12 Group B Streptococcal Infections

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CHAPTER OUTLINE

Lancefield group B β-hemolytic streptococci were first recorded as a cause of human infection in 1938, when Fry[1] described three patients with fatal puerperal sepsis. Sporadic cases were reported until the 1970s, when a dramatic increase in the incidence of septicemia and meningitis in neonates caused by group B streptococci (GBS) was documented from geographically diverse regions.[2-4] Emergence of GBS infections in neonates was accompanied by an increasing number of these infections in pregnant women and nonpregnant adults. In pregnant women, infection commonly manifested as localized uterine infection or chorioamnionitis, often with bacteremia, and had an almost uniformly good outcome with antimicrobial therapy. The incidence of perinatal infection associated with GBS remained stable through the early 1990s. Case-fatality rates had declined by then but remained substantial compared with case-fatality rates reported for other invasive bacterial infections in infants. The implementation of the consensus guidelines published in 2002 that are designed to prevent early-onset disease in neonates through universal antenatal culture-based screening at 35 to 37 weeks of gestation, and accompanying intrapartum antibiotic prophylaxis was associated with a substantial decline in the incidence of early-onset neonatal infection by 2008 and has reached a plateau since then.[5]

In recent years, the genomes of several GBS types have been sequenced, opening new avenues for the identification of novel potential vaccine targets.[6,7] The discovery that surface-associated pili are widely distributed among GBS and that a vaccine based on combinations of the three pilus-island variants protects mice against lethal challenge with a wide variety of GBS strains paves the way for the design of pilus-based and perhaps other putative surface protein vaccines for testing in humans.[8-10]

Testing of glycoconjugate GBS candidate vaccines in healthy adults and in pregnant women is ongoing, offering promise that immunization to prevent maternal and infant invasive GBS disease could become a reality.

Organism

Streptococcus agalactiae is the species designation for streptococci belonging to Lancefield group B. This bacterium is a facultative gram-positive diplococcus with an ultrastructure similar to that of other gram-positive cocci. Before

Lancefield's classification of hemolytic streptococci in 1933,[11] this microorganism was identified by its characteristic colonial morphology, its narrow zone of β-hemolysis surrounding colonies on blood agar plates, and its double zone of hemolysis that appeared when plates were refrigerated an additional 18 hours beyond the initial incubation. Occasional strains (1%-4%) are α-hemolytic or nonhemolytic. GBS are readily cultivated in various bacteriologic media. Isolation from respiratory, genital, and gastrointestinal tracts can be enhanced by use of selective enrichment broth containing antimicrobial agents that inhibit growth of other bacterial species indigenous to these sites.[12,13]

COLONIAL MORPHOLOGY AND IDENTIFICATION

Colonies of GBS grown on sheep blood agar are 3 to 4 mm in diameter, produce a narrow zone of β-hemolysis, are gray-white, and are flat and mucoid. β-Hemolysis for some strains is apparent only when colonies are removed from the agar.

Tests for presumptive identification include bacitracin and trimethoprim-sulfamethoxazole disk susceptibility testing (92%-98% of strains are resistant), hydrolysis of sodium hippurate broth (99% of strains are positive), hydrolysis of bile esculin agar (99%-100% of strains fail to react), pigment production during anaerobic growth on certain media (96%-98% of strains produce an orange pigment), and CAMP (Christie-Atkins-Munch-Petersen) testing (98%-100% of strains are CAMP positive).[14] The CAMP factor is a thermostable extracellular protein that, in the presence of the β toxin of *Staphylococcus aureus,* produces synergistic hemolysis when grown on sheep blood agar. Hippurate hydrolysis is an accurate method for presumptive identification of GBS, but the incubation time required limits its usefulness. Definitive identification of GBS requires detection of the group B antigen common to all strains. Lancefield's original method required acid treatment of broth-grown cells to extract the group B antigen from the cell wall.[15] Supernatants brought to neutral pH were mixed with hyperimmune rabbit antiserum prepared by immunization with the group B–variant strain, and precipitins in capillary tubes were recorded. Less time-consuming techniques are now used. Conventional means for presumptive identification of isolates subcultured to blood-agar plates include use of the CAMP test or latex agglutination with GBS antisera. Chromogenic agars that undergo color change in the presence of β-hemolytic colonies of GBS have become available. Most of these do not detect the small percentage of strains that are nonhemolytic. In addition, more rapid techniques have been developed for identifying GBS directly from enrichment broth or after subculture. These include DNA probes and nucleic acid amplification tests (NAAT), such as polymerase chain reaction (PCR).[16] The sensitivity of NAAT, when an enrichment step is included, ranges from 93% to 100%.

Strains of Human and Bovine Origin

Group B streptococci were known to cause bovine mastitis before they were appreciated as pathogenic in humans.[17] Modern veterinary practices have largely controlled epidemics of bovine mastitis, but sporadic cases still occur. Substantial biochemical, serologic, and molecular differences exist between human and bovine isolates.[18] Among typeable bovine strains, patterns of distribution distinct from the patterns of human isolates are noted. Other distinguishing characteristics for bovine strains include their unique fermentation reactions, decreased frequency of pigment production, and susceptibility to bacitracin. Protein X, rarely found in human strains, is commonly present in pathogenic bovine isolates.[19]

The relationship between GBS strains of human and bovine origin has been queried for years. There is no compelling evidence that cattle serve as a reservoir for human disease, and transmission from cows to humans is exceedingly rare. In addition, during the decades when GBS has been a dominant human pathogen, the risk of exposure through direct contact with dairy cattle or ingestion of unpasteurized milk has been unlikely. Application of molecular techniques to strains from bovine sources and those infecting human neonates supports the assertion that these lineages are unrelated. Phylogenetic lineage determination does indicate, however, that some clonal complexes of invasive or colonizing strains in humans are related to ancestral lineages of bovine GBS.[20]

CLASSIFICATION

Lancefield defined two cell wall carbohydrate antigens by using hydrochloric acid–extracted cell supernatants and hyperimmune rabbit antisera: the group B–specific, or "C," substance common to all strains and the type-specific, or "S," substance that allowed classification into types—initially types I, II, and III.[21-23] Strains designated as type I were later shown to have cross-reactive and antigenically distinct polysaccharides designated type Ia and type Ib.[22] GBS historically designated type Ic possessed type Ia capsular polysaccharide (CPS) and a protein antigen common to type Ib, most type II, and rare type III strains.[24] This protein now is designated C protein. Rabbit antibodies directed against CPS protected mice against lethal challenge with homologous, but not heterologous, GBS types, and cross-protection was also afforded when antibodies against C protein were tested.

Current nomenclature designates polysaccharide antigens as type antigens and protein antigens as additional markers for characterization.[25,26] The former type Ic now is designated type Ia/c. Type IV was identified as a new type in 1982, when 62 strains were described that possessed type IV polysaccharide alone or with additional protein antigens.[27] Antigenically distinct types, V through IX, now are characterized. Strains not expressing one of the CPS-specific antigens are designated as nontypeable by serologic methods but often can be assigned a GBS type by PCR-based methods.

C protein is composed of two unrelated components, the trypsin-resistant α C protein and the trypsin-sensitive β C protein. α C protein is expressed on many type Ia, Ib, and II strains.[28] Strains expressing α C protein are more opsonoresistant than are α C–negative strains. α C protein consists of a series of tandem repeating units, and in naturally occurring strains, the repeat numbers can vary. The number of repeating units expressed alters antigenicity and influences the repertoires of antibodies elicited.[29] The use of one or two repeat units of α C proteins elicits antibodies that bind all α C proteins with equal affinity, suggesting its potential

as a vaccine candidate.[30,31] β C protein is a single protein with a molecular mass of 124 to 134 kDa that is present in about 10% of isolates. β C protein binds the Fc region of human IgA.[32,33] Strains bearing α and β C proteins possess increased resistance to opsonization in vitro.

GBS express numerous additional surface proteins. Designation of additional α-like repetitive proteins (Alp) numerically (e.g., Alp2 and Alp3) is being considered. Most strains have the gene for just one Alp family protein. Genes encoding Alp1 (also designated "epsilon") are associated with type Ia, and genes encoding Alp3 are associated with type V strains.[34] Alp also are referred to as R proteins, with R1 and R4 as the major ones found on clinical isolates.[34] Rib protein, expressed by most type III strains, has an identical sequence to R4. The gene sequence of a protein initially designated R5 has been renamed group B protective surface protein (BPS).[35] In one large collection, BPS was found in 3.5% of invasive or colonizing isolates, most often in type Ia, II, or V GBS and never in type III.[36] Some GBS contain surface proteins designated as X antigens.[37] The X and R antigens are immunologically cross-reactive. A laddering protein from type V GBS shares sequence homology with α C protein.[38] A protein designated Sip (surface immunogenic protein) is distinct from other known surface proteins. It is produced by all GBS types and confers protection against experimental infection; its role in human infection is unknown.[39]

Genome analysis has shown that GBS produce long pilus-like structures. These structures extend from the bacterial surface and beyond CPS (Fig. 12-1).[8] Formed by proteins with adhesive functions, these structures are implicated in host colonization, attachment, and invasion.[40] The pilus-like structures are encoded in genomic pilus islands that have an organization similar to that of pathogenicity islands. Three types of pilus islands have been identified through genomic analysis; these are composed of partially homogeneous covalently linked proteins (pilus islands 1, 2a, and 2b). These pilus proteins are highly surface-expressed and are involved in paracellular translocation through epithelial cells. At least one pilus island is present on all GBS clinical strains tested to date.

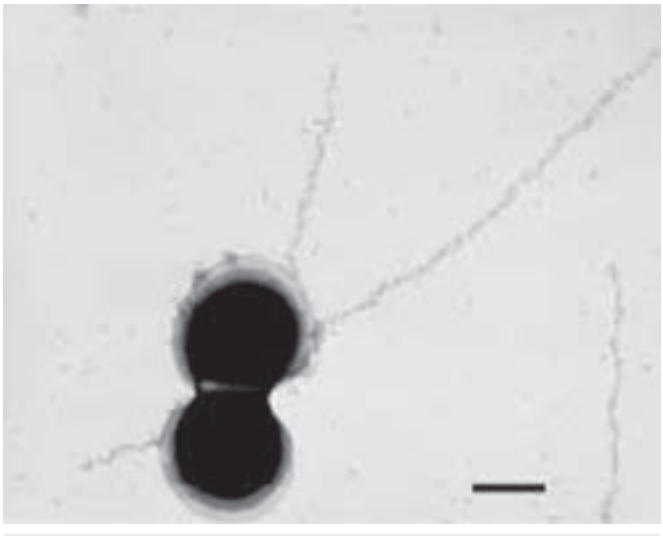

Figure 12-1 Immunogold labeling and transmission electron microscopy of group B streptococcal organisms showing long pilus-like structures extending from the cell surface. (From Lauer P, Rinaudo CD, Soriani M, et al: Genome analysis reveals pili in group B streptococcus, *Science* 309:105, 2005.)

ULTRASTRUCTURE

Early concepts suggested a thick, rigid peptidoglycan layer external to the cytoplasmic membrane surrounded by concentric layers of cell wall antigens. Evidence now supports a model in which the group B carbohydrate and the CPS are linked independently to cell wall peptidoglycan.[41] Immunoelectron techniques reveal abundant capsule on Lancefield prototype strains Ia, II, and III, whereas less dense capsules are found on type Ib strains (Fig. 12-2).[42] Similarly, incubation of the reference strains with homologous type-specific antisera reveals a thick capsular layer on types IV, V, and VI.[43,44] Ultrastructural studies show that the C protein also has a surface location.[42] CPS capsule expression can be

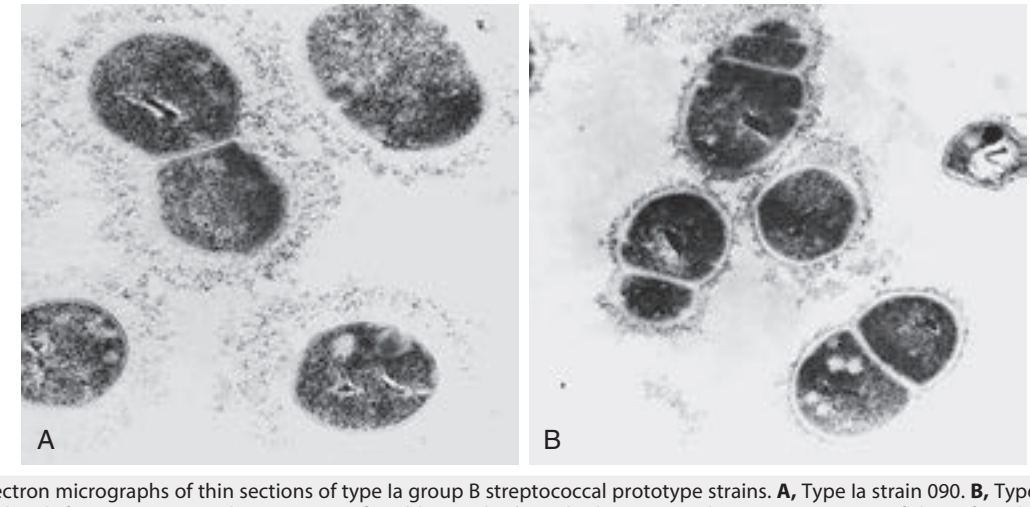

Figure 12-2 Electron micrographs of thin sections of type Ia group B streptococcal prototype strains. **A,** Type Ia strain 090. **B,** Type Ia/c strain A909. Both are stained with ferritin-conjugated type Ia–specific rabbit antibodies. The larger capsule is representative of those found also in Lancefield prototype II strain (18RS21) and type III isolates from infants with meningitis, whereas the smaller capsule is representative of that also found on Lancefield prototype strain Ib (H36B). (Micrographs courtesy Dennis L. Kasper, MD.)

regulated by altering cell growth rate.[45] Immunogold labeling and transmission electron microscopy show that the GBS pilus-like structures extend from the bacterial surface.[8]

IMMUNOCHEMISTRY OF POLYSACCHARIDE ANTIGENS

Lancefield's initial serologic definition used hydrochloric acid and heat treatment, resulting in degraded antigens of small molecular mass. Gentler techniques isolated large molecular mass or "native" polysaccharides that contained sialic acid. Human immunity correlates with antibody to the sialic acid–containing type III structure.[46] With the use of contemporary methods for determination, L-rhamnose, D-galactose, 2-acetamido-2-deoxy-D-glucose, and D-glucitol have been identified as the constituent monosaccharides of the group B antigen. It is composed of four different oligosaccharides, designated I though IV, and linked by a phosphodiester bond to form a complex, highly branched multiantennary structure.[47]

The repeating unit structures of the type-specific CPSs are schematically represented in Figure 12-3. Types Ia, Ib, and III have a five-sugar repeating unit containing galactose, glucose, *N*-acetylglucosamine, and sialic acid in a ratio of 2:1:1:1.[46,48-50] Type II and type V have a seven-sugar repeating unit, type IV and type VII have six-sugar repeats, and type VIII polysaccharide has a four-sugar repeating unit.[43,51-55] Molar ratios vary, but the component monosaccharides are the same among the polysaccharide types except that type VI lacks *N*-acetylglucosamine and type VIII contains rhamnose in the backbone structure.[56]

Each antigen has a backbone repeating unit of two (Ia, Ib), three (III, IV, V, VII, VIII), or four (II) monosaccharides to which one or two side chains are linked. Sialic acid is the exclusive terminal side chain sugar except for the type II polysaccharide, which also has a terminal galactose. The structures of the type Ia and type Ib polysaccharides differ only in a single side-chain linkage, although there are differences in the tertiary configuration of the molecules.[57] These linkages are critical to immunologic specificity and explain the observed immunologic cross-reactivity.[22,58] The desialylated type III polysaccharide is immunologically identical to that of type 14 *Streptococcus pneumoniae*.[59] This observation stimulated investigations concerning the immunodeterminant specificity of human immunity to type III GBS and of antibody recognition of conformational epitopes as a facet of the host immune response.[60] The type III polysaccharide also can form extended helices. The position of the conformational epitope along these helices is potentially important to binding site interactions.[61]

GROWTH REQUIREMENTS AND BACTERIAL PRODUCTS

Group B streptococci are quite homogeneous in their amino acid requirements during aerobic or anaerobic growth.[62] A glucose-rich environment enhances the number of viable GBS during stationary phase and the amount of CPS elaborated.[63] In a modified chemically defined medium, the expression of capsule during continuous growth is regulated by the growth rate.[45] Invasiveness is enhanced by a fast growth rate and is optimal in the presence of at least 5% oxygen.[64,65]

GBS elaborate many products during their growth. Among these is the hemolysin that produces the β-hemolysis surrounding colonies on blood agar. Hemolysin is a surface-associated toxin active against the erythrocytes from several mammalian species. The GBS hemolysin recently has been characterized as the ornithine rhamnolipid pigment and shown to function as a virulence factor, promoting invasion of placental cells.[66] GBS can hydrolyze hippuric acid to benzoic acid and glycine. The hippuricase of GBS is cell associated and is trypsin and heat labile.[67] It is antigenic in rabbits, but its relationship to bacterial virulence, if any, has not been studied.

Most strains of GBS have an enzyme that inactivates complement component C5a by cleaving a peptide at the carboxyl terminus.[68] GBS C5a-ase seems to be a serine esterase; it is distinct from the C5a-cleaving enzyme produced by group A streptococci,[69] although the genes that encode these enzymes are similar.[70] C5a-ase contributes to pathogenesis by rapidly inactivating the neutrophil agonist C5a, preventing the accumulation of neutrophils at the site of infection.[71]

Another group of enzymes elaborated by nearly all GBS are the extracellular nucleases.[72] Three distinct nucleases have been physically and immunologically characterized. All are maximally activated by divalent cations of calcium plus manganese. These nucleases are immunogenic in animals, and neutralizing antibodies to them are detectable in sera from pregnant women known to be genital carriers of GBS. Their role in the pathogenesis of human infection is unknown.

An extracellular product that can contribute to virulence of GBS was originally defined as a neuraminidase and subsequently characterized as a hyaluronate lyase.[73] Maximal levels are detected during late exponential growth in a chemically defined medium. Elaboration of large quantities can be a virulence factor for type III GBS. Musser and coworkers[74] identified a high neuraminidase–producing subset of type III strains that were responsible for most serious GBS infections. Later studies indicated that these were from a single clonal complex, designated ST 17, that has been designated as "hypervirulent." ST 17 is almost exclusively found in type III strains.

GBS synthesize acylated (lipoteichoic) and deacylated glycerol teichoic acids that are cell associated and can be readily extracted and purified.[75] Strains from infants with early- or late-onset disease have higher levels of cell-associated and native deacylated lipoteichoic acid, and this product seems to contribute to attachment to human cells.[76]

Epidemiology and Transmission

ASYMPTOMATIC INFECTION (COLONIZATION) IN ADULTS

Group B streptococcal infection limited to mucous membrane sites is designated as asymptomatic infection, colonization, or carriage. Comparisons of the prevalence of colonization are related to differences in ascertainment techniques. Factors that influence the accuracy of colonization detection include density of colonization, choice of bacteriologic media, body sites sampled, number of culture specimens obtained, and time interval of study.

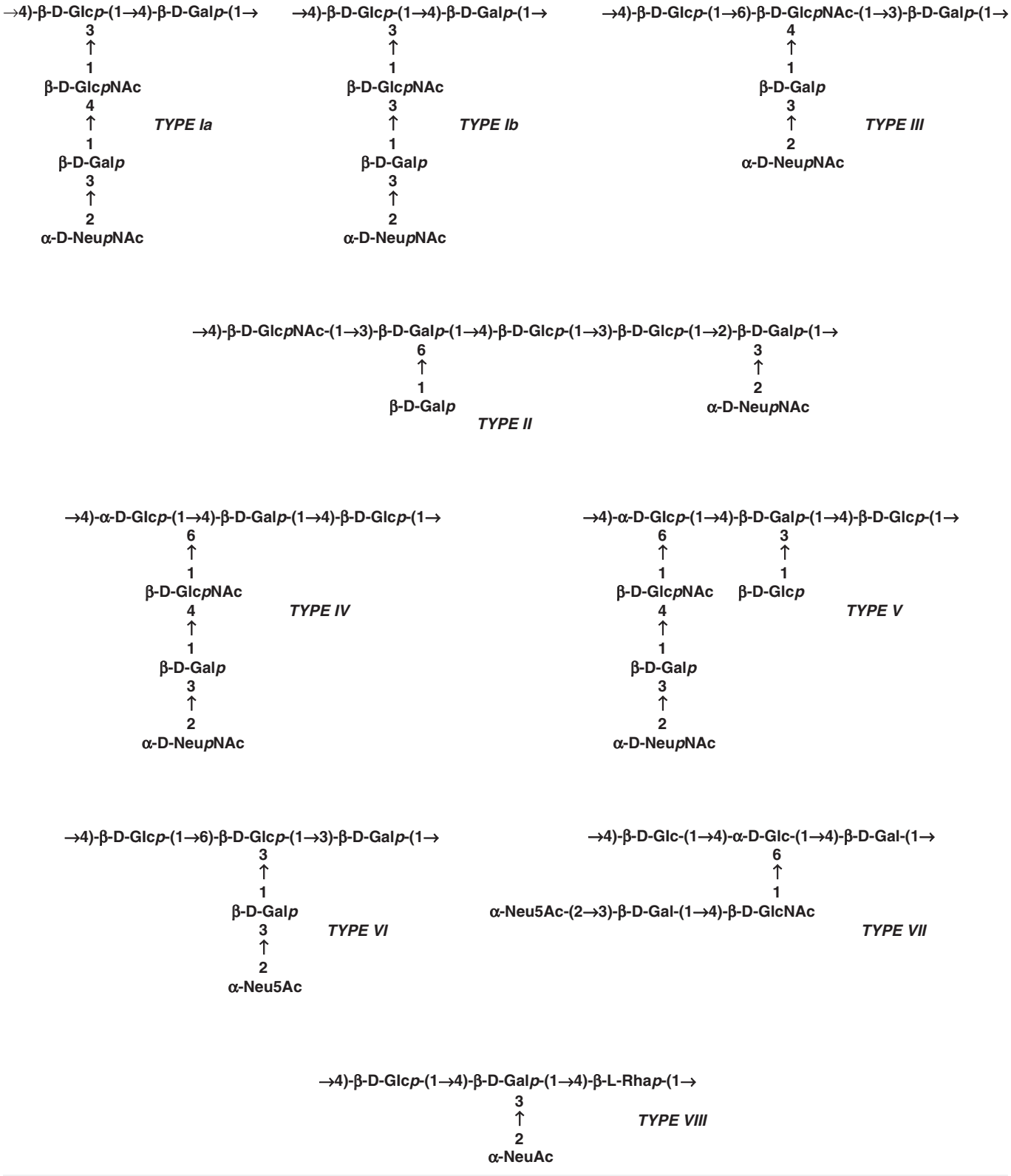

Figure 12-3 Repeating unit structures of group B streptococcal capsular polysaccharides type Ia,[57] type Ib,[57,58] type II,[53,55] type III,[49,50] type IV,[54] type V,[51] type VI,[568] type VII,[52] and type VIII.[56]

Isolation rates are higher with use of an enrichment broth, rather than solid agar media, and with enrichment broth containing substances inhibitory for normal flora (usually antimicrobials). Selective enrichment broths, include Todd-Hewitt broth supplemented either with gentamicin (8 μg/mL) and nalidixic acid (15 μg/mL; TransVag broth) or with colistin (10 μg/mL) and nalidixic acid (15 μg/mL; Lim broth). Addition of 5% sheep blood to TransVag broth or Lim broth can increase the recovery of GBS. Such media inhibit the growth of most gram-negative

enteric bacilli and other normal flora that make isolation of streptococci from these sites difficult. Use of selective enrichment broth promotes detection of low numbers of organisms that escape detection when inoculation of swabs is directly onto solid agar.

Isolation rates also are influenced by body sites selected for culture. Female genital culture isolation rates double with progression from the cervical os to the vulva. In addition, culture sampling of lower genital tract and rectal sites increases GBS colonization rates 10% to 15% beyond that found if a single site is cultured. The urinary tract is an important site of infection, especially during pregnancy, which usually manifests as asymptomatic bacteriuria. To predict accurately the likelihood of neonatal exposure to GBS at delivery, maternal culture specimens from the lower vagina and rectum (not perianal area) should be collected.

The prevalence of GBS colonization is influenced by the number of cultures obtained from a site and the interval of sampling. Vaginal colonization patterns can be chronic, transient, or intermittent. A longitudinal cohort study of nonpregnant young women found that almost one half of those who are culture negative at enrollment acquired vaginal colonization during three 4-month intervals of assessment.[77] The duration of colonization among college students is estimated to be 14 weeks for women and 9 weeks for men.[78] The predictive value of a positive second trimester vaginal or rectal culture for colonization at delivery is only 67%.[79] The predictive value of a positive prenatal culture result is highest (73%) in women with vaginal and rectal colonization and lowest (60%) in women with rectal colonization only. Cultures performed 1 to 5 weeks before delivery have a positive predictive value of 87% (95% confidence interval [CI], 83 to 92) for colonization status at delivery in term parturients. The negative predictive value is 96% (95% CI, 95 to 98). Culture specimens collected within this interval perform significantly better than specimens collected 6 or more weeks before delivery.[80]

The primary reservoir for GBS is the lower gastrointestinal tract.[3] The recovery of GBS from the rectum is three to five times more common than recovery from the vagina, and the rectal site predicts persistence or chronicity of carriage.[81] GBS in the gastrointestinal tract is a risk factor for vaginal GBS. Additional support for the lower gastrointestinal tract as the primary reservoir is the association of GBS with infections resulting from gastrointestinal tract surgical procedures. Several factors influence genital carriage of GBS. Among healthy young men and women living in a college dormitory, sexually experienced subjects had colonization rates twice those of sexually inexperienced subjects.[82] In a longitudinal cohort study of nonpregnant young women, African-American ethnicity, having multiple sex partners during a preceding 4-month interval, having frequent sexual intercourse within the same interval, and having sexual intercourse within the 5 days before a follow-up visit were independently associated with vaginal acquisition of GBS.[77] These latter findings suggest either that the organism is sexually transmitted or that sexual activity alters the microenvironment to make it more permissive to colonization. In another study of college women, GBS were isolated significantly more often from sexually experienced women, women studied during the first half of the menstrual cycle, women with an intrauterine device, and women 20 years of age or younger.[83] Colonization with GBS also occurs at a high rate in healthy college students and is associated with having engaged in sexual activity, tampon use, milk consumption, and hand washing done four times daily or less.[84] Fish consumption increased the risk of acquiring some, but not all, capsular types.[85]

A higher prevalence of colonization with GBS is found among pregnant diabetic patients than among nondiabetic controls.[86] Carriage over a prolonged interval reportedly occurs more often in women who use tampons than women who do not.[87] Colonization is more frequent among teenage women than among women 20 years of age or older[83] and among women with three or fewer pregnancies than in women with more than three pregnancies. Hispanic women of Caribbean origin have a high rate of colonization, and African-American women have a higher rate of colonization at delivery than do other racial or ethnic groups.[77,88,89] A large inoculum of vaginal GBS colonization also is more common among African-American than among Hispanic or non-Hispanic white women.[90] Factors that do *not* influence the prevalence of genital colonization in nonpregnant women include use of oral contraceptives[83]; marital status; presence of vaginal discharge or other gynecologic signs or symptoms[83]; carriage of *Chlamydia trachomatis, Ureaplasma urealyticum, Trichomonas vaginalis,* or *Mycoplasma hominis*; and infection with *Neisseria gonorrhoeae*.

Colonization with GBS can elicit an immune response. In a group of pregnant women evaluated at admission for delivery, vaginal or rectal colonization with types Ia, II, III, or V was associated with significantly higher serum concentrations of immunoglobulin (IgG) specific for the colonizing type compared with noncolonized women.[88] Moderate concentrations of Ia, Ib, II, III, and V CPS-specific IgG also were found in association with colonization during pregnancy.[91] Maternal colonization with type III was least likely to be associated with these CPS-specific antibodies. In contrast to infection with organisms such as *N. gonorrhoeae* or genital mycoplasmas, genital infection with GBS is not related to genital symptoms.[83]

GBS have been isolated from vaginal or rectal sites or both in 15% to 40% of pregnant women. The range in colonization rates relates to intrinsic differences in populations (age, ethnicity, parity, socioeconomic status, geographic location) and to lack of standardization in culture methods used for ascertainment. True population differences account for some of the disparity in reported prevalence rates. When selective enrichment broth is used and vaginal and rectal sites are sampled, the prevalence of maternal colonization with GBS by region is 12% in India and Pakistan, 19% in Asia and the Pacific Islands, 19% in sub-Saharan Africa, 22% in the Middle East and North Africa, 14% in Central and South America, and 26% in the United States.[88,92] The rates of colonization among pregnant women range from 20% to 29% in Eastern Europe, 11% to 21% in Western Europe, 21% to 36% in Scandinavia, and 7% to 32% in Southern Europe.[93] The rate of recurrence of GBS colonization in a subsequent pregnancy is higher compared with women negative for colonization in their prior pregnancy.[94] Pharyngeal carriage rates are low and are similar among pregnant and nonpregnant women and heterosexual men[95,96]; however, rates approach 20% in men who have

sex with men.[97] No definite relationship between isolation of GBS from throat cultures and symptoms of pharyngitis has been proved,[98] but some investigators have suggested that these organisms can cause acute pharyngitis.[96]

ASYMPTOMATIC INFECTION IN INFANTS AND CHILDREN

Cultures from the throat and rectum are the best sites for detection of GBS during childhood and until the start of sexual activity.[99,100] In a study of 100 girls ranging in age from 2 months to 16 years, Hammerschlag and coworkers[99] isolated GBS from pharyngeal, rectal or vaginal sites, or both, in 20% of children. The prevalence of positive pharyngeal cultures resembled the prevalence of adults in girls 11 years or older (5%) but approached the prevalence reported for neonates in younger girls (15%). Rectal colonization was detected frequently in girls younger than 3 or older than 10 years of age (about 25%), but was uncommon in girls 3 to 10 years of age. Mauer and colleagues[100] isolated GBS from cultures of vaginal, anal, or pharyngeal specimens or all three in 11% of prepubertal boys and girls. Pharyngeal (5% each) and rectal (10% and 7%) isolation rates were similar for boys and for girls. Persson and coworkers[101] detected fecal carriage of GBS in 4% of healthy boys and girls, and Cummings and Ross[102] found that 2% of English schoolchildren had pharyngeal carriage. Genital colonization in girls is uncommon before puberty.[103] Whether this relates to environmental influences in the prepubertal vagina or to lack of sexual experience before puberty, or both, awaits further study.

TRANSMISSION OF GROUP B STREPTOCOCCI TO NEONATES

The presence of GBS in the maternal genital tract at delivery is the major determinant of colonization and infection in the neonate. Exposure of the neonate to the organism occurs by the ascending route in utero through translocation through intact membranes, through ruptured membranes, or by contamination during passage via the birth canal. Prospective studies have indicated vertical transmission rates of 29% to 85%, with a mean rate of approximately 50% among neonates born to women from whom GBS were isolated from cultures of vagina or rectum or both at delivery. Conversely, only about 5% of healthy infants delivered to culture-negative women become colonized at one or more sites during the first 48 hours of life.

The risk of a neonate acquiring colonization by the vertical route correlates directly with the density of colonization (inoculum size). Neonates born to heavily colonized women are more likely to acquire carriage at mucous membrane sites than neonates born to women with low colony counts of GBS in vaginal cultures at delivery.[104] Boyer and associates [79] found that rates of vertical transmission were substantially higher in women with heavy than in women with light colonization (65% vs. 17%) and that colonization at multiple sites and development of early-onset disease were more likely among infants born to heavily colonized mothers. The likelihood of colonization in a neonate born to a woman who is culture-positive at delivery is unrelated to maternal age, race, parity, or blood type or to duration of labor or method of delivery.[79] It is unclear whether preterm or low-birth-weight neonates are at higher risk for colonization from maternal sources than term infants.

Most neonates exposed to GBS by their mothers have infection that is limited to surface or mucous membrane sites (colonization), which results from contamination of the oropharynx, gastric contents, or gastrointestinal tract by swallowing of infected amniotic fluid or maternal vaginal secretions. In neonates, external auditory canal cultures are more likely to yield GBS than cultures from anterior nares, throat, umbilicus, or rectum in first 24 hours of life,[3,105] and isolation of organisms from the ear canal is a surrogate for the degree of contamination from amniotic fluid and vaginal secretions sequestered during the birth process. After the first 48 hours of life, throat and rectal sites are the best sources for detection of GBS, and positive cultures indicate true colonization (multiplication of organisms at mucous membrane sites), not just maternal exposure.[106]

Other sources for acquisition of GBS in neonates have been established. Horizontal transmission from hospital or community sources is an important, albeit uncommonly proved, mode for transmission of infection.[105,107,108] Acquisition can occur from hands of nursery personnel. In contrast to group A streptococci, which can cause epidemic disease in nurseries, GBS rarely exhibits this potential, and isolation of colonized neonates is not routinely indicated. An epidemic cluster of five infants with late-onset bacteremia infection caused by type Ib GBS occurred among very-low-birth-weight infants in a neonatal intensive care unit in the 1980s.[109] None of the index cases was colonized at birth, establishing that acquisition during hospitalization had occurred. Epidemiologic analysis suggested infant-to-infant spread by means of the hands of personnel, although acquisition from two nurses colonized with the same phage type Ib strain was not excluded. The infection control measures instituted, including cohorting of culture-positive infants and strict hand hygiene, prevented additional cases. Community sources afford a likely potential for transmission of GBS to the neonate. Indirect evidence has suggested that this mode of infection is infrequent.[107] Only 2 of 46 neonates culture negative for GBS when discharged from the newborn nursery acquired mucous membrane infection at 2 months of age.[110] The mode of transmission likely is fecal-oral. Healthy infants colonized from a maternal source or postnatally show persistence of infection at mucous membrane sites for weeks or months.[107,111]

SEROTYPE DISTRIBUTION OF ISOLATES

The differentiation of GBS into CPS types has provided a valuable tool in defining the epidemiology of human infection. In the 1970s and 1980s, virtually all evaluations of GBS isolated from healthy neonates, children, or adults revealed an even distribution into types Ia or Ib, II, and III. This distribution also was reported for isolates from neonates with early-onset infection without meningitis and their mothers.[112] In the 1990, types other than I, II, or III accounted for less than 5% of all isolates.

