller, variable number become colonised from non-maternal sources. Babies are colonised in the ear and upper-respiratory tract, umbilical stump and rectum. The rectum soon becomes the primary site of carriage and, in the woman, acts as a source of organisms for the genital tract. Although maternal GBS carriage rates and transmission rates to babies seem to be similar wherever studied, infection rates in neonates vary from 0.03/1000 live births in the UK (Mayon-White, 1982) to more than 10 times as high in some centres in the USA. The reasons for such variation are not fully understood.

This cycle of transmission and pattern of carriage accounts for the difficulty experienced in trying to achieve long-term eradication of GBS by antibiotic treatment and for the patterns of GBS infection seen in mother and neonate. Future epidemiological surveys of GBS should use standardised sensitive bacteriological methods with broth enrichment and selective indicator solid media. Systemic short-term intrapartum chemoprophylaxis seems to be an effective way of breaking the cycle of GBS transmission from mother to baby. It does, however, require extensive antepartum screening. An alternative approach that is now being studied is the use of intrapartum topical chlorhexidine.

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Typing methods for group-B streptococci

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Several methods are available for the subdivision of *Streptococcus agalactiae* (Lancefield's group B). Brown (1953) used the reactions in blood agar and sugars to create five varieties or biotypes in this Lancefield group. Human strains, however, fall almost always into the biotype defined by haemolysis on blood agar and failure to ferment lactose. Preliminary experiments (Tagg and Martin, 1980) have suggested that the sensitivity of GBS to bacteriocins could form the basis of a typing scheme but this method has not yet been investigated fully.

Serotyping

Lancefield (1934) used precipitin tests to distinguish three types that she named I, II and III. Later (Lancefield, 1938) by cross-protection experiments in mice and by absorbing different batches of type-I sera, she was able to subdivide the original type I into Ia and Ib. All four antigens are polysaccharides and the type antigens Ia and Ib differ by a single sugar linkage (Kasper and Jennings, 1983). Of 1694 GBS strains typed at the Streptococcal Reference Laboratory, Colindale, 1472 (86.9%) carried one or other of these polysaccharide type antigens.

TABLE
Reactions of vaccine strains with absorbed sera

Type strain	Reaction of the type strains with serum						
	Ia	Ib	Ic	II	III	R	X
Ia (NCTC9993)	+	-	-	-	-	-	-
Ib (NCTC8187)	-	+	+	-	-	-	-
Ic (NCTC11078)	+	-	+	-	-	-	-
II (NCTC11079)	-	-	-	+	-	-	-
III (NCTC11080)	-	-	-	-	+	-	-
R (NCTC9828)	-	-	-	-	-	+	-
X (NCTC9829)	-	-	-	-	-	-	+

Pattison, Matthews and Maxted (1955) found two protein antigens in their collection of bovine strains. One, the R antigen, occurs also in strains of other Lancefield groups (Maxted, 1949). The other, the X antigen, is particularly useful when considering bovine strains (Jensen and Berg, 1981). A third protein antigen was first described among strains carrying the Ia or the Ib carbohydrate (Wilkinson, 1975). When this antigen, the Ic protein (Icp) occurs in strains with the Ia polysaccharide they are called type Ic. The Icp antigen can be subdivided further by the detection of α and β determinants (Bevanger, 1983). The Icp antigen is sometimes called the Ibc protein but it can be found in association with other antigens notably the type II polysaccharide.

The protein antigens can occur alone or with one or other polysaccharide antigen. The combination IIIR, for example, is particularly common. With a set of seven absorbed sera (table), one or two type antigens were detected in 1629 (96%) of the 1694 strains. Attempts have been made to increase further the proportion of strains that can be serotyped but the additional typing sera have restricted usefulness (Jensen and Berg, 1981).

An independent scheme containing 16 serotypes was described by Stableforth (1946) but has fallen into disuse.

Association with disease

One half of 85 strains received at Colindale from patients with meningitis carried the Lancefield III antigen whereas this type formed a quarter of 918 control cultures isolated from superficial sites (Stringer *et al.*, 1981). This association of type III with meningitis has been observed also in other laboratories (Wilkinson, 1978). No additional positive association between serotype and disease was observed in the Colindale data but Jensen (1980) has some evidence that strains carrying the Icp antigen may be more common in the urogenital tracts of patients other than pregnant women.

Phage-typing

A serotyping scheme in which up to one quarter of the strains can fall into a single type lacks discriminatory power and a phage-typing scheme was developed (Stringer,

1980). Bacteriophages were isolated by exposing growing cultures to mitomycin C. The routine typing set of 24 bacteriophages usually produces patterns of lysis of lawns of test strains and so resembles phage-typing schemes for staphylococci.

In all, some 80% of 1700 cultures isolated in the UK were lysed strongly by at least one phage in the set. In a limited investigation employing strains from Nigeria the typability rate was 50% (Stringer, 1980). This suggests that like strains of group-A streptococci those of group B may have a restricted geographical distribution.

Phage-typing is not done without preliminary serotyping. This gives a working total of 94 different sero-phage types (Stringer, 1980). Of these the most frequently observed single pattern is lysis by phage no. 12 of serotype-III strains. In routine use, this result occurred 18 times (6%) in 281 separate incidents. The strains could, however, be subdivided further by testing for the R protein.