In the early 1990s, GBS type V emerged as a frequent cause of colonization and invasive disease in neonates and adults.[113-115] Most type V isolates have one pulse-field gel electrophoresis pattern that has been present in the United

States since 1975.[116] Type V now causes a substantial proportion of cases of invasive early-onset disease and infection during pregnancy. Type Ia has increased in prevalence and a corresponding decline has occurred in type II strains causing perinatal disease.[114] Type III strains account for about 70% of isolates from infants with meningitis and continue to be isolated from at least two thirds of infants with late-onset disease globally.[117,118,119] Type IV, which accounts for occasional cases, could be emerging as a more important cause of early-onset infection.[120] Types VI, VII, VIII, and IX rarely cause human disease in the United States or the United Kingdom, but types VI and VIII are the most common serotypes isolated from healthy Japanese women.[121,122]

The contemporary CPS type distribution of GBS from different patient groups is shown in Figure 12-4. Prospective population-based surveillance through the Active Bacterial Core Surveillance/Emerging Infections Program Network of the U.S. Centers for Disease Control and Prevention (CDC) defined the epidemiology of invasive GBS disease in the United States from 1999-2005.[123] The GBS types represented in 528 early-onset disease cases were Ia (30%), III (28%), V (18%), and II (13%). The distribution for 172 pregnancy-associated cases was similar. The type distribution among 469 late-onset cases was Ia (24%), III (51%), and V (14%). Type V predominated among cases in nonpregnant adults, accounting for 31%, followed by Ia (24%), II (12%), and III (12%).

MOLECULAR EPIDEMIOLOGY

Tools such as multilocus enzyme electrophoresis,[74,124] restriction-enzyme fragment-length polymorphism analysis, pulsed-field gel electrophoresis (PFGE),[125] random-amplified polymorphic DNA assay[126] and multiplex PCR[127] have been used for molecular characterization of GBS isolates. Multilocus sequence typing (MLST) and PFGE are reportedly more appropriate than a semiautomated repetitive sequence-based PCR DiversiLab system (bioMérieux, Durham, NC) for determining the relatedness of invasive GBS strains.[128] These molecular typing techniques have indicated that some geographically and epidemiologically distinct GBS isolates have identical patterns, suggesting dissemination of a limited number of clones in the United States. Molecular techniques also have confirmed the molecular relatedness of mother and infant strains, strains from twins and those from sexual partners.[121,126] Multilocus sequence typing and capsular gene cluster (*cps*) genotyping have been used to investigate the dynamics of perinatal colonization. Changes in capsule expression and recolonization with antigenically distinct GBS clones were detected in culture-positive women over time by applying MLST.[129]

Molecular characterization has been used to explore the role of virulence clones in contributing to invasive disease. Type III strains were classified into three major phylogenetic lineages, with most cases of invasive neonatal disease caused by strains with one restriction digest pattern (type III-3) on the basis of bacterial DNA restriction digest patterns.[130] The genetic variation that distinguishes restriction digest pattern type III-3 strains seems to occur within localized areas of the genome that contain known or putative

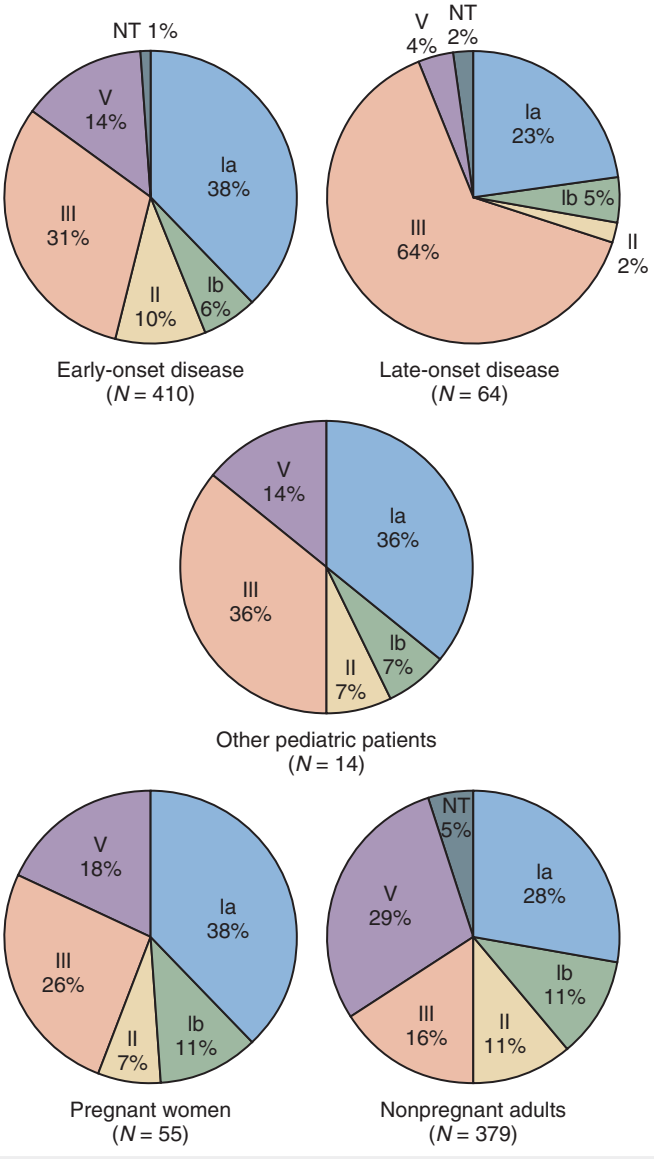

Figure 12-4 Schematic representation of group B streptococcal serotypes isolated from various patient groups. *N,* Number of patient isolates studied; *NT,* nontypeable strains. (Data from Blumberg HM, Stephens DS, Modansky M, et al: Invasive group B streptococcal disease: the emergence of serotype V, *J Infect Dis* 173:365-373. 1996; Zaleznik DF, Rench MA, Hillier S, et al: Invasive disease due to group B *Streptococcus* in pregnant women and neonates from diverse population groups, *Clin Infect Dis* 30:276-281, 2000; and Lin FY, Clemens JD, Azimi PH, et al: Capsular polysaccharide types of group B streptococcal isolates from neonates with early-onset systemic infection, *J Infect Dis* 177:790-792, 1998.)

virulence genes.[131,132] Using genomic subtractive hybridization to identify regions of the genome unique to virulent restriction fragment digest pattern type III-3 strains, a surface protein was identified that mediates epithelial cell invasion.[133] Using MLST, 10 allelic profiles that converged into three groups on concatenation were identified among type III isolates recovered from neonates with invasive disease and from colonized pregnant women.[134] One PFGE group bearing a gene from the capsular synthesis operon has been shown in type III strains causing neonatal meningitis, but not in type III colonizing strains.[135] Clustering of most invasive neonatal isolates into major PFGE groups also has been noted.[136] Among type III strains evaluated by MLST,

a single clone, ST17, is reported to be hypervirulent. Additional studies are required to elucidate the differences in virulence among clones identified by these techniques.[137] However, genetic analysis of GBS isolates from worldwide sources demonstrates that epidemic clones, such as clonal complex 17 (CC17) have adapted specifically to the human host.[138] The finding that isolates with different capsular serotypes have the same sequence type suggests that capsular switching can occur.[134,139] Bellais and colleagues[140] have demonstrated that capsular switching from CPS type III to IV does occur within the highly homogeneous CC17 hypervirulent clone. Sequence analysis showed that this capsular switch was due to the exchange of a DNA fragment containing the whole *cps* operon.

INCIDENCE OF INFECTION IN NEONATES AND PARTURIENTS

Two clinical syndromes occur among young infants with GBS disease that are epidemiologically distinct and relate to age at onset.[2,3] The attack rate for the first of these syndromes, designated early onset, because it occurs within the first 6 days of life (mean onset, 12-18 hours), ranged historically from 0.7 to 3.7 per 1000 live births. The attack rate for late-onset infection (onset 7-89 days of age) ranged from 0.5 to 1.8 per 1000 live births. The burden of early-onset disease is disproportionately high in African-American infants for reasons that are not well defined but might include higher maternal colonization rates, higher density of colonization, and higher rates of preterm deliveries compared with white women.[141] There has been a dramatic decline in the incidence of early-onset disease in the United States in association with implementation of universal antenatal screening and use of intrapartum antibiotic prophylaxis (IAP). From 1993 to 1998, when risk-based and GBS culture-based methods were in use, incidence of early-onset disease declined by 65%, from 1.7 to 0.6 per 1000 live births.[5] Comparison of the two approaches showed the superiority of a culture-based approach.[142] The incidence of early-onset disease declined further in association with implementation of guidelines, published in 2002 and revised in 2010, that advocate a culture-based approach for prevention of early-onset disease.[16,123] The national estimate of early-onset invasive disease in 2012 was 0.24 per 1000 live births, but this represented a plateau since 2008.[143] Despite the decline in incidence, GBS remains the most commonly reported pathogen causing early-onset disease, accounting for approximately 40% of cases in the United States.[144] In contrast to its impact on early-onset disease, IAP has had no impact on the incidence of late-onset disease, which has remained stable at approximately 0.3 per 1000 live births since 2002.[123]

Globally, GBS is a leading cause of neonatal sepsis in developed countries, but the burden of disease in the developing world is less clear, and more high-quality studies are needed. The reported incidence of neonatal GBS disease in developing countries ranges from 0 to 3.1 per 1000 live births, with variation within and between geographic regions.[145] Incidence rates are higher when automated culture methods are used. A systemic review and meta-analysis to examine the global burden reported a mean incidence of GBS in infants from birth to 89 days of age of 0.53 per 1000 live births.[146] Substantial heterogeneity existed between studies. Studies that reported use of any intrapartum antibiotic prophylaxis were associated with a lower incidence of early-onset GBS (0.23/1000 live births [95% CI, 0.13 to 0.59]) than those in which prophylaxis was not used (0.75/1000 live births [95% CI, 0.58 to 0.089]).

The male-to-female ratio for early-onset and late-onset GBS disease is equal. Before 1996, 20% to 25% of all infants with GBS disease had onset after the first 6 days of life. In 2012, 57% of infants had disease with onset after 6 days of life.[143] Infants born prematurely constitute approximately one fourth of the total with early-onset disease and one half of the total with late-onset disease.

The importance of GBS as a common pathogen for the perinatal period relates to the pregnant woman as well as her infant. The risk of intraamniotic infection is greater in women with heavy colonization. Implementation of IAP has been associated with a significant decline in the incidence of invasive disease in pregnant women, from 0.29 per 1000 live births in 1993 to 0.23 per 1000 live births in 1998, and a further decline to 0.12 per 1000 live births during 1999 to 2005.[123,142] One half of these infections were associated with infection of the upper genital tract, placenta, or amniotic sac and resulted in fetal death. Among the other infections, bacteremia without a focus (31%), endometritis without fetal death (8%), and chorioamnionitis without fetal death (4%) were the most common manifestations.

Immunology and Pathogenesis

HOST-BACTERIAL INTERACTIONS RELATED TO PATHOGENESIS

The prevalence and severity of GBS diseases in neonates have stimulated intensive investigation to elucidate the pathogenesis of infection. The unique epidemiologic and clinical features of GBS disease pose several basic questions that provide a framework for hypothesis development and experimental testing: How does the organism colonize pregnant women and gain access to the infant before or during delivery? Why are newborns, especially infants born prematurely, uniquely susceptible to infection? What allows GBS to evade host innate immune defenses? How do these organisms gain entry to the bloodstream and then cross the blood-brain barrier to produce meningitis? What specific GBS factors injure host tissues or induce the sepsis syndrome?

Advances in knowledge of pathogenesis have been achieved through analysis of GBS behavior in cell-culture systems and animal models. Advanced molecular genetic techniques have yielded isogenic mutant strains varying solely in the production of a particular component (e.g., capsular polysaccharide). Such mutants are important in establishing the biologic relevance of a given trait and its requirement for virulence in vivo. The sequencing of numerous complete GBS genomes has provided additional context for interpretation of experimental data and comparison with other well-studied pathogens.[6,7]

Although GBS have adapted well to asymptomatic colonization of healthy adults, they remain a potentially devastating pathogen to susceptible infants. This section reviews

Table 12-1 Group B Streptococcal Virulence Factors in Pathogenesis of Neonatal Infection

Virulence Factor	Molecular or Cellular Action(s)	Proposed Role in Pathogenesis
HOST CELL ATTACHMENT/INVASION		
C surface protein	Binds glycosaminoglycans	Epithelial/endothelial invasion
Fibrinogen receptor, FbsA	Binds fibrinogen in extracellular matrix	Epithelial cell attachment
Lipoteichoic acid	Binds host cell surfaces	Epithelial cell attachment
C5a peptidase, ScpB	Binds fibronectin in extracellular matrix	Epithelial cell adherence, invasion
Surface protein Lmb	Binds laminin in extracellular matrix	Epithelial cell attachment
Spb1 surface protein	Promotes epithelial cell uptake	Invasion of epithelial barriers
iagA gene	?Alteration in bacterial cell surface	Blood-brain barrier invasion
Glycoprotein Srr1	Binds fibronectin in extracellular matrix	Vaginal and brain capillary binding
CovRS two-component regulator	Global transcriptional regulator	Regulation of adherence factors
Surface protein HgvA	Endothelial cell binding/uptake	Promotes blood-brain barrier invasion
Pili	Promotes cell surface binding	Epithelial and endothelial cell invasion
INJURY TO HOST TISSUES		
β-Hemolysin/cytolysin	Lyses epithelial and endothelial cells	Damage and spread through tissues causes neuronal apoptosis
Hyaluronate lyase	Cleaves hyaluronan or chondroitin sulfate	Promotes spread through host tissues
CAMP factor	Lyses host cells (co-hemolysin)	Direct tissue injury
RESISTANCE TO IMMUNE CLEARANCE		
Exopolysaccharide capsule	Impairs C3 deposition/activation	Blocks opsonophagocytic clearance
C5a peptidase, ScpB	Cleaves and inactivates human C5a	Inhibits neutrophil recruitment
CAMP factor	Binds to Fc portion of IgG, IgM	Impairment of antibody function
Serine protease, CspA	Cleaves fibrinogen, coats GBS surface with fibrin	Blocks opsonophagocytosis
Fibrinogen receptor, FbsA	? Steric interference with C3 function	Blocks opsonophagocytosis
C protein	Nonimmune binding of IgA	Blocks opsonophagocytosis
β-Hemolysin/cytolysin	Lyses neutrophils macrophages, proapoptotic	Impairment of phagocyte killing
Superoxide dismutase	Inactivates superoxide	Impairment of oxidative burst killing
Carotenoid pigment	Antioxidant effect blocks H_2O_2, singlet oxygen	Impairment of oxidative burst killing
dlt operon genes	Alanylation of lipoteichoic acid	Blocks antimicrobial peptides
Penicillin-binding protein 1a	Alteration in cell wall composition	Blocks antimicrobial peptides
Nuclease A	Degrades DNA	Escape neutrophil extracellular taps
CovRS two-component regulator	Global transcriptional regulator	Phagolysomal survival
ACTIVATION OF INFLAMMATORY MEDIATORS		
Cell wall lipoteichoic acid	Binds pattern recognition receptors (TLRs)	Cytokine activation
Cell wall peptidoglycan	Binds pattern recognition receptors (TLRs)	Cytokine activation
β-Hemolysin/cytolysin	Activation of host cell stress response pathways, inflammasome	iNOS, IL-10, IL-1β release
GAPDH	Triggers IL-10 release	Suppression of neutrophil migration

CAMP, Christie-Atkins-Munch-Petersen; *GAPDH,* glyceraldehyde-3-phosphate dehydrogenase; *GBS,* group B streptococci; *H_2O_2,* hydrogen peroxide; *IgG, IgM,* and *IgA,* immunoglobulin G, M, and A, respectively; *IL,* interleukin; *iNOS,* inducible nitric oxide synthase.

the current understanding of virulence mechanisms, many of which are revealed or magnified by the unique circumstances of the birth process and the deficiencies of neonatal immune defense. Many of the GBS virulence factors defined to date, with mode of action and proposed role in pathogenesis, are summarized in Table 12-1. Key stages in the molecular, cellular, and immunologic pathogenesis of newborn infection are summarized schematically in Figure 12-5.

Maternal Colonization

The presence of GBS in the genital tract of the mother at delivery determines whether or not a newborn is at risk for invasive disease. Among infants born to colonized women, the risk of early-onset disease is approximately 30-fold that for infants born to women with a negative result on prenatal cultures.[147] A direct relationship exists between the degree (inoculum size) of GBS vaginal colonization, the risk of vertical transmission, and the likelihood of serious disease in the newborn.[104] Consequently, a crucial step in the pathogenesis of invasive disease in the newborn caused by GBS is colonization of pregnant women.

To establish colonization of the female genital tract, GBS must adhere successfully to the vaginal epithelium. Compared with other microorganisms, GBS bind very efficiently to exfoliated human vaginal cells or vaginal tissue culture cells,[148,149] with maximal biofilm formation at the acidic pH characteristic of vaginal mucosa.[150] A low-affinity interaction with epithelial cells is mediated by its amphiphilic cell wall–associated lipoteichoic acid, whereas higher-affinity interactions with host cells are mediated by hydrophobic

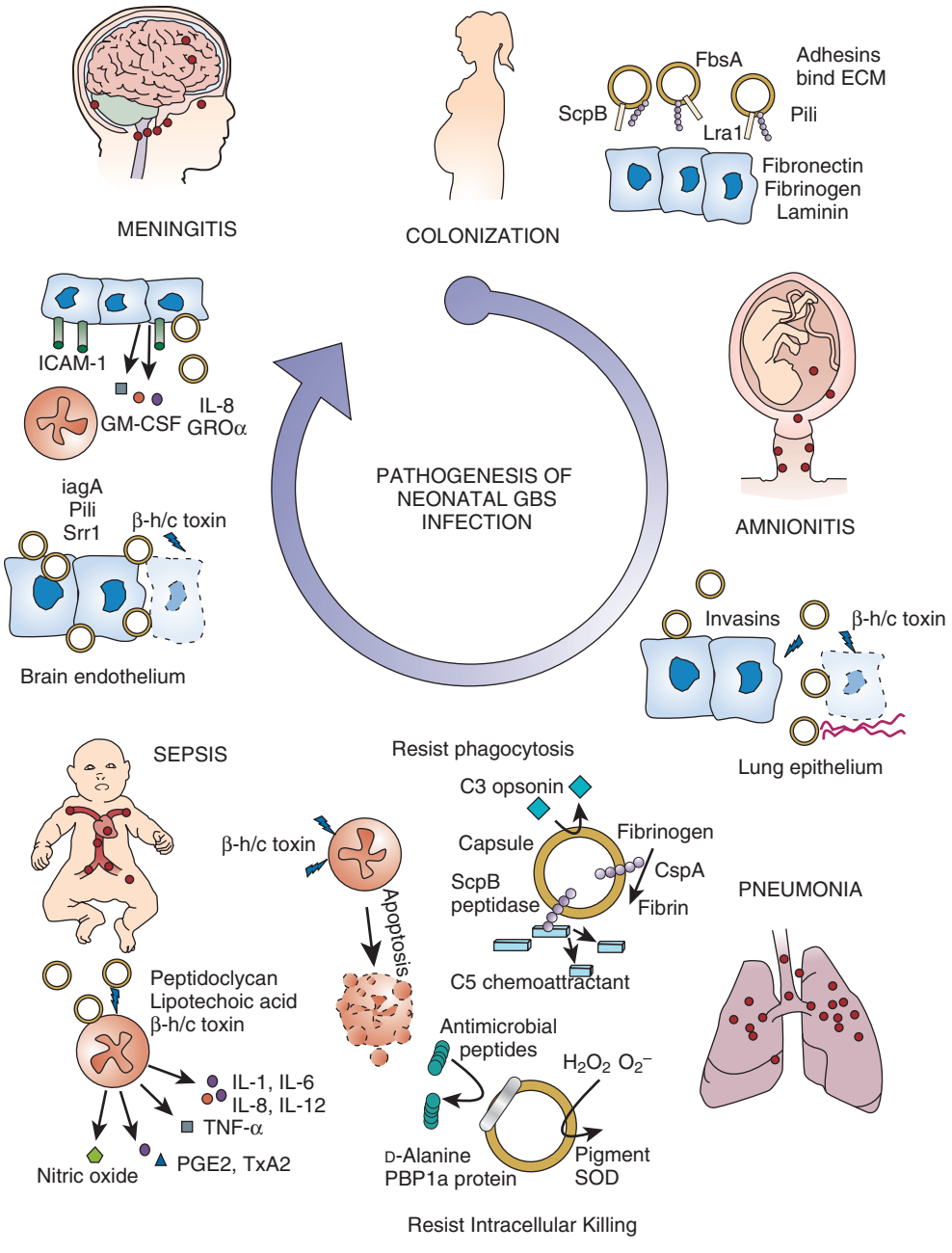

Figure 12-5 Pathologic mechanisms for different types of neonatal group B streptococcal (GBS) infection. *ECM,* Extracellular matrix; *GM-CSF,* granulocyte-macrophage colony-stimulating factor; *GROα,* growth-regulated protein α; *β-h/c,* β-hemolysin/cytolysin; *ICAM-1,* intercellular adhesion molecule-1; *IL-8,* interleukin-8; *PBP1a,* penicillin-binding protein 1a; *PGE2,* prostaglandin E2; *SOD,* superoxide dismutase; *TNF-α,* tumor necrosis factor-α; *TxA2,* thromboxane A2. (Modified from Doran KS, Nizet V: Molecular pathogenesis of neonatal group B streptococcal infection: no longer in its infancy, *Mol Microbiol* 54:23-31, 2004.)

surface proteins. Soluble lipoteichoic acid competitively inhibits epithelial cell adherence[151,152] and decreases vaginal colonization of pregnant mice.[153]

High-affinity protein-mediated interactions of GBS with epithelium are mediated largely through extracellular matrix components, such as fibronectin, fibrinogen, and laminin, which interact with host cell–anchored proteins such as integrins. Binding occurs to immobilized but not soluble fibronectin, suggesting that this interaction requires close proximity of multiple fibronectin molecules and GBS adhesins.[154] For example, a genome-wide phage display technique revealed a fibronectin-binding property associated with the surface-anchored GBS C5a peptidase ScpB, a dual functionality confirmed by decreased fibronectin binding of isogenic ScpB mutants and the direct interaction of recombinant ScpB with solid-phase fibronectin.[155,156] Similar targeted mutagenesis studies showed that adherence of GBS to laminin involves a protein adhesin called Lmb,[157] attachment to fibrinogen is mediated by repetitive motifs within the surface-anchored protein FbsA,[158] and binding to human keratin 4 is carried out by the serine-rich repeat domain protein Srr-1.[159]

Group B streptococci express filamentous cell surface appendages known as pili.[8] Group B streptococcal pili contain three subunits: a backbone pilin protein (PilB), a pilus-associated adhesion (PilA) and a component anchoring the protein assembly to the cell wall (PilC); these components are encoded by two distinct loci in the genome, called pilus islands 1 and 2 (PI-1 and PI-2), the latter presenting two distinct variants, PI-2a and PI-2b.[160,161] Epithelial cell adherence was reduced in isogenic GBS mutants lacking PilA or PilC, but not mutants lacking the PilB backbone.[161] Elucidation of the

crystal structure of PilC reveals a specific IgG-like fold domain (N2) required for epithelial cell binding.[162] In a mouse model of GBS vaginal colonization, both pili and Srr-1 protein are required for efficient colonization.[163] The regulation of these and other GBS adherence factors that determine vaginal epithelial cell and extracellular matrix binding is dynamically controlled by environmental pH and the two-component gene regulation system CovRS (CsrRS).[164] Deletion of CovRS results in increased bacterial adherence but decreased invasion of vaginal epithelial cells. In the vaginal colonization model, the host mounts a more robust inflammatory response to the GBS CovRS mutant, accelerated clearance.[165]

Ascending Amniotic Infection

Group B streptococci can reach the fetus in utero through ascending infection of the placental membranes and amniotic fluid. Alternatively, the newborn may become contaminated with the organism on passage through the birth canal. Infection by the ascending route plays a pivotal role in early-onset disease. A direct relationship exists between the duration of membrane rupture before delivery and attack rate for early-onset disease,[166] whereas an inverse relationship exists between the duration of membrane rupture and the age at which clinical signs of early-onset pneumonia and sepsis first appear.[167] When the duration of membrane rupture was 18 hours or less, the attack rate was 0.7 per 1000 live births; when it was more than 30 hours, the attack rate increased to 18.3 per 1000,[166] Histologic examination of placentas from women with GBS chorioamnionitis showed bacterial infiltration along a choriodecidual course, implying that ascending infection may be a primary trigger in many instances of premature rupture.[168]

GBS may promote membrane rupture and premature delivery by several mechanisms. Isolated chorioamniotic membranes exposed to the organism have decreased tensile strength and elasticity and are prone to rupture.[169] GBS also can modify the arachidonic acid metabolism of cultured human amnion cells, favoring production of prostaglandin E2,[170] which is known to stimulate the onset of labor. Stimulation of placental release of macrophage inflammatory protein-lα (MIP-lα), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), and matrix metalloproteases can recruit inflammatory cell and trigger alternative molecular signaling pathways that may provoke infection-associated preterm labor.[171,172]

This GBS β-hemolysin/cytolysin (β-h/c) toxin is responsible for the characteristic β-hemolytic phenotype displayed by the organism when grown on sheep blood agar. Mutagenesis and heterologous expression studies have identified the *cyl* gene locus to encode β-h/c and production of an orange carotenoid pigment; a single gene within this locus, *cylE*, is necessary for GBS β-h/c expression and sufficient to confer β-hemolysis when cloned in *E. coli*,[173,174] and one group has recently suggested that the pigment itself may function to form pores in target cell membranes.[175] The β-h/c activity targets a wide variety of host epithelial, endothelial, and immune cell membranes, provoking cellular dysfunction and, ultimately, a necrotic or apoptotic cell death.[176] GBS mutants lacking the CovRS regulator overexpress the toxin and penetrate isolated human chorioamniotic membranes more aggressively in a β-h/c–dependent manner, a finding which appears to correlate with increased hemolysis (and frequent CovRS mutation) among GBS isolated from women in preterm labor.[175] Findings in a novel mouse model of GBS chorioamniotis confirm a critical role for the β-h/c in breaching maternal-fetal barriers to trigger preterm birth and intrauterine fetal demise.[177]

GBS occasionally seem to penetrate into the amniotic cavity through intact membranes. Clinically, this mechanism of entry is suggested by reports of neonates with fulminant early-onset infection after cesarean section and no identifiable obstetric risk factors.[133,178] Migration of the organism through freshly isolated chorioamniotic membranes has been documented by scanning and transmission electron microscopy,[179] and GBS invade primary chorion cells efficiently in vitro and are capable of transcytosing through intact chorion cell monolayers without disruption of intracellular junctions.[180] They also secrete an enzyme that degrades hyaluronic acid, an important component of the extracellular matrix that is abundant in placental tissues and may facilitate amniotic invasion.[73] Placental expression of antimicrobial peptides such as human β-defensins and lactoferrins are induced by GBS and may constitute a key aspect of innate host defense against ascending infection by the pathogen.[181]

Amniotic fluid supports the proliferation of GBS,[182,183] such that when the organism gains access to the uterine cavity, a large inoculum can be delivered to the fetal lung; this results in a continuum of intrapartum (stillbirth) to early postpartum infant death.[183,184] In utero infection probably accounts for the 40% to 60% of newborns with early-onset disease who have poor Apgar scores and in whom pulmonary signs develop within a few hours of birth because these infants almost invariably display clinical or histologic evidence of congenital pneumonia.[183] Conversely, when GBS are encountered in the immediate peripartum period or on passage through the birth canal, a lesser inoculum is delivered to the neonate. Although a small but meaningful risk of subsequent invasive disease exists, most of these newborns have asymptomatic colonization limited to mucosal surfaces and remain healthy.

Fascinating new data collected in a nonhuman primate model of GBS choriodecidual inoculation suggest that the bacterium can induce fetal lung injury without ever crossing the placenta to produce intraamniotic infection or preterm delivery. Proinflammatory cytokines, including TNF-α and IL-8, elicited during transient choriodecidual infection appear to mediate this pathology, which included influx of neutrophils into the fetal intraalveolar space and thickening of the pulmonary interstitium.[185] Analysis of the gene expression profile in these primate fetal lung tissues showed an upregulation in immune response genes but downregulation of key genes associated with cellular growth, angiogenesis, morphogenesis, and development.[186]

Pulmonary and Bloodstream Entry

Early-onset GBS disease is heralded by respiratory symptoms, including tachypnea, hypoxia, cyanosis, and pulmonary hypertension.[187] One third to more than half of infants are symptomatic at birth or within 4 to 6 hours after delivery. Autopsies in fatal early-onset cases reveal that 80% have histologic evidence of lobar or multilobar pneumonia,[188] characterized by dense bacterial infiltration, epithelial cell damage, alveolar hemorrhage, interstitial inflammatory

exudate, and hyaline membrane formation.[189] When pneumonia develops in newborn primates exposed by intraamniotic injection of GBS, bacterial density reaches 10^9 to 10^{11} organisms per gram of lung tissue.[190] As shown in rabbits, the poorer resolution of pneumonia in preterm versus term newborns reflects quantitative deficiency of pulmonary alveolar macrophages, mandating the recruitment of neutrophils as a secondary phagocytic defense mechanism.[191]

Group B streptococcal disease rarely is limited to the initial pulmonary focus but spreads to the bloodstream and is circulated through other organs and tissues. The capacity of GBS to cause disruption of the lung epithelial and endothelial barrier evidently involves the process of intracellular invasion, direct cytolytic injury, and damage induced by the inflammatory response of the newborn host. Intracellular invasion of alveolar epithelial and pulmonary endothelial cells by GBS was first noted in newborn macaques after intraamniotic challenge[190] and later confirmed in human tissue culture lines derived from both cellular barriers.[192,193] In vivo and in vitro electron microscopy studies show that host cytoskeletal changes are triggered that lead to endocytotic uptake of the bacterium within a membrane-bound vacuole. Uptake requires induction of signal transduction pathways in the host cell that are mediated by Rho-family guanosine triphosphatases[194] and phosphatidylinositol-3-kinase.[195]

Cellular invasion is correlated with virulence potential. Clinical isolates of GBS from infants with bloodstream infections invade epithelial cells better than strains from the vaginal mucosa of asymptomatic women.[196] FbsA, a GBS fibrinogen-binding protein[197]; Lmb, which mediates laminin binding[198]; and ScpB, which interacts with fibronectin,[156] each play a role in promoting efficient epithelial or endothelial cell invasion. In addition, surface-anchored α C protein specifically interacts with host cell glycosaminoglycan on the epithelial cell surface to promote GBS internalization.[195-199] By contrast, CPS decreases intracellular invasion, presumably through steric interference of certain receptor-ligand interactions.[200] Finally, a specific role for pilus proteins in GBS lung epithelial cell adherence and invasion was recently identified.[201]

Although cellular invasion may play a principal role in bloodstream penetration in late-onset GBS infection, damage to the lung barrier often is evident in severe early-onset infection. Alveolar exudate and hemorrhage in autopsy studies of infants with GBS pneumonia attest to significant pulmonary epithelial and endothelial cell injury.[202] Recent studies in a fetal rat lung explant model show GBS profoundly alters lung morphology and caspase-dependent macrophage apoptosis within the lung interstitium.[203] The cellular damage may result largely from the actions of the pore-forming β-h/c that can lyse lung epithelial and endothelial cells and compromises their barrier function.[204,205] At subcytolytic doses, it promotes intracellular invasion and triggers the release of IL-8, the principal chemoattractant for human neutrophils.[206] Mutants lacking hemolysin expression are less virulent than the corresponding wild-type strains in a rabbit model of GBS pneumonia.[207]

The cytolytic, proinvasive, and proinflammatory effects of GBS β-h/c all are neutralized by dipalmitoyl phosphatidylcholine, the major phospholipid constituent of human lung surfactant.[204,206] This finding may partly explain the increased risk in premature, surfactant-deficient neonates for severe lung injury and invasive disease from GBS infection. Treatment with exogenous surfactant reduces histologic evidence of lung inflammation, improves lung compliance, and mitigates bacterial growth in preterm rabbits infected with GBS.[208,209] Clinical studies exploring the effect of surfactant administration on human infants with GBS sepsis also suggest a beneficial effect.[210,211]

Capsular Polysaccharide and Immune Resistance

On penetration of GBS into the lung tissue or bloodstream of the newborn infant, an immunologic response is recruited to clear the organism. Central to this response are host phagocytic cells, including neutrophils and macrophages. Effective uptake and killing by these cells require opsonization of the bacterium by specific antibodies in the presence of complement.[212-214] Neonates are particularly prone to invasive disease because of their quantitative or qualitative deficiencies in phagocytic cell function, specific antibody, or classical and alternative complement pathways. In addition to these newborn host susceptibilities, GBS possess numerous virulence determinants that seek to thwart each of the key components of effective opsonophagocytic killing. Chief among these factors is the sialylated GBS polysaccharide capsule.

The serotype-specific epitopes of group B streptococcal CPS are created by different arrangements of four monosaccharides (glucose, galactose, *N*-acetylglucosamine, and sialic acid) into a unique repeating unit (see "Immunochemistry of Polysaccharide Antigens"), but unfailingly, these structures contain a terminal sialic acid bound to galactose in an α2→3 linkage. The enzymatic machinery for capsule biosynthesis is encoded in the single long transcript of a 16-gene operon. Heterologous expression of a single polymerase gene (*cpsH*) from this operon can cause a GBS type Ia strain to express type III capsule epitopes, and vice versa.[215]

The conserved GBS terminal α2→3 sialic acid capsular component is identical to a sugar epitope widely displayed on the surface of all mammalian cells.[216] The terminal α2→3-linked sialic acid is overexpressed in humans, who in evolution have lost the genes to produce the alternative sialic acid, *N*-glycolylneuraminic acid (Neu5Gc). It is suggested that GBS may be a particularly troublesome human pathogen because its sialylated capsule has undergone selection to resemble host "self" and avoid immune recognition. Compared with wild-type strains, isogenic capsule-deficient mutants of GBS elicit greater degrees of proinflammatory cytokine release from human cells.[217] Group B streptococci can use this molecular mimicry to engage a sialic acid–binding surface receptor, Siglec-9, expressed on human neutrophils, leading to negative cell signaling cascades that dampen the oxidative burst and bactericidal activities of phagocytic cell.[218]

The properties of group B streptococcal CPS have been studied most thoroughly in serotype III organisms. Sialic acid is a critical element in the epitope of the type III capsule that confers protective immunity. After treatment with sialidase, the altered CPS fails to elicit protective antibodies against GBS infection. Protective antibodies derived from native type III capsule do not bind to the altered (asialo) capsule backbone structure.[219] Sialidase-treated type III

GBS are opsonized more effectively by complement through the alternative pathway and are more readily phagocytosed by human neutrophils in vitro, and exhibits diminished lethality in neonatal rats.[220,221]

Definitive evidence for the role of type III capsule in virulence is provided by the study of isogenic capsule-deficient mutants.[222,223] Compared with the parent strains, isogenic capsule mutants are susceptible to opsonophagocytosis in the presence of complement and healthy adult neutrophils.[224] Opsonization by complement is a pivotal element in host defense against invasive infections; however, the extent of C3 deposition on GBS by the alternative complement pathway is inversely related to the size and density of their polysaccharide capsule present.[224] C3 fragments bound to the acapsular mutant are predominantly in the active form, C3b, whereas the inactive form, C3bi, is predominantly bound to the surface of the parent strain. Moreover, by limiting phagocytic uptake into dendritic cells, GBS capsule decreases release of proinflammatory cytokines important in the bridge between innate and adaptive immunity.[225]

The type III GBS acapsular mutants lose virulence in animal models of infection. In a model of pneumonia and bacteremia, neonatal rats were inoculated with either the parent strain or an acapsular mutant by intratracheal injection. In animals that received the acapsular mutant, fewer GBS were recovered per gram of lung, more bacteria were associated with resident alveolar macrophages, and the animals became significantly less bacteremic than animals that received the parent strain.[226] Subcutaneous injection of the acapsular mutants in neonatal rats resulted in 50% lethal dose (LD_{50}) values that were at least 100-fold greater than the values obtained with the parent strain.[222,227] Mouse passage of various serotypes of GBS was followed by increases in sialylated capsule content that correlated with increased virulence.[228] Taken together, these data provide compelling evidence that the capsule protects the organism from phagocytic clearance during the initial pulmonary phase and the later bacteremic phase of early-onset infection.