Use of sero-phage typing system

The scheme met the needs of the Public Health Laboratory Service survey of neonatal group-B streptococcal diseases (Mayon-White, 1982) but the first published report of its use was in a study of nosocomial acquisition (Anthony *et al.*, 1979). The normal manner of neonatal acquisition is for a colonised baby to carry a single strain of the same sero-phage type as that present in the vagina of its mother (C. S. F. Easmon—this review). A similar pattern is seen with cultures isolated from cases of early-onset infection. During the years 1981 and 1982, for instance, 53 sets of organisms isolated from babies with early-onset infection and their mothers were studied at Colindale. In every instance the paired organisms from mother and baby could not be distinguished because they gave identical patterns. Strains were not received from the mothers of an additional 17 infected babies.

The mother, the baby or both have sometimes left hospital before late-onset infection occurs and of the 27 strains sero-phage typed from such infections during 1981 and 1982, cultures were available from one mother only. Twice during those years two pairs of babies being nursed in special care baby units developed late-onset meningitis within a day of each other. Cultures from within a pair could not be distinguished by sero-phage typing and the possibility of nosocomial infection could not be excluded. Similar infections have been described by Boyer *et al.* (1980).

Apart from the epidemiological studies just described, the combined sero-phage typing scheme has been used for the examination of random isolates, but except for confirming the association of strains of serotype III with meningitis, no clinically relevant information was obtained. The addition of a phage-typing method for GBS to the established serotyping procedures has, however, provided a system that is sufficiently discriminating for the study of transmission of these organisms in hospitals.

The binding of group-B streptococci to epithelial cells

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The importance of adherence of bacteria to mucosal surfaces as a first step in the pathogenesis of many infectious diseases is now well established. This finding has interesting clinical implications, because if a means of blocking bacterial attachment to host cells could be perfected, it would offer a fresh approach to the prevention and treatment of infections. Therapy of this kind would be particularly relevant to GBS disease, because if the interaction between GBS and vaginal epithelial cells could be prevented, it would provide a powerful new approach to the eradication of maternal colonisation with GBS before parturition. The problems encountered with prophylactic antibiotic treatment in this situation are numerous, and there is a continuing problem of neonatal GBS disease acquired by vertical transmission from the mother.

Adherence of GBS to buccal epithelial cells

The results of adherence studies summarised below are from the study of Bagg *et al.* (1982). Attachment of a strain of type-III GBS (NCTC11080) to buccal epithelial cells (BEC) was studied in an in-vitro adherence assay, similar to that described by Gibbons and van Houte (1971). The salient feature of this binding reaction was that the maximum degree of adherence of the GBS to BEC was attained after incubation of the bacteria-epithelial cell mixtures at 37°C for 45 min. Therefore, all mixtures were subsequently incubated for this time and at this temperature.

The first finding of physicochemical interest was that pretreatment of BEC with sodium metaperiodate before the adherence assay significantly reduced the attachment of GBS to the BEC. Periodate treatment of the GBS, however, did not affect their ability to adhere to BEC. Mild heat treatment (75°C for 30 min) of the bacteria did not affect attachment either, but stronger heat treatment (121°C for 15 min) reduced the binding to virtually zero. Trypsin treatment of GBS produced a significant reduction in their subsequent adherence to BEC, whereas neuraminidase treatment of the bacteria caused a highly significant increase in the number of organisms that attached to BEC.

Attachment mechanism was further investigated by studying the adherence-inhibiting properties of a sonicate prepared from type-III GBS. Preparation of the sonicate was a very mild procedure, in which a suspension of GBS was treated in a sonic water bath for 5 min and the bacterial cells centrifuged to leave a clear supernate, termed the "sonicate".

Pretreatment of BEC with the sonicate caused dose-dependent inhibition of subsequent binding of the homologous strain. Heat treatment of the sonicate significantly reduced its ability to block binding, whereas periodate oxidation of the sonicate had very little, if any, effect on its activity. Preparation of the sonicate did not

disrupt the bacterial cells to any marked degree, as shown by the constancy of the chamber count (microscopy) and viable count before and after sonication. However, the attachment of sonicated GBS to BEC was reduced in comparison with that of untreated GBS, indicating some interference with the binding mechanism, possibly by depletion of adhesin.

Because many of the previous results had indicated that a carbohydrate molecule may be the receptor for GBS on the BEC, inhibition studies were performed with a variety of sugars. Type-III GBS were pretreated with D-glucose, D-galactose, L-fucose, methyl-D-mannoside, lactose, maltose, N-acetyl-D-glucosamine and N-acetyl-D-galactosamine. Only N-acetyl-D-glucosamine caused a significant reduction in binding and the response was dose dependent.

The fatty-acid portion of membrane lipoteichoic acid (LTA) has been strongly implicated in the attachment of group-A streptococci to BEC. In view of this, LTA was prepared from type-III GBS (NCTC11080) by cold, aqueous phenol extraction of cell membranes. The extracted polymer was a type-specific antigen and had the chemical composition expected of a lipoteichoic acid-like molecule. However, pretreatment of BEC with this LTS did not significantly reduce subsequent binding of the homologous strain.

Discussion

The results gained from the assays in which whole bacterial or epithelial cells were chemically and physically pretreated indicated that the bacterial adhesin was likely to be proteinaceous; the receptor on the BEC had the characteristics of a carbohydrate molecule. The bacterial adhesin did not appear to be disrupted by periodate treatment, but heat treatment or trypsinisation of the bacteria resulted in a decrease in the number of organisms attaching to BEC. Conversely, the receptor on the BEC was markedly disrupted by periodate treatment. Sodium metaperiodate cleaves the C—C bond between vicinal hydroxyl groups of sugars, and because periodate treatment of BEC greatly reduced subsequent attachment of GBS to the cells, it was clear that a carbohydrate was implicated as the epithelial cell receptor.

The effects of mild and severe heat treatment of GBS on adherence were interesting. In both cases the organisms were killed, but the mild treatment did not reduce attachment to BEC. It appears, therefore, that viability is not a prerequisite for binding of GBS, and this finding is in agreement with those of Zawaneh *et al.* (1979) who showed that killing GBS with ultra-violet light or penicillin did not affect their adherence to human vaginal epithelial cells.

Some lectins are remarkably thermostable compared with other proteins (Gilboa-Garber, Mizrahi and Garber, 1972), thus the adhesin on the GBS may be a fairly thermostable protein, a characteristic that would correlate well with its proposed lectin-like activity.

The increase in attachment of GBS after treatment with neuraminidase is significant in view of the work of Milligan *et al.* (1978), which related high production of neuraminidase by certain strains of GBS to an enhanced pathogenic potential. Thus, the neuraminidase may be increasing the pathogenicity of these strains by enhancing their attachment to host cells. The adhesin may be masked to some extent by sialic acid residues, which are removed by the neuraminidase, thereby increasing the

degree of interaction with host cell receptors. The overall negative charge of the bacterial cells will also be reduced in this way which would favour attachment of the organism.

The results gained from studying the effects of pretreatment of whole bacterial cells on their subsequent attachment to BEC were reinforced by the results obtained with the sonicate. Pretreatment of BEC with the sonicate caused a dose-dependent inhibition of subsequent attachment of GBS, this activity being reduced by heat treatment but unaffected by periodate oxidation. It is believed that the sonicate contains group-B streptococcal adhesin, which binds to receptor sites, preventing subsequent bacterial attachment. The sonicate did not affect binding of a strain of group-A streptococcus to BEC, indicating a degree of specificity. The fact that activity of this sonicate was reduced by heat but not periodate again implies that the bacterial adhesin is a protein. SDS-PAGE showed that the sonicate contained a number of proteins, but purification of this heterogeneous mixture, to define the adhesin, has not been achieved.

The apparent involvement of a host-cell surface carbohydrate as the receptor for GBS was very interesting, because a number of bacteria-host cell interactions have now been shown to be mediated by lectin binding. Various specificities have been reported for these bacterial lectins, including D-mannose (Eshdat *et al.*, 1978), L-fucose (Jones and Freter, 1976), D-galactose or N-acetyl-D-galactosamine (Mongiello and Falkler, 1979; Yamazaki, Ebisu and Okada, 1981) and lactose (Saunders and Miller, 1980). Of the bank of sugars tested as potential inhibitors of the interaction between GBS and BEC, only N-acetyl-D-glucosamine proved effective, which may indicate a role for an N-acetyl-D-glucosamine-specific lectin in adherence of this organism.

Wheat-germ agglutinin (WGA) is an example of a lectin that is specific for N-acetyl-D-glucosamine (Burger and Goldberg, 1967), and Stanley and Carver (1978) have shown that Chinese hamster ovary cells possess binding sites with high and low affinities for WGA. Recently, Gibbons and Dankers (1983) have shown that WGA binds to human oral epithelial cells *in vivo*. It seems reasonable, therefore, to suggest that a bacterial lectin with the same specificity could mediate adhesion of a micro-organism to mammalian cells. Indeed, recent work has shown that N-acetyl-D-glucosamine is probably the receptor in a number of microbial interactions with host cells. These include the binding of some strains of *Bordetella bronchiseptica* to rabbit pharyngeal cells (Bemis and Plotkin, 1982), adhesion of type-A *Pasteurella multocida* to rabbit pharyngeal cells (Glorioso *et al.*, 1982) and the attachment of *Chlamydia psittaci* and *Chlamydia trachomatis* to mouse fibroblasts (Levy, 1979). Thus, the data outlined in this paper regarding attachment of GBS to BEC may be explained by a similar mechanism whereby a lectin on the bacterial surface recognises and binds to N-acetyl-D-glucosamine residues on the host cell surface.

The involvement of a lectin in GBS attachment is also suggested by the work of Elbein *et al.* (1981), who have shown that GBS will adhere to canine kidney (MDCK) epithelial cells only after the mammalian cells have been infected with influenza virus. The bacteria will not attach to uninfected kidney cells. It appears that GBS recognise and adhere to influenza virus glycoproteins that have been synthesised in infected MDCK cells and inserted into the cell membrane (Elbein *et al.*, 1981). These influenza-virus glycoproteins have been partially characterised (Collins and Knight, 1978) and shown to contain two types of oligosaccharide. It may be significant that

both types of oligosaccharide contain N-acetyl glucosamine because if, as suggested, GBS possess a lectin-like adhesin which recognises N-acetyl-D-glucosamine, then the requirement for infection of MDCK cells with influenza virus to allow GBS attachment may be explained by the subsequent availability of N-acetyl-D-glucosamine residues expressed by the viral glycoproteins.