Noncapsular Factors That Interfere with Immune Clearance

The ability of GBS to avoid opsonophagocytosis is enhanced by surface proteins that can act in concert with CPS. Serotype II strains displaying both components of the C protein antigen are more resistant to phagocytic killing than are serotype II strains lacking C protein.[229,230] The β antigen of C protein binds human IgA,[231,232] and IgA deposited nonspecifically on the bacterial surface probably inhibits interactions with complement or IgG.[233] A cell surface protease, CspA, targets host fibrinogen, producing adherent fibrin-like cleavage products that coat the bacterial surface and interfere with opsonophagocytic clearance.[234] The GBS BibA protein binds human C4bp, a component of the classical complement pathway, and increases resistance to phagocytic killing.[235] Finally, certain type Ia GBS strains can also use the surface-anchored β protein to engage Siglec-5 on macrophages and neutrophils and downregulate their innate immune function, a unique example of protein-mediated subversion of a host lectin receptor.[236]

After phagocytic uptake of pathogens, neutrophils and macrophages seek to kill the engulfed bacteria by generation of reactive oxygen products and other antimicrobial substances. Streptococci are often thought of as "extracellular pathogens," but these organisms can survive for prolonged periods within the phagolysosome of macrophages.[237,238] Although GBS lack the neutralizing enzyme catalase, they are 10 times more resistant to killing by hydrogen peroxide than is catalase-positive *Staphylococcus aureus*.[239] Several mechanisms for enhanced intracellular survival have been identified. The organism possesses an endogenous source of the oxygen metabolite scavenger glutathione.[239] Another defense against oxidative burst killing is the enzyme superoxide dismutase (SodA), as evidenced by the fact that a SodA mutant is highly susceptible to macrophage killing and survives poorly in vivo.[240] Finally, the orange carotenoid pigment genetically linked to the *cyl* operon encoding β-h/c can scavenge free radicals and neutralize hydrogen peroxide, superoxide, hypochlorite, and singlet oxygen, providing a shield against several elements of phagocyte oxidative burst killing.[176] The antioxidant effects of glutathione, SodA, and carotenoid pigment apparently compensate for the lack of catalase and explain the unexpected persistence of GBS within host phagolysosomes. The acid-responsive CovRS regulator is central to regulation of these phenotypes because CovR knockout mutants are unable to survive inside the phagosome of the macrophage.[241]

Cationic antimicrobial peptides, such as defensins and cathelicidins produced by host phagocytes, also are an important component of innate immune defense against invasive bacterial infection.[242] The GBS *ponA* gene encodes an extracytoplasmic penicillin-binding protein (PBP1a) that promotes resistance to phagocytic killing independent of capsule.[243] GBS mutants with deletion of the *PBP1a* gene are less virulent after lung and systemic challenge, and this is correlated to an increased susceptibility to defensins and cathelicidins.[244] Another way in which the organism avoids antimicrobial peptide clearance is through the D-alanylation of lipoteichoic acid in the bacterial cell wall; this requires activity of gene products that are encoded by the *dlt* operon. A *dltA* mutant exhibits decreased negative surface charge, which impedes cationic host defense peptides from reaching their cell membrane target of action.[245]

Direct cytotoxicity to host phagocytes represents another important virulence mechanism for immune resistance. The GBS β-h/c toxin produces direct cytolytic injury to macrophages and induces macrophage apoptosis over a longer interval. With highly hemolytic strains or with a large bacterial inoculum, killing of the phagocyte seems to outpace the phagocyte's microbicidal mechanisms, allowing bacterial proliferation in vitro in a murine bacteremia model.[241] Addition of an inhibitor of β-h/c blocks cytolysis and reduces apoptosis of macrophages, restoring phagocytic killing.[241] Signaling pathways involved in GBS-induced programmed cell death of macrophages seem to involve either caspase-3 or calpain activation.[246,247]

Deficiencies in the neutrophil response to GBS have been documented in newborn infants. Neutropenia and depletion of the marrow neutrophil storage pool are frequent findings in infants with septicemia[248] and are correlated with poor clinical outcome.[187] Although neutrophilia and an increase in granulocytic stem cells develop in adult rats infected with GBS, severe neutropenia without a change in stem cell counts develops in neonatal rats.[249] Fatal infection in neonatal rats is associated with failure of recovery of depleted myeloid

storage pools.[250] The explanation for this finding may be that the proliferative rate of neutrophils in noninfected neonatal animals already is maximal or near-maximal and cannot increase further in response to bacterial challenge[251] Recent data show that extracellular release of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by GBS induces macrophage IL-10 production, blunting neutrophil recruitment to infected organs.[252] The pathogen also produces an extracellular nuclease that degrades DNA-based neutrophil extracellular traps (NETs), increasingly recognized as a key component of neutrophil antibacterial function.[253]

GBS actively contribute to poor mobilization of neutrophils by production of an enzyme that cleaves and inactivates human C5a, a complement component that stimulates neutrophil chemotaxis.[71] Expression of C5a peptidase reduces the acute neutrophil response to sites of infection in C5a knockout mice reconstituted with human C5a.[254] Expression of GBS C5a peptidase is induced in normal human serum[255]; however, its enzymatic activity is often neutralized, in large part because of naturally occurring IgG antibodies present in many adults.[71] IgG also neutralizes C5a peptidase on the surface of a capsule-deficient GBS mutant but fails to neutralize the enzyme on the surface of the intact encapsulated type III parent strain. The capsule serves to protect the cell-associated C5a peptidase from inactivation by naturally occurring antibodies.

Inflammatory Mediators and Sepsis

When failures in epithelial barrier function and immunologic clearance allow GBS to establish bacteremia in the neonate, sepsis or septic shock develops. Intravenous infusion of GBS in animal models produces similar pathophysiologic changes to human newborn infection, including hypotension, persistent pulmonary hypertension, tissue hypoxemia and acidosis, temperature instability, disseminated intravascular coagulation, neutropenia, and, ultimately, multiple–organ system failure. These similarities have allowed in vivo experiments to elucidate the patterns in which the organism activates host inflammatory mediators to induce sepsis and circulatory shock.

Animal models in which GBS are infused intravenously exhibit a biphasic host inflammatory response.[256-258] The acute phase (≤1 hour after infusion) is manifested by increased pulmonary artery pressure and decreased arterial oxygenation and is associated with an increase in serum levels of thromboxanes. Pulmonary hypertension and hypoxemia persist through the late phase (2-4 hours), in which a progressive pattern of systemic hypotension, decreased cardiac output, and metabolic acidosis develops together with hematologic abnormalities; organ-system dysfunction; and increase in inflammatory markers, such as thromboxanes, TNF-α, and prostacyclins. If production of thromboxane and prostacyclin is blocked by inhibition of the cyclooxygenase pathway in rabbits or lambs infused with GBS, decreased myocardial dysfunction and a significant increase in systemic blood pressure are observed.[259-261]

Infusion of GBS produces pulmonary hypertension in piglets and isolated piglet lung preparations, suggesting a direct interaction of the organism with target cells in lung microvasculature.[262,263] GBS induce release of vasoactive eicosanoids prostacyclin and prostaglandin E2 from lung microvascular cells[264] and stimulate the host inflammatory mediators leukotriene D4[265] and thromboxane A2.[266]

The cytokine IL-12 has an important role in the systemic response to GBS infection. Elevation of IL-12 occurs 12 to 72 hours after challenge in the neonatal rat. Pretreatment with a monoclonal antibody against IL-12 results in greater mortality and intensity of bacteremia, whereas therapeutic administration of IL-12 is associated with a lower mortality rate and bloodstream replication of the organism.[267] By contrast, IL-1β, a known stimulator of cyclooxygenase and lipoxygenase pathways, has a more complex role depending on the stage and magnitude of infection. Activation of the inflammasome by the GBS β-h/c stimulated macrophage IL-1β release, and mice deficient in inflammasome components or the IL-1 receptor were considerably more susceptible to infection than wild-type mice.[268] In contrast, treatment with an IL-1 receptor antagonist improved cardiac output and mean arterial pressure, and increased duration of survival in piglets receiving a continuous infusion of GBS.[269]

TNF-α often is detected in the blood, urine, or cerebrospinal fluid (CSF) of infants with invasive GBS disease.[270] Although infusion of GBS in piglets is associated with TNF-α release during the late phase of hemodynamic response, the TNF-α inhibitor pentoxifylline has only modest effects on pulmonary hypertension, hypoxemia, and systemic hypotension.[271] Marked improvement in these hemodynamic parameters is seen only when pentoxifylline treatment is combined with indomethacin inhibition of thromboxane and prostacyclin synthesis.[272] Serum TNF-α also rises after challenge in rodent models; however, administration of polyclonal or monoclonal anti–TNF-α antibody does not affect overall mortality rate in these animal models.[272,273]

Inhibitor studies have shown that the mitogen-activated protein kinase (MAPK)/Jun *N*-terminal kinase (JNK) signaling pathway is required for the nuclear factor kappa B (NF-κB)–dependent inflammatory response of phagocytes to GBS.[274] Knockout mouse studies indicate that cell wall peptidoglycan–induced activation of p38 and NF-κB depends on the cytoplasmic Toll-like receptor (TLR) adapter protein myeloid differentiation primary response protein 88 (MyD88) but does not require the pattern recognition receptor TLR2 or TLR4.[275] The nitric oxide pathway is implicated in overproduction of proinflammatory cytokines, such as IL-6, and initiation of cellular injury during GBS lung infection.[276] Inducible cyclooxygenase-2 is also stimulated on GBS infection in human monocytes, likely through the MAPK pathway.[277] Infection also stimulates cyclooxygenase-2 and prostaglandin E2 expression in lung tissue in vitro and in vivo. GBS-induced cyclooxygenase-2 and prostaglandin E2 inflammatory response is reduced on treatment with an inducible nitric oxide synthase inhibitor and restored by addition of a nitric oxide donor, showing at least partial regulation by the nitric oxide pathway.[278]

The β-h/c toxin has several immunomodulatory properties that strongly influence the host response to GBS. β-H/c stimulates inducible nitric oxide synthase in macrophages, leading to release of nitric oxide.[279] In a mouse model of bacteremia and arthritis, β-h/c expression is associated with higher mortality, increased bacterial loads, greater degrees of joint injury, and release of the proinflammatory cytokines IL-6 and IL-1α systemically and intraarticularly.[280] Challenge of rabbits with isogenic GBS mutants showed that β-h/c production was associated with significantly higher degrees of hypotension, increased mortality, and evidence of liver necrosis with

hepatocyte apoptosis.[281] Partially purified β-h/c preparations produce significant hypotensive actions when infused in rats and rabbits, including death from shock.[282] β-H/c also contributes directly to cardiomyocyte dysfunction and apoptosis, which may magnify its role in the pathophysiology of GBS sepsis.[283] Finally, recent data reveal that p38 MAPK activation by β-h/c contributes to evasion of host defense through induction of the antiinflammatory cytokine IL-10, thereby blunting macrophage activation and immune resistance.[284]

Blood-Brain Barrier Penetration and Meningitis

The pathophysiology of GBS meningitis varies according to age at onset. In early-onset disease, autopsy studies show little or no evidence of leptomeningeal inflammation, despite the presence of abundant bacteria, vascular thrombosis, and parenchymal hemorrhage.[2,204] By contrast, infants with late-onset disease usually have diffuse purulent arachnoiditis with prominent involvement of the base of the brain.[285] Similar age-related differences in central nervous system (CNS) pathology are evident in the infant rat model of invasive disease.[286] These histopathologic differences reflect underdevelopment of the host immunologic response in the immediate neonatal period, with a higher proportion of deaths resulting from overwhelming septicemia.

To produce meningitis, GBS must penetrate human brain microvascular endothelial cells, the single-cell layer constituting the blood-brain barrier. Intracellular invasion and transcytosis of human brain microvascular endothelial cell tissue culture monolayers have been shown in vitro.[287] A number of specific virulence factors have been implicated in blood-brain barrier invasion. At high bacterial densities, invasion by GBS of brain microvascular endothelial cells is accompanied by evidence of β-h/c–induced cellular injury.[206] Correspondingly, β-h/c knockout mutants show decreased blood-brain barrier penetration and decreased lethality from meningitis in vivo.[206] Mutants with a defect in a diglucosyldiacylgycerol synthase (IagA) required for lipoteichoic acid anchoring to the cell wall show decreased brain endothelial invasion and are attenuated in their ability to produce meningitis in mice.[288]

Furthermore, GBS mutants lacking the fibrinogen receptor FbsA, laminin-binding protein Lmb, or pilus backbone subunit protein PilB also showed reduced adherence or invasion of human brain microvascular endothelial cells in vitro.[40,198,289] The pilus adhesin PilA binds collagen and allows GBS to interact with brain endothelial cell integrins, triggering chemokine expression and neutrophil recruitment; a GBS PilA-knockout mutant is associated with reduced neutrophil infiltration and diminished bacterial dissemination into the central nervous system.[290]

More recently, a GBS mutant lacking the surface-anchored, serine-rich repeat motif glycoprotein Srr-1 was attenuated for brain endothelial cell invasion and for production of meningitis in the murine model.[291,292] Similarly, the α C protein expressed by certain GBS strains binds to glycosaminoglycans on brain endothelium, promoting invasion and central nervous system entry.[293] Finally, a newly described surface protein, HgvA, characteristic of the hypervirulent ST-17 GBS clone binds brain capillary cells and choroid plexus and promotes the development of meningitis in these strains.[294]

The host inflammatory response to GBS contributes significantly to the pathogenesis of meningitis and CNS injury. The initiation of the inflammatory response is triggered through the sentinel function of the blood-brain barrier endothelium, which activates a specific pattern of gene transcription for neutrophil recruitment, including production of chemokines (e.g., IL-8, Groα), endothelial receptors (intercellular adhesion molecule-1), and neutrophil activators (granulocyte-macrophage colony-stimulating factor), with the β-h/c representing a major provocative factor.[206] A vascular distribution of cortical lesions in neonatal rats with GBS meningitis indicates that disturbances of cerebral blood flow contribute to neuronal damage.[295] Inflammation of individual brain vessels can lead to focal lesions, whereas diffuse alterations of cerebral blood flow could cause generalized hypoxic-ischemic injury and cerebral edema.[295]

TNF-α production by astrocytes, microglial cells, and infiltrating leukocytes seems to contribute to apoptosis of hippocampal neurons.[296] Intraventricular inoculation of newborn piglets with GBS results in an early sharp increase in CSF TNF-α levels, followed shortly by prostaglandin release and neutrophil influx.[297] GBS signal through TLR2 to activate and stimulate nitric oxide production by microglia cells, resulting in neuronal destruction.[298] In the course of experimental GBS meningitis, microglial apoptosis is triggered via the cysteine protease caspase-8 and is hypothesized to represent a self-dampening mechanism that prevents overstimulation of brain inflammation.[299] However, the GBS β-h/c toxin provokes neuronal apoptosis independently of caspase activation, which worsens clinical outcome in a neonatal rat intracisternal injection model.[300]

Risk Factors for Early-Onset Infection

Infant and maternal factors that increase risk for early-onset GBS infection are listed in Table 12-2. The most important risk determinant is exposure through maternal colonization at delivery. Maternal race or ethnicity correlates significantly with early-onset GBS disease, with enhanced risk for infants

Table 12-2 Risk Factors for Early-Onset Group B Streptococcal Disease

Risk Factor	Representative References
Maternal colonization at delivery	3, 83
High-density maternal colonization	104, 184
Rupture of membranes before onset of labor	184, 301
Preterm delivery < 37-wk gestation	301
Prolonged rupture of membranes ≥ 18 hr	301, 569
Chorioamnionitis	546
Intrapartum fever ≥38° C ≥100.4° F)	301
Intrauterine monitoring	301, 569
Maternal postpartum bacteremia	304
Multiple pregnancy	302, 303
Group B streptococcal bacteriuria or urinary tract infection	301
Cesarean section	3, 184
Low level of antibody to infecting CPS type	310
Young maternal age (<20 yr)	301, 569
Previous infant with invasive group B streptococcal infection	570
Maternal race/ethnicity	84, 114

CPS, Capsular polysaccharide.

born to African-American and Hispanic mothers compared with infants born to white mothers,[88,114,301] Risk correlates directly with density of maternal genital inoculum.[104] Symptomatic early-onset disease develops in 1% to 2% of infants born to colonized women who do not receive IAP, and the rate increases if there is premature onset of labor (before 37 weeks of gestation), chorioamnionitis or interval between rupture of membranes and delivery longer than 18 hours (11%),[184] twin pregnancy (35%),[302,303] or maternal postpartum bacteremia (10%).[304]

Maternal GBS bacteriuria and urinary tract infection are markers for high-inoculum ("heavy") colonization, which enhances infant risk for invasive infection.[305] Heavy colonization in the second trimester of pregnancy also is associated with increased risk of delivering a preterm infant.[306] Among infants born to mothers with premature rupture of membranes at term gestation, maternal chorioamnionitis and colonization with GBS enhance risk for neonatal infection.[307] Vaginal colonization with GBS is an independent risk factor for the development of chorioamnionitis.[308]

Prolonged interval after rupture of membranes (≥18 hours) before delivery and preterm delivery (<37 weeks of gestation) often are concomitant risk factors in neonates with early-onset GBS infection. The estimated incidence of early-onset GBS infection is 10 times higher in preterm than in term neonates.[184] The increased risk in twins likely relates to genetic factors regulating host susceptibility, lack of specific antibody to the infecting strain in the mother, similar density of maternal colonization, and virulence of disease-producing strains.[302,303]

Antibody to Capsular Polysaccharide

Lancefield showed that antibodies directed against capsular type-specific surface antigens of GBS protected mice from lethal challenge.[309] Baker and Kasper[310] showed in 1976 that neonatal risk for type III GBS disease correlated with a deficiency of antibody to type III CPS in maternal sera. Women with type III GBS genital colonization at delivery whose infants remained well more often had antibody concentrations exceeding 2 μg/mL of type III–specific antibodies in their sera than women whose infants developed type III early-onset disease.[311] These antibodies were predominantly IgG.[312] A similar correlation exists between low concentrations of type Ia– and type II–specific antibodies in maternal delivery sera and susceptibility of infants to invasive infection.[313,314] Approximately 15% to 20% of pregnant women have a concentration of IgG to CPS in their delivery serum presumed to protect against invasive disease. These higher concentrations are present significantly more often in sera of women colonized with the homologous GBS type than in sera of noncolonized women.[88,91]

Attempts have been made to quantify the concentration of antibody to group B streptococcal CPS in maternal serum conferring protection against invasive disease in infants. A prospective, multicenter, hospital-based, case-control study of mothers delivering infants with type Ia, III, or V early-onset sepsis and matched colonized control mothers delivering healthy infants quantified the maternal serum concentrations of type Ia, III, and V CPS-specific IgG at delivery that protected neonates from early-onset disease. For types Ia and III, maternal IgG concentrations of 0.5 μg/mL or greater corresponded to a 90% risk reduction. For type V, the same antibody concentration corresponded to 70% risk reduction.[315] The findings of Lin and colleagues[316,317] agreed in principle but described a higher concentration of CPS-specific IgG as the correlate for protection against type Ia or III GBS. Neonates whose mothers had at least a 5 μg/mL concentration of IgG to type Ia CPS in their sera had an 88% lower risk of developing early-onset disease compared with neonates whose mothers had concentrations less than 0.5 μg/mL. Neonates whose mothers had at least a 10 μg/mL concentration of IgG to type III CPS in their sera by enzyme-linked immunosorbent assay had a 91% lower risk for early-onset disease compared with neonates whose mothers had concentrations less than 2 μg/mL. Low concentrations of antibody to type III CPS are uniformly found in acute sera of infants with late-onset type III infection. Among 28 infants with late-onset bacteremia and 51 with meningitis, low levels of antibodies to type III CPS were found in acute sera from all infants. These low levels in term infants with late-onset type III GBS infection correlated with maternal levels at delivery.[311,318]

Antigens with "native" or intact type III polysaccharide specificity must be used to accurately assess human immunity to GBS.[319] Kasper and colleagues[219] used gently extracted (native) and acid-extracted (core) type III GBS and pneumococcal type 14 antigens to study sera from infants with invasive type III infection and their mothers. Concentrations of type III–specific antibodies in sera of sick infants and their mothers had uniformly low binding to intact polysaccharide. Opsonic immunity and immune responses during recovery from invasive disease correlated with antibodies to the native, fully sialylated, but not to the core, desialylated type III polysaccharide or to type 14 pneumococcal antigen.

Mucosal Immune Response

Genital colonization with GBS may elicit antibody responses in cervical secretions. Women with GBS type Ia, II, or III rectal or cervical colonization have elevated levels of IgA and IgG to the colonizing serotype in their cervical secretions compared with cervical secretions from noncolonized women. Elevated amounts of IgA and IgG to the protein antigen R4 also have been found in women colonized with type III strains (most type III strains contain R4 antigen) compared with noncolonized women.[320,321] These findings suggest that a mucosal immune response occurs in response to colonization with GBS. Induction of mucosal antibodies to surface GBS polysaccharide or protein antigens may prevent genital colonization, diminishing vertical transmission of infection from mothers to infants.

Complement and Antibody Interactions

Specific antibody is required, and the classical complement pathway maximizes opsonization of types I, II, and III GBS and facilitates alternative complement pathway–mediated opsonization of type III GBS.[220] A linear relationship exists between antibody concentration and the rate constant of killing of type III strains that is determined, at least in part, by the number of antibody molecules bound per organism.[322,323] IgG subclasses 1, 2, and 3 and IgM support opsonic activity in vitro,[324-327] and an IgA monoclonal antibody activated C3 and conferred protection against lethal infection.[328] Encapsulated and genetically derived acapsular mutants of type III GBS deposit C3 and support

its degradation, but an inverse correlation exists between extent of encapsulation and C3 deposition by the alternative pathway.[224,329] Among infants surviving type III GBS meningitis, transient development of specific IgM antibodies supported opsonophagocytosis during convalescence.[330] Clinical isolates of type Ia GBS can be efficiently opsonized by the classical complement pathway in the absence of antibodies.[331] Surface-bound CPS of type Ia strains mediates C1 binding and activation.[332,333] For type Ib GBS, a role for capsule size and density in modulating C3 deposition is reported. Variability among these strains in their capacity for C3 deposition by the alternative pathway also has been shown.[334]

Type II strains possessing both components of the C protein antigen are more resistant to opsonization than strains lacking both components.[229] Strains lacking type II polysaccharide but having C protein are readily opsonized. R protein or an IgA-mediated blocking effect may modulate phagocytosis of some type II strains. Despite the complexity of type II opsonins, it is clear that complement is essential and that integrity of the classical complement pathway is critical. Evaluation of neutrophil-mediated killing of types IV and V GBS also reveals the importance of complement and CPS-specific antibodies.[335,336] When complement is limited, type-specific antibodies facilitate killing. In sufficient concentration, agammaglobulinemic serum promotes opsonization, phagocytosis, and killing of types IV and V GBS.

During the course of septic shock caused by GBS, complement components are consumed. Cairo and associates[337] found a significant association between low levels of total hemolytic complement and fatal outcome from neonatal bacterial sepsis, including GBS. A critical role for C3 activation through the alternative pathway has been shown for potent GBS-induced TNF-α release.[338] This finding and the observation that complement-dependent uptake of CPS by marginal zone B cells seems necessary for an effective immune response to CPS[339] may partially explain this finding.

Pathology

Pathologic findings in early-onset infection depend on the duration of exposure to GBS before or during birth. Intrauterine death has been attributed to GBS infection[180,340] and is considered to be a contributing cause to midgestational fetal loss in women who have experienced either vaginal hemorrhage or septic abortion.[340,341] Fetal membrane infection with GBS can result in spontaneous abortion or premature rupture of membranes, or both, as suggested by Hood and associates in 1961 and others.[342-344]

Becroft and colleagues[345] noted histologic changes consistent with congenital pneumonia in live-born neonates whose autopsy lung cultures yielded GBS. Placentas showed amnionitis in mothers whose infants had fulminant pneumonia and died within 36 hours after birth. Findings were sufficient in stillborn infants to indicate that death occurred as a direct consequence of GBS intraamnionitis infection and intrauterine pneumonia. deSa and Trevenen[341] described pneumonitis with pulmonary interstitial and intraalveolar inflammatory exudates in 15 infants weighing less than 1000 g who had intraamniotic infection; 6 infants were stillborn, and 9 died within hours of birth. Placental examination revealed chorioamnionitis. In a primate model of infection, intraamniotic inoculation of GBS elicited fulminant early-onset neonatal infection.[186] Microscopy of lung tissue revealed organisms within membrane-bound vacuoles of alveolar epithelial cells; interstitial fibroblasts; and organisms present within tissue macrophages of the liver, spleen, and brain, documenting their rapid dissemination.

Amnionitis in association with early-onset GBS sepsis is more frequently detected when death occurs shortly after birth, is a common finding when membranes have been ruptured 24 hours or longer before delivery,[182,341,342] and can be clinically silent in some women. GBS can enter the amniotic fluid cavity through ruptured or intact membranes, allowing fetal aspiration of infected fluid and subsequent pulmonary lesions or bacteremia, without eliciting a local inflammatory response or maternal signs of intraamniotic infection.

Among neonates with fatal early-onset GBS disease, pulmonary lesions are the predominant pathologic feature. The association between pulmonary inflammation and formation of hyaline membranes was first noted by Franciosi and coworkers.[2] Subsequently, autopsy findings in early-onset disease cases revealed "atypical" pulmonary hyaline membranes, and these corresponded with radiographic features consistent with respiratory distress syndrome in some neonates. GBS were frequently present within these membranes, and in some infants these were composed almost entirely of streptococci. Katzenstein and colleagues[189] postulated that invasion of alveolar cells and capillary endothelial cells by GBS resulted in exudation of plasma proteins into the alveoli, deposition of fibrin, and hyaline membrane formation. Immune complex–mediated injury to the lung was proposed as a mechanism for this hyaline membrane formation.

Evidence for pneumonia was found historically in most infants with fatal early-onset GBS congenital pneumonia. The typical histologic features of congenital pneumonia (i.e., alveolar exudates composed of neutrophils, erythrocytes, and aspirated squamous cells, with edema and congestion) were observed either independently or in association with hyaline membrane formation. In neonates with fulminant, rapidly fatal GBS infection, the cellular inflammatory response was less pronounced. An interstitial inflammatory exudate is a common feature of fatal infection, as is pulmonary hemorrhage, which can range from focal interstitial to extensive intraalveolar bleeding.

In central nervous system infection, age at onset predicts distinctive morphologic findings in the brain and meninges. In early-onset meningitis, little or no evidence of leptomeningeal inflammation is seen in three quarters of infants, although purulent meningitis can be observed occasionally. This lack of inflammatory response can be the result of rapidly progressive infection, with an interval of only a few hours from onset of clinical illness until death, or can reflect inadequate host response to infection, or both. Bacteria generally are found in large numbers, and perivascular inflammation, thrombosis of small vessels, and parenchymal hemorrhage frequently are noted. In some preterm infants surviving septic shock caused by early-onset GBS infection,

periventricular leukomalacia, a condition characterized by infarction of the white matter surrounding the lateral ventricles, develops.[346] Infants with fatal late-onset meningitis almost always have a diffuse purulent leptomeningitis, especially prominent at the base of the brain, with or without perivascular inflammation and hemorrhage.[2,347] Infants surviving severe meningitis often have multiple areas of infarction or encephalomalacia.

The age-related inflammatory response in infants with GBS infection has a parallel in an experimental model of meningitis.[286] Young infant rats 5 to 10 days of age have numerous bacteria distributed in a perivascular pattern, and organisms can extend transmurally into vessel lumina. These animals generally have no evidence of acute leptomeningeal inflammation or edema. By contrast, 11- to 15-day-old animals have leptomeningitis and cerebritis with a pronounced infiltration of neutrophils and macrophages around meningeal vessels and in perivascular spaces within the cerebral cortex. Because response to infection becomes more efficient within a few weeks after birth, the absence of inflammation in the brain and meninges of infant rats and of human neonates with early-onset GBS infection may relate to chemotactic defects, exhaustion of neutrophil stores, reticuloendothelial system immaturity, or to other deficits in the host response to infection.

Clinical Manifestations and Outcome

EARLY-ONSET INFECTION

When the incidence of neonatal infection caused by GBS increased dramatically in the 1970s, a bimodal distribution became apparent. Two syndromes related to age were described by Franciosi and associates[2] (acute and delayed) and by Baker and Barrett[3] (early and late). Early-onset infection typically manifests within 24 hours of birth (an estimated 85% of cases; median age, 12 hours), but it can become evident during the second 24 hours of life (an estimated 10% of cases) or at any time during the subsequent 5 days. Premature infants often experience onset at or within 6 hours of birth; infants with onset after the first 24 hours of life usually are of term gestation. Late-onset infections occur at 7 to 89 days of age (median age, 37 days). Classification of syndromes by age at onset is useful, but there also is a continuum in age at onset. A few patients with early-onset disease can present at 5 or 6 days of age, and late late-onset infection can affect 3- to 6-month-old infants, especially infants with gestational age of less than 28 weeks. Onset beyond 6 months of age can herald the presentation of human immunodeficiency virus infection or other immune system abnormalities.[348]

Early-onset GBS infection often affects neonates whose mothers have obstetric complications associated with risk for neonatal sepsis: onset of labor before 37 weeks of gestation, prolonged interval at any gestation between rupture of membranes and delivery, rupture of membranes greater than or equal to 18 hours before delivery, intrapartum fever greater than 38° C (>100.4° F), intraamniotic infection, early postpartum febrile morbidity, and twin births (Table 12-3). A nearly threefold increase in risk of early-onset infection has been observed when six or more vaginal examinations are performed before delivery.[349] The incidence of infection correlates inversely with the degree of preterm birth, and GBS is the most frequent pathogen associated with early-onset sepsis in neonates with very low birth weight (<1500 g).[350] One fourth of infants with early-onset disease historically were born before 37 weeks of gestation, but this number has increased since the introduction of prenatal culture screening and IAP for women colonized with GBS.[123] Nevertheless, approximately three fourths of infants with early-onset GBS sepsis are born at term.[351]

Early-onset GBS infection in term neonates often occurs among infants with no defined maternal risk factors other than colonization. In such cases, recognition is often delayed until the appearance of definite signs of sepsis (e.g., tachypnea, apnea, hypotension), but more subtle signs usually precede these overt manifestations. One report found that one third of healthy term neonates with early-onset GBS infection were identified solely on the basis of evaluation for maternal intrapartum temperature exceeding 38° C (100.4° F).[352]

The three most common expressions of early-onset infection are bacteremia without a defined focus of infection, pneumonia, and meningitis. In the 21st century, bacteremia without a focus occurs in 80% to 85%, pneumonia

Table 12-3 Features of Group B Streptococcal Disease in Neonates and Infants

Feature	Early Onset (<7 days)	Late Onset (7-89 days)	Late–Late Onset (>89 days)
Median age at onset	1 day	37 days	>3 months
Incidence of prematurity	Increased	Increased	Common
Maternal obstetric complications	Frequent (70%)	Preterm delivery	Varies
Common manifestations	Septicemia (80%-85%)	Meningitis (25%-30%)	Bacteremia without focus (common)
	Meningitis (5%-10%)	Bacteremia without focus (65%)	Bacteremia with focus (uncommon)
	Pneumonia (10%-15%)	Soft tissue, bone/joint (5%-10%)	
CPS types isolated	Ia (≈30%)	III (≈60%)	Several
	II (≈15%)	Ia (≈25%)	
	III (30%)	V (≈15%)	
	V (20%)		
Case-fatality rate	3%-10%	1%-6%	Low

CPS, Capsular polysaccharide.

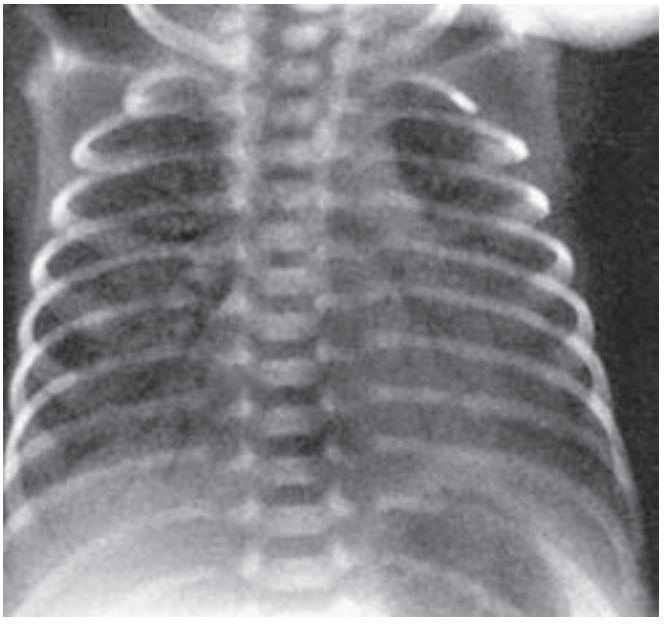

Figure 12-6 Chest radiograph from an infant with early-onset group B streptococcal septicemia shows features consistent with respiratory distress syndrome of the newborn.

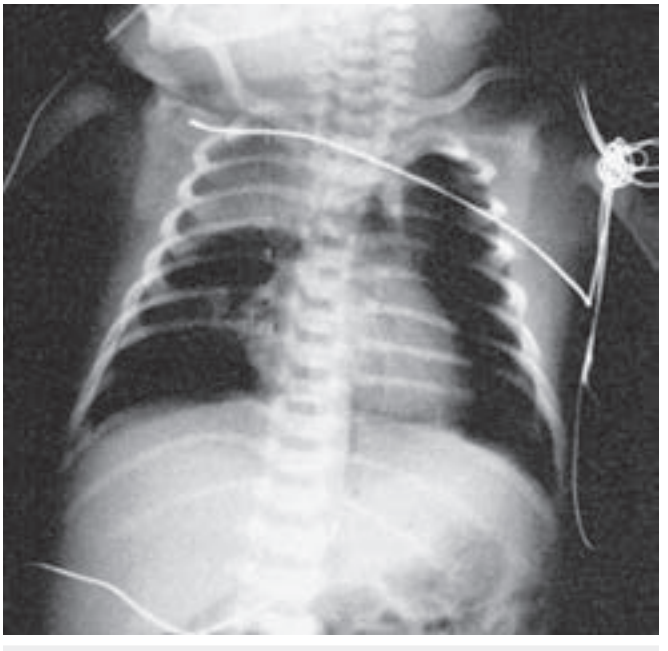

Figure 12-7 Chest radiograph shows right upper and lower lobe infiltrates as manifestations of early-onset group B streptococcal pneumonia in an infant.

occurs in 10% to 15%, and meningitis occurs in 5% to 10% of infants.[123] Bacteremia is often detected in neonates with the latter two presentations, but not always. Regardless of site of involvement, respiratory signs (apnea, grunting respirations, tachypnea, or cyanosis) are the initial clinical findings in more than 80% of neonates. Hypotension is an initial finding in approximately 25%. Infants with fetal asphyxia related to GBS infection in utero can have shock and respiratory failure at delivery.[353] Additional signs include lethargy, poor feeding, hypothermia or fever, abdominal distention, pallor, tachycardia, and jaundice.

Pneumonia occurs in 10% to 15% of infants with early-onset infection, and virtually all of these infants have acute respiratory signs. Most have these respiratory findings in the first few hours of life, many at birth. Among 19 infants with GBS congenital pneumonia at autopsy, 89% had 1-minute Apgar scores of 4 or less, indicating in utero onset of infection. Radiographic features consistent with and indistinguishable from those of surfactant deficiency are commonly found (Fig. 12-6). Treatment with surfactant improves gas exchange in most, although the response is slower than in noninfected infants, and repeated surfactant doses often are needed.[210] Infiltrates suggesting congenital pneumonia (Fig. 12-7) are present in one third of infants. Increased vascular markings suggesting the diagnosis of transient tachypnea of the newborn or pulmonary edema can occur. On occasion, respiratory distress is present in the absence of radiographic abnormalities, appearing as persistent fetal circulation and pulmonary hypertension. Small pleural effusions and cardiomegaly can occur.

Meningitis occurs in 5% to 10% of neonates with early-onset infection. Neonates with meningitis often have a clinical presentation early in the course that is identical to presentation of neonates without meningeal involvement. Respiratory distress can be the most common initial sign, and in 27 infants with early-onset meningitis, seizures were never a presenting feature.[354] Examination of CSF is the only means to exclude meningitis, a finding that requires modification of supportive and specific chemotherapy (see "Treatment" later). Seizures occur during the first 24 hours of therapy in nearly 50% of infants with meningitis. Persistent seizures, semicoma or coma, and a CSF protein concentration greater than 300 mg/dL are associated with a poor prognosis.[355,356]

The case-fatality rate for early-onset infection in the 1970s approached 50%. Current data indicate much lower rates of 2% to 10%. Features associated with fatal outcome include a low 5-minute Apgar score, shock, neutropenia, pleural effusion, apnea, and delay in treatment after onset of symptoms.[353,357] Fatal infection also occurs significantly more often among premature than term neonates (Table 12-4).

Table 12-4 Fatality Rates in Early-Onset Group B Streptococcal Infection

	CASE-FATALITY RATE (%) BY BIRTH WEIGHT (G) OR GESTATIONAL AGE (WK)				
Study	**500-1000**	**1001-1500**	**1501-2000**	**2001-2500**	**<2500**
Boyer et al[571] (1973-1981)	90	25	29	33	3
Baker[504] (1982-1989)	60	25	26	18	5
Weisman et al[353] (1987-1989)	75	40	20	15	6
Schrag et al[5] (1993-1998)		30 (≤33 wk)		10 (34-36 wk)	2 (≥37 wk)
Phares et al[123] (1999-2005)		20 (<37wk)			3 (≥37 wk)

Contemporary data document that the risk of death among preterm cases is 20%, nearly eightfold that of term infants for whom infection was fatal in 3% of cases.[123]

LATE-ONSET INFECTION

Late-onset GBS infection historically affected term infants 7 to 89 days of age who had had an unremarkable maternal obstetric and early neonatal history. Contemporary data indicate that at least half of infants with late-onset disease are born before 37 weeks of gestation.[123] Term infants are younger at presentation for late-onset disease (mean, 30 days) than are preterm infants (mean, 41 days).[358] Late-onset disease has a lower fatality rate (1%-6%) than early-onset disease. Clinical expressions of late-onset disease include bacteremia without a focus of infection (65% of infants), meningitis (25%) and cellulitis or osteoarthritis (2%-3% each) (see Table 12-3).[123]

Bacteremia without a detectable focus of infection is the most common clinical expression of late-onset GBS disease. Bacteremia without a focus typically manifests with nonspecific signs (i.e., fever, poor feeding, irritability). Diagnosis results from the practice of obtaining a blood culture in febrile infants during the first few weeks of life to exclude serious bacterial infection. These infants often are mildly ill, but failure to initiate antimicrobial therapy in a timely manner can result in progression to shock, especially in preterm infants, or extension of infection to distant sites such as the CNS. Either transient or persistent bacteremia can occur. Approximately 3% of infants with late-onset bacteremia without a focus die; survivors typically recover without sequelae after treatment.

The presenting signs in infants with late-onset meningitis almost always include fever; irritability or lethargy, or both; poor feeding; and tachypnea. Upper respiratory tract infection precedes late-onset meningitis in 20% to 30% of infants, suggesting that alteration of mucosal barrier by respiratory viral illness might facilitate entry of GBS into the bloodstream.[2,3] In contrast to early-onset infection, grunting respirations and apnea are less frequent initial findings, and their presence suggests rapidly progressive, fulminant infection. Apnea or hypotension is observed in less than 15% of patients, but there is a spectrum in clinical severity of illness at presentation. Some infants appear clinically well a few hours before initial evaluation and present with seizures, poor perfusion, neutropenia, and large numbers of gram-positive cocci in the CSF. These patients often have a rapidly fatal course, or, if they survive, they are left with devastating neurologic sequelae. Leukopenia or neutropenia at the time of diagnosis has been correlated with fatal outcome in these infants.[357]

Other initial findings associated with increased risk for fatal outcome or permanent neurologic sequelae include seizures at admission, coma or semicoma, need for pressor support, and CSF protein level greater than 300 mg/dL.[356,357,359] These findings most likely reflect a high bacterial inoculum in the CSF and cerebritis. Subdural effusions, which usually are small, unilateral, and asymptomatic, are found in some infants, but these are not associated with permanent sequelae. Subdural empyema, obstructive ventriculitis, large infarctions, and encephalomalacia are uncommon complications.

LATE LATE–ONSET INFECTION

Infections in infants older than 89 days of age account for approximately 10% of cases in infancy.[360] Most of these infants have a gestational age of less than 35 weeks. The need for prolonged hospitalization and the immature host status probably contribute to infection beyond the interval for term neonates. Bacteremia without a focus is a common presentation. On occasion, a focus for infection, such as the CNS, intravascular catheter, or soft tissues, is identified (see Table 12-3). In the outpatient setting, infants older than 89 days of age are likely to have a temperature greater than 39° C (>102.2° F) and a white blood cell count exceeding 15,000/mm^3.[361] A viral infection can precede the onset of bacteremia.[362] When there are no other apparent risk factors for late late–onset infection in a term infant, immunodeficiency should be considered.[363-365]

SEPTIC ARTHRITIS AND OSTEOMYELITIS

The clinical features of 20 infants with GBS septic arthritis alone and 45 infants with osteomyelitis (with or without concomitant septic arthritis) are shown in Table 12-5. The mean age at diagnosis of osteomyelitis (31 days) is greater than that for septic arthritis (20 days). The mean duration of clinical sign is shorter for septic arthritis than for osteomyelitis (2 vs. 9 days). In some infants with osteomyelitis, failure to move the involved extremity since hospital discharge after birth, or shortly thereafter, may be noted; this lack of movement can persist for weeks before the diagnosis is established.[366]

Decreased motion of the involved extremity and evidence of pain with manipulation, such as lifting or diaper changing, are common signs of bone infection. Warmth or erythema can occur occasionally; a history of fever is uncommon. The paucity of signs suggesting infection and the finding of pseudoparalysis have led to an initial diagnosis of Erb palsy and to assessment for possible child abuse.[366,367] In some infants, osteomyelitis of the proximal humerus has been associated with findings on nerve conduction studies consistent with brachial plexus neuropathy,[368,369] and in one infant, sciatic nerve injury at the level of the pelvis caused footdrop in association with iliac osteomyelitis.[370]

Physical findings include fixed flexion of the involved extremity, mild swelling, evidence of pain with passive

Table 12-5 Clinical Features of Group B Streptococcal Bone and Joint Infections

Feature	Septic Arthritis without Osteomyelitis	Osteomyelitis
Mean age at diagnosis (days)	20	31
Mean duration of symptoms (days)	2	9
Most common site	Hip	Humerus
Other common sites	Knee, ankle	Femur, tibia
Usual duration of parenteral therapy (range)	2 weeks (2-3)	3 weeks (2-6)

motion, decreased spontaneous movement, and, in a few infants, erythema and warmth. Most infants with osteomyelitis of the humerus have concomitant infection in the shoulder joint. Lack of associated systemic involvement is the rule, although osteomyelitis can occur in association with sepsis and in infants with other foci of infection such as meningitis.

Infants with septic arthritis often have lower extremity involvement, with the hip joint predominating. By contrast, more than half of the reported infants with osteomyelitis have had involvement of the humerus, and in infants for whom the location was specified, the proximal humerus predominated.[366] Involvement of the femur, vertebrae, or small bones occurs occasionally.[371,372] Usually, only one bone is affected, although infection involving two adjacent bones or multiple nonadjacent bones can occur rarely.[373] GBS bone and joint infections have a good prognosis. At evaluation months to years after diagnosis, most infants with osteomyelitis have had normal function in the affected extremity.

Residual shortening and limitation of motion of the humerus can be observed after osteomyelitis. Growth disturbance can result as a consequence of subluxation of the hip joint after septic arthritis.

Osteomyelitis seems to represent a clinically silent early-onset bacteremia with seeding of a bone and then later onset of clinical expression of infection. An episode of asymptomatic bacteremia with a birth trauma–induced nidus in the proximal humerus could allow localization of bacteria to the bone. Because lytic lesions take more than 10 to 14 days to become radiographically visible, the presence of such lesions on radiographs obtained at hospital admission suggests long-standing infection (Fig. 12-8).

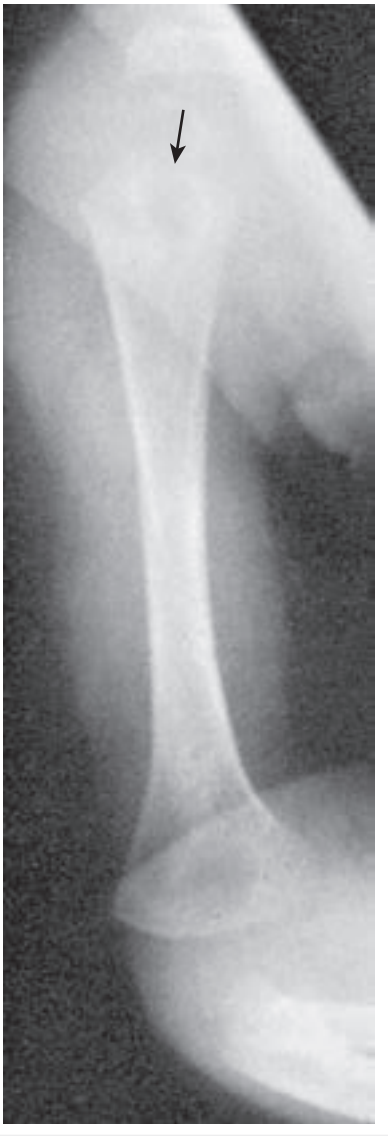

Figure 12-8 Radiograph shows lytic lesion *(arrow)* of the proximal humerus in an infant whose bone biopsy showed osteomyelitis caused by type III group B streptococci.

CELLULITIS OR ADENITIS

The manifestation of late-onset GBS infection, designated as facial cellulitis,[374] submandibular cellulitis,[375] cellulitis/adenitis syndrome,[376] or lymphadenitis,[377] has been reported in at least 25 infants.[378-381] Presenting signs include poor feeding; irritability; fever; and unilateral facial, preauricular, or submandibular swelling, usually, but not always, accompanied by erythema. The mean age at onset is 5 weeks (range, 2-11 weeks), and in contrast to all other expressions of late-onset infection, there is a striking male predominance. The most common sites are the submandibular and parotid, and enlarged adjacent nodes become palpable within 2 days after onset of the soft tissue infection. Four of the five infants with facial or submandibular cellulitis described by Baker[376] had ipsilateral otitis media at the time of diagnosis. Less common sites of involvement with cellulitis are the face, preauricular or inguinal areas, scrotum, anterior neck region, and prepatellar spaces (Fig. 12-9).[376,380,381] In one patient, cellulitis of the neck occurred in association with an infected thyroglossal duct cyst.[376]

Bacteremia almost always is detected in these infants, and cultures of soft tissue or lymph node aspirates have yielded GBS in approximately 90% of infants in whom this procedure was performed. These infants usually are not seriously ill, few have associated meningitis, and recovery within a few days of initiation of appropriate antimicrobial therapy is the rule.[382] Fulminant and fatal facial cellulitis has been described in a 7-hour-old neonate,[4] however, and associated meningitis has been described.[383]

UNUSUAL MANIFESTATIONS OF INFECTION

Numerous uncommon clinical manifestations of early-onset and late-onset GBS infection have been recorded (Table 12-6). Peritonitis[384] and adrenal abscess[385-387] have been described as abdominal manifestations of early- and late-onset infection. Adrenal abscess is thought to result from bacteremic seeding associated with adrenal hemorrhage and subsequent abscess formation. One neonate thought to have neuroblastoma underwent en bloc resection of a large mass with nephrectomy before the diagnosis of adrenal abscess was established.[386] Gallbladder distention is a nonspecific manifestation of early-onset sepsis that usually resolves with medical management of the infection.[388] Late-onset bacteremia can occur in association with

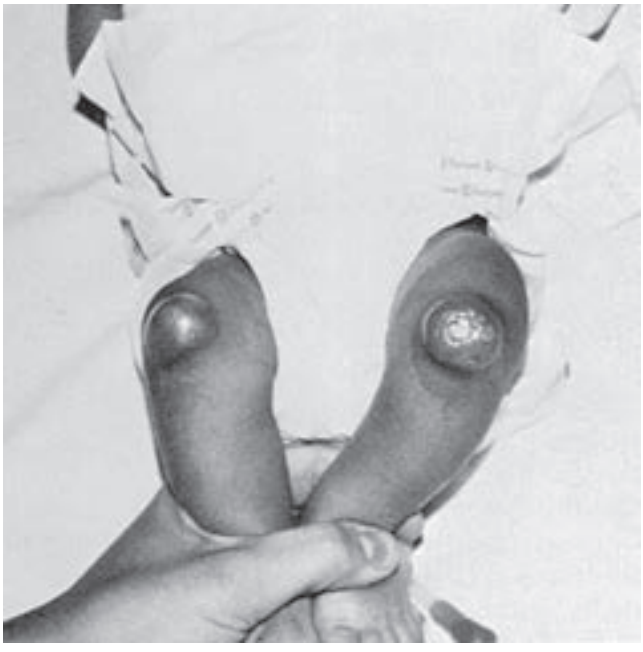

Figure 12-9 Prepatellar bursitis of both knees in an infant who had abraded his knees on the bed sheets. Aspiration of purulent material from the prepatellar space yielded type III group B streptococci. The knee joints were not affected.

jaundice, elevated levels of liver enzymes, and increased direct-reacting bilirubin fraction. Hemolysis and hepatocellular inflammation possibly contribute to the development of jaundice.

Brain abscess rarely occurs in association with recurrence of GBS meningitis. One infant recovered after craniotomy and excision of a well-encapsulated frontal mass but had neurologic sequelae.[389] Another infant presented at 5 weeks of age with a cerebellar cyst believed to represent an astrocytoma.[390] This infant proved to have obstructive hydrocephalus and chronic GBS ventriculitis. Rarely, anterior fontanelle herniation can complicate severe meningitis. The presence of a noncystic doughy mass over the fontanelle indicates that brain herniation may have occurred, and cranial imaging can confirm this diagnosis. One patient with cervical myelopathy initially had absence of extremity movement, but made a good recovery and was able to walk at age 3 years.[391]

Subdural empyema is another unusual complication of GBS meningitis described in patients with early- and late-onset infections.[392,393] The diagnosis is established by needle aspiration of the subdural space.[393] Irritability, vomiting, seizures, increasing head circumference, focal neurologic signs, a tense anterior fontanelle, or a combination of these should prompt evaluation.[392,394] Sterilization of the subdural space was accomplished by open or closed drainage in conjunction with antimicrobial therapy. Basal ganglia and massive cerebral infarction also have been described.[395]

Cardiovascular manifestations of GBS infection are rare. Endocarditis,[109,396,397] pericarditis,[398] myocarditis,[399] and mycotic aneurysm of the aorta[400] have been documented. Echocardiography can be useful in delineating the nature of cardiac involvement, and this technique was used

Table 12-6 Unusual Clinical Manifestations of Group B Streptococcal Infections

Site and Manifestation	Associated with Early-Onset or Late-Onset Infection	Reference
ABDOMEN		
Peritonitis	Both	384
Adrenal abscess	Both	385-387
Gallbladder distention	Early	388
BRAIN		
Abscess	Late	389
Anterior fontanelle herniation	Both	571
Chronic meningitis	Late	390
Subdural empyema	Both	392, 393
Cerebritis	Late	395
Myelopathy/myelitis	Early	391, 573
Ventriculitis of myelomeningocele	Both	574
Oculomotor nerve paralysis	Late	575
Ventriculoperitoneal shunt infection	Late	576
CARDIOVASCULAR		
Asymptomatic bacteremia	Both	4, 577, 578
Endocarditis	Both	109, 396, 397
Pericarditis	Not specified	398
Myocarditis	Late	399
Mycotic aneurysm	Late	400
EAR AND SINUS		
Ethmoiditis	Late	4
Otitis media/mastoiditis	Both	401-404
EYE		
Conjunctivitis/ophthalmia neonatorum	Early	2, 405, 579
Endophthalmitis	Late	406
Retrobulbar abscess	Early	580
RESPIRATORY TRACT		
Diaphragmatic hernia	Both	411
Supraglottitis	Late	407
Pleural empyema	Both	4, 409, 410
Tracheitis	Late	408
SKIN AND SOFT TISSUE		
Abscess of cystic hygroma	Late	425
Breast abscess	Late	428, 581
Bursitis	Late	582
Cellulitis/adenitis	Both	4, 374-381, 583
Dactylitis	Late	584
Fasciitis	Late	418-420
Impetigo neonatorum	Early	421, 422
Purpura fulminans	Both	415-417
Omphalitis	Both	423
Rhabdomyolysis	Late	585
Retropharyngeal cellulitis	Late	426, 427
Scalp abscess	Both	424
Urinary Tract Infection	Both	429

successfully to detect a 0.7-cm vegetation on the anterior leaflet of the mitral valve in a 4-week-old infant with endocarditis caused by a type III strain.[396] Paroxysmal atrial tachycardia can be a presenting feature of GBS septicemia in the absence of focal infection of the heart.[133]

GBS are an uncommon cause of otitis media in the first few weeks of life (2%-3% of cases).[401] Otitis media is more often associated with late-onset disease manifesting as meningitis or submandibular cellulitis.[402-404] The finding of acute mastoiditis at autopsy in one infant with otitis media and meningitis suggests that the middle ear can serve as a portal of entry in a few patients.[404]

Conjunctivitis related to GBS occurs only rarely. Exudative conjunctivitis has been reported, however, in association with early-onset bacteremia.[405] More severe ocular involvement is rare, but endophthalmitis has been noted in infants with septicemia and meningitis.[406] As is the case for other agents producing endophthalmitis, high-grade bacteremia is a likely prelude to this unusual metastatic focus of GBS infection.

Supraglottitis was described in a 3-month-old infant with acute onset of stridor.[407] Swelling of the left aryepiglottic fold, but not the epiglottis, was noted at laryngoscopy. An infant with bacterial tracheitis had a similar presentation.[408] Although pulmonary infection caused by GBS is common, pleural involvement is rare, but it has been reported as a complication of early-onset[409] and late-onset[410] pneumonia. An interesting but unexplained association is delayed development of right-sided diaphragmatic hernia and early-onset GBS sepsis.[411] In affected infants, the onset of respiratory distress invariably occurs at or within 48 hours after birth, whereas the mean age at diagnosis of right-sided diaphragmatic hernia in the 40 reported cases is 11 days (range, 4-91 days). One speculation is that GBS pneumonia causes necrosis of the adjacent diaphragm and results in herniation of viscera into the pleural space. Another is that ventilation increases intrathoracic pressure to mask or delay herniation through a congenital diaphragmatic defect. This phenomenon should be a consideration in an infant whose condition deteriorates despite appropriate management for early-onset disease. Radiographic features include increased density in the right lower lung or irregular aeration or both, followed by progression to elevation of right bowel gas and liver shadow.

In addition to cellulitis and adenitis, GBS uncommonly can produce various unusual skin and soft tissue manifestations, including violaceous cellulitis,[412] perineal cellulitis and septicemia after circumcision,[413] scrotal ecchymosis as a sign of intraperitoneal hemorrhage,[414] purpura fulminans,[415-417] necrotizing fasciitis,[418-420] impetigo neonatorum,[421,422] omphalitis,[423] scalp abscess secondary to fetal scalp electrode,[424] abscess complicating cystic hygroma,[425] retropharyngeal cellulitis,[426,427] and breast abscess.[428] In patients with impetiginous lesions and abscess formation, bacteremia is unusual, but it is a frequent accompaniment to omphalitis and necrotizing fasciitis.

Among infants with early-onset bacteremia, isolation of GBS from the urine is frequent when this body fluid is cultured, but primary urinary tract infection with these organisms is rare. An infant with severe bilateral ureterohydronephrosis and GBS in his urine has been described.[429] The isolation of GBS from a urine culture of a patient without bacteremia is an indication for evaluation for possible structural anomalies of the genitourinary tract.

RELAPSE OR RECURRENCE OF INFECTION

Relapse or recurrence of GBS infection occurs in an estimated 0.5% to 3% of term or preterm infants.[430,431] Signs can develop during treatment for the initial episode or at an interval of 3 days to 3 months after completion of therapy.[432-434] In one review, eight of nine infants with a recurrence were born at 25 to 36 weeks of gestation, and male infants predominated.[433] The first episode occurred at a mean age of 10 days (range, 1-27 days) and the recurrence at a mean age of 42 days (range, 23-68 days) of life. In another report that included a set of fraternal twins, seven of eight infants were preterm (mean, 30 weeks of gestation), each had a birth weight of less than 2500 g, and all infections were late onset.[434] The mean age at initial presentation was 38 days (range, 13-112 days), and at recurrence, it was 57 days (range, 34-130 days). Recurrence often occurs within days after discontinuing antibiotic treatment. Two relapses in one infant have been documented.[430,431,435,436]

Relapse or recurrence of infection can be the result of an undrained focus of infection, such as a brain abscess, or can occur in association with congenital heart disease. Identical isolates recovered from maternal genital and breast milk cultures suggest that breast milk can serve as a source of repeated infant exposure.[437,438] Recurrent infection can have a clinical expression similar to that of the initial episode or can involve new sites (meninges, ventricular or subdural fluid, or both; brain parenchyma; and soft tissue). In most instances, the second episode of GBS disease responds to retreatment with penicillin or ampicillin, but typically the duration of treatment for the recurrence is extended empirically; evidence for longer duration of therapy in this circumstance is lacking.[434]

Because infants who receive treatment for invasive infection often remain colonized with GBS at mucous membrane sites, pharyngeal or gastrointestinal colonization can be the source for recurrence. In addition, infants recovering from invasive infection with type III strains usually lack protective levels of antibody during convalescence. Moylett and colleagues[434] and others[435] used pulsed-field gel electrophoresis to document that isolates from patients with recurrent episodes were identical and were derived from a single clone. Sets of isolates analyzed from first and second episodes and from maternal and infant-colonizing and invasive strains were genotypically identical.[433,434] Recurrent infection in most infants likely is a consequence of reinvasion from persistently colonized mucous membrane sites or from reexposure to a household carrier. Association with transient hypoglobulinemia of infancy has been described.[439] Uncommonly, infants have had a second infection with a strain that is genetically unrelated to the original isolate.

MATERNAL INFECTIONS

In 1938, Fry[1] described three fatal cases of endocarditis in postpartum women. This was the initial insight that GBS was a human pathogen and could cause puerperal infection. Postpartum infections, including septic abortion, bacteremia, chorioamnionitis, endometritis, pneumonia, and

septic arthritis, were recorded sporadically, but infections in postpartum women, as in neonates, were uncommonly reported before 1970.[342,440,441] The dramatic increase in incidence of neonatal infections in the 1970s was paralleled by an increased incidence of infections in pregnant women.

Before the institution of IAP in the 1990s, GBS accounted for 10% to 20% of blood culture isolates from febrile women on obstetric services.[442] In the IAP era, GBS is infrequently documented as a cause of maternal peripartum bacteremia, accounting for only 4% of cases in one retrospective cohort study.[443] The clinical presentation of GBS bacteremic infection is characterized by fever, malaise, uterine tenderness with normal lochia, and occasionally chills. Among 40 women with GBS endometritis and endoparametritis described by Faro,[304] GBS were isolated from the endometrium in pure culture in one third of cases, and one third of the women had concomitant bacteremia. In most, signs of infection developed within 24 hours after cesarean section. Clinical features included chills, tachycardia, abdominal distention, and exquisite uterine, parametrial, or adnexal tenderness. Higher fever correlated with risk for concomitant bacteremia. Six infants born to these women developed GBS septicemia, and infection was fatal to three. Contemporary data continue to support the concept that maternal febrile morbidity from chorioamnionitis often is associated with early-onset infection.[444]

The incidence of invasive disease in pregnant women now is 0.12 per 1000 live births, having declined significantly in association with implementation of IAP.[5,114,123] Half of the 409 pregnancy-associated disease cases identified in the United States from 1999 to 2005 by an active population-based surveillance system were associated with infection of the upper genital tract, placenta, or amniotic sac and resulted in fetal death. Among the remainder, manifestations of disease included bacteremia without a focus (31%), endometritis without fetal death (8%), chorioamnionitis without fetal death (4%), pneumonia (2%), and puerperal sepsis (2%). Isolates in pregnancy-associated infections were obtained from blood in 52% of women and from the placenta, amniotic fluid, or conceptus in most of the remainder. When pregnancy outcome was known, most of the women (61%) had a spontaneous abortion or stillborn infant, 5% had infants who developed clinical infection, 4% had induced abortions, and 30% had infants who remained clinically well.[123]

Most obstetric patients with GBS infection, even in the presence of bacteremia, show a rapid response after initiation of antimicrobial therapy. Potentially fatal complications can occur, however, including meningitis,[445] ventriculoperitoneal shunt infection,[446] abdominal abscess,[447] endocarditis,[113,448-450] vertebral osteomyelitis,[451] epidural abscess,[452] or necrotizing fasciitis.[453]

Group B streptococcal bacteriuria during pregnancy is a risk factor for intrauterine or neonatal infection. Asymptomatic bacteriuria, cystitis, or pyelonephritis occurs in 6% to 8% of women during pregnancy. In women with asymptomatic bacteriuria, approximately 20% are caused by GBS.[454] Bacteriuria is a marker for heavy vaginal colonization and indicates enhanced risk for maternal and neonatal infection.[79] In a report series of cases that predated IAP, a cohort of 68 women with asymptomatic GBS bacteriuria had significantly increased risk of preterm delivery compared with nonbacteriuric controls.[343] Stillbirth because of congenital GBS infection can occur even in the current IAP era, and all women with GBS bacteriuria during pregnancy should receive IAP.[455]

Diagnosis

ISOLATION AND IDENTIFICATION OF THE ORGANISM

The definitive diagnosis of invasive GBS infection is established by isolation of the organism from culture of blood, CSF, or a site of suppurative focus (e.g., bone, joint fluid, empyema fluid). Isolation of GBS from surfaces, such as the skin or umbilicus or from mucous membranes, is of no clinical significance.

Lumbar puncture is required to exclude meningeal involvement in infants with invasive GBS infection because clinical features cannot reliably distinguish between meningeal and nonmeningeal involvement. GBS often are isolated from blood at the time of initial evaluation of infants with meningitis, but the blood culture is sterile in 20% to 30%. Wiswell and colleagues[456] found that if lumbar puncture were omitted as part of the sepsis evaluation, the diagnosis of meningitis was missed or delayed in more than one third of infants. Infants with late-onset infection can have meningitis even when focal infection, such as cellulitis, is apparent. If lumbar puncture must be deferred because an infant is clinically unstable, penicillin G or ampicillin at the doses recommended for treatment of GBS meningitis (see "Treatment") should be administered until meningeal involvement can be assessed.

Antigen Detection Methods

Antigen detection is not a substitute for appropriately performed bacterial cultures and now is rarely used to establish a provisional diagnosis. A positive result indicates that GBS antigen is detectable, but not that viable organisms are present. Serum and CSF are the only specimens recommended for testing.[457] In neonates with meningitis, the sensitivity of antigen detection is 72% to 89%. The estimated sensitivity for serum is 30% to 40%. False-positive results have been encountered. The estimated specificity of commercial assays ranges from 95% to 98%. Antigen assays should not be used to assess treatment efficacy.

NAAT such as PCR can have a role in intrapartum testing of vaginal-rectal samples from women with unknown GBS colonization status (see "Prevention"), but they are not routinely used for the diagnosis of infant infection. As a research tool however, a PCR assay targeting the *cylB* gene was shown to be superior to culture methods for detecting GBS in CSF and blood samples from infants with probable GBS sepsis or meningitis.[458] The use of PCR to exclude GBS colonization in neonates, allowing for early discharge of infants, also has been proposed.[459] A fluorescent PCR assay was sensitive and specific for early detection within 4 hours of incubation of GBS in neonatal blood cultures, but such testing is not available commercially.[460]

Other Laboratory Tests

A single measurement of an acute-phase reactant, such as C-reactive protein (CRP), can be elevated during GBS

infection, but its usefulness is limited because CRP can be elevated in a number of noninfectious inflammatory conditions. Sequential assessment of CRP values is useful in supporting a diagnosis of sepsis and in excluding sepsis if levels remain persistently normal. The return to normal of the CRP level can be helpful in guiding the duration of antibiotic exposure in suspected GBS infection, minimizing antibiotic exposure in the nursery setting. Levels of inflammatory cytokines, such as IL-6, are elevated acutely during GBS sepsis. In one report, production of IL-6 was noted in all 16 neonates with bacteremic early- or late-onset GBS infection when samples were collected within 48 hours of initiation of antimicrobial therapy.[461] These assays are not routinely used because of the cost of testing, and they are not generally available in clinical laboratories.

Abnormalities in the white blood cell count, including leukopenia, neutropenia, leukocytosis, increase in band forms, or decline in the total white blood cell count in the first 24 hours of life, can be suggestive of GBS infection.[462] A multicenter study found that a low white blood cell count, absolute or relative neutropenia, or an elevated immature to total neutrophil count was more helpful as a predictor of sepsis when the complete blood cell count is obtained after 4 hours of age.[463] Fatal early-onset GBS sepsis can occur with normal leukocyte parameters, however.[464] Measurements of peripheral blood leukocyte parameters generally have a low sensitivity and should be used only as an adjunct to results from blood and CSF cultures.

DIFFERENTIAL DIAGNOSIS

The clinical features in neonates with early-onset GBS infection mimic the features in infants with sepsis caused by other etiologic agents and by some noninfectious illnesses. Radiographic findings of pneumonia are present in some neonates with early-onset GBS sepsis. Neonates with early-onset pneumonia can have apnea and shock within the first 24 hours of life, a 1-minute Apgar score of 5 or less, and an unusually rapid progression of pulmonary disease.[183] Infection also should be considered in neonates with persistent fetal circulation associated with respiratory distress, neutropenia, and systemic hypotension.[357]

The differential diagnosis for late-onset GBS infection depends on the clinical presentation. For infants with meningitis, the characteristic CSF Gram-stain findings can provide a presumptive diagnosis. When this method is inconclusive, other bacteria, including *S. pneumoniae, Escherichia coli, Listeria monocytogenes*, *Neisseria meningitidis,* as well as viruses must be considered. Fever usually is a presenting feature in term infants, and empirical therapy with broad-spectrum antibiotics customarily is used until culture results are available. Finally, the lengthy list of uncommon manifestations of infection between 1 week and 3 months of age and beyond indicates that GBS should be suspected as an etiologic agent, regardless of site of infection, for infants in this age group.

Treatment

GBS have been a frequent cause of infection in neonates for 4 decades, resulting in increased awareness of associated risk factors and need for prompt and aggressive therapy. Despite striking declines, however, death and disability from these infections still occur. In addition, relapses or reinfections, although uncommon, occur in the face of optimal therapy. These facts should prompt efforts to develop improved treatment modalities.

IN VITRO SUSCEPTIBILITY

Uniform susceptibility of GBS to penicillin G has continued for more than 50 years of use.[465-470] Recently, reduced susceptibility of certain strains of GBS to penicillin and other β-lactam antibiotics has been documented in the United States[471] and Japan[472] and traced to point mutations in penicillin-binding proteins reminiscent of first-step mutations in the evolution of pneumococcal penicillin resistance decades ago. The clinical implications of this finding are as yet unclear. None among almost 2000 isolates of GBS from 2008 to 2009 were penicillin nonsusceptible.[473] In vitro susceptibility of GBS to ampicillin, semisynthetic penicillins, vancomycin, teicoplanin, linezolid, quinopristin/dalfopristin, gatifloxacin, levofloxacin, tigecycline and cephalosporins also is the rule, although the degree of in vitro activity varies.[468,469,474,475] Ceftriaxone is the most active of the cephalosporins in vitro. Imipenem and meropenem are highly active.[466] Resistance to quinolones can occur through mutations in the gyrase and topoisomerase IV genes, usually in patients who have received prior quinolone therapy.[476]

Resistance to erythromycin and clindamycin is increasing. Contemporary data from multiple studies indicate that 20% to 40% of isolates are erythromycin-resistant, and 10% to 30% are resistant to clindamycin.[468,469,477] Rates of resistance in colonizing isolates can be 40% for erythromycin and clindamycin.[478] These high rates of resistance are reported from geographically diverse regions.[479-481]

Macrolide resistance mechanisms include ribosomal modification by a methylase encoded by *erm* genes and drug efflux by a membrane-bound protein encoded by the *mef* gene.[482] The presence of *erm* genes results in the macrolide–lincosamide–streptogramin B resistance phenotype.[483] Erythromycin-resistant isolates that are constitutively resistant, inducibly resistant, or susceptible to clindamycin are described.[484] Alone or in combination, *ermA, ermB,* and *mefA* genes are responsible for resistance in GBS. An *ermT* gene has been identified in a few strains of GBS inducibly resistant to clindamycin.[485] The presence of a composite transposon in GBS and pneumococci suggests that *ermB*-mediated macrolide resistance could be due to the horizontal transfer of a mobile transposable element.[486] A particularly high proportion of strains resistant to erythromycin has been reported for type V.[469,487] Tigecycline and telithromycin are active in vitro against macrolide-resistant GBS, but data confirming their clinical effectiveness are scant.[488,489] The percentage of tetracycline-resistant strains is 75% to nearly 90%. Resistance of GBS to bacitracin, nalidixic acid, trimethoprim-sulfamethoxazole, metronidazole, and aminoglycosides is uniform.