The role of lipoteichoic acid (LTA) in the attachment of group-A streptococci to mucosal cells (Beachey, 1975) is well documented. It has been reported that LTA purified from group-A streptococci inhibits attachment of GBS to pharyngeal epithelial cells (Botta, 1981), but the specificity of this inhibition is questionable, because the same pretreatment also reduces adherence of *Staphylococcus aureus* (Botta, 1981). To overcome this objection, membrane LTA was prepared from type-III GBS (NCTC11080) for use as an inhibitor of attachment of the organism to BEC. However, under the experimental conditions of the assay described this material did not appear to inhibit significantly the attachment of the homologous strain to BEC. The extracted polymer was shown to be a LTA-like molecule (analysis performed by Dr C. G. Cumming, Department of Oral Medicine and Oral Pathology, University of Edinburgh), and it was of interest that it also proved to be a type-specific antigen, reacting only with antiserum raised against type-III GBS.

Our data suggest, therefore, that LTA does not play such a significant role in attachment of GBS to BEC as it does in the binding of group-A streptococci to BEC. The findings of Zawaneh *et al.* (1979) also suggest that LTA is not important in the adherence of GBS to vaginal epithelial cells. They have shown that treatment of GBS with high concentrations of penicillin, which stimulates the organisms to lose their LTS, did not inhibit subsequent adherence of the bacteria to vaginal epithelial cells (Zawaneh *et al.*, 1979). Similar treatment of group-A streptococci causes a concomitant loss of the organisms' ability to bind to BEC (Alkan and Beachey, 1978).

In addition, Zawaneh *et al.* (1979) showed that attachment of GBS to vaginal epithelial cells was not inhibited by either anti-group or anti-type antisera, suggesting that neither group-specific nor type-specific antigenic determinants was involved in the adherence mechanism. As described earlier, our studies indicate that the membrane LTA of GBS is a type-specific antigen; this provides another piece of confirmatory evidence for the lack of involvement of LTA in adherence of GBS to buccal or vaginal epithelial cells.

Recently it has been shown that clinical isolates of GBS from infants with early- or late-onset disease possess significantly higher levels of cell-associated teichoic acids than do strains from asymptomatic infants (Nealon and Mattingly, 1983), and the same authors report that GBS bind to primary passaged human embryonic and fetal epithelial cells by a specific LTA-mediated mechanism. Cox (1982) has also demonstrated that topically applied LTA prevents group-B streptococcal colonisation in a maternal-newborn mouse model. Therefore, adherence of GBS may be mediated by more than one mechanism. From the available evidence it would appear likely that the lectin type of interaction plays a major role in group-B streptococcal colonisation of adult epithelial cells, whereas the LTA-mediated adherence mechanisms is of greater importance in the colonisation of neonatal tissue surfaces.

Group-B streptococcal cell surfaces

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One of the major problems encountered by early workers was to devise a reliable method of identification and to establish subdivisions within streptococcal groups. Differentiation by cultural and biochemical characteristics of the organisms had proved of little value, although Brown (1920) claimed that human and bovine strains could be distinguished by variations in haemolytic patterns on blood agar.

The method of preferentially staining bacterial cell walls described by Gram (1884) 100 years ago provided great incentives to examine the nature of the surface structures of bacteria. Moreover, significant advances at the turn of this century occurred in the art of serological techniques. Hitchcock (1924) was the first to demonstrate that most streptococci from human infections possess a serologically active surface carbohydrate antigen. In 1928 and 1933, Lancefield reported that β -haemolytic streptococci could be differentiated into serological groups and she recognised seven such groups, labelled A to G. *Str. agalactiae* was designated group B (GBS).

Since these studies, streptococcal taxonomy has been expanded to accommodate 13 additional serological groups labelled H to V, omitting I and J (Wilkinson, 1978). Serological subdivision of GBS was proposed by Lancefield (1934). Three distinct types (I, II and III) were recognised by precipitation tests with hot-acid extracts. These findings were confirmed by passive protection tests in mice, and the type-specific antigens, called "S substances", were identified as polysaccharides. Lancefield (1938) reported two distinct sub-types of type-I GBS and called them Ia and Ib. During this time an alternative scheme of serological typing for GBS was proposed by Stableforth (1932 and 1937). To avoid further confusion, however, the International Congress for Microbiology (1936) suggested that Lancefield's scheme be adopted.

Pattison *et al.* (1955) isolated GBS strains of bovine origin which lacked a polysaccharide type antigen, but possessed protein antigens that could be extracted with hot acid and detected in precipitation tests. They designated these antigens X and Y. The Y protein was trypsin resistant, but pepsin sensitive, and indistinguishable from the R antigen of group A streptococcus type 28 (Wilkinson, 1972). The X protein was sensitive to treatment with both trypsin and pepsin. Further reports (Butter and de Moor, 1967; Jelínková, Neubauer and Duben, 1970) showed that most bovine GBS strains have protein type-antigens, but that many are devoid of polysaccharide type-antigens, whereas human strains nearly always have polysaccharide type-antigens and fewer have protein type-antigens. Wilkinson and Eagon (1971) identified another subtype within the type-I series of strains. This was called the Ic serotype and possessed a polysaccharide antigen in common with Ia strains, and a protein antigen similar to that found in the Ib strain. Although Lancefield, McCarty and Everly (1975) reported a polysaccharide antigen common to all type-I strains, the Iabc complex, this finding is disputed by Cumming, Ross and Poxton (1981*b*, 1982).