Despite resistance of most GBS strains to aminoglycosides, synergy often is observed when an aminoglycoside (especially gentamicin) and penicillin or ampicillin are used in combination.[490] The best combination theoretically to accelerate the killing of GBS in vivo is penicillin or ampicillin plus gentamicin. Therapeutic concentrations of gentamicin in the serum are not required to achieve synergy.

By contrast, the rapid and predictable bactericidal effect of penicillin or ampicillin on GBS in vitro is ablated by the addition of rifampin.[491] Although in vivo data are lacking, the in vitro antagonism of rifampin when combined with penicillins suggests that they should not be used concurrently in the treatment of proven or suspected GBS disease.

Among the newer β-lactam antibiotics reputed to attain high concentrations of drug in the CSF, only cefotaxime, ceftriaxone, meropenem, and imipenem achieve minimal bactericidal concentrations (MBCs) comparable with MBCs of penicillin G and ampicillin (0.01-0.4 μg/mL),[466,482] and limited data suggest that their efficacy is equivalent to that of penicillin G.[492,493] Despite their uniform susceptibility to penicillin G, GBS require higher concentrations for growth inhibition in vitro than strains belonging to group A. The minimal inhibitory concentration (MIC) of penicillin G to GBS is 4-fold to 10-fold greater than the MIC for group A strains (range, 0.003-0.4 μg/mL).[465] This observation, combined with the observation indicating the significant influence of inoculum size on in vitro susceptibility to penicillin G, may have clinical relevance.[465,494]

When the inoculum of GBS is reduced from 10^5 to 10^4 colony-forming units (CFU)/mL, a twofold lower concentration of penicillin G is sufficient to inhibit in vitro growth. Similarly, if the inoculum is increased from 10^4 to 10^7 CFU/mL, the MBC of ampicillin is increased from 0.06 to 3.9 μg/mL. Such in vitro observations may have in vivo correlates because some infants with GBS meningitis have CSF bacterial inocula of 10^7 to 10^8 CFU/mL.[494] At the initiation of therapy for meningitis, achievable CSF levels of penicillin G or ampicillin may be only one tenth of serum levels. This inoculum effect also has been noted with cefotaxime and imipenem. The dose chosen to treat GBS meningitis can be crucial to the prompt sterilization of CSF.

ANTIMICROBIAL THERAPY

Penicillin G is the drug of choice for treatment of GBS infections. The recommended dosage for treatment of meningitis is high because of the relatively high MIC of penicillin G for GBS (median, 0.06 μg/mL) with respect to attainable levels of this drug in the CSF, the high inoculum in the CSF of some infants, reports of relapse in infants with meningitis treated for 14 days with 200,000 U/kg/day of penicillin G, and the safety of high doses of penicillin G in the newborn. To ensure rapid bactericidal effects, particularly in the CSF, we recommend penicillin G (450,000-500,000 U/kg/day) or ampicillin (300-400 mg/kg/day) for the treatment of meningitis (Table 12-7). There is no evidence to suggest increased risk for adverse reactions at these higher doses even in premature infants.

In the usual clinical setting, antimicrobial therapy is initiated before definitive identification of the organism. Initial therapy should include ampicillin and an aminoglycoside appropriate for the treatment of early-onset neonatal pathogens, including GBS. Such a combination is more effective than penicillin G or ampicillin alone for killing of GBS.[490] We continue combination therapy until the isolate has been identified as GBS and, in patients with meningitis, until a CSF specimen obtained 24 to 48 hours into therapy is sterile. MIC and MBC determinations may be considered in the following settings: (1) a poor bacteriologic response to antimicrobial therapy, (2) relapse or recurrence of infection without a discernible cause, and (3) infections manifested as meningitis or endocarditis.[495] For an infant with late-onset disease in whom CSF reveals gram-positive cocci in pairs or short chains, initial therapy should include ampicillin and gentamicin or ampicillin and cefotaxime. GBS is a frequent cause of meningitis in infants 1 to 8 weeks of age, and combination therapy can improve efficacy early in the course of infection. If pneumococcal meningitis is a consideration, cefotaxime and vancomycin would be a reasonable regimen pending culture confirmation. Because GBS meningitis is uncommon beyond 8 weeks of age, no change is suggested from the use of conventional agents as the initial treatment of meningitis in term infants older than 2 months. For preterm infants remaining hospitalized from birth, empirical therapy can include vancomycin and an aminoglycoside. If meningitis is suspected, cefotaxime should be included in the regimen because vancomycin achieves low CSF concentrations and has a substantially higher MBC against GBS than ceftriaxone or cotaxime.

When the diagnosis of GBS infection is confirmed, and CSF for patients with meningitis obtained 24 to 48 hours into therapy is sterile, treatment can be completed with penicillin G monotherapy. Good outcomes have been achieved

Table 12-7 Antimicrobial Regimens Recommended for Treatment of Group B Streptococcal Infections in Infants*

Manifestation of Infection	Drug	Daily Dose (Intravenous)	Duration
Bacteremia without meningitis	Ampicillin plus gentamicin	150-200 mg/kg plus 7.5 mg/kg	Initial treatment before culture results (48-72 hr)
	Penicillin G	200,000 units/kg	Complete a total treatment course of 10 days
Meningitis	Ampicillin plus gentamicin	300-400 mg/kg plus 7.5 mg/kg	Initial treatment (until cerebrospinal fluid is sterile)
	Penicillin G	450,000-500,000 units/kg	Complete a minimum total treatment course of 14 days†
Septic arthritis	Penicillin G	200,000 units/kg	2-3 wk
Osteomyelitis	Penicillin G	200,000 units/kg	3-4 wk
Endocarditis	Penicillin G	400,000 units/kg	4 wk‡

*No modification of dose by postnatal age is recommended. Oral therapy is never indicated.
†In combination with gentamicin for the first 14 days.
‡Longer treatment (up to 4 wk) may be required for ventriculitis.

when parenteral therapy is given for 10 days for bacteremia without a focus or with most soft tissue infections, 2 to 3 weeks for meningitis or pyarthrosis, and 3 to 4 weeks for osteomyelitis or endocarditis (see Table 12-7). Limited evidence suggests that a 7-day course of therapy can suffice for uncomplicated bacteremia, but additional data would be required to support a change in current recommendations.[496] For infants with meningitis, failure to achieve CSF sterility suggests an unsuspected suppurative focus (subdural empyema, brain abscess, obstructive ventriculitis, septic thrombophlebitis) or failure to administer an appropriate drug in sufficient dosage.

At the completion of therapy in an infant with complicated meningitis, a lumbar puncture should be considered to evaluate whether the CSF findings are compatible with resolution of the inflammatory process or are of sufficient concern to warrant extending treatment or additional diagnostic evaluation. Neutrophils counted as greater than 30% of the total cells and a protein concentration greater than 200 mg/dL warrant consideration of an additional week of treatment. Cranial imaging by magnetic resonance imaging with contrast enhancement toward the end of a planned course of therapy is advisable to document adequate resolution of cerebritis, subdural empyema, and so forth, and for prognostic purposes (see "Prognosis"). Infants with septic arthritis should receive at least 2 weeks and those with osteomyelitis 3 to 4 weeks of parenteral therapy to optimize an uncomplicated outcome. Drainage of the suppurative focus is an adjunct to antibiotic therapy. In infants with septic arthritis, excluding the hip, one-time needle aspiration of the involved joint usually achieves adequate drainage. With hip involvement, immediate open drainage is warranted. For most infants with osteomyelitis, aspiration assists in diagnosis because blood cultures typically are sterile. Diagnostic procedures must be performed early in the course of therapy to optimize isolation of the infecting organism.

With recurrent infection, three points should be considered. First, appropriate antimicrobial therapy fails to eliminate mucous membrane colonization with GBS in 50% of infants. Second, community exposure can result in colonization with a new strain that subsequently invades the bloodstream. Systemic infection in neonates does not elicit protective levels of CPS type-specific antibodies. Recurrent infections do occur in healthy infants. In this event, an evaluation to exclude an immune abnormality can be considered, but detection of abnormalities is rare. Therapy for recurrent infection need not be extended beyond that appropriate to the clinical expression of the recurrent infection. Finally, although it is desirable to eliminate colonization, an efficacious regimen has not been identified. One small prospective study revealed that administration of oral rifampin (20 mg/kg/day for 4 days) to infants after completion of parenteral therapy eliminated mucous membrane colonization in some subjects.[497] Further study is needed to identify a more reliable approach to eliminating colonization.

SUPPORTIVE CARE

Supportive care is important to the successful outcome of most GBS infections. It is imperative that all infants with suspected or proven meningitis receive intensive care observation at least for 24 hours, irrespective of the initial stable presentation, because clinical deterioration is frequent in this circumstance. When early-onset disease is accompanied by respiratory distress, the need for ventilatory assistance should be anticipated before onset of apnea. Early treatment of shock, often not suspected during its initial phase, when systolic pressure is maintained by peripheral vasoconstriction, is crucial. Persistent metabolic acidosis and reasonably normal color are characteristic of this early phase. Persistent perfusion abnormalities after initial attempts to achieve adequate volume expansion warrant placement of a central venous pressure monitoring device and treatment with appropriate inotropic agents. This concept applies also to patients with late-onset meningitis. In patients with meningitis, effective seizure control is required to achieve proper oxygenation, to decrease metabolic demands, prevent additional cerebral edema, and optimize cerebral blood flow. Monitoring of urine output and attention to electrolyte balance and osmolality are needed to detect and manage the early complications of meningitis, such as inappropriate secretion of antidiuretic hormone and increased intracranial pressure. Such intense and careful supportive management requires treatment in an intensive care unit of a tertiary care facility.

Prognosis

Clinical scoring systems have attempted to predict at the time of initial evaluation infants likely to die as a consequence of neonatal GBS infection.[356,357,498] One such score derived from five variables, together with an initial blood pH of less than 7.25, predicted outcome accurately in 93% of infants with early-onset GBS infection. These features were birth weight less than 2500 g, absolute neutrophil count less than 1500 cells/mm^3, hypotension, apnea, and pleural effusion on the initial chest radiograph.[357]

One group at potential risk for sequelae from GBS sepsis is preterm infants with septic shock, who can develop periventricular leukomalacia. Among survivors, substantial neurodevelopmental sequelae have been identified at evaluation during the second year of life. The correlates of severity and duration of shock with periventricular leukomalacia and with long-term morbidity have not been assessed. Prospective, active surveillance of neonatal GBS infections in Germany conducted from 2001 to 2003 found that 14% of 347 infants had neurologic sequelae of infection at the time of discharge from the hospital.[499]

Long-term outcomes for survivors of GBS meningitis are guarded. Among neonates cared for in the 1970s and 1980s, one quarter died in the hospital and 20% of survivors had major neurologic sequelae.[354,355,356] The death rate has declined but outcomes have not improved in the intervening years. Among 41 survivors from a cohort born in 1996 to 1997 in England and Wales, 34% had moderate or severe disability, 27% had mild disability, and 39% were functioning normally at 5 years of age.[500] Stoll and colleagues[501] showed for extremely-low-birth-weight infants that meningitis with or without sepsis was associated with poor neurodevelopmental and growth outcomes and impairment of vision and hearing in early childhood.

Features predictive of death or adverse outcome from GBS meningitis as assessed at hospital discharge include

seizures within hours of hospital admission, coma or semicoma, pressor support, and initial CSF protein of 300 mg/dL or greater and CSF glucose of less than 20 mg/dL.[359] Among 90 term and near-term infants diagnosed with GBS meningitis from 1998 through 2006, five died acutely and five died at 6 months to 3 years of age. Twenty-four of 43 survivors were evaluated at a mean of 7 years of age. Fifty-six percent were functioning normally. Among the remainder, 25% had mild-to-moderate impairment and 19% had severe neurodevelopmental impairment.[502] Because subtle deficits, such as delayed language development and mild hearing loss, may not be detected by routine examination meningitis survivors should undergo audiometric testing during convalescence as well as careful long-term neurologic and developmental assessments.

Prevention

Theoretically, early- and late-onset GBS disease could be prevented if susceptible hosts were not exposed to the microorganism or if exposure occurred in the setting of protective immunity. Several approaches to prevention have been advocated; conceptually, these are directed at eliminating exposure or enhancing host resistance by chemoprophylaxis or immunoprophylaxis. Both strategies have limitations with respect to implementation but could be targeted for the prevention of maternal and neonatal infections and are theoretically achievable.[503,504]

CHEMOPROPHYLAXIS

Historical Precedents

Because maternal genital colonization was recognized to expose infants to the organism, oral penicillin treatment for colonized women was proposed in the early 1970s. Approximately 20% to 30% of third-trimester GBS carriers remained colonized after this treatment, and in most of these women, GBS were isolated from vaginal cultures at delivery.[505-507] Reacquisition from colonized sexual partners was suggested as an explanation for these high failure rates, but failure rates remained high when colonized pregnant women and their spouses received concurrent treatment with penicillin by the oral or the parenteral route.[2,505,508] Yow and colleagues[509] gave intravenous ampicillin at hospital admission to 34 GBS vaginally colonized women and successfully interrupted vertical transmission of GBS to all neonates. In 1986, Boyer and Gotoff[510] provided the first documentation that IAP could prevent invasive early-onset neonatal infection. Women colonized with GBS were randomly assigned to receive routine labor and delivery care or routine care plus intrapartum ampicillin intravenously until delivery. Group B streptococcal sepsis developed in 5 of 79 neonates in the routine care group (1 died), whereas 85 infants born to women in the ampicillin treatment group remained well. Intrapartum ampicillin prophylaxis for GBS carriers also resulted in reduced maternal morbidity.[511] These data established the efficacy of IAP for prevention of early-onset neonatal disease and reduction of GBS-associated febrile maternal morbidity. The cost-effectiveness of this approach subsequently was validated.[512]

In 1992, the American College of Obstetricians and Gynecologists (ACOG)[513] and the American Academy of Pediatrics (AAP)[514] published separate documents regarding maternal IAP for the prevention of early-onset GBS infection. The ACOG technical bulletin was educational, whereas the AAP guidelines were directive. The AAP guidelines specified that if culture screening was performed antenatally, specimens should be obtained from lower vaginal and rectal sites, and GBS culture-positive women should be given intravenous penicillin G or ampicillin intrapartum. The ACOG proposed that culture screening could be avoided by providing treatment for all women with risk factors. Neither the AAP nor ACOG approach was widely implemented, and although invasive disease rates began to diminish, they remained unacceptably high.

Rapid Assays for Antenatal Detection of Group B Streptococci

Bergeron and colleagues[515] described a fluorogenic real time (RT)-PCR technique for rapid identification of women colonized with GBS at admission for delivery. The sensitivity of RT-PCR and of conventional PCR was 97%, the negative predictive value was 99%, and the specificity and positive predictive value were 100%. Results were available from RT-PCR in 45 minutes; by comparison, conventional PCR required 100 minutes, and conventional cultures required 36 hours minimum. Field testing of commercially available assays, such as the Xpert GBS Assay (Cepheid, Sunnyvale, Calif), which uses automated rapid RT-PCR technology has been conducted.[516-518] The performance of RT-PCR in one study was deemed sufficiently robust for possible use in point-of-care settings.[519] One cost-benefit analysis suggested that widespread implementation of RT-PCR would afford benefit over the current culture-based strategy (decrease antimicrobial use), but another concluded that molecular point-of-care testing was cost neutral compared with recommended culture techniques.[520,521]

More recent studies evaluating RT-PCR versus antenatal cultures suggest that PCR testing is superior to antenatal testing, but typically the investigators have sampled only the vaginal without the recommended rectal site or have put swabs directly onto agar media without preceding inoculation into enrichment broth, or both. Studies that use recommended site sampling (vaginal and rectal specimens) and compare RT-PCR with culture processing that includes placing swabs into enrichment broth media, with incubation and then inoculation onto agar media, indicate equivalent sensitivity and specificity of antenatal culture screening and intrapartum PCR testing.[522-524]

There continue to be benefits and difficulties inherent to ascertainment of GBS colonization using RT-PCR even when assays can be processed rapidly 24 hours a day. Currently, RT-PCR detection of GBS colonization should be considered an adjunct to antenatal culture screening at 35 to 37 weeks of gestation.[16]

Intrapartum Antibiotic Prophylaxis

The current era of IAP dates from 1996, when consensus recommendations for the prevention of early-onset GBS disease were endorsed by the CDC, AAP, and ACOG.[457,525,526] These recommendations indicated that obstetric care

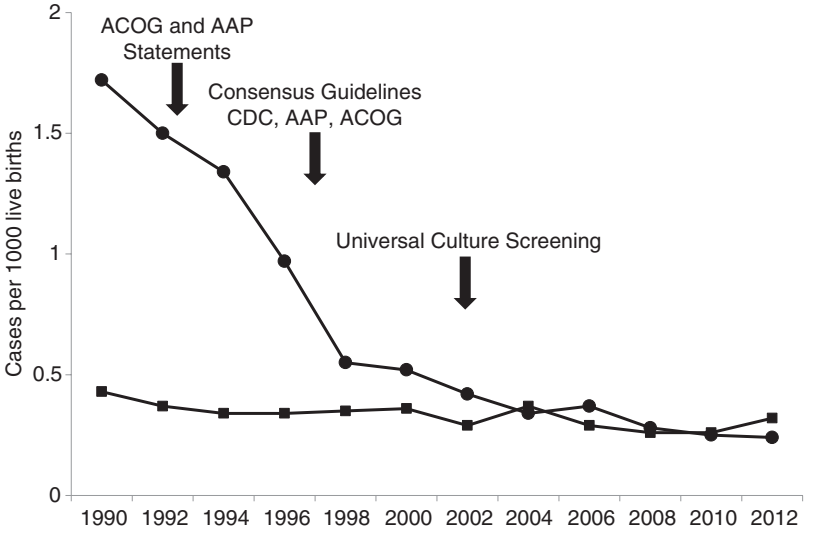

Figure 12-10 Incidence of early-onset *(solid black bullet)* and late-onset *(solid black square)* group B streptococcal disease from 1990 to 2012. The dates of the initial prevention statements from the American College of Obstetricians and Gynecologists (ACOG) and the American Academy of Pediatrics (AAP),[513,514] the 1996 consensus guidelines from the Centers for Disease Control and Prevention (CDC),[525] and the revised 2002 CDC guidelines for universal culture screening[503] are shown.

providers and hospitals should adopt a culture-based or a risk-based policy to identify women to receive IAP. The culture-based approach used lower vaginal and rectal cultures obtained at 35 to 37 weeks of gestation to identify candidates for IAP. The risk-based strategy identified IAP recipients by factors known to increase the likelihood of neonatal GBS disease: labor onset or membrane rupture before 37 weeks of gestation, intrapartum fever greater than or equal to 38° C (±100.4° F), or rupture of membranes 18 or more hours before delivery. In both strategies, women with GBS bacteriuria or previous delivery of an infant with GBS disease were to receive IAP. These strategies each resulted in the administration of IAP to approximately one in four pregnant women.

The incidence of early-onset disease declined by 70% from 1.7 per 1000 live births to 0.5 per 1000 live births by 1999 in association with implementation of one of these two IAP methods[503,527] (Fig. 12-10). A resulting 3900 to 4500 early-onset infections and 200 to 225 neonatal deaths were estimated to be prevented annually.[503,528] By contrast, the rate of late-onset disease remained constant at 0.5 to 0.6 per 1000 live births. Also, the incidence of invasive GBS disease, primarily bacteremia with or without intraamniotic infection or endometritis among pregnant women declined significantly, from 0.29 per 1000 live births in 1993 to 0.23 in 1998.[503,528] By 1999, two thirds of U.S. hospitals in a multistate survey had a formal prevention policy, and numerous individual practitioners had adopted one of the two strategies proposed in 1996.[503,529]

By 2002, it was evident that further reduction in the incidence of early-onset disease could be accomplished by adoption of universal culture screening. A direct comparison in 5144 births showed that the culture-based strategy was 50% more effective than the risk-based strategy in preventing early-onset disease in neonates.[142] Culture-based screening more often resulted in administration of IAP for at least 4 hours before delivery. The 2002 revised CDC guidelines recommending a universal culture-based approach to prevention of perinatal GBS disease are endorsed by the AAP and the ACOG.[503] Early-onset disease incidence declined an additional 29% after issuance of the revised guidelines in 2002, to 0.34 cases per 1000 live births from 2003 to 2007.[141] An additional reduction in incidence occurred in 2008, but since then, as of 2012, incidence has reached a plateau at 0.25 per 1000 live births.

Currently, all pregnant women should be screened in each pregnancy for GBS carriage at 35 to 37 weeks of gestation. The risk-based approach is an acceptable alternative *only* in circumstances in which the culture has not been performed or results are unavailable before delivery. The 2010 revised recommendations from CDC, ACOG, and AAP are essentially the same as those from 2002, but further clarifications have been given for six topics: (1) expanded information on laboratory methods for GBS identification, (2) definition of colony-count threshold for reporting GBS bacteriuria, (3) algorithms for GBS screening and IAP for women with preterm labor or preterm premature rupture of membranes, (4) a minor change in the second dose of penicillin from 3 million U to 2.5 to 3 million U intravenously, (5) updated prophylaxis regimens for women with allergy to penicillin, and (6) a simplified algorithm for management of neonates with respect to risk for early-onset GBS disease. Culture specimens from pregnant women should be obtained from the lower vagina and the rectum using the same or two different swabs. These swabs should be placed in a nonnutritive transport medium, transferred to a laboratory where the swabs are incubated overnight in a selective enrichment broth, and subcultured onto suitable agar medium for isolation of GBS.[16] At the time of labor or rupture of membranes, IAP should be given to all pregnant women identified antenatally as carriers of GBS. The indications for IAP are shown in Figure 12-11. Group B streptococcal bacteriuria during the current pregnancy or prior delivery of an infant with invasive GBS disease always is an indication for IAP, so antenatal screening is unnecessary for these women.[16] If culture results are unknown at the onset of labor or rupture of membranes, the risk factors listed in Figure 12-11 should be used to determine the need to institute IAP. Women who present with preterm labor before antenatal GBS screening should have cultures obtained and IAP initiated. If labor ceases and cultures are negative, IAP is discontinued, and antenatal screening is performed at 35 to 37 weeks of gestation. If labor ceases and cultures are positive, some experts recommend oral amoxicillin for another 5 to 7 days.

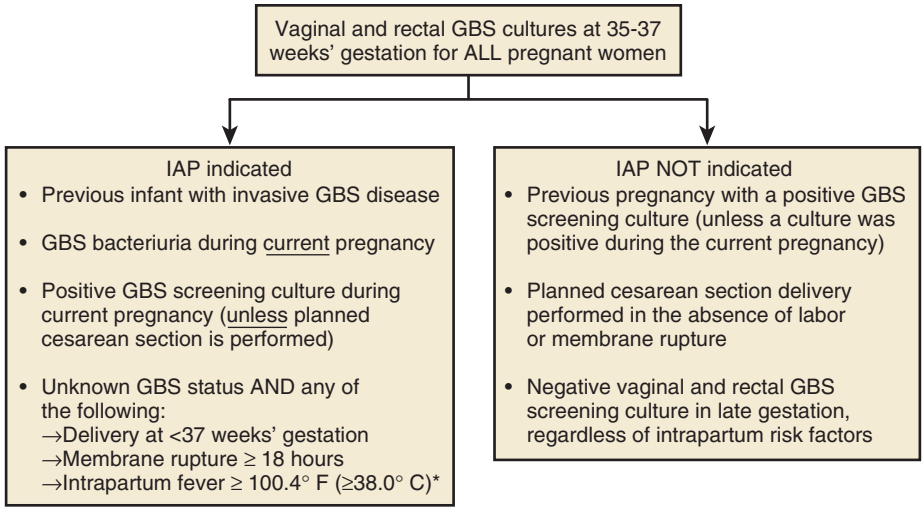

Figure 12-11 Recommendations for culture-based screening for maternal colonization with group B streptococci (GBS) and administration of intrapartum antibiotic prophylaxis (IAP). *If chorioamnionitis is suspected, broad-spectrum antibiotic therapy that includes an agent known to be active against GBS should replace GBS IAP. (Modified from Centers for Disease Control and Prevention: Prevention of perinatal group B streptococcal disease: revised guidelines from CDC, *MMWR Morb Mortal Wkly Rep* 59[RR-10]:1-32, 2010.)

Planned cesarean section before rupture of membranes and onset of labor constitute an exception to the need for IAP for women colonized with GBS. These women are at extremely low risk for having an infant with early-onset disease. Culture-negative women who are delivered at 37 weeks of gestation or later need not receive IAP routinely. Therapeutic use of broad-spectrum antibiotics in labor should be used as is appropriate for maternal indications, such as intraamniotic infection.

The recommended maternal IAP regimen consists of penicillin G (5 million U initially, then 2.5-3 million U every 4 hours thereafter until delivery).[16] Penicillin or ampicillin given 4 or more hours before delivery reliably prevents vertical transmission and early-onset disease.[530] Ampicillin administered as a 2-g intravenous loading dose and then 1 g every 4 hours until delivery is an alternative to penicillin.[503] The rationale for the high initial dose of the β-lactam antibiotic relates to the desired drug concentrations needed in the amniotic and vaginal fluids (peak approximately 3 hours after completion of the initial dose) to reduce substantially the number of GBS at either site. IAP "failures" typically occur when penicillin or ampicillin has been initiated 2 or less hours before delivery; clindamycin has been given without susceptibility testing, and clindamycin-resistant early-onset GBS neonatal sepsis ensued; or appropriate IAP is given in the setting of clinically apparent or silent intraamniotic infection.

Prophylaxis for penicillin-allergic women must take into account increasing resistance among GBS to erythromycin and clindamycin. Women *not* at high risk for anaphylaxis (e.g., a rash without anaphylaxis or respiratory compromise) should receive cefazolin, 2 g intravenously as an initial dose and then 1 g every 8 hours until delivery. Cefazolin has pharmacokinetics similar to penicillin with respect to peak concentrations in serum and amniotic fluid of pregnant women. Women whose GBS isolates are tested and found to be clindamycin susceptible by a D test and who are at high risk for anaphylaxis with penicillin can receive clindamycin at a dose of 900 mg every 8 hours. However, the efficacy of clindamycin in preventing early-onset GBS disease is not known, and clindamycin prophylaxis failures have occurred.[531] If susceptibility testing is unavailable or the results are unknown, or when isolates are resistant to clindamycin, vancomycin, 1 g intravenously every 12 hours until delivery, is an alternative for women with serious penicillin hypersensitivity reactions. Neither the pharmacokinetics of vancomycin in amniotic nor vaginal fluids nor its efficacy in preventing early-onset disease has been investigated.

The risk of anaphylaxis from administration of penicillin is low. Estimates range from 4 events per 10,000 to 4 per 100,000 patients. Anaphylaxis associated with administration of a β-lactam antibiotic as IAP for the prevention of early-onset GBS infection has been reported but is rare.[142,532,533] Most pregnant women reporting a penicillin allergy that is not anaphylaxis have negative testing for hypersensitivity and are able to receive IAP with penicillin safely.[534] A fetal demise in association with new-onset penicillin allergy during IAP has been reported in a woman with rheumatoid arthritis.[535] No adult fatalities in association with IAP are reported, and the risk of a fatal event is low because the antimicrobials are administered in a hospital setting where medical intervention is readily available. Despite these data and the 2010 guidelines, adherence to appropriate use of IAP in penicillin-allergic women is poor.[536]

Numerous residual problems, barriers to implementation, and missed opportunities must be overcome to achieve maximal benefit from IAP. Procedural issues, such as suboptimal culture processing and collection of cultures earlier than 5 weeks before delivery, constitute one set of problems. Laboratories may not adhere to recommended methods for isolation of GBS, a problem that remains despite the 2010 consensus recommendations and one that results in colonized women delivering infants with early-onset disease. Even optimal antenatal culture methods miss some women who are colonized at delivery, exposing their neonate to GBS and resulting in colonization or illness. Another problem is that women who are not screened adequately more often are medically underserved; women in their teens, blacks, and Hispanics are more likely than whites to receive

inadequate prenatal care and prenatal testing, and are less likely to receive recommended prevention interventions.

Problems surround lack of recommended IAP in certain circumstances. The most prominent is lack of adherence to the 2010 recommendation for routine IAP in women who deliver before antenatal screening occurs (i.e., 35-37 weeks of gestation). These women should have vaginal and rectal cultures performed and routinely receive IAP, but this recommendation is the one least commonly implemented. Whether this is because delivery ensues too quickly to administer IAP or the recommendation is unclear to obstetric providers, or both, is unknown. Also, adherence to guidelines in penicillin-allergic women is suboptimal, and cefazolin as the appropriate IAP for women with a nonserious penicillin allergy is administered uncommonly. Reliance on clindamycin as the alternative agent in women without serious penicillin allergy results in inadequate IAP in at least 20% of patients when antimicrobial susceptibility testing of colonizing isolates is not performed antenatally.[16]

A final issue is a need for increased awareness of perinatal GBS infection. In one report, only 47% of women younger than 50 years reported having heard of GBS. Women with a high school education or less; with low household income; or reporting black, Asian/Pacific Islander, or "other" race had lower awareness than that noted in other women. Efforts to raise awareness should target women from groups that traditionally are medically underserved. Hospital infection control teams can contribute to these efforts by spearheading educational efforts toward effective implementation among hospital obstetric staff and laboratory personnel.[528]

Impact of Intrapartum Antibiotic Prophylaxis on Neonatal Sepsis

The efficacy of IAP in preventing early-onset GBS infection has been shown in numerous observational studies and in countries other than the United States when guidelines have been implemented.[142,349] The impact of increased use of IAP on the occurrence of sepsis caused by organisms other than GBS is a subject of ongoing evaluation. Concern existed that neonatal sepsis caused by organisms other than GBS, especially *E. coli*, would increase and that the organisms causing non–GBS sepsis were likely to be ampicillin resistant. Several studies have demonstrated that these concerns are unfounded.[537-539]

Management of Neonates Born to Mothers Receiving Intrapartum Antibiotic Prophylaxis

Management of infants is based on the neonate's clinical status, whether the mother had chorioamnionitis, an indication for IAP, or adequate duration of IAP, and gestation (Fig. 12-12).[16] If an infant has any signs of sepsis, a full diagnostic evaluation should be conducted, including complete blood cell count, differential and platelet count, blood culture; chest radiograph if the neonate has respiratory signs; and if the infant is clinically stable, a lumbar puncture. Then empirical therapy should be initiated pending laboratory results.[16] Although published reports vary, a minimum of 10% and a maximum of nearly 40% of infants with meningitis have a negative blood culture.[456] If lumbar puncture is deferred and therapy is continued for more than 48 hours because of suspected infection, CSF should be obtained for routine studies and culture. Depending on the CSF results, therapy appropriate for sepsis or presumed meningitis is given.

If a woman has suspected chorioamnionitis, her healthy appearing infant should have a limited diagnostic evaluation, including a blood culture at birth and complete blood count, differential and platelet count at birth or 6 to 12 hours later. Most experts would then initiate empirical therapy pending culture results. This approach is based on the infant's exposure to suspected or established infection. The duration of therapy is based on results of cultures and the infant's clinical course (see "Treatment"). If the mother was

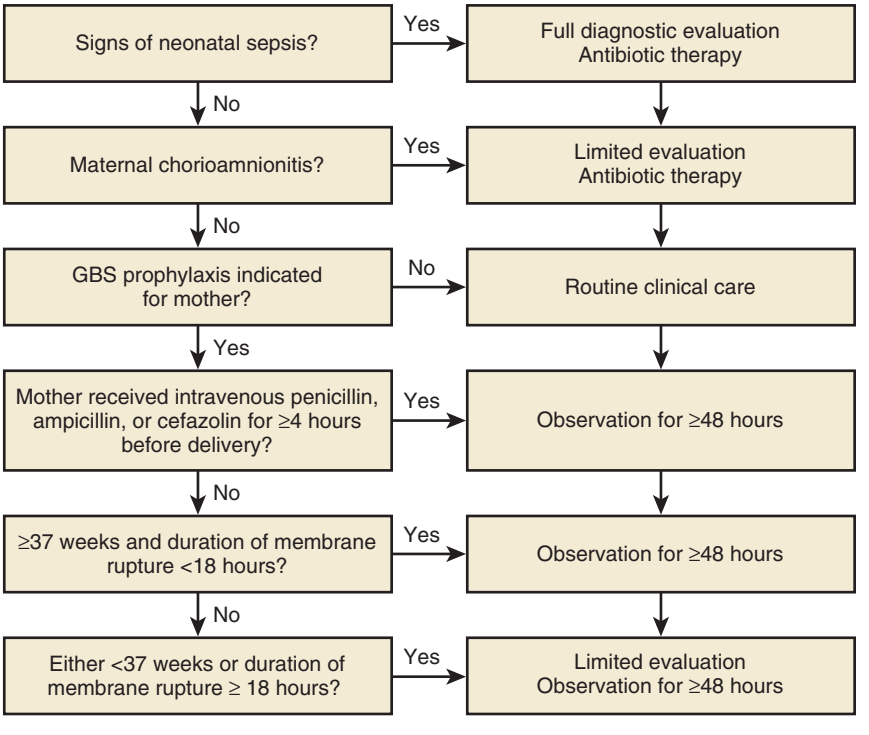

Figure 12-12 Algorithm for prevention of early-onset GBS disease among newborns. (Modified from Centers for Disease Control and Prevention: Prevention of perinatal group B streptococcal disease: revised guidelines from CDC, *MMWR Morb Mortal Wkly Rep* 59[RR-10]:1-32, 2010.)

a candidate for IAP and received adequate IAP (4 or more hours of penicillin, ampicillin, or cefazolin) before delivery, her healthy appearing infant should receive routine clinical care with 48 hours observation. If the mother has not received adequate IAP and the rupture of membranes has been less than 18 hours, the healthy appearing infant who has a gestational age of 37 weeks or more should receive observation in the hospital for a minimum of 48 hours. However, if the infant is either less than 37 weeks of gestation or delivery occurs 18 or more hours before delivery, the infant should have a limited evaluation and observation in the hospital for 48 hours. If in any of these circumstances, the neonate develops signs of sepsis, a full diagnostic evaluation should be performed and empirical broad-spectrum antibiotic therapy initiated, typically with ampicillin and gentamicin. The approach presented in Figure 12-12 is not to be taken as an exclusive management pathway. Hospital discharge at 24 hours of age can be reasonable under certain circumstances, specifically when the infant is born after the mother has received a β-lactam as IAP for 4 hours or longer before delivery, has a gestational age of 37 weeks or more, and is healthy appearing. Other discharge criteria also should be met, and the infant should be under the care of a person able to comply with instructions for home observation, with ready access to medical care.[16] The risk of bacterial infection in healthy appearing newborns is low. The revised 2010 CDC guidelines for prevention of early-onset GBS disease[16] also were endorsed by ACOG and AAP.[540] Because some recommendations from neonatologists were in conflict with the 2010 recommended infant management guidelines,[463] further clarification was needed to establish Figure 12-12 as the optimal care pathway.[541]

The influence of maternal IAP on the clinical spectrum of early-onset infection in term infants has been evaluated.[542,543] Exposure to antibiotics in labor does not change the clinical spectrum of disease or the onset of clinical signs of infection within 24 to 48 hours of birth for infants with early-onset GBS infection. Infants whose mothers have received IAP are less likely to be ill, to require assisted ventilation, or to have proven bacterial infection. These infants are not more likely to undergo invasive procedures or to receive antibiotics.[542] The number of infants undergoing evaluation for sepsis has decreased in association with implementation of IAP guidelines, and among GBS-negative women, ordering of laboratory tests diminished by almost 40% after the 2002 guidelines[544] and are anticipated to decrease even more if the 2010 infant management recommendations are fully implemented.[541]

Chemoprophylaxis for the Neonate

In the special circumstance of an apparently nonaffected sibling in a twin or multiple birth with early-onset[302,303] or late-onset[303] GBS disease, the well appearing sibling of a neonate with invasive infection is at increased risk of developing GBS disease. At the time of diagnosis of GBS disease in the index patient of a multiple birth, the other infant or infants should be assessed clinically.[303] If signs of infection are noted, cultures of blood and CSF should be obtained, and empirical antimicrobial treatment should be initiated until laboratory results become available. If cultures yield GBS, a full course of treatment is appropriate. If findings from the clinical assessment are unremarkable, management should be undertaken on a case-by-case basis. The risk for a poor outcome when the second twin is not evaluated until clinical signs of infection are apparent warrants caution in this circumstance. Even when empirical therapy is given and invasive infection is excluded, later onset is possible.[545]

IMMUNOPROPHYLAXIS

A promising approach to prevention of GBS disease is immunoprophylaxis.[504,546] The underlying principle is that IgG directed against CPS of GBS, critical for protection against invasive disease, is provided by active or passive immunization. Human sera containing a sufficient concentration of CPS-specific antibody have been shown in animal models of infection to protect against lethal challenge with each of the major group B streptococcal CPS types.[547] Provision of protective levels of CPS type-specific immunity to the newborn could be achieved by active maternal immunization with passive protection of the neonate.