Recently, Perch, Kjems and Henriksen (1979) have raised antiserum to a previously unrecognised serotype of GBS. They suggested that the new antigen was probably polysaccharide in nature and that strains possessing this complex be termed type IV. The complete antigenic constitution of GBS serotypes are shown elsewhere in this review.

The group-specific antigens

Cummins and Harris (1956) extensively reviewed the chemical composition of cell walls in many gram-positive bacteria. They described the cell wall as being "very tough and extremely insoluble in a wide variety of solvents". The main components of the walls were sugars and amino acids, and were therefore called "mucosubstances". The cell wall from one strain of GBS (NCTC6175) was examined and found to contain rhamnose, galactose, glucosamine and an unknown hexosamine, as well as the amino acids alanine, glutamic acid, and lysine. No attempts were made in this study to quantify the components relative to each other.

By 1961, it was realised that the cell walls of most gram-positive bacteria were broadly similar and that the basic structural component responsible for maintaining rigidity of the cell was the cell-wall mucopeptide, now known as peptidoglycan (Krause and McCarty, 1961). This component was shown to be composed of N-acetylglucosamine, N-acetylmuramic acid, and a variable number of amino acids. Krause and McCarty also suggested that the peptidoglycan component, although associated with the other cell wall component in GBS, the group-specific antigen or "C substance", was chemically distinct from it.

Slade and Slamp (1962) reported on the composition of cell walls of bacteria from 17 serological groups of streptococci. Rhamnose was found to be the main constituent of GBS walls but trace amounts of glucose and galactose were also detected. Wittner and Hayashi (1965) concentrated on GBS cell walls only. Carbohydrate composition of cell walls of GBS serotypes Ia, Ib, II and III were analysed and found to contain almost identical proportions of sugars, namely rhamnose (10%), hexoses (18%) which were mainly galactose and glucose, and hexosamines (9%) which were mainly glucosamine and galactosamine. These workers also separated carbohydrates from the wall of serotype II into two distinct fractions. When tested serologically, one fraction exhibited group-specific activity and was rich in rhamnose, while the other fraction showed type-specific activity and contained mainly galactose and no rhamnose. The importance of rhamnose as an antigenic determinant in the group-specific carbohydrate of GBS was also noted by Curtis and Krause (1964a, 1964b). Sugar inhibition tests revealed that at a final concentration of 1%, rhamnose markedly inhibited the GBS precipitin reaction, whereas blocking the other constituent sugars had no effect.

Recently Cumming *et al.* (1981a, 1981b, 1982 and 1983) examined the immunochemical profiles of the secondary cell-wall carbohydrates or "C substances" from GBS serotypes Ia, Ib and III. Cell walls were collected and purified by extensive sodium dodecyl sulphate (SDS) treatment, thereby removing all non-covalently bound polymers. To extract carbohydrate, walls were suspended in either trichloroacetic acid (TCA) at 4°C for 48 h, or treated with 0.5 M NaOH for 2 h at 20°C (Anderson and Archibald, 1975). After further purification, antigen-antibody reactions were demon-

strated by ELISA (Cumming *et al.*, 1980), and by crossed-immunoelectrophoresis techniques (Cumming *et al.*, 1981*a* and *b*). Generally it was found that from all the serotypes the NaOH extraction technique released a single antigenic complex mainly comprising rhamnose, with small amounts of galactose, glucose, glucosamine and sialic acid. Traces of phosphorus were also detected. The TCA-extracted antigen was partially degraded, and lacked sialic acid. A difference in serological behaviour of this degraded complex was noted in crossed-immunoelectrophoresis studies. The results indicated that the serotypes of GBS possess a carbohydrate antigen which is covalently linked to the cell-wall peptidoglycan. The type-specific antigens are not covalently bound to peptidoglycan, as they can be removed by treating cell walls with SDS. Wagner *et al.* (1980) agreed with the findings of these studies and demonstrated by immunoelectronmicroscopy that the group-specific antigen of GBS traverses the whole of the cell wall, whereas the type-specific antigens are present only as surface complexes.

The biological significance of streptococcal peptidoglycan has yet to be clarified. In appropriate conditions, peptidoglycan exhibits some of the biological properties of endotoxin. Rotta *et al.* (1965) showed that streptococcal cell-wall peptidoglycan induced fever in rabbits and enhanced non-specific resistance in mice against subsequent challenge with streptococci. Abdulla and Schwab (1965) observed dermal necrosis after injection of peptidoglycan into the skin of rabbits. These authors suggested that these properties depended mainly on the inherent toxicity of peptidoglycan. More recently it has been reported that peptidoglycan fractions extracted from cell walls may exert a diversity of biological effects such as adjuvant activity (Holton and Schwab, 1966), induction of polyarthritis (Hunter *et al.*, 1980), production of local Shwartzman reactions (Rotta *et al.*, 1982) and release of histamine from mast cells and platelets by membrane damage (Kimural *et al.*, 1982).

The "type-specific" antigens

The type-specific antigens of GBS consist of surface or capsular polysaccharides and proteins which are chemically and serologically distinct from the group-specific antigen contained within the cell wall (Lancefield and Freimer, 1966). Analysis of the carbohydrate moiety was originally performed on HCl-extracts of cells; glucose, galactose and glucosamine were detected in all GBS serotypes (Lancefield, 1934; Lancefield, 1938; Wilkinson and Eagon, 1971). The same three polymers were released from cell walls by hydrolysis with cold TCA as well as an additional serologically-active component which was not identified (Lancefield and Freimer, 1966). This component was destroyed by heating the TCA-extracted antigen with dilute HCl, leaving an antigen chemically and serologically identical with that obtained by HCl extraction. In the report of Wilkinson and Eagon (1971), sugar inhibition tests with the three previously identified polymers failed to inhibit precipitin reactions between GBS antigens and homologous antisera significantly, indicating that an important determinant group on the antigen had been lost during the extraction procedure. Russel and Norcross (1972) described the components of the GBS type-III antigen. The predominant constituents were galactose, glucose and glucosamine in a molar ratio of 2:1:1. Glucuronic acid amounting to 3.1% of the total antigen was detected. Serological inhibition tests with glucuronic acid resulted in a 34% inhibition

of precipitation, which suggests that glucuronic acid may play a role as a determinant group. Wilkinson (1975) confirmed her earlier findings (Wilkinson and Eagon, 1971) of the chemical composition of type-specific antigens of serotypes Ia, Ib and Ic using a different extraction technique that involved washing whole cells in a saline solution only. This method enabled the detection of sialic acid in the extract. Little importance was attached to this finding at the time.

By 1976 it was realised that the classical methods of extracting polysaccharide type-specific antigens from GBS by hot HCl treatment of whole organisms resulted in partially degraded antigen complexes which were of low mol. wt and were immunologically incomplete (Baker, Kasper and Davis, 1976). Isolation of the complete native antigen by more subtle extraction techniques, such as washing cells in buffered saline, enabled the isolation of antigen complexes that were, theoretically, similar to those which would elicit an immune response in the host. Using the saline-wash technique, they isolated from GBS type-III cell walls mannose and sialic acid in addition to the three monosaccharides previously mentioned. They suggested that the presence of sialic acid in the antigen was the predominant factor in overcoming host resistance in neonatal meningitis. Kane and Karakawa (1977) noted that sialic acid was present in significant proportions in the Ia serotype and they presented evidence to suggest that sialic acid was just one of two antigenic determinants present in the Ia antigen (Kane and Karakawa, 1978). Rabbits immunised with type-Ia cells produced two distinct populations of protective antibody. One set of antibodies was active against the terminal sialic acid residue, whereas the other antibody population reacted with a galactosyl oligosaccharide determinant. Further recent studies have highlighted the importance of sialic acid as a determinant in the type-specific antigens of GBS (Tai, Gotschlich and Lancefield, 1979; Carey *et al.*, 1980; Jennings, Rosell and Kasper, 1980). Kasper and Jennings (1983) reviewed many of the recent American studies on the type-specific antigenic structure of GBS. The complete structure of the native antigen and the partially degraded (core) complex lacking in sialic acid of type III is amply shown in this review. (The immunological differences produced by these two antigens are discussed by Hastings in this review.)

Cumming *et al.* (1983) recently reported the presence of a membrane-bound lipocarbohydrate in GBS type II. This antigen was extracted by phenol from cell membranes and contained galactose, glucose, glucosamine, phosphorus, and fatty acid in a molar ratio of 1.6:1.0:0.35:2.6:0.016. This polymer should not be regarded as a teichoic acid, however, because it does not contain ribitol or glycerol phosphate. It is interesting that the composition of the carbohydrate portion of the molecule is similar to the type-specific antigen of GBS type II described by Freimer (1967), except for the absence of sialic acid in the membrane polymer. Serological studies with this antigen indicated that it is specific for type-II GBS.

It is outside the scope of this review to discuss the interactions between the host-defence systems and surface antigens of GBS. However, recent studies (Kowolik, Cumming and Grant, 1982; Kowolik and Cumming, 1983) showing significant differences in activation of protective neutrophils against the different serotypes of GBS imply that there are yet unidentified bacterial cell-surface factors to be recognised. Because the polysaccharide type-specific antigens have consistently been shown to be associated with the virulence of the organism, comparatively little attention has been directed towards the immunochemical properties of the protein

antigens of GBS serotypes. It is known that the Ibc protein fraction from the prototype Ic strain A909 contains two precipitinogens called the α and β antigens. In a study to determine the prevalence of these antigens in various GBS serotypes, Bevanger, Iversen and Naess (1982) found that of a total of 31 strains known to have the Ibc complex, only 11 (35%) strains contained the β antigen, whereas all strains had the α antigen. In addition, serotype Ib strains contained the β antigen more frequently than strains of the other serotypes. The significance of these protein antigens and their contribution to the virulence of GBS serotypes requires further elucidation before the complexity of the host-bacteria relationship can be understood.