The first candidate GBS vaccine, a purified type III CPS, underwent testing in healthy adults in 1978.[319] Subsequently, types Ia and II CPS vaccines were studied. Although these vaccines were well tolerated and elicited a primarily IgG class response within 2 to 4 weeks, the immunogenicity was variable. It was discovered that nearly 90% of healthy young adults had very low preimmunization serum concentrations of CPS-specific antibodies in association with presumed immunologic naïveté. These low levels predicted a poor immune response in many so that only 40% and 60% developed significant type-specific antibody responses after immunization with type Ia and type III CPS vaccines, respectively. By contrast, 88% of adults immunized with type II CPS vaccine responded with fourfold or greater increases in type II CPS–specific antibodies. These early trials verified the feasibility of immunization as an approach to prevent GBS disease and revealed the need to develop candidate vaccines with enhanced immunogenicity. The first study conducted in pregnant women was an encouragement to the ultimate potential success of a GBS vaccine program. Among 25 pregnant responders to a type III CPS GBS vaccine, 90% delivered infants with substantial levels of specific antibody to the type III CPS in cord sera that promoted functional activity in vitro throughout the first 3 months of life in most instances.

Development of the first group B streptococcal CPS conjugate vaccine, type III CPS–tetanus toxoid, was driven by the prominence of type III among infants with early- and late-onset disease and by its dominance as a cause of meningitis. Type III CPS was linked covalently to monomeric tetanus toxoid by reductive amination coupling chemistry.[548] Group B streptococcal CPS–protein conjugate vaccines of all clinically important types in developed countries (Ia, Ib, II, III, and V) subsequently were developed and found to be immunogenic and protective in experimental animals.[548-552] The first clinical evaluation of the type III CPS–tetanus toxoid conjugate showed greater than fourfold increases in postimmunization CPS-specific IgG in 90% of healthy nonpregnant women.[553] The vaccine was well tolerated, and the antibodies, predominantly of the IgG class, were functional in vitro and protective in a murine model of infection.

Conjugate vaccines to each of the clinically relevant group B streptococcal CPS types causing invasive disease have been tested in nearly 500 healthy adults 18 to 50 years of age.[553-556] Systemic responses, such as low-grade fever, chills, headache or myalgias, always short-lived, were observed in less than 2% of volunteers. Local reactions were frequent but mild, typically consisting of pain without erythema or swelling, and resolved within 48 to 72 hours. Immune responses to each of the conjugate vaccines, with the exception of type V, are dose dependent. Doses of 4 to 15 μg of the CPS component have elicited greater than fourfold increases in CPS-specific IgG in 80% to 93% of recipients of type Ia, Ib, II, III, and V conjugates at 8 weeks after immunization. Evaluation of a vaccine combining type II and type III CPS, each conjugated to tetanus toxoid, showed no immune interference compared with response after administration of one of the monovalent vaccines.[557]

A phase I randomized placebo-controlled, double-blinded trial of type III CPS–tetanus toxoid conjugate vaccine was conducted in 30 healthy pregnant women at 30 to 32 weeks of gestation.[558] Immunization was well tolerated. Geometric mean concentrations of IgG elicited to type III CPS from immunized women were significantly increased from preimmunization values and correlated well with infant cord values. Sera from the infants of vaccinated women collected at 1 and 2 months of age promoted in vitro opsonization and killing of type III GBS by human neutrophils.

Although glycoconjugates are the most likely first GBS vaccine candidates, the precise formulation will be challenging. Currently, the most prevalent types causing global GBS perinatal disease are III and Ia. Bellais and colleagues[140] identified the presence of the CC17-specific surface protein encoding the *hvgA* gene of a type III GBS strain from France. Using a variety of molecular techniques, they were able to demonstrate a CPS switch from type III to type IV that used the entire *cps* operon. Use of surface proteins that are conserved across most GBS serotypes offers another strategy for vaccine development. The C protein could be an alternative to tetanus toxoid as the protein component of a conjugate vaccine.[547,551,559] Invasive disease, but not colonization, elicits α C–specific and β C–specific IgM and IgG in adults.[560,561] A type III polysaccharide–C protein conjugate vaccine theoretically could prevent most systemic infections. A recombinant β C protein modified to eliminate its IgA-binding site conjugated to type III CPS has been shown to be immunogenic in mice, inducing polysaccharide and C protein–specific functional IgG.[562] The GBS surface proteins, Rib, Sip, and C5a peptidase, each have been shown to elicit antibodies that are protective in experimental models of GBS infection.[563-565] Their roles in human infection are not established, however.

The discovery that surface-associated pilus-like islands are distributed widely among GBS clinical isolates potentially paves the way for the development of pilus island–based vaccines. An entire pilus island has been transferred from group B *Streptococcus* to a nonpathogenic species. Mucosally delivered *Lactococcus*-expressing pilus island 1 protected mice from challenge with pilus 1–containing GBS strains.[9] Pilus islands 1, 2a, and 2b, alone or in combination, were identified on each of 289 GBS isolates from infants and adults with invasive disease, and most were highly surface expressed.[10] A combination of the three pilus-island components conferred protection against all tested GBS challenge strains. A vaccine exclusively constituted by pilus components in concept could be broadly efficacious in preventing infections caused by GBS.[10,566]

Recent studies emphasize the importance of perinatal GBS disease not only in resource-rich but also developing countries, with a substantial impact on global neonatal mortality.[145,146] IAP is not feasible in many countries and does not prevent late-onset disease. Maternal immunization, which has eliminated maternal and neonatal tetanus, is an attractive prevention method. In the United States and some European countries, which now recommend routine influenza and tetanus and diphtheria toxoids plus acellular pertussis vaccine for pregnant women, the platform for a GBS vaccine appears to be growing. Recent phase I and II trials of a trivalent Ia, Ib, III GBS glycoconjugate in pregnant women conducted by Novartis Vaccines (Basel) is encouraging and it is hoped that further industry development will progress. Meanwhile, physicians, their patients and pharmaceutical industry leaders must perceive this mode of prevention to be of high benefit and negligible risk, especially when pregnant women are the target population for immunization. The cost of developing suitable vaccines, although substantial, is considerably less than the death, disability, and treatment associated with these infections.[512,567] If the prevention of GBS disease is to become a reality, however, physicians, public health officials, parents, and patients must join together as advocates for pregnant women, neonates, and young infants.

References

1. Fry RM: Fatal infections by haemolytic streptococcus group B, *Lancet* 1:199-201, 1938.
2. Franciosi RA, Knostman JD, Zimmerman RA: Group B streptococcal neonatal and infant infections, *J Pediatr* 82:707-718, 1973.
3. Baker CJ, Barrett FF: Transmission of group B streptococci among parturient women and their neonates, *J Pediatr* 83:919-925, 1973.
4. Howard JB, McCracken GH Jr: The spectrum of group B streptococcal infections in infancy, *Am J Dis Child* 128:815-818, 1974.
5. Schrag SJ, Zywicki S, Farley MM, et al: Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis, *N Engl J Med* 342:15-20, 2000.
6. Tettelin H, Masignani V, Cieslewicz MJ, et al: Complete genome sequence and comparative genomic analysis of an emerging human pathogen, serotype V *Streptococcus agalactiae*, *Proc Natl Acad Sci U S A* 99:12391-12396, 2002.
7. Glaser P, Rusniok C, Buchrieser C, et al: Genome sequence of *Streptococcus agalactiae*, a pathogen causing invasive neonatal disease, *Mol Microbiol* 45:1499-1513, 2002.
8. Lauer P, Rinaudo CD, Soriani M, et al: Genome analysis reveals pili in group B *Streptococcus*, *Science* 309:105, 2005.
9. Buccato S, Maione D, Rinaudo CD, et al: Use of *Lactococcus lactis* expressing pili from group B *Streptococcus* as a broad-coverage vaccine against streptococcal disease, *J Infect Dis* 194:331-340, 2006.
10. Margarit I, Rinaudo CD, Galeotti CL, et al: Preventing bacterial infections with pilus-based vaccines: the group B streptococcus paradigm, *J Infect Dis* 199:108-115, 2009.
11. Lancefield RC: A serological differentiation of human and other groups of hemolytic streptococci, *J Exp Med* 57:571-595, 1933.
12. Baker CJ, Clark DJ, Barrett FF: Selective broth medium for isolation of group B streptococci, *Appl Microbiol* 26:884-885, 1973.
13. Lim DV, Morales WJ, Walsh AF: Lim group B Strep Broth and coagglutination for rapid identification of group B streptococci in preterm pregnant women, *J Clin Microbiol* 25:452-453, 1987.
14. Christie R, Atkins NE, Munch-Petersen E: A note on a lytic phenomenon shown by group B streptococci, *Aust J Exp Biol Med Sci* 22: 197-200, 1944.

15. Lancefield RC: A microprecipitin technic for classifying hemolytic streptococci, and improved methods for producing antisera, *Proc Soc Exp Biol Med* 38:473-478, 1938.
16. Centers for Disease Control and Prevention: Prevention of perinatal group B streptococcal disease—revised guidelines from CDC, *MMWR Recomm Rep* 59(RR-10):1-32, 2010.
17. Stableforth AW: Incidence of various serological types of *Streptococcus agalactiae* in herds of cows in Great Britain, *J Pathol Bacteriol* 46:21-119, 1938.
18. Finch LA, Martin DR: Human and bovine group B streptococci: two distinct populations, *J Appl Bacteriol* 57:273-278, 1984.
19. Wibawan IWT, Lämmler C: Properties of group B streptococci with protein surface antigens X and R, *J Clin Microbiol* 282:834-2836, 1990.
20. Bohnsack JF, Whiting AA, Martinez G, et al: Serotype III *Streptococcus agalactiae* from bovine milk and human neonatal infections, *Emerg Infect Dis* 10:1412-1419, 2004.
21. Lancefield RC, Hare R: The serological differentiation of pathogenic and non-pathogenic strains of hemolytic streptococci from parturient women, *J Exp Med* 61:335-349, 1935.
22. Lancefield RC: Two serological types of group B hemolytic streptococci with related, but not identical, type-specific substances, *J Exp Med* 67:25-40, 1938.
23. Freimer EH: Type-specific polysaccharide antigens of group B streptococci. II. The chemical basis for serological specificity of the type II HCI antigen, *J Exp Med* 125:381-392, 1967.
24. Wilkinson HW, Eagon RG: Type-specific antigens of group B type Ic streptococci, *Infect Immun* 4:596-604, 1971.
25. Jelínková J, Motlová J: The nomenclature of GBS, *Antibiot Chemother* 35:49-52, 1985.
26. Henrichsen J, Ferrieri P, Jelinkova J, et al: Nomenclature of antigens of group B streptococci, *Int J Syst Bacteriol* 34:500, 1984.
27. Perch B, Kjems E, Henrichsen J: New serotypes of group B streptococci isolated from human sources, *J Clin Microbiol* 10:109-110, 1979.
28. Johnson DR, Ferrieri P: Group B streptococcal Ibc protein antigen: distribution of two determinants in wild-type strains of common serotypes, *J Clin Microbiol* 19:506-510, 1984.
29. Madoff LC, Michel JL, Gong EW, et al: Group B streptococci escape host immunity by deletion of tandem repeat elements of the alpha C protein, *Proc Natl Acad Sci U S A* 93:4131-4136, 1996.
30. Kling DE, Gravekamp C, Madoff LC, et al: Characterization of two distinct opsonic and protective epitopes within the alpha C protein of the group B *Streptococcus*, *Infect Immun* 65:1462-1467, 1997.
31. Gravekamp C, Horensky DS, Michel JL, et al: Variation in repeat number within the alpha C protein of group B streptococci alters antigenicity and protective epitopes, *Infect Immun* 64:3576-3583, 1996.
32. Jerlström PG, Chhatwal GS, Timmis KN: The IgA-binding 3 antigen of the C protein complex of group B streptococci: sequence determination of its gene and detection of two binding regions, *Mol Microbiol* 54:843-849, 1991.
33. Jerlström PG, Talay SR, Valentin-Weigand P, et al: Identification of an immunoglobulin A binding motif located in the beta-antigen of the c protein complex of group B streptococci, *Infect Immun* 64: 2787-2793, 1996.
34. Ferrieri P, Baker CJ, Hillier SL, et al: Diversity of surface protein expression in group B streptococcal colonizing and invasive isolates, *Indian J Med Res* 119(Suppl):191-196, 2004.
35. Erdogan S, Fagan PK, Talay SR, et al: Molecular analysis of group B protective surface protein, a new cell surface protective antigen of group B streptococci, *Infect Immun* 70:803-811, 2002.
36. Flores AE, Chhatwal GS, Hillier SL, et al: Expression of group B protective surface protein (BPS) by invasive and colonizing isolates of group B streptococci, *Curr Microbiol* 69:894-898, 2014.
37. Pattison IH, Matthews PRJ, Howell DG: The type classification of group B streptococci with special reference to bovine strains apparently lacking in type polysaccharide, *J Pathol Bacteriol* 69:41-50, 1955.
38. Lachenauer CS, Madoff LC: A protective surface protein from type V group B streptococci shares N-terminal sequence homology with the alpha C protein, *Infect Immun* 64:4255-4260, 1996.
39. Rioux S, Martin D, Ackermann HW, et al: Localization of surface immunogenic protein on group B *Streptococcus*, *Infect Immun* 69:5162-5265, 2001.
40. Maisey HC, Hensler M, Nizet V, et al: Group B streptococcal pilus proteins contribute to adherence to and invasion of brain microvascular endothelial cells, *J Bacteriol* 189:1464-1467, 2007.
41. Deng L, Kasper DL, Krick TP, et al: Characterization of the linkage between the type III capsular polysaccharide and the bacterial cell wall of group B *Streptococcus*, *J Biol Chem* 275:7497-7504, 2000.
42. Kasper DL, Baker CJ: Electron microscopic definition of surface antigens of group B *Streptococcus*, *J Infect Dis* 139:147-151, 1979.
43. von Hunolstein C, D'Ascenzi S, Wagner B, et al: Immunochemistry of capsular type polysaccharide and virulence properties of type VI *Streptococcus agalactiae* (group B streptococci), *Infect Immun* 61:1272-1280, 1993.
44. Ráyc M, Jelínková J, Motlová J, et al: Immuno-electronmicroscopic demonstration of capsules on group-B streptococci of new serotypes and type candidates, *J Med Microbiol* 25:147-149, 1988.
45. Paoletti LC, Ross RA, Johnson KD: Cell growth rate regulates expression of group B *Streptococcus* type III capsular polysaccharide, *Infect Immun* 64:1220-1226, 1996.
46. Baker CJ, Kasper DL, Davis CE: Immunochemical characterization of the native type III polysaccharide of group B *Streptococcus*, *J Exp Med* 143:258-270, 1976.
47. Michon F, Brisson JR, Dell A, et al: Multiantennary group-specific polysaccharide of group B *Streptococcus*, *Biochemistry* 27: 5341-5351, 1988.
48. Tai JY, Gotschlich EC, Lancefield RC: Isolation of type-specific polysaccharide antigen from group B type Ib streptococci, *J Exp Med* 149:58-66, 1979.
49. Jennings HJ, Rosell KG, Kasper DL: Structural determination and serology of the native polysaccharide antigen of the type III group B *Streptococcus*, *Can J Biochem* 58:112-120, 1980.
50. Wessels MR, Pozsgay V, Kasper DL, et al: Structure and immunochemistry of an oligosaccharide repeating unit of the capsular polysaccharide of type III group B *Streptococcus*, *J Biol Chem* 262:8262-8267, 1987.
51. Wessels MR, DiFabio JL, Benedì VJ, et al: Structural determination and immunochemical characterization of the type V group B *Streptococcus* capsular polysaccharide, *J Biol Chem* 266:6714-6719, 1991.
52. Kogan G, Brisson JR, Kasper DL, et al: Structural elucidation of the novel type VII group B *Streptococcus* capsular polysaccharide by high resolution NMR spectroscopy, *Carbohydr Res* 277:1-9, 1995.
53. Kasper DL, Baker CJ, Galdes B, et al: Immunochemical analysis and immunogenicity of the type II group B streptococcal capsular polysaccharide, *J Clin Invest* 72:260-269, 1983.
54. Wessels MR, Benedí WJ, Jennings HJ, et al: Isolation and characterization of type IV group B *Streptococcus* capsular polysaccharide, *Infect Immun* 57:1089-1094, 1989.
55. Jennings HJ, Rosell KG, Katzenellenbogen E, et al: Structural determination of the capsular polysaccharide antigen of type II group B *Streptococcus*, *J Biol Chem* 258:1793-1798, 1983.
56. Kogan G, Uhrín D, Brisson JR, et al: Structural and immunochemical characterization of the type VIII group B *Streptococcus* capsular polysaccharide, *J Biol Chem* 271:8786-8790, 1996.
57. Jennings HJ, Katzenellenbogen E, Lugowski C, et al: Structure of native polysaccharide antigens of type Ia and type Ib group B *Streptococcus*, *Biochemistry* 22:1258-1264, 1983.
58. Schifferle RE, Jennings HJ, Wessels MR, et al: Immunochemical analysis of the types Ia and Ib group B streptococcal polysaccharides, *J Immunol* 135:4164-4170, 1985.
59. Lindberg B, Lönngren J, Powell DA: Structural studies of the specific type 14 pneumococcal polysaccharide, *Carbohydr Res* 58:117-186, 1977.
60. Wessels MR, Paoletti LC, Guttormsen HK, et al: Structural properties of group B streptococcal type III polysaccharide conjugate vaccines that influence immunogenicity and efficacy, *Infect Immun* 66: 2186-2192, 1998.
61. Zou W, Jennings HJ: The conformational epitope of type III group B *Streptococcus* capsular polysaccharide, *Adv Exp Med Biol* 491: 473-484, 2001.
62. Milligan TW, Doran TI, Straus DC, et al: Growth and amino acid requirements of various strains of group B streptococci, *J Clin Microbiol* 7:28-33, 1978.
63. Baker CJ, Kasper DL: Microcapsule of type III strains of group B *Streptococcus*: production and morphology, *Infect Immun* 13:189-194, 1976.

64. Malin G, Paoletti LC: Use of a dynamic in vitro attachment and invasion system (DIVAS) to determine influence of growth rate on invasion of respiratory epithelium by group B *Streptococcus*, *Proc Natl Acad Sci U S A* 98:13335-13340, 2001.
65. Johri AK, Padilla J, Malin G, et al: Oxygen regulates invasiveness and virulence of group B *Streptococcus*, *Infect Immun* 71:6707-6711, 2003.
66. Whidbey C, Harrell MI, Burnside K, et al: A hemolytic pigment of group B *Streptococcus* allows bacterial penetration of human placenta, *J Exp Med* 210:1265-1281, 2013.
67. Ferrieri P, Wannamaker LW, Nelson J: Localization and characterization of the hippuricase activity of group B streptococci, *Infect Immun* 7:747-752, 1973.
68. Bohnsack JF, Mollison KW, Buko AM, et al: Group B streptococci inactivate complement component C5a by enzymic cleavage at the C-terminus, *Biochem J* 273:635-640, 1991.
69. Bohnsack JF, Zhou XN, Williams PA, et al: Purification of a protease from group B streptococci that inactivates human C5a, *Biochim Biophys Acta* 1079:222-228, 1991.
70. Cleary PP, Handley J, Suvorov AN, et al: Similarity between the group B and A streptococcal C5a peptidase genes, *Infect Immun* 60:4239-4244, 1992.
71. Hill HR, Bohnsack JF, Morris EZ, et al: Group B streptococci inhibit the chemotactic activity of the fifth component of complement, *J Immunol* 141:3551-3556, 1988.
72. Ferrieri P, Gray ED, Wannamaker LW: Biochemical and immunological characterization of the extracellular nucleases of group B streptococci, *J Exp Med* 151:56-68, 1980.
73. Pritchard DG, Lin B, Willingham TR, et al: Characterization of the group B streptococcal hyaluronate lyase, *Arch Biochem Biophys* 315:431-437, 1994.
74. Musser JM, Mattingly SJ, Quentin R, et al: Identification of a high-virulence clone of type III *Streptococcus agalactiae* (group B *Streptococcus*) causing invasive neonatal disease, *Proc Natl Acad Sci U S A* 86:4731-4735, 1989.
75. Nealon TJ, Mattingly SJ: Association of elevated levels of cellular lipoteichoic acids of group B streptococci with human neonatal disease, *Infect Immun* 39:1243-1251, 1983.
76. Goldschmidt JC Jr, Panos C: Teichoic acids of *Streptococcus agalactiae*: chemistry, cytotoxicity, and effect on bacterial adherence to human cells in tissue culture, *Infect Immun* 43:670-677, 1984.
77. Meyn L, Moore DM, Hillier SL, et al: Association of sexual activity with colonization and vaginal acquisition of group B *Streptococcus* in nonpregnant women, *Am J Epidemiol* 155:949-957, 2002.
78. Foxman B, Gillespie B, Manning SD, et al: Incidence and duration of group B *Streptococcus* by serotype among male and female college students living in a single dormitory, *Am J Epidemiol* 163:544-551, 2006.
79. Boyer KM, Gadzala CA, Kelly PD, et al: Selective intrapartum chemoprophylaxis of neonatal group B streptococcal early-onset disease. II. Predictive value of prenatal cultures, *J Infect Dis* 148:802-809, 1983.
80. Yancey MK, Schuchat A, Brown LK, et al: The accuracy of late antenatal screening cultures in predicting genital group B streptococcal colonization at delivery, *Obstet Gynecol* 88:811-815, 1996.
81. Persson K, Bjerre B, Elfström L, et al: Longitudinal study of group B streptococcal carriage during late pregnancy, *Scand J Infect Dis* 19:325-329, 1987.
82. Manning SD, Neighbors K, Tallman PA, et al: Prevalence of group B *Streptococcus* colonization and potential for transmission by casual contact in healthy young men and women, *Clin Infect Dis* 39: 380-388, 2004.
83. Baker CJ, Goroff DK, Alpert S, et al: Vaginal colonization with group B *Streptococcus*: a study in college women, *J Infect Dis* 135:392-397, 1977.
84. Bliss SJ, Manning SD, Tallman P, et al: Group B *Streptococcus* colonization in male and non-pregnant female university students: a cross-sectional prevalence study, *Clin Infect Dis* 34:184-190, 2002.
85. Foxman B, Gillespie BW, Manning SD, et al: Risk factors for group B streptococcal colonization: potential for different transmission systems by capsular type, *Ann Epidemiol* 17:854-862, 2007.
86. Ramos E, Gaudier FL, Hearing LR, et al: Group B *Streptococcus* colonization in pregnant diabetic women, *Obstet Gynecol* 89:257-260, 1997.
87. Christensen KK, Dykes AK, Christensen P: Relation between use of tampons and urogenital carriage of group B streptococci, *BMJ* 289:731-732, 1984.
88. Campbell JR, Hillier SL, Krohn MA, et al: Group B streptococcal colonization and serotype-specific immunity in pregnant women at delivery, *Obstet Gynecol* 96:498-503, 2000.
89. Hickman ME, Rench MA, Ferrieri P, et al: Changing epidemiology of group B streptococcal (GBS) colonization, *Pediatrics* 104:203-209, 1999.
90. Newton ER, Butler MC, Shain RN: Sexual behavior and vaginal colonization by group B *Streptococcus* among minority women, *Obstet Gynecol* 88:577-582, 1996.
91. Davies HD, Adair C, McGeer A, et al: Antibodies to capsular polysaccharides of group B *Streptococcus* in pregnant Canadian women: relationship to colonization status and infection in the neonate, *J Infect Dis* 184:285-291, 2001.
92. Stoll BJ, Schuchat A: Maternal carriage of group B streptococci in developing countries, *Pediatr Infect Dis J* 17:499-503, 1998.
93. Barcaite E, Bartusevicius A, Tameliene R, et al: Prevalence of maternal group B streptococcal colonisation in European countries, *Acta Obstet Gynecol Scand* 87:260-271, 2008.
94. Turrentine MA, Ramirez MM: Recurrence of group B streptococci colonization in subsequent pregnancy, *Obstet Gynecol* 112:259-264, 2008.
95. Ferrieri P, Blair LL: Pharyngeal carriage of group B streptococci: detection by three methods, *J Clin Microbiol* 6:136-139, 1977.
96. Chretien JH, McGinniss CG, Thompson J, et al: Group B beta-hemolytic streptococci causing pharyngitis, *J Clin Microbiol* 10:263-266, 1979.
97. Sackel SG, et al: *Isolation of group B Streptococcus from 18th Interscience Conference on Antimicrobial Agents and Chemotherapy*, Atlanta, 1978, [abstract 467]. October 1-4, 1978.
98. Hayden GF, Murphy TF, Hendley JO: Non-group A streptococci in the pharynx. Pathogens or innocent bystanders? *Am J Dis Child* 143:794-797, 1989.
99. Hammerschlag MR, Baker CJ, Alpert S, et al: Colonization with group B streptococci in girls under 16 years of age, *Pediatrics* 60:473-477, 1977.
100. Mauer M, Thirumoorthi MC, Dajani AS: Group B streptococcal colonization in prepubertal children, *Pediatrics* 64:65-67, 1979.
101. Persson KS, Bjerre B, Elfström L, et al: Faecal carriage of group B streptococci, *Eur J Clin Microbiol* 5:156-159, 1986.
102. Cummings CG, Ross PW: Group B streptococci (GBS) in the upper respiratory tract of schoolchildren, *Health Bull* 40:81-86, 1982.
103. Shafer MA, Sweet RL, Ohm-Smith MJ, et al: Microbiology of the lower genital tract in postmenarchal adolescent girls: differences by sexual activity, contraception, and presence of nonspecific vaginitis, *J Pediatr* 107:974-981, 1985.
104. Ancona RJ, Ferrieri P, Williams PP: Maternal factors that enhance the acquisition of group B streptococci by newborn infants, *J Med Microbiol* 13:273-280, 1980.
105. Ferrieri P, Cleary PP, Seeds AE: Epidemiology of group B streptococcal carriage in pregnant women and newborn infants, *J Med Microbiol* 10:103-114, 1976.
106. Anthony BF, Okada DM, Hobel CJ: Epidemiology of the group B *Streptococcus*: maternal and nosocomial sources for infant acquisitions, *J Pediatr* 95:431-436, 1979.
107. Paredes A, Wong P, Mason EO Jr, et al: Nosocomial transmission of group B streptococci in a newborn nursery, *Pediatrics* 59:679-682, 1976.
108. Easmon CSF, Hastings MJ, Clare AJ, et al: Nosocomial transmission of group B streptococci, *BMJ* 283:459-461, 1981.
109. Noya FJD, Rench MA, Metzger TG, et al: Unusual occurrence of an epidemic of type Ib/c group B streptococcal sepsis in a neonatal intensive care unit, *J Infect Dis* 155:1135-1144, 1987.
110. Gardner SE, Mason EO Jr, Yow MD: Community acquisition of group B *Streptococcus* by infants of colonized mothers, *Pediatrics* 66: 873-875, 1980.
111. Hansen SM, Uldbjerg N, Kilian M, et al: Dynamics of *Streptococcus agalactiae* colonization in women during and after pregnancy and in their infants, *J Clin Microbiol* 42:83-89, 2004.
112. Baker CJ, Barrett FF: Group B streptococcal infection in infants: the importance of the various serotypes, *JAMA* 230:1158-1160, 1974.
113. Blumberg HM, Stephens DS, Modansky M, et al: Invasive group B streptococcal disease: the emergence of serotype V, *J Infect Dis* 173:365-373, 1996.

114. Zaleznik DF, Rench MA, Hillier S, et al: Invasive disease due to group B *Streptococcus* in pregnant women and neonates from diverse population groups, *Clin Infect Dis* 30:276-281, 2000.
115. Davies HD, Raj S, Adair C, et al: Population-based active surveillance for neonatal group B streptococcal infections in Alberta, Canada: implications for vaccine formulation, *Pediatr Infect Dis J* 20:879-884, 2001.
116. Elliott JA, Farmer KD, Facklam RR: Sudden increase in isolation of group B streptococci, serotype V, is not due to emergence of a new pulsed-field gel electrophoresis type, *J Clin Microbiol* 36:2115-2116, 1998.
117. Fluegge K, Supper S, Siedler A, et al: Serotype distribution of invasive group B streptococcal isolates in infants: results from a nationwide active laboratory surveillance study over 2 years in Germany, *Clin Infect Dis* 40:760-763, 2005.
118. Madzivhandila M, Adrian PV, Cutland CL, et al: Serotype distribution and invasive potential of group B *Streptococcus* isolates causing disease in infants and colonizing maternal-newborn dyads, *PLoS One* 6:e17861, 2011.
119. Lamagni TL, Keshishian C, Efstratiou A, et al: Emerging trends in the epidemiology of invasive group B streptococcal disease in England and Wales, 1991-2010, *Clin Infect Dis* 57:682-688, 2013.
120. Ferrieri P, Lynfield R, Creti R, et al: Serotype IV and invasive group B *Streptococcus* disease in neonates, Minnesota, USA, 2000-2010, *Emerg Infect Dis* 19:551-558, 2013.
121. Matsubara K, Katayama K, Baba K, et al: Seroepidemiologic studies of serotype VIII group B *Streptococcus* in Japan, *J Infect Dis* 186: 855-858, 2002.
122. Matsubara K, Mikamo H, Numa M, et al: Three fatal cases of invasive serotype VI group B streptococcal infection, *J Infect* 53:e139-e142, 2006.
123. Phares CR, Lynfield R, Farley MM, et al: Epidemiology of invasive group B streptococcal disease in the United States, 1999–2005, *JAMA* 299:2056-2065, 2008.
124. Quentin R, Huet H, Wang FS, et al: Characterization of *Streptococcus agalactiae* strains by multilocus enzyme genotype and serotype: identification of multiple virulent clone families that cause invasive neonatal disease, *J Clin Microbiol* 33:2576-2581, 1995.
125. Gordillo ME, Singh KV, Baker CJ, et al: Comparison of group B streptococci by pulsed field gel electrophoresis and by conventional electrophoresis, *J Clin Microbiol* 31:1430-1434, 1993.
126. Limansky AS, Sutich EG, Guardati MC, et al: Genomic diversity among *Streptococcus agalactiae* isolates detected by a degenerate oligonucleotide-primed amplification assay, *J Infect Dis* 177: 1308-1313, 1998.
127. Imperi M, Pataracchia M, Alfarone G, et al: A multiplex PCR assay for the direct identification of the capsular type (Ia to IX) of *Streptococcus agalactiae*, *J Microbiol Meth* 80:212-214, 2010.
128. Al Nakib M, Longo M, Tazi A, et al: Comparison of the Diversilab system with multi-locus sequence typing and pulsed-gel electrophoresis for the characterization of *Streptococcus agalactiae* invasive strains, *J Microbiol Meth* 85:137-142, 2011.
129. Luan SL, Granlund M, Sellin M, et al: Multilocus sequence typing of Swedish invasive group B streptococcus isolates indicates a neonatally associated genetic lineage and capsule switching, *J Clin Microbiol* 43:3727-3733, 2005.
130. Adderson EE, Takahashi S, Bohnsack JF: Bacterial genetics and human immunity to group B streptococci, *Mol Genet Metab* 71: 451-454, 2000.
131. Bohnsack JF, Whiting AA, Bradford RD, et al: Long-range mapping of the *Streptococcus agalactiae* phylogenetic lineage restriction digest pattern type III-3 reveals clustering of virulence genes, *Infect Immun* 70:134-139, 2002.
132. Takahashi S, Detrick S, Whiting AA, et al: Correlation of phylogenetic lineages of group B streptococci, identified by analysis of restriction-digestion patterns of genomic DNA, with *infB* alleles and mobile genetic elements, *J Infect Dis* 186:1034-1038, 2002.
133. Adderson EE, Takahashi S, Wang Y, et al: Subtractive hybridization identifies a novel predicted protein mediating epithelial cell invasion by virulent serotype III group B *Streptococcus agalactiae*, *Infect Immun* 71:6857-6863, 2003.
134. Davies HD, Jones N, Whittam TS, et al: Multilocus sequence typing of serotype III group B *Streptococcus* and correlation with pathogenic potential, *J Infect Dis* 189:1097-1102, 2004.
135. Bidet P, Brahimi N, Chalas C, et al: Molecular characterization of serotype III group B *Streptococcus* isolates causing neonatal meningitis, *J Infect Dis* 188:1132-1137, 2003.
136. von Both U, John A, Fluegge K, et al: Molecular epidemiology of invasive neonatal *Streptococcus agalactiae* isolates in Germany, *Pediatr Infect Dis J* 27:903-906, 2008.
137. Dore N, Bennett D, Kaliszer M, et al: Molecular epidemiology of group B streptococci in Ireland: associations between serotype, invasive status and presence of genes encoding putative virulence factors, *Epidemiol Infect* 131:823-833, 2003.
138. Sørensen UBS, Poulsen K, Ghezzo C, et al: Emergence and global dissemination of host-specific *Streptococcus agalactiae* clones, *mBio* 1: 1-9, 2010.
139. Jones N, Bohnsack JF, Takahashi S, et al: Multilocus sequence typing system for group B Streptococcus, *J Clin Microbiol* 41:2530-2536, 2003.
140. Bellais S, Six A, Fouet A, et al: Capsular switching in group B *Streptococcus* CC17 hypervirulent clone: a future challenge for polysaccharide vaccine development, *J Infect Dis* 206:1745-1752, 2012.
141. Perinatal group B streptococcal disease after universal screening recommendations—United States, 2003–2005, *MMWR Morb Mortal Wkly Rep* 56:701-705, 2007.
142. Schrag SJ, Zell ER, Lynfield R, et al: A population-based comparison of strategies to prevent early-onset group B streptococcal disease in neonates, *N Engl J Med* 34:7233-7239, 2002.
143. Centers for Disease Control and Prevention: Active bacterial core surveillance report, emerging infections program network, group B streptococcus, 2012. Available at http://www.cdc.gov/abcs/reports-findings/survreports/gbs12.pdf. Accessed Feb. 24, 2014.
144. Weston EJ, Pondo T, Lewis MM, et al: The burden of invasive early-onset neonatal sepsis in the United States, 2005-2008, *Pediatr Infect Dis J* 30:937-941, 2011.
145. Dagnew AF, Cunnington MC, Dube Q, et al: Variation in reported neonatal group B streptococcal disease incidence in developing countries, *Clin Infect Dis* 55:91-102, 2012.
146. Edmond KM, Kortsalioudaki C, Scott S, et al: Group B streptococcal disease in infants aged younger than 3 months: systemic review and meta-analysis, *Lancet* 379:547-556, 2012.
147. Boyer KM, Gotoff SP: Strategies for chemoprophylaxis of GBS early-onset infections, *Antibiot Chemother* 35:267-280, 1985.
148. Sobel JD, Myers P, Levison ME, et al: Comparison of bacterial and fungal adherence to vaginal exfoliated epithelial cells and human vaginal epithelial tissue culture cells, *Infect Immun* 35:697-701, 1982.
149. Jelínková J, Grabovskaya KB, Ryc M, et al: Adherence of vaginal and pharyngeal strains of group B streptococci to human vaginal and pharyngeal epithelial cells, *Zentralbl Bakteriol Mikrobiol Hyg [A]* 262:492-499, 1986.
150. Hoet YR, Li CM, Yu CH, et al: The enhancement of biofilm formation in group B streptococcal isolates at vaginal pH, *Med Microbiol Immunol* 202:105-115, 2013.
151. Nealon TJ, Mattingly SJ: Role of cellular lipoteichoic acids in mediating adherence of serotype III strains of group B streptococci to human embryonic, fetal, and adult epithelial cells, *Infect Immun* 43:523-530, 1984.
152. Teti G, Tomasello F, Chiofalo MS, et al: Adherence of group B streptococci to adult and neonatal epithelial cells mediated by lipoteichoic acid, *Infect Immun* 55:3057-3064, 1987.
153. Cox F: Prevention of group B streptococcal colonization with topically applied lipoteichoic acid in a maternal-newborn mouse model, *Pediatr Res* 16:816-819, 1982.
154. Tamura GS, Rubens CE: Group B streptococci adhere to a variant of fibronectin attached to a solid phase, *Mol Microbiol* 15:581-589, 1995.
155. Beckmann C, Waggoner JD, Harris TO, et al: Identification of novel adhesins from group B streptococci by use of phage display reveals that C5a peptidase mediates fibronectin binding, *Infect Immun* 70:2869-2876, 2002.
156. Cheng Q, Stafslien D, Purushothaman SS, et al: The group B streptococcal C5a peptidase is both a specific protease and an invasin, *Infect Immun* 70:2408-2413, 2002.
157. Spellerberg B, Rozdzinski E, Martin S, et al: Lmb, a protein with similarities to the LraI adhesin family, mediates attachment of *Streptococcus agalactiae* to human laminin, *Infect Immun* 67:871-878, 1999.
158. Schubert A, Zakikhany K, Schreiner M, et al: A fibrinogen receptor from group B *Streptococcus* interacts with fibrinogen by repetitive units with novel ligand binding sites, *Mol Microbiol* 46:557-569, 2002.