Immunopathology of group-B streptococcal infection

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The ratio of neonates colonised with GBS to those who actually become infected is high, which suggests that either a small minority of babies have critical defects in their immune defence mechanisms or that there are a few GBS strains of increased virulence. The possibility that some neonates may be acutely susceptible to GBS infection has led to the development of immunoassays for the measurement of antibody to GBS and to their use in the study of babies colonised and infected by GBS. The other main approach to the investigation of the pathophysiology of GBS disease has been the search for a "virulence factor" common to invasive GBS isolates. Such studies have used animal models and various in-vitro opsonisation techniques.

Antibody response to the type polysaccharides

Mouse-protection studies have demonstrated that protective antibody to GBS is type specific (Lancefield, 1934). A degree of cross-protection between the type-I subtypes has also been shown, presumably related to their shared immunodeterminants, Ibc protein and Iabc carbohydrate (Lancefield, 1938; Lancefield *et al.*, 1975). Based on this evidence from work in animals, several research groups have attempted to measure type-specific antibody to GBS in neonates and their mothers. Before considering this work, it would first be appropriate to emphasise the basic immunochemical make-up of the type polysaccharides. The classical procedure for extracting these type antigens uses hot acid treatment and reveals a three-sugar repeating unit comprising galactose, glucose and glucosamine. This is true for all five serotypes (Lancefield and Freimer, 1966; Russell and Norcross, 1972; Wilkinson, 1975; Kane and Karakawa, 1977). Extraction of these type antigens with neutral buffer, however, produces a larger, four-sugar repeating sequence consisting of the same three sugars but including a further endgroup residue, sialic acid, (Lancefield, 1972; Kane and Karakawa, 1977; Kasper, Goroff and Baker, 1978; Tai *et al.*, 1979). The acid-labile

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sialic-acid endgroups are presumably lost during acidic extraction procedures. The hot acid extracts have become known as "core" antigens and the more complete, buffer-extracted determinants as "native" antigens. The immunological specificity of these type antigens appears to reside in the whole four-sugar repeating unit (Jennings *et al.*, 1980).

Baker and Kasper (1976) demonstrated that women who were colonised with GBS but whose babies did not become infected, had a significantly higher mean level of type-specific antibody than mothers of infected infants. They also showed a good correlation between antibody concentrations in matched maternal and cord serum samples, indicating transplacental transfer of antibody (Baker *et al.*, 1977). In both studies, a radioimmunoassay was employed with antigen prepared by neutral buffer extraction—the complete or native type-III antigen.

In contrast, Wilkinson (1978) reported levels of type-specific antibody in babies with GBS sepsis similar to those in healthy, colonised infants. The antigen used in her radioimmunoassay was prepared by hot-acid extraction and, consequently, only antibody to core antigen was detected. The correlation between levels of antibody to native antigen and clinical sepsis suggests that this antibody is protective. However, there is considerable contradictory evidence to suggest a significant role for antibody directed against core antigen:

- (i) Lancefield's original animal-protection studies were performed with sera raised against a vaccine that contained both core and native antigen. Antibodies to both determinants appeared to protect mice (Lancefield and Freimer, 1966).
- (ii) Similarly, antibodies to both the core and native type-Ia determinant have been shown to possess opsonic capabilities *in vivo* and *in vitro* (Kane and Karakawa, 1977).
- (iii) Fischer *et al.* (1978) demonstrated that the core antigen of type-III GBS was indistinguishable immunochemically from the polysaccharide antigen of the type-XIV pneumococcus. They immunised rabbits with the pneumococcus and showed that the resulting sera gave protection to infant rats against challenge with GBS type III.
- (iv) Immunisation of pregnant guinea pigs with pneumococcal vaccine has been found to protect their newborn infants from intraperitoneal challenge with type-III GBS (Cusick and Reid, 1982).

In an attempt to clarify the relationship between antibody production and protection, Baker, Kasper and Schiffman (1979) immunised two groups of human volunteers with either type-III core (pneumococcal type XIV) or native antigen. The first group showed an increase in antibody titres to core antigen, with no corresponding rise in the opsonic capacity of their serum towards GBS type III. The second group showed a rise in antibody titres to both core and native antigens, but only the latter correlated with a concomitant rise in opsonic function against type-III organisms. The evidence in man thus appears to indicate that native type-antigens are the principal determinants of pathogenicity in GBS. However, animal protection studies and *in-vitro* opsonisation assays suggest that antibody to core antigen may play some protective role.

The preliminary findings of Baker and Kasper (1976) of lower mean levels of type-specific antibody to GBS amongst mothers of infected babies, in comparison with

a population of mothers of infants colonised with GBS, have been confirmed in later studies (Vogel *et al.*, 1980; Baker, Edwards and Kasper, 1981). However, it has also become apparent that within each of these groups the scatter of antibody titres to GBS type antigens is wide. Many mothers of healthy, colonised infants appear to have only low titres of type-specific antibody, similar to those levels found in mothers of infected babies. This would imply that although absent or reduced humoral immunity to GBS may be a major component of the pathogenesis of GBS sepsis it is probably not the only one. Other host and microbial factors may also be significant in determining when GBS invasive sepsis will occur.