159. Samen U, Eikmanns BJ, Reinscheid DJ, et al: The surface protein Srr-1 of *Streptococcus agalactiae* binds human keratin 4 and promotes adherence to epithelial HEp-2 cells, *Infect Immun* 75:5405-5414, 2007.
160. Rosini R, Rinaudo CD, Soriani M, et al: Identification of novel genomic islands coding for antigenic pilus-like structures in *Streptococcus agalactiae*, *Mol Microbiol* 61:126-141, 2006.
161. Dramsi S, Caliot E, Bonne I, et al: Assembly and role of pili in group B streptococci, *Mol Microbiol* 60:1401-1413, 2006.
162. Krishnan V, Gaspar AH, Ye N, et al: An IgG-like domain in the minor pilin GBS52 of *Streptococcus agalactiae* mediates lung epithelial cell adhesion, *Structure* 15:893-903, 2007.
163. Sheen Jimenez A, Wang NY, et al: Serine-rich repeat proteins and pili promote *Streptococcus agalactiae* colonization of the vaginal tract, *J Bacteriol* 193:6834-6842, 2011.
164. Park Jiang S, Wessels MR: CsrRS and environmental pH regulate group B streptococcus adherence to human epithelial cells and extracellular matrix, *Infect Immun* 80:3975-3984, 2012.
165. Patras Wang NY, Fletcher EM, et al: Group B *Streptococcus* CovR regulation modulates host immune signalling pathways to promote vaginal colonization, *Cell Microbiol* 15:1154-1167, 2013.
166. Stewardson-Krieger PB, Gotoff SP: Risk factors in early-onset neonatal group B streptococcal infections, *Infection* 6:50-53, 1978.
167. Tseng PI, Kandall SR: Group B streptococcal disease in neonates and infants, *N Y State J Med* 74:2169-2173, 1974.
168. Evaldson GR, Malmborg AS, Nord CE: Premature rupture of the membranes and ascending infection, *Br J Obstet Gynaecol* 89:793-801, 1982.
169. Schoonmaker JN, Lawellin DW, Lunt B, et al: Bacteria and inflammatory cells reduce chorioamniotic membrane integrity and tensile strength, *Obstet Gynecol* 74:590-596, 1989.
170. Bennett PR, Rose MP, Myatt L, Elder MG: Preterm labor: stimulation of arachidonic acid metabolism in human amnion cells by bacterial products, *Am J Obstet Gynecol* 156:649-655, 1987.
171. Dudley DJ, Edwin SS, Van Wagoner J, et al: Regulation of decidual cell chemokine production by group B streptococci and purified bacterial cell wall components, *Am J Obstet Gynecol* 177:666-672, 1997.
172. Estrada-Gutierrez G, Gomez-Lopez N, Zaga-Clavellina V, et al: Interaction between pathogenic bacteria and intrauterine leukocytes triggers alternative molecular signaling cascades leading to labor in women, *Infect Immun* 78:4792-4799, 2010.
173. Spellerberg B, Martin S, Franken C, et al: Identification of genetic determinants for the hemolytic activity of *Streptococcus agalactiae* by ISS1 transposition, *J Bacteriol* 181:3212-3219, 1999.
174. Pritzlaff CA, Chang JC, Kuo SP, et al: Genetic basis for the beta-haemolytic/cytolytic activity of group B *Streptococcus*, *Mol Microbiol* 39:236-247, 2001.
175. G. Whidbey, Harrell MI, Burnside K, et al: A hemolytic pigment of group B *Streptococcus* allows bacterial penetration of human placenta, *J Exp Med* 210:1265-1281, 2013.
176. Liu GY, Doran KS, Lawrence T, et al: Sword and shield: linked group B streptococcal beta-hemolysin/cytolysin and carotenoid pigment act synergistically to subvert host phagocyte defenses, *Proc Natl Acad Sci USA* 10:14491-14496, 2004.
177. Randis TM, Gelber SE, Hooven TA, et al: Group B *Streptococcus* β-hemolysin/cytolysin breaches maternal fetal barriers to cause preterm birth and intrauterine demise in vivo, *J Infect Dis* 210:265-273, 2014.
178. Ferrieri P, Cleary PP, Seeds AE: Epidemiology of group B streptococcal carriage in pregnant women and newborn infants, *J Med Microbiol* 10:103-114, 1977.
179. Galask RP, Varner MW, Petzold CR, et al: Bacterial attachment to the chorioamniotic membranes, *Am J Obstet Gynecol* 148:915-928, 1984.
180. Winram SB, Jonas M, Chi E, et al: Characterization of group B streptococcal invasion of human chorion and amnion epithelial cells in vitro, *Infect Immun* 66:4932-4941, 1998.
181. Boldenow E, Jones S, Lieberman RW, et al: Antimicrobial peptide response to group B *Streptococcus* in human extraplacental membranes in culture, *Pediatrics* 34:480-485, 2013.
182. Hemming VG, Nagarajan K, Hess LW, et al: Rapid in vitro replication of group B *Streptococcus* in term human amniotic fluid, *Gynecol Obstet Invest* 19:124-129, 1985.
183. Baker CJ: Early onset group B streptococcal disease, *J Pediatr* 93: 124-125, 1978.
184. Pass MA, Gray BM, Khare S, et al: Prospective studies of group B streptococcal infections in infants, *J Pediatr* 95:437-443, 1979.
185. Adams-Waldorf KM, Gravett MG, McAdams RM, et al: Choriodecidual group B streptococcal inoculation induces fetal lung injury without intra-amniotic infection and preterm labor in *Macaca nemestrina*, *PLoS One* 6:e28972, 2011.
186. McAdams RM, Vanderhoeven J, Beyer RP, et al: Choriodecidual infection downregulates angiogenesis and morphogenesis pathways in fetal lungs from *Macaca nemestrina*, *PLoS One* 7:e46863, 2012.
187. Payne NR, Burke BA, Day DL, et al: Correlation of clinical and pathologic findings in early onset neonatal group B streptococcal infection with disease severity and prediction of outcome, *Pediatr Infect Dis J* 7:836-847, 1988.
188. Hemming VG, McCloskey DW, Hill HR: Pneumonia in the neonate associated with group B streptococcal septicemia, *Am J Dis Child* 130:1231-1233, 1976.
189. Katzenstein A, Davis C, Braude A: Pulmonary changes in neonatal sepsis due to group B beta-hemolytic streptococcus: relation to hyaline membrane disease, *J Infect Dis* 133:430-435, 1976.
190. Rubens CE, Raff HV, Jackson JC, et al: Pathophysiology and histopathology of group B streptococcal sepsis in *Macaca nemestrina* primates induced after intraamniotic inoculation: evidence for bacterial cellular invasion, *J Infect Dis* 164:320-330, 1991.
191. Sherman MP, Johnson JT, Rothlein R, et al: Role of pulmonary phagocytes in host defense against group B streptococci in preterm versus term rabbit lung, *J Infect Dis* 166:818-826, 1992.
192. Rubens CE, Smith S, Hulse M, et al: Respiratory epithelial cell invasion by group B streptococci, *Infect Immun* 60:5157-5163, 1992.
193. Gibson RL, Lee MK, Soderland C, et al: Group B streptococci invade endothelial cells: type III capsular polysaccharide attenuates invasion, *Infect Immun* 61:478-485, 1993.
194. Burnham CA, Shokoples SE, Tyrrell GJ: Rac1, RhoA, and Cdc42 participate in HeLa cell invasion by group B streptococcus, *FEMS Microbiol Lett* 272:8-14, 2007.
195. Shin S, Paul-Satyaseela M, Lee JS, et al: Focal adhesion kinase is involved in type III group B streptococcal invasion of human brain microvascular endothelial cells, *Microb Pathog* 4:168-173, 2006.
196. Valentin-Weigand P, Chhatwal GS: Correlation of epithelial cell invasiveness of group B streptococci with clinical source of isolation, *Microb Pathog* 19:83-91, 1995.
197. Schubert A, Zakikhany K, Pietrocola G, et al: The fibrinogen receptor FbsA promotes adherence of *Streptococcus agalactiae* to human epithelial cells, *Infect Immun* 72:6197-6205, 2004.
198. Tenenbaum T, Spellerberg B, Adam R, et al: *Streptococcus agalactiae* invasion of human brain microvascular endothelial cells is promoted by the laminin-binding protein, *Lmb Microbes Infect* 9:714-720, 2007.
199. Baron MJ, Bolduc GR, Goldberg MB, et al: Alpha C protein of group B *Streptococcus* binds host cell surface glycosaminoglycan and enters cells by an actin-dependent mechanism, *J Biol Chem* 279: 24714-24723, 2004.
200. Hulse ML, Smith S, Chi EY, et al: Effect of type III group B streptococcal capsular polysaccharide on invasion of respiratory epithelial cells, *Infect Immun* 61:4835-4841, 1993.
201. Sharma P, Lata H, Arya DK, et al: Role of pilus proteins in adherence and invasion of *Streptococcus agalactiae* to the lung and cervical epithelial cells, *J Biol Chem* 288:4023-4034, 2013.
202. Quirante J, Ceballos R, Cassady G: Group B beta-hemolytic streptococcal infection in the newborn. I. Early onset infection, *Am J Dis Child* 128:659-665, 1974.
203. Kling DE, Tsvang I, Murphy MP, et al: Group B *Streptococcus* induces a caspase-dependent apoptosis in fetal rat lung interstitium, *Microb Pathogen* 61-62:1-10, 2013.
204. Nizet V, Gibson RL, Chi EY, et al: Group B streptococcal beta-hemolysin expression is associated with injury of lung epithelial cells, *Infect Immun* 64:3818-3826, 1996.
205. Gibson RL, Nizet V, Rubens CE: Group B streptococcal beta-hemolysin promotes injury of lung microvascular endothelial cells, *Pediatr Res* 45:626-634, 1999.
206. Doran KS, Chang JC, Benoit VM, et al: Group B streptococcal beta-hemolysin/cytolysin promotes invasion of human lung epithelial cells and the release of interleukin-8, *J Infect Dis* 185:196-203, 2002.
207. Hensler ME, Liu GY, Sobczak S, et al: Virulence role of group B *Streptococcus* beta-hemolysin/cytolysin in a neonatal rabbit model of early-onset pulmonary infection, *J Infect Dis* 191:1287-1291, 2005.

208. Herting E, Jarstrand C, Rasool O, et al: Experimental neonatal group B streptococcal pneumonia: effect of a modified porcine surfactant on bacterial proliferation in ventilated near-term rabbits, *Pediatr Res* 36:784-791, 1994.
209. Herting E, Sun B, Jarstrand C, et al: Surfactant improves lung function and mitigates bacterial growth in immature ventilated rabbits with experimentally induced neonatal group B streptococcal pneumonia, *Arch Dis Child Fetal Neonatal Ed* 76:F3-F8, 1997.
210. Auten RL, Notter RH, Kendig JW, et al: Surfactant treatment of full-term newborns with respiratory failure, *Pediatrics* 87:101-107, 1991.
211. Herting E, Gefeller O, Land M, et al: Surfactant treatment of neonates with respiratory failure and group B streptococcal infection, *Pediatrics* 106:957-964, 2000.
212. Shigeoka AO, Hall RT, Hemming VG, et al: Role of antibody and complement in opsonization of group B streptococci, *Infect Immun* 21:34-40, 1978.
213. Anderson DC, Hughes BJ, Edwards MS, et al: Impaired chemotaxigenesis by type III group B streptococci in neonatal sera: relationship to diminished concentration of specific anticapsular antibody and abnormalities of serum complement, *Pediatr Res* 17:496-502, 1983.
214. Edwards MS, Nicholson-Weller A, Baker CJ, et al: The role of specific antibody in alternative complement pathway-mediated opsonophagocytosis of type III, group B *Streptococcus*, *J Exp Med* 151: 1275-1287, 1980.
215. Chaffin DO, Beres SB, Yim HH, et al: The serotype of type Ia and III group B streptococci is determined by the polymerase gene within the polycistronic capsule operon, *J Bacteriol* 182:4466-4477, 2000.
216. Angata T, Varki A: Chemical diversity in the sialic acids and related alpha-keto acids: an evolutionary perspective, *Chem Rev* 102: 439-469, 2002.
217. Doran KS, Liu GY, Nizet V: Group B streptococcal beta-hemolysin/cytolysin activates neutrophil signaling pathways in brain endothelium and contributes to development of meningitis, *J Clin Invest* 112:736-744, 2003.
218. Carlin AF, Uchiyama S, Chang YC, et al: Molecular mimicry of host sialylated glycans allows a bacterial pathogen to engage neutrophil Siglec-9 and dampen the innate immune response, *Blood* 113: 3333-3336, 2009.
219. Kasper DL, Baker CJ, Baltimore RS, et al: Immunodeterminant specificity of human immunity to type III group B streptococcus, *J Exp Med* 149:327-339, 1979.
220. Edwards MS, Kasper DL, Jennings HJ, et al: Capsular sialic acid prevents activation of the alternative complement pathway by type III, group B streptococci, *J Immunol* 128:1278-1283, 1982.
221. Shigeoka AO, Rote NS, Santos JI, et al: Assessment of the virulence factors of group B streptococci: correlation with sialic acid content, *J Infect Dis* 147:857-863, 1983.
222. Rubens CE, Wessels MR, Heggen LM, et al: Transposon mutagenesis of type III group B *Streptococcus*: correlation of capsule expression with virulence, *Proc Natl Acad Sci U S A* 84:7208-7212, 1987.
223. Yim HH, Nittayarin A, Rubens CE: Analysis of the capsule synthesis locus, a virulence factor in group B streptococci, *Adv Exp Med Biol* 418:995-997, 1997.
224. Marques MB, Kasper DL, Pangburn MK, et al: Prevention of C3 deposition by capsular polysaccharide is a virulence mechanism of type III group B streptococci, *Infect Immun* 60:3986-3993, 1992.
225. Lemire P, Houde M, Lecours MP, et al: Role of capsular polysaccharide in group B *Streptococccus* interactions with dendritic cells, *Microb Infect* 14:1064-1076, 2012.
226. Martin TR, Ruzinski JT, Rubens CE, et al: The effect of type-specific polysaccharide capsule on the clearance of group B streptococci from the lungs of infant and adult rats, *J Infect Dis* 165:306-314, 1992.
227. Wessels MR, Rubens CE, Benedí VJ, et al: Definition of a bacterial virulence factor: sialylation of the group B streptococcal capsule, *Proc Natl Acad Sci USA* 86:8983-8987, 1989.
228. Orefici G, Recchia S, Galante L: Possible virulence marker for Streptococcus agalactiae (Lancefield group B), *Eur J Clin Microbiol Infect Dis* 7:302-305, 1988.
229. Payne NR, Ferrieri P: The relation of the Ibc protein antigen to the opsonization differences between strains of type II group B streptococci, *J Infect Dis* 151:672-681, 1985.
230. Baker CJ, Webb BJ, Kasper DL, et al: The role of complement and antibody in opsonophagocytosis of type II group B streptococci, *J Infect Dis* 154:47-54, 1986.
231. Russell-Jones GJ, Gotschlich EC, Blake MS: A surface receptor specific for human IgA on group B streptococci possessing the Ibc protein antigen, *J Exp Med* 160:1467-1475, 1984.
232. Jerlström PG, Chhatwal GS, Timmis KN: The IgA-binding beta antigen of the C protein complex of group B streptococci: sequence determination of its gene and detection of two binding regions, *Mol Microbiol* 5:843-849, 1991.
233. Payne NR, Kim YK, Ferrieri P: Effect of differences in antibody and complement requirements on phagocytic uptake and intracellular killing of "C" protein-positive and -negative strains of type II group B streptococci, *Infect Immun* 55:1243-1251, 1987.
234. Harris TO, Shelver DW, Bohnsack JF, et al: A novel streptococcal surface protease promotes virulence, resistance to opsonophagocytosis, and cleavage of human fibrinogen, *J Clin Invest* 111:61-70, 2003.
235. Santi I, Scarselli M, Mariani M, et al: BibA: a novel immunogenic bacterial adhesin contributing to group B *Streptococcus* survival in human blood, *Mol Microbiol* 63:754-767, 2007.
236. Carlin AF, Chang YC, Areschoug T, et al: Group B *Streptococcus* suppression of phagocyte functions by protein-mediated engagement of human Siglec-5, *J Exp Med* 206:1691-1699, 2009.
237. Cornacchione P, Scaringi L, Fettucciari K, et al: Group B streptococci persist inside macrophages, *Immunology* 93:86-95, 1998.
238. Teixeira CF, Azevedo NL, Carvalho TM, et al: Cytochemical study of *Streptococcus agalactiae* and macrophage interaction, *Microsc Res Tech* 54:254-259, 2001.
239. Wilson CB, Weaver WM: Comparative susceptibility of group B streptococci and *Staphylococcus aureus* to killing by oxygen metabolites, *J Infect Dis* 152:323-329, 1985.
240. Poyart C, Pellegrini E, Gaillot O, et al: Contribution of Mn-cofactored superoxide dismutase (SodA) to the virulence of *Streptococcus agalactiae*, *Infect Immun* 69:5098-5106, 2001.
241. Cumley NJ, Smith LM, Anthony M, et al: The CovS/CovR acid response regulator is required for intracellular survival of group B *Streptococcus* in macrophages, *Infect Immun* 80:1650-1661, 2012.
242. Nizet V, Ohtake T, Lauth X, et al: Innate antimicrobial peptide protects the skin from invasive bacterial infection, *Nature* 414:454-457, 2001.
243. Jones AL, Needham RH, Clancy A, et al: Penicillin-binding proteins in *Streptococcus agalactiae*: a novel mechanism for evasion of immune clearance, *Mol Microbiol* 4:7247-7256, 2003.
244. Hamilton A, Popham DL, Carl DJ, et al: Penicillin-binding protein 1a promotes resistance of group B streptococcus to antimicrobial peptides, *Infect Immun* 74:6179-6187, 2006.
245. Poyart C, Pellegrini E, Marceau M, et al: Attenuated virulence of *Streptococcus agalactiae* deficient in D-alanyl-lipoteichoic acid is due to an increased susceptibility to defensins and phagocytic cells, *Mol Microbiol* 49:1615-1625, 2003.
246. Ulett GC, Maclean KH, Nekkalapu S, et al: Mechanisms of group B streptococcal-induced apoptosis of murine macrophages, *J Immunol* 175:2555-2562, 2005.
247. Fettucciari K, Fetriconi I, Mannucci R, et al: Group B *Streptococcus* induces macrophage apoptosis by calpain activation, *J Immunol* 176:7542-7556, 2006.
248. Wheeler JG, Chauvenet AR, Johnson CA, et al: Neutrophil storage pool depletion in septic, neutropenic neonates, *Pediatr Infect Dis* 3:407-409, 1984.
249. Christensen RD, MacFarlane JL, Taylor NL, et al: Blood and marrow neutrophils during experimental group B streptococcal infection: quantification of the stem cell, proliferative, storage and circulating pools, *Pediatr Res* 16:549-553, 1982.
250. Zeligs BJ, Armstrong CD, Walser JB, et al: Age-dependent susceptibility of neonatal rats to group B streptococcal type III infection: correlation of severity of infection and response of myeloid pools, *Infect Immun* 37:255-263, 1982.
251. Christensen RD, Hill HR, Rothstein G: Granulocytic stem cell (CFUc) proliferation in experimental group B streptococcal sepsis, *Pediatr Res* 17:278-280, 1983.
252. Madureira P, Andrade EB, Gama B, et al: Inhibition of IL-10 production by maternal antibodies against group B *Streptococcus* GAPDH confers immunity to offspring by favoring neutrophil recruitment, *PLoS Pathog* 7:e1002363, 2011.
253. Derre-Bobillot A, Cortes-Perez NG, Yamamoto Y, et al: Nuclease A (Gbs0661), an extracellular nuclease of *Streptococcus agalactiae*, attacks the neutrophil extracellular traps and is needed for full virulence, *Mol Microbiol* 89:518-531, 2013.

254. Bohnsack JF, Widjaja K, Ghazizadeh S, et al: A role for C5 and C5a-ase in the acute neutrophil response to group B streptococcal infections, *J Infect Dis* 175:847-855, 1997.
255. Gleich-Theurer U, Aymanns S, Haas G, et al: Human serum induces streptococcal c5a peptidase expression, *Infect Immun* 77: 3817-3825, 2009.
256. Rojas J, Larsson LE, Hellerqvist CG, et al: Pulmonary hemodynamic and ultrastructural changes associated with group B streptococcal toxemia in adult sheep and newborn lambs, *Pediatr Res* 17: 1002-1008, 1983.
257. Hemming VG, O'Brien WF, Fischer GW, et al: Studies of short-term pulmonary and peripheral vascular responses induced in oophorectomized sheep by the infusion of a group B streptococcal extract, *Pediatr Res* 18:266-269, 1984.
258. Gibson Truog WE, Henderson WRRL, Redding GJ: Group B streptococcal sepsis in piglets: effect of combined pentoxifylline and indomethacin pretreatment, *Pediatr Res* 31:222-227, 1992.
259. Peevy KJ, Chartrand SA, Wiseman HJ, et al: Myocardial dysfunction in group B streptococcal shock, *Pediatr Res* 19:511-513, 1985.
260. Peevy KJ, Panus P, Longenecker GL, et al: Prostaglandin synthetase inhibition in group B streptococcal shock: hematologic and hemodynamic effects, *Pediatr Res* 20:864-866, 1986.
261. O'Brien WF, Golden SM, Bibro MC, et al: Short-term responses in neonatal lambs after infusion of group B streptococcal extract, *Obstet Gynecol* 65:802-806, 1985.
262. Gibson RL, Redding GJ, Truog WE, et al: Isogenic group B streptococci devoid of capsular polysaccharide or beta-hemolysin: pulmonary hemodynamic and gas exchange effects during bacteremia in piglets, *Pediatr Res* 26:241-245, 1989.
263. Bowdy BD, Aziz SM, Marple SL, et al: Organ-specific disposition of group B streptococci in piglets: evidence for a direct interaction with target cells in the pulmonary circulation, *Pediatr Res* 27:344-348, 1990.
264. Gibson RL, Soderland C, Henderson WR Jr, et al: Group B streptococci (GBS) injure lung endothelium in vitro: GBS invasion and GBS-induced eicosanoid production is greater with microvascular than with pulmonary artery cells, *Infect Immun* 63:271-279, 1995.
265. Schreiber MD, Covert RF, Torgerson LJ: Hemodynamic effects of heat-killed group B beta-hemolytic streptococcus in newborn lambs: role of leukotriene D4, *Pediatr Res* 31:121-126, 1992.
266. Pinheiro JM, Pitt BR, Gillis CN: Roles of platelet-activating factor and thromboxane in group B *Streptococcus*-induced pulmonary hypertension in piglets, *Pediatr Res* 26:420-424, 1989.
267. Mancuso G, Cusumano V, Genovese F, et al: Role of interleukin 12 in experimental neonatal sepsis caused by group B streptococci, *Infect Immun* 65:3731-3735, 1997.
268. Costa A, Gupta R, Signorino G, et al: Activation of the NLRP3 inflammasome by group B streptococci, *J Immunol* 188, 1953-1960.
269. Vallette JD, Goldberg RN, Suguihara C, et al: Effect of an interleukin-1 receptor antagonist on the hemodynamic manifestations of group B streptococcal sepsis, *Pediatr Res* 38:704-708, 1995.
270. Williams PA, Bohnsack JF, Augustine NH, et al: Production of tumor necrosis factor by human cells in vitro and in vivo, induced by group B streptococci, *J Pediatr* 123:292-300, 1993.
271. Gibson RL, Redding GJ, Henderson WR, et al: Group B streptococcus induces tumor necrosis factor in neonatal piglets: effect of the tumor necrosis factor inhibitor pentoxifylline on hemodynamics and gas exchange, *Am Rev Respir Dis* 143:598-604, 1991.
272. Teti G, Mancuso G, Tomasello F, et al: Production of tumor necrosis factor-alpha and interleukin-6 in mice infected with group B streptococci, *Circ Shock* 38:138-144, 1992.
273. Teti G, Mancuso G, Tomasello F: Cytokine appearance and effects of anti-tumor necrosis factor alpha antibodies in a neonatal rat model of group B streptococcal infection, *Infect Immun* 61:227-235, 1993.
274. Kenzel S, Mancuso G, Malley R, et al: c-Jun kinase is a critical signaling molecule in a neonatal model of group B streptococcal sepsis, *J Immunol* 176:3181-3188, 2006.
275. Henneke P, Takeuchi O, Malley R, et al: Cellular activation, phagocytosis, and bactericidal activity against group B streptococcus involve parallel myeloid differentiation factor 88-dependent and independent signaling pathways, *J Immunol* 169:3970-3977, 2002.
276. Raykova VD, Glibetic M, Ofenstein JP, et al: Nitric oxide-dependent regulation of pro-inflammatory cytokines in group B streptococcal inflammation of rat lung, *Ann Clin Lab Sci* 33:62-67, 2003.
277. Maloney CG, Thompson SD, Hill HR, et al: Induction of cyclooxygenase-2 by human monocytes exposed to group B streptococci, *J Leukoc Biol* 67:615-621, 2000.
278. Natarajan G, Glibetic M, Raykova V, et al: Nitric oxide and prostaglandin response to group B streptococcal infection in the lung, *Ann Clin Lab Sci* 37:170-176, 2007.
279. Ring A, Braun JS, Nizet V, et al: Group B streptococcal beta-hemolysin induces nitric oxide production in murine macrophages, *J Infect Dis* 182:150-157, 2000.
280. Puliti M, Nizet V, von Hunolstein C, et al: Severity of group B streptococcal arthritis is correlated with beta-hemolysin expression, *J Infect Dis* 182:824-832, 2000.
281. Ring A, Depnering C, Pohl J, et al: Synergistic action of nitric oxide release from murine macrophages caused by group B streptococcal cell wall and beta-hemolysin/cytolysin, *J Infect Dis* 186:1518-1521, 2002.
282. Griffiths BB, Rhee H: Effects of haemolysins of groups A and B streptococci on cardiovascular system, *Microbios* 69:17-27, 1992.
283. Hensler ME, Miyamoto S, Nizet V: Group B streptococcal beta-hemolysin/cytolysin directly impairs cardiomyocyte viability and function, *PLoS One* 3:e2446, 2008.
284. Bebien M, Hensler ME, Davanture S, et al: The pore-forming toxin beta hemolysin/cytolysin triggers p38 MAPK-dependent IL-10 production in macrophages and inhibits innate immunity, *PLoS Pathog* 8:e1002812, 2012.
285. Berman PH, Banker BQ: Neonatal meningitis: a clinical and pathological study of 29 cases, *Pediatrics* 38:6-24, 1966.
286. Ferrieri P, Burke B, Nelson J: Production of bacteremia and meningitis in infant rats with group B streptococcal serotypes, *Infect Immun* 27:1023-1032, 1980.
287. Nizet V, Kim KS, Stins M, et al: Invasion of brain microvascular endothelial cells by group B streptococci, *Infect Immun* 65:5074-5081, 1997.
288. Doran KS, Engelson EJ, Khosravi A, et al: Blood-brain barrier invasion by group B *Streptococcus* depends upon proper cell-surface anchoring of lipoteichoic acid, *J Clin Invest* 115:2499-2507, 2005.
289. Tenenbaum T, Bloier C, Adam R, et al: Adherence to and invasion of human brain microvascular endothelial cells are promoted by fibrinogen-binding protein FbsA of *Streptococcus agalactiae*, *Infect Immun* 73:4404-4409, 2005.
290. Banerjee A, Kim BJ, Carmona EM, et al: Bacterial pili exploit integrin machinery to promote immune activation and efficient blood-brain barrier penetration, *Nat Comm* 2:462, 2011.
291. van Sorge NM, Quach D, Gurney MA, et al: The group B streptococcal serine-rich repeat 1 glycoprotein mediates penetration of the blood-brain barrier, *J Infect Dis* 199:1479-1487, 2009.
292. Seo HS, Mu R, Kim BJ, et al: Binding of glycoprotein Srr1 of *Streptococcus agalactiae* to fibrinogen promotes attachment to brain endothelium and the development of meningitis, *PLoS Pathog* 10:e1002947, 2013.
293. Chang YC, Wang Z, Flax LA, et al: Glycosaminoglycan binding facilitates entry of a bacterial pathogen into central nervous systems, *PLoS Pathog* 7:e1002082, 2011.
294. Tazi A, Disson O, Bellais S, et al: The surface protein HvgA mediates group B streptococcus hypervirulence and meningeal tropism in neonates, *J Exp Med* 207:2313-2322, 2010.
295. Kim YS, Sheldon RA, Elliott BR, et al: Brain injury in experimental neonatal meningitis due to group B streptococci, *J Neuropathol Exp Neurol* 54:531-539, 1995.
296. Bogdan I, Leib SL, Bergeron M, et al: Tumor necrosis factor-alpha contributes to apoptosis in hippocampal neurons during experimental group B streptococcal meningitis, *J Infect Dis* 176:693-697, 1997.
297. Ling EW, Noya FJ, Ricard G, et al: Biochemical mediators of meningeal inflammatory response to group B streptococcus in the newborn piglet model, *Pediatr Res* 38:981-987, 1995.
298. Lehnardt S, Henneke P, Lien E, et al: A mechanism for neurodegeneration induced by group B streptococci through activation of the TLR2/MyD88 pathway in microglia, *J Immunol* 177:583-592, 2006.
299. Lehnardt S, Wennekamp J, Freyer D, et al: TLR2 and caspase-8 are essential for group B *Streptococcus*-induced apoptosis in microglia, *J Immunol* 179:6134-6143, 2007.
300. A. Reiss Braun JS, Jäger K, et al: Bacterial pore-forming cytolysins induce neuronal damage in a rat model of neonatal meningitis, *J Infect Dis* 203:393-400, 2011.