Opsonisation of GBS in vitro

Host defence against GBS depends on serum opsonisation, followed by phagocytosis and intracellular killing (Hemming *et al.*, 1976). It is generally agreed that both type-specific antibody and complement are required for efficient opsonisation of this organism (Shigeoka *et al.*, 1978; Edwards *et al.*, 1980; Hastings and Easmon, 1981). However, the relative importance of the two complement pathways is less clear. Shigeoka *et al.* (1978) found that only the classical pathway was involved in the opsonisation of a serotype-III isolate. In contrast, Edwards *et al.* (1980), also using a serotype-III strain, demonstrated activation of the alternative pathway, but only in the presence of high titres of type-specific antibody. In studies at the Wright-Fleming Institute the opsonic requirement of a range of GBS type-III isolates was examined using luminol-dependent neutrophil chemiluminescence (Hastings and Easmon,

FIG. 1.—Opsonisation of GBS type-III strain 11080 (a; open columns) and strain 344 (b; hatched columns) as demonstrated by phagocytic chemiluminescence. Organisms were opsonised with pooled normal human serum (NHS), serum chelated with magnesium-EGTA (MgEGTA), heated serum (56°C, 30 min; HHS) or serum absorbed with the homologous strain at 4°C (Abs). Results are expressed as a percentage of the chemiluminescence produced by strain 11080 opsonised with NHS.

1981). This technique is based upon the ability of neutrophils to produce light, which can be measured in a luminometer as a voltage change, when presented with opsonised particles. Well-opsonised bacteria stimulate a large response; poorly opsonised or non-opsonised bacteria a very low response. The GBS type -III isolates tested were from colonised and infected neonates. For the majority, efficient opsonisation depended upon the presence of both type-specific antibody and complement. Blocking of the classical pathway with chelated serum had only a minimal effect on the opsonisation of these strains, indicating activation of the alternative pathway (strain 11080; fig. 1a). However, three other patterns of response are also found: (i) opsonisation in the absence of type-specific antibody (strain 344; fig. 1b); (ii) resistance to opsonisation by the activation pathway (strain 15136, Fig. 2a); and (iii) resistance to opsonisation by whole untreated serum (strain 675; fig. 3a). Unfortunately, there was no clear correlation between resistance to opsonisation and the source of the isolate. Of six type-III strains that were poorly opsonised by whole serum, four were isolated from neonates with GBS infection and two from colonised babies.

The capsular type-specific antigens of GBS contain sialic acid. Particles such as zymosan that activate the alternative pathway directly are relatively deficient in surface-associated sialic acid, whereas surfaces rich in sialic acid (e.g., sheep red cells) appear to be poor activators of the alternative pathway (Fearon, 1978; Kazatchkine, Fearon and Austen, 1979). Removal of sialic acid from the surface of the latter with the enzyme neuraminidase converts these particles to activators of the alternative

FIG. 2.—Opsonisation of GBS type-III strain 15136 as demonstrated by phagocytic chemiluminescence. Organisms were grown in Todd-Hewitt broth (a; open columns) or in Todd-Hewitt broth supplemented with neuraminidase (b; hatched columns) and opsonised with serum treated or untreated as indicated (see fig. 1). Results are expressed as a percentage of the chemiluminescence produced by organism grown in Todd-Hewitt broth and opsonised with NHS.

pathway (Pangburn and Müller-Eberhard, 1978). Sialic acid modulates the alternative pathway by increasing the affinity of $\beta 1H$ relative to Factor B for C3b. This results in blocking of the formation of the alternative pathway convertase C3bBb (Kazatchkine *et al.*, 1979). It has been suggested that the sialic acid of the GBS capsule might force a requirement for type-specific antibody in order to mask this moiety before complement activation can occur (Edwards *et al.*, 1980).

In further studies at the Wright-Fleming Institute, growth of the alternative pathway-resistant type-III strains in the presence of neuraminidase rendered the organisms sensitive to alternative pathway opsonisation (strain 15136; fig. 2b). Similarly, several of the strains shown to be resistant to opsonisation with untreated serum were found to be sensitive after treatment with neuraminidase (strain 675; fig. 3b). Using a bacterial killing assay, Fischer, Hunter and Wilson (1981) found a similar decrease in resistance to opsonisation with a GBS type-III strain after growth in broth supplemented with neuraminidase. A correlation between the removal of surface-associated sialic acid from GBS and decreased virulence in neonatal rats has been demonstrated recently (Shigeoka *et al.*, 1983).

The enzyme trypsin appeared to have little effect on resistance to opsonisation in the strains described above. However, there is evidence to suggest that protein moieties on the GBS cell surface may mediate resistance to phagocytosis in some GBS strains (Shigeoka, Hall and Hill, 1979). Two separate mechanisms of GBS resistance to host defences may therefore exist—one related to surface-associated sialic acid, and the other to as yet unspecified moieties.

FIG. 3.—Opsonisation of GBS type-III strain 675 as demonstrated by phagocytic chemiluminescence. Organisms were grown in Todd-Hewitt broth (a; open columns) or in Todd-Hewitt broth supplemented with neuraminidase (b; hatched columns) and opsonised with serum treated or untreated as indicated (see fig. 1). Results are expressed as a percentage of the chemiluminescence produced by strain 11080 (control) opsonised with NHS (stripped column).

Summary

Protection against invasive GBS disease involves complex interactions between bacteria and host. Prophylaxis against GBS sepsis by active immunisation, and the treatment of such infections with GBS immune serum are being considered as future therapeutic options. The differences in opsonic requirement outlined in this review require careful evaluation before such immunotherapy is considered for use in routine clinical practice.

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