301. Schuchat A, Deaver-Robinson K, Plikaytis BD, et al: Multistate case-control study of maternal risk factors for neonatal group B streptococcal disease, *Pediatr Infect Dis J* 13:623-629, 1994.
302. Pass MA, Khare S, Dillon HC: Twin pregnancies: incidence of group B streptococcal colonization and disease, *J Pediatr* 97:635-637, 1980.
303. Edwards MS, Jackson CV, Baker CJ: Increased risk of group B streptococcal disease in twins, *JAMA* 245:2044-2046, 1981.
304. Faro S: Group B beta-hemolytic streptococci and puerperal infections, *Am J Obstet Gynecol* 139:686-689, 1981.
305. Schuchat A: Group B *Streptococcus*, *Lancet* 353:51-56, 1999.
306. Regan JA, Klebanoff MA, Nugent RP, et al: Colonization with group B streptococci in pregnancy and adverse outcome, *Am J Obstet Gynecol* 174:1354-1360, 1996.
307. Seaward PG, Hannah ME, Myhr TL, et al: International multicenter term PROM study: evaluation of predictors of neonatal infection in infants born to patients with premature rupture of membranes at term, *Am J Obstet Gynecol* 179:635-639, 1998.
308. Yancey MK, Duff P, Clark P, et al: Peripartum infection associated with vaginal group B streptococcal colonization, *Obstet Gynecol* 84:816-819, 1994.
309. Lancefield RC, McCarty M, Everly WN: Multiple mouse-protective antibodies directed against group B streptococci, *J Exp Med* 142: 165-179, 1975.
310. Baker CJ, Kasper DL: Correlation of maternal antibody deficiency with susceptibility to neonatal group B streptococcal infection, *N Engl J Med* 294:753-756, 1976.
311. Baker CJ, Edwards MS, Kasper DL: Role of antibody to native type III polysaccharide of group B *Streptococcus* in infant infection, *Pediatrics* 68:544-549, 1981.
312. Guttormsen HK, Baker CJ, Edwards MS, et al: Quantitative determination of antibodies to type III group B streptococcal polysaccharide, *J Infect Dis* 173:142-150, 1996.
313. Gray BM, Pritchard DG, Dillon HC Jr: Seroepidemiological studies of group B *Streptococcus* type II, *J Infect Dis* 151:1073-1080, 1985.
314. Gotoff SP, Papierniak CK, Klegerman ME, et al: Quantitation of IgG antibody to the type-specific polysaccharide of group B *Streptococcus* type 1b in pregnant women and infected infants, *J Pediatr* 105:628-630, 1984.
315. Baker CJ, Carey VJ, Rench MA, et al: Maternal antibody at delivery protects neonates from early-onset group B streptococcal disease, *J Infect Dis* 209:781-788, 2014.
316. Lin FY, Weisman LE, Azimi PH, et al: Level of maternal IgG anti-group B streptococcus type III antibody correlated with protection of neonates against early-onset disease caused by this pathogen, *J Infect Dis* 190:928-934, 2004.
317. Lin FY, Philips JB 3rd, Azimi PH, et al: Level of maternal antibody required to protect neonates against early-onset disease caused by group B *Streptococcus* type Ia: a multicenter, seroepidemiology study, *J Infect Dis* 184:1022-1028, 2001.
318. Baker CJ, Kasper DL, Tager IRAB, et al: Quantitative determination of antibody to capsular polysaccharide in infection with type III strains of group B *Streptococcus*, *J Clin Invest* 59:810-818, 1977.
319. Baker CJ, Edwards MS, Kasper DL: Immunogenicity of polysaccharides from type III group B *Streptococcus*, *J Clin Invest* 61:1107-1110, 1978.
320. Hordnes K, Tynning T, Kvam AI, et al: Cervical secretions in pregnant women colonized rectally with group B streptococci have high levels of antibodies to serotype III polysaccharide capsular antigen and protein, *R Scand J Immunol* 47:179-188, 1998.
321. Hordnes K, Tynning T, Kvam AI, et al: Colonization in the rectum and uterine cervix with group B streptococci may induce specific antibody responses in cervical secretions of pregnant women, *Infect Immun* 64:1643-1652, 1996.
322. De Cueninck BJ, Eisenstein TK, McIntosh TS, et al: Quantitation of in vitro opsonic activity of human antibody induced by a vaccine consisting of the type III-specific polysaccharide of group B streptococcus, *Infect Immun* 39:1155-1160, 1983.
323. Pincus SH, Shigeoka AO, Moe AA, et al: Protective efficacy of IgM monoclonal antibodies in experimental group B streptococcal infection is a function of antibody avidity, *J Immunol* 140:2779-2785, 1988.
324. Givner LB, Baker CJ, Edwards MS: Type III group B *Streptococcus*: functional interaction with IgG subclass antibodies, *J Infect Dis* 155:532-539, 1987.
325. Kim JS, Kim KS, Wass CA, et al: A human IgG 3 is opsonic in vitro against type III group B streptococci, *J Clin Immunol* 10:154-159, 1990.
326. Anthony BF, Concepcion NF: Opsonic activity of human IgG and IgM antibody for type III group B streptococci, *Pediatr Res* 26: 383-387, 1989.
327. Campbell JR, Baker CJ, Metzger TG, et al: Functional activity of class-specific antibodies to type III, group B *Streptococcus*, *Pediatr Res* 23:31-34, 1988.
328. Bohnsack JF, Hawley MM, Pritchard DG, et al: An IgA monoclonal antibody directed against type III antigen on group B streptococci acts as an opsonin, *J Immunol* 143:3338-3342, 1989.
329. Campbell JR, Baker CJ, Edwards MS: Deposition and degradation of C3 on type III group B streptococci, *Infect Immun* 59:1978-1983, 1991.
330. Edwards MS, Hall MA, Rench MA, Baker CJ: Patterns of immune response among survivors of group B streptococcal meningitis, *J Infect Dis* 161:65-70, 1990.
331. Baker CJ, Edwards MS, Webb BJ, Kasper DL: Antibody-independent classical pathway-mediated opsonophagocytosis of type Ia, group B *Streptococcus*, *J Clin Invest* 69:394-404, 1982.
332. Levy NJ, Kasper DL: Surface-bound capsular polysaccharide of type Ia group B *Streptococcus* mediates C1 binding and activation of the classic complement pathway, *J Immunol* 136:4157-4162, 1986.
333. Levy NJ, Kasper DL: Antibody-independent and -dependent opsonization of group B *Streptococcus* requires the first component of complement C1, *Infect Immun* 49:19-24, 1985.
334. Smith CL, Smith AH: Strain variability of type Ib group B streptococci: unique strains are resistant to C3 deposition by the alternate complement pathway, *Clin Res* 40:823A, 1992.
335. Hall MA, Edwards MS, Baker CJ: Complement and antibody participation in opsonophagocytosis of type IV and V group B streptococci, *Infect Immun* 60:5030-5035, 1992.
336. Hall MA, Hickman ME, Baker CJ, et al: Complement and antibody in neutrophil-mediated killing of type V group B *Streptococcus*, *J Infect Dis* 170:88-93, 1994.
337. Cairo MS, Worcester C, Rucker R, et al: Role of circulating complement and polymorphonuclear leukocyte transfusion in treatment and outcome in critically ill neonates with sepsis, *J Pediatr* 110: 935-941, 1987.
338. Levy O, Jean-Jacques RM, Cywes C, et al: Critical role of the complement system in group B *Streptococcus*-induced tumor necrosis factor alpha release, *Infect Immun* 71:6344-6353, 2003.
339. Pozdnyakova O, Guttormsen HK, Lalani FN, et al: Impaired antibody response to group B streptococcal type III capsular polysaccharide in C3- and complement receptor 2-deficient mice, *J Immunol* 170: 84-90, 2003.
340. Singer DB, Campognone P: Perinatal group B streptococcal infection in midgestation, *PediatrPathol* 52:71-276, 1986.
341. deSa DJ, Trevenen CL: Intrauterine infections with group B beta-haemolytic streptococci, *Br J Obstet Gynaecol* 91:237-239, 1984.
342. Hood M, Janney A, Dameron G: Beta-hemolytic *Streptococcus* group B associated with problems of perinatal period, *Am J Obstet Gynecol* 82:809-818, 1961.
343. Moller M, Thomsen AC, Borch K, et al: Rupture of fetal membranes and premature delivery associated with group B streptococci in urine of pregnant women, *Lancet* 2:69-70, 1984.
344. Novak RW, Platt MS: Significance of placental findings in early-onset group B streptococcal neonatal sepsis, *Clin Pediatr* 24: 256-258, 1985.
345. Becroft DMO, Farmer K, Mason GH, et al: Perinatal infections by group B 4-hemolytic streptococci, *Br J Obstet Gynaecol* 83:960-965, 1976.
346. Faix RG, Donn SM: Association of septic shock caused by early-onset group B streptococcal sepsis and periventricular leukomalacia in the preterm infant, *Pediatrics* 76:415-419, 1985.
347. Van Peenen PF, Cannon RE, Seibert DJ: Group B beta-hemolytic streptococci causing fatal meningitis, *Mil Med* 130:65-67, 1965.
348. DiJohn D, Krasinski K, Lawrence R, et al: Very late onset of group B streptococcal disease in infants infected with the human immunodeficiency virus, *Pediatr Infect Dis J* 9:925-928, 1990.
349. Schuchat A, Zywicki SS, Dinsmoor MJ, et al: Risk factors and opportunities for prevention of early-onset neonatal sepsis: a multicenter case-control study, *Pediatrics* 105:21-26, 2000.

350. Stoll BJ, Gordon T, Korones SB, et al: Early-onset sepsis in very low birth weight neonates: a report from the National Institute of Child Health and Human Development Neonatal Research Network, *J Pediatr* 129:72-80, 1996.
351. Stoll BJ, Hansen NI, Sánchez PJ, et al: Early onset neonatal sepsis: the burden of group B streptococcal and *E. coli* disease continues, *Pediatrics* 127:817-826, 2011.
352. Chen KT, Ringer S, Cohen AP, et al: The role of intrapartum fever in identifying asymptomatic term neonates with early-onset neonatal sepsis, *J Perinatol* 22:653-657, 2002.
353. Weisman LE, Stoll BJ, Cruess DF, et al: Early-onset group B streptococcal sepsis: a current assessment, *J Pediatr* 121:428-433, 1992.
354. Chin KC, Fitzhardinge PM: Sequelae of early-onset group B streptococcal neonatal meningitis, *J Pediatr* 106:819-822, 1985.
355. Haslam RHA, Allen JR, Dorsen MM, et al: The sequelae of group B beta-hemolytic streptococcal meningitis in early infancy, *Am J Dis Child* 131:845-849, 1977.
356. Edwards MS, Rench MA, Haffar AA, et al: Long-term sequelae of group B streptococcal meningitis in infants, *J Pediatr* 106:717-722, 1985.
357. Payne NR, Burke BA, Day DL, et al: Correlation of clinical and pathologic findings in early onset neonatal group B streptococcal infection with disease severity and prediction of outcome. *Pediatr Infect Dis J* 7:836-847, 1988.
358. Berardi A, Rossi C, Lugli L, et al: Group B *Streptococcus* late-onset disease, *Pediatrics* 131:e361-e368, 2013.
359. Levent F, Baker CJ, Rench MA, et al: Early outcomes of group B streptococcal meningitis in the 21st century, *Pediatr Infect Dis J* 29:1009-1012, 2010.
360. Yagupsky P, Menegus MA, Powell KR: The changing spectrum of group B streptococcal disease in infants: an eleven-year experience in a tertiary care hospital, *Pediatr Infect Dis J* 10:801-808, 1991.
361. Garcia Peña BM, Harper MB, Fleisher GR: Occult bacteremia with group B streptococci in an outpatient setting, *Pediatrics* 102:67-72, 1998.
362. Raymond J, Armengaud JB, Lambe C, et al: Late-onset neonatal infections caused by group B *Streptococcus* associated with viral infection, *Pediatr Infect Dis J* 26:963-1955, 2007.
363. Hussain SM, Luedtke GS, Baker CJ, et al: Invasive group B streptococcal disease in children beyond early infancy, *Pediatr Infect Dis J* 14:278-281, 1995.
364. De Witt CC, Ascher DP, Winkelstein J: Group B streptococcal disease in a child beyond early infancy with a deficiency of the second component of complement (C2), *Pediatr Infect Dis J* 18:77-78, 1999.
365. Krause JC, Ghandil P, Chrabieh M, et al: Very late-onset group B streptococcus meningitis, sepsis, and systemic shigellosis due to interleukin-1 receptor-associated kinase-4 deficiency, *Clin Infect Dis* 49:1393-1396, 2009.
366. Edwards MS, Baker CJ, Wagner ML, et al: An etiologic shift in infantile osteomyelitis: the emergence of the group B *Streptococcus*, *J Pediatr* 93:578-583, 1978.
367. Baevsky RH: Neonatal group B beta-hemolytic *Streptococcus* osteomyelitis, *Am J Emerg Med* 17:619-622, 1999.
368. Clay SA: Osteomyelitis as a cause of brachial plexus neuropathy, *Am J Dis Child* 136:1054-1056, 1982.
369. Sadleir LG, Connolly MB: Acquired brachial-plexus neuropathy in the neonate: a rare presentation of late-onset group-B streptococcal osteomyelitis, *Dev Med Child Neurol* 40:496-499, 1998.
370. Ammari LK, Offit PA, Campbell AB: Unusual presentation of group B *Streptococcus* osteomyelitis, *Pediatr Infect Dis J* 11:1066-1067, 1992.
371. Obando I, Martin E, Alvarez-Aldean J, et al: Group B *Streptococcus* pelvic osteomyelitis presenting as footdrop in a newborn infant, *Pediatr Infect Dis J* 10:703-705, 1991.
372. Barton LL, Villar RG, Rice SA: Neonatal group B streptococcal vertebral osteomyelitis, *Pediatrics* 98:459-461, 1996.
373. Broughton RA, Edwards MS, Haffar A, et al: Unusual manifestations of neonatal group B streptococcal osteomyelitis, *Pediatr Infect Dis J* 1:410-412, 1982.
374. Hauger SB: Facial cellulitis: an early indicator of group B streptococcal bacteremia, *Pediatrics* 67:376-377, 1981.
375. Patamasucon P, Siegel JD, McCracken GH Jr: Streptococcal submandibular cellulitis in young infants, *Pediatrics* 67:378-380, 1981.
376. Baker CJ: Group B streptococcal cellulitis/adenitis in infants, *Am J Dis Child* 136:631-633, 1982.
377. Fluegge K, Greiner P, Berner R: Late onset group B streptococcal disease manifested by isolated cervical lymphadenitis, *Arch Dis Child* 88:1019-1020, 2003.
378. Pathak A, Hwu HH: Group B streptococcal cellulitis, *South Med J* 78:67-68, 1985.
379. Haque KN, Bashir O, Kambal AMM: Delayed recurrence of group B streptococcal infection in a newborn infant: a case report, *Ann Trop Paediatr* 6:219-220, 1986.
380. Rand TH: Group B streptococcal cellulitis in infants: a disease modified by prior antibiotic therapy or hospitalization? *Pediatrics* 81: 63-65, 1988.
381. Brady MT: Cellulitis of the penis and scrotum due to group B *Streptococcus*, *J Urol* 137:736-737, 1987.
382. Shetty SK, Hindley D: Late onset group B streptococcal disease manifests as submandibular cellulitis, *BMJ Case Rep*, 2010. pii:bcr0220102726.
383. Albanyan EA, Baker CJ: Is lumbar puncture necessary to exclude meningitis in neonates and young infants: lessons from group B *Streptococcus* cellulitis-adenitis syndrome, *Pediatrics* 102:985-986, 1998.
384. Chadwick EG, Shulman ST, Yogev R: Peritonitis as a late manifestation of group B streptococcal disease in newborns, *Pediatr Infect Dis J* 2:142-143, 1983.
385. Walker KM, Coyer WF: Suprarenal abscess due to group B streptococcus, *J Pediatr* 94:970-971, 1979.
386. Atkinson GO Jr, Kodroff MB, Gay BB Jr, et al: Adrenal abscess in the neonate, *Radiology* 155:101-104, 1985.
387. Carty A, Stanley P: Bilateral adrenal abscesses in a neonate, *Pediatr Radiol* 1:63-64, 1973.
388. Peevy KJ, Wiseman HJ: Gallbladder distension in septic neonates, *Arch Dis Child* 57:75-76, 1982.
389. Siegel JD, Shannon KM, De Passe BM: Recurrent infection associated with penicillin-tolerant group B streptococci: a report of two cases, *J Pediatr* 99:920-924, 1981.
390. Sokol DM, Demmler GJ, Baker CJ: Unusual presentation of group B streptococcal ventriculitis, *Pediatr Infect Dis J* 9:525-527, 1990.
391. Coker SB, Muraskas JK, Thomas C: Myelopathy secondary to neonatal bacterial meningitis, *Pediatr Neurol* 10:259-261, 1994.
392. Ferguson L, Gotoff SP: Subdural empyema in an infant due to group B beta-hemolytic *Streptococcus, Am J Dis Child* 131:97, 1977.
393. McReynolds EW, Shane R: Diabetes insipidus secondary to group B beta streptococcal meningitis, *J Tenn Med Assoc* 67:117-120, 1974.
394. Dorand RD, Adams G: Relapse during penicillin treatment of group B streptococcal meningitis, *J Pediatr* 89:188-190, 1976.
395. Kim KS, Kaye KL, Itabashi HH, et al: Cerebritis due to group B *Streptococcus, Scand J Infect Dis* 14:305-308, 1982.
396. Barton CW, Crowley DC, Uzark K, et al: A neonatal survivor of group B beta-hemolytic streptococcal endocarditis, *Am J Perinatol* 1: 214-215, 1984.
397. Horigome H, Okada Y, Hirano T, et al: Group B streptococcal endocarditis in infancy with a giant vegetation on the pulmonary valve, *Eur J Pediatr* 153:140-142, 1994.
398. Harper IA: The importance of group B streptococci as human pathogens in the British Isles, *J Clin Pathol* 244:38-441, 1971.
399. Ancona RJ, McAuliffe J, Thompson TR, et al: Group B streptococcal sepsis with osteomyelitis and arthritis, *Am J Dis Child* 133:919-920, 1979.
400. Agarwala BN: Group B streptococcal endocarditis in a neonate, *Pediatr Cardiol* 9:51-53, 1988.
401. Shurin PA, Howie VM, Pelton SI, et al: Bacterial etiology of otitis media during the first six weeks of life, *J Pediatr* 92:893-896, 1978.
402. Sapir-Ellis S, Johnson A, Austin TL: Group B streptococcal meningitis associated with otitis media, *Am J Dis Child* 130:1003-1004, 1976.
403. Tetzlaff TR, Ashworth C, Nelson JD: Otitis media in children less than 12 weeks of age, *Pediatrics* 59:827-832, 1977.
404. Ermocilla R, Cassady G, Ceballos R: Otitis media in the pathogenesis of neonatal meningitis with group B beta-hemolytic *Streptococcus*, *Pediatrics* 54:643-644, 1974.
405. Armstrong JH, Zacarias F, Rein MF: Ophthalmia neonatorum: a chart review, *Pediatrics* 57:884-892, 1976.
406. Sparks JR, Recchia FM, Weitkamp JH: Endogenous group B streptococcal endophthalmitis in a preterm infant, *J Perinatol* 27:392-394, 2007.
407. Lipson A, Kronick JB, Tewfik L, et al: Group B streptococcal supraglottitis in a 3-month-old infant, *Am J Dis Child* 140:411-412, 1986.
408. Park JW: Bacterial tracheitis caused by *Streptococcus agalactiae*, *Pediatr Infect Dis J* 9:450-451, 1990.
409. Sokal MM, Nagaraj A, Fisher BJ, et al: Neonatal empyema caused by group B beta-hemolytic *Streptococcus*, *Chest* 81:390-391, 1982.

410. LeBovar Y, Trung PH, Mozziconacci P: Neonatal meningitis due to group B streptococci, *Ann Pediatr* 17:207-213, 1970.
411. Strunk T, Simmer K, Kikiros C, et al: Late-onset right-sided diaphragmatic hernia in neonates—case report and review of the literature, *Eur J Pediatr* 166:521-526, 2007.
412. Nudelman R, Bral M, Sakhai Y, et al: Violaceous cellulitis, *Pediatrics* 70:157-158, 1982.
413. Barton LL, Kapoor NK: Recurrent group B streptococcal infection, *Clin Pediatr* 21:100-101, 1982.
414. Amoury RA, Barth GW, Hall RT, et al: Scrotal ecchymosis: sign of intraperitoneal hemorrhage in the newborn, *South Med J* 75: 1471-1478, 1982.
415. Isaacman SH, Heroman WM, Lightsey A: Purpura fulminans following late-onset group B beta-hemolytic streptococcal sepsis, *Am J Dis Child* 138:915-916, 1984.
416. Lynn NJ, Pauly TH, Desai NS: Purpura fulminans in three cases of early-onset neonatal group B streptococcal meningitis, *J Perinatol* 11:144-146, 1991.
417. Zenciroglu A, Karagol BS, Ipek MS, et al: Neonatal purpura fulminans secondary to group B streptococcal infection, *Pediatr Hematol Oncol* 8:620-625, 2010.
418. Ramamurthy RS, Srinivasan G, Jacobs NM: Necrotizing fasciitis and necrotizing cellulitis due to group B *Streptococcus*, *Am J Dis Child* 131:1169-1170, 1977.
419. Goldberg GN, Hansen RC, Lynch PJ: Necrotizing fasciitis in infancy: report of three cases and review of the literature, *Pediatr Dermatol* 2:55-63, 1984.
420. Lang ME, Vaudry W, Robinson JL: Case report and literature review of late-onset group B streptococcal disease manifesting as necrotizing fasciitis in preterm infants. Is this a new syndrome? *Clin Infect Dis* 37:e132-e135, 2003.
421. Lopez JB, Gross P, Boggs TR: Skin lesions in association with beta-hemolytic *Streptococcus* group B, *Pediatrics* 58:859-860, 1976.
422. Belgaumkar TK: Impetigo neonatorum congenita due to group B beta-hemolytic *Streptococcus* infection, *J Pediatr* 86:982-983, 1975.
423. Jacobs MR, Koornhof HJ, Stein H: Group B streptococcal infections in neonates and infants, *S Afr Med J* 54:154-158, 1978.
424. Feder AM Jr, MacLean WC, Moxon R: Scalp abscess secondary to fetal scalp electrode, *J Pediatr* 89:808-809, 1976.
425. Wiswell TE, Miller JA: Infections of congenital cervical neck masses associated with bacteremia, *J Pediatr Surg* 21:173-174, 1986.
426. Bourgeois FT, Shannon MW: Retropharyngeal cellulitis in a 5-week-old infant, *Pediatrics* 109:e51-e53, 2002.
427. Kelly CP, Isaacman DJ: Group B streptococcal retropharyngeal cellulitis in a young infant: a case report and review of the literature, *J Emerg Med* 23:179-182, 2002.
428. Nelson JD: Bilateral breast abscess due to group B *Streptococcus*, *Am J Dis Child* 130:567, 1976.
429. St. Laurent-Gagnon T, Weber ML: Urinary tract *Streptococcus* group B infection in a 6-week-old infant, *JAMA* 240:1269, 1978.
430. Shoda T, Miyagi N, Shirai K, et al: Double recurrence of group B streptococcus bacteremia in an immunocompetent infant, *Pediatr International* 54:701-703, 2012.
431. Soukka H, Rantakokko-Jalava K, Vähäkuopus S, et al: Three distinct episodes of GBS septicemia in a healthy newborn during the first month of life, *Eur J Pediatr* 169:1275-1277, 2010.
432. Atkins JT, Heresi GP, Coque TM, et al: Recurrent group B streptococcal disease in infants: who should receive rifampin? *J Pediatr* 132:537-539, 1998.
433. Green PA, Singh KV, Murray BE, Baker CJ: Recurrent group B streptococcal infections in infants: clinical and microbiologic aspects, *J Pediatr* 125:931-938, 1994.
434. Moylett EH, Fernandez M, Rench MA, et al: A 5-year review of recurrent group B streptococcal disease: lessons from twin infants, *Clin Infect Dis* 30:282-287, 2000.
435. Denning DW, Bressack M, Troup NJ, et al: Infant with two relapses of group B streptococcal sepsis documented by DNA restriction enzyme analysis, *Pediatr Infect Dis J* 7:729-732, 1988.
436. Simón JL, Bosch J, Puig A, et al: Two relapses of group B streptococcal sepsis and transient hypogammaglobulinemia, *Pediatr Infect Dis J* 8:729-730, 1989.
437. Kotiw M, Zhang GW, Daggard G, et al: Late-onset and recurrent neonatal group B streptococcal disease associated with breast-milk transmission, *Pediatr Dev Pathol* 6:251-256, 2003.
438. Wang LY, Chen CT, Liu WH, et al: Recurrent neonatal group B streptococcal disease associated with infected breast milk, *Clin Pediatr (Phila)* 46:547-549, 2007.
439. Jones SM, Steele RW: Recurrent group B streptococcal bacteremia, *Clin Pediatr* 51:884-887, 2012.
440. Ramsay AM, Gillespie M: Puerperal infection associated with haemolytic streptococci other than Lancefield's group A, *J Obstet Gynaecol Br Emp* 48:569-585, 1941.
441. Butter MNW, de Moor CE: *Streptococcus agalactiae* as a cause of meningitis in the newborn, and of bacteremia in adults, *Antonie van Leeuwenhoek* 33:439-450, 1967.
442. Ledger WJ, Norman M, Gee C, et al: Bacteremia on an obstetric-gynecologic service, *Am J Obstet Gynecol* 121:205-212, 1975.
443. Cape A, Tuomala RE, Taylor C, et al: Peripartum bacteremia in the era of group B *Streptococcus* prophylaxis, *Obstet Gynecol* 121: 812-818, 2013.
444. Tudela CM, Stewart RD, Roberts SW, et al: Intrapartum evidence of early-onset group B *Streptococcus*, *Obstet Gynecol* 119:626-629, 2012.
445. Aharoni A, Potasman I, Levitan Z, et al: Postpartum maternal group B streptococcal meningitis, *Rev Infect Dis* 12:273-276, 1990.
446. Kane JM, Jackson K, Conway JH: Maternal postpartum group B beta-hemolytic streptococcus ventriculoperitoneal shunt infection, *Arch Gynecol Obstet* 269:139-141, 2004.
447. Sexton DJ, Rockson SG, Hempling RE, et al: Pregnancy-associated group B streptococcal endocarditis: a report of two fatal cases, *Obstet Gynecol* 66:44-47S, 1985.
448. Backes RJ, Wilson WR, Geraci JE: Group B streptococcal infective endocarditis, *Arch Intern Med* 145:693-696, 1985.
449. Seaworth BJ, Durack DT: Infective endocarditis in obstetric and gynecologic practice, *Am J Obstet Gynecol* 154:180-188, 1986.
450. Vartian CV, Septimus EJ: Tricuspid valve group B streptococcal endocarditis following elective abortion, *Rev Infect Dis* 13:997-998, 1991.
451. Lischke JH, McCreight PHB: Maternal group B streptococcal vertebral osteomyelitis: an unusual complication of vaginal delivery, *Obstet Gynecol* 76:489-491, 1990.
452. Jenkin G, Woolley IJ, Brown GV, et al: Postpartum epidural abscess due to group B *Streptococcus*, *Clin Infect Dis* 25:1249, 1997.
453. Sutton GP, Smirz LR, Clark DH, et al: Group B streptococcal necrotizing fasciitis arising from an episiotomy, *Obstet Gynecol* 66:733-736, 1985.
454. Wood EG, Dillon HC: A prospective study of group B streptococcal bacteriuria in pregnancy, *Am J Obstet Gynecol* 140:515-520, 1981.
455. Gibbs RS, Roberts DJ: Case records of the Massachusetts General Hospital. Case 27-2007. A 30-year-old pregnant woman with intrauterine fetal death, *N Engl J Med* 357:918-925, 2007.
456. Wiswell TE, Baumgart S, Gannon CM, et al: No lumbar puncture in the evaluation for early neonatal sepsis. Will meningitis be missed? *Pediatrics* 95:803-806, 1995.
457. American Academy of Pediatrics Committee on Infectious Diseases and Committee on Fetus and Newborn: revised guidelines for prevention of early-onset group B streptococcal (GBS) infection, *Pediatrics* 99:489-496, 1997.
458. de Zoysa A, Edwards K, Gharbia S, et al: Non-culture detection of *Streptococcus agalactiae* (Lancefield group B *Streptococcus*) in clinical samples by real-time PCR, *J Clin Microbiol* 61:1086-1090, 2012.
459. Natarajan G, Johnson YR, Zhang F, et al: Real-time polymerase chain reaction for the rapid detection of group B streptococcal colonization in neonates, *Pediatrics* 118:14-22, 2006.
460. Golden SM, Stamilio DM, Faux BM, et al: Evaluation of a real-time fluorescent PCR assay for rapid detection of group B streptococci in neonatal blood, *Diagn Microbiol Infect Dis* 50:7-13, 2004.
461. Vallejo JG, Baker CJ, Edwards MS: Interleukin-6 production by human neonatal monocytes stimulated by type III group B streptococci, *J Infect Dis* 174:332-337, 1996.
462. Greenberg DN, Yoder BA: Changes in the differential white blood cell count in screening for group B streptococcal sepsis, *Pediatr Infect Dis J* 9:886-889, 1990.
463. Polin RA; Committee on Fetus and Newborn: Management of neonates with suspected or proven early-onset bacterial sepsis, *Pediatrics* 129:1006-1015, 2012.
464. Christensen RD, Rothstein G, Hill HR, et al: Fatal early onset group B streptococcal sepsis with normal leukocyte counts, *Pediatr Infect Dis J* 4:242-245, 1985.

465. Baker CJ, Webb BJ, Barrett FF: Antimicrobial susceptibility of group B streptococci isolated from a variety of clinical sources, *Antimicrob Agents Chemother* 10:128-131, 1976.
466. Fernandez M, Hickman ME, Baker CJ: Antimicrobial susceptibilities of group B streptococci isolated between 1992 and 1996 from patients with bacteremia or meningitis, *Antimicrob Agents Chemother* 42:1517-1519, 1998.
467. Meyn LA, Hillier SL: Ampicillin susceptibilities of vaginal and placental isolates of group B *Streptococcus* and *Escherichia coli* obtained between 1992 and 1994, *Antimicrob Agents Chemother* 41: 1173-1174, 1997.
468. Biedenbach DJ, Stephen JM, Jones RN: Antimicrobial susceptibility profile among β-haemolytic *Streptococcus* spp. collected in the SENTRY Antimicrobial Surveillance Program-North America, 2001, *Diag Microbiol Infect Dis* 46:291-294, 2003.
469. Manning SD, Foxman B, Pierson CL, et al: Correlates of antibiotic-resistant group B *Streptococcus* isolated from pregnant women, *Obstet Gynecol* 101:74-79, 2003.
470. Chen KT, Puopolo KM, Eichenwald EC, et al: No increase in rates of early-onset neonatal sepsis by antibiotic-resistant group B *Streptococcus* in the era of intrapartum antibiotic prophylaxis, *Am J Obstet Gynecol* 192:1167-1171, 2005.
471. Dahesh S, Hensler ME, Van Sorge NM, et al: Point mutation in the group B streptococcal pbp2x gene conferring decreased susceptibility to beta-lactam antibiotics, *Antimicrob Agents Chemother* 52: 2915-2918, 2008.
472. Nagano N, Nagano Y, Kimura K, et al: Genetic heterogeneity in pbp genes among clinically isolated group B streptococci with reduced penicillin susceptibility, *Antimicrob Agents Chemother* 52: 4258-4267, 2008.
473. Kasahara K, Baltus AJ, Lee SH, et al: Prevalence of non-penicillin-susceptible group B *Streptococcus* in Philadelphia and specificity of penicillin resistance screening methods, *J Clin Microbiol* 48: 1468-1469, 2010.
474. Manning SD, Pearlman MD, Tallman P, et al: Frequency of antibiotic resistance among group B *Streptococcus* isolated from healthy college students, *Clin Infect Dis* 33:e137-e139, 2001.
475. Brandon M, Dowzicky MJ: Antimicrobial susceptibility among gram-positive organisms collected from pediatric patients globally between 2004 and 2011: results from the tigecycline evaluation and surveillance trial, *J Clin Microbiol* 51:2371-2378, 2013.
476. Wehbeh W, Rojas-Diaz R, Li X, et al: Fluoroquinolone-resistant *Streptococcus agalactiae*: epidemiology and mechanism of resistance, *Antimicrob Agents Chemother* 49:2495-2497, 2005.
477. Capanna F, Emonet SP, Cherkaoui A, et al: Antibiotic resistance patterns among group B *Streptococcus* isolates: implications for antibiotic prophylaxis for early-onset neonatal sepsis, *Swiss Med Wkly* 143:w13778, 2013.
478. Borchardt SM, DeBusscher JH, Tallman PA, et al: Frequency of antimicrobial resistance among invasive and colonizing group B streptococcal isolates, *BMC Infect Dis* 6:57, 2006.
479. Fitoussi F, Loukil C, Gros I, et al: Mechanisms of macrolide resistance in clinical group B streptococci isolated in France, *Antimicrob Agents Chemother* 45:1889-1891, 2001.
480. Betriu C, Culebras E, Gómez M, et al: Erythromycin and clindamycin resistance and telithromycin susceptibility in Streptococcus agalactiae, *Antimicrob Agents Chemother* 47:1112-1114, 2003.
481. Acikgoz ZC, Almayanlar E, Gamberzade S, et al: Macrolide resistance determinants of invasive and noninvasive group B streptococci in a Turkish hospital, *Antimicrob Agents Chemother* 48:1410-1412, 2004.
482. de Azavedo JCS, McGavin M, Duncan C, et al: Prevalence and mechanisms of macrolide resistance in invasive and noninvasive group B *Streptococcus* isolates from Ontario, Canada, *Antimicrob Agents Chemother* 45:3504-3508, 2001.
483. Marimón JM, Valiente A, Ercibengoa M, et al: Erythromycin resistance and genetic elements carrying macrolide efflux genes in Streptococcus agalactiae, *Antimicrob Agents Chemother* 49:5069-5074, 2005.
484. Heelan JS, Hasenbein ME, McAdam AJ: Resistance of group B *Streptococcus* to selected antibiotics, including erythromycin and clindamycin, *J Clin Microbiol* 42:1263-1264, 2004.
485. DiPersio LP, DiPersio JR: Identification of an *erm(T)* gene in strains of inducibly clindamycin-resistant group B *Streptococcus, Diagn Microbiol Infect Dis* 57:189-193, 2007.
486. Puopolo KM, Klinzing DC, Lin MP, et al: A composite transposon associated with erythromycin and clindamycin resistance in group B *Steptococcus, J Med Microbiol* 56:947-955, 2007.
487. Lin FY, Azimi PH, Weisman LE, et al: Antibiotic susceptibility profiles for group B streptococci isolated from neonates, 1995–1998, *Clin Infect Dis* 31:76-79, 2000.
488. Betriu C, Culebras E, Rodríguez-Avial I, et al: In vitro activities of tigecycline against erythromycin-resistant *Streptococcus pyogenes* and *Streptococcus agalactiae*: mechanisms of macrolide and tetracycline resistance, *Antimicrob Agents Chemother* 48:323-325, 2004.
489. Bingen E, Doit C, Bidet P, et al: Telithromycin susceptibility and genomic diversity of macrolide-resistant serotype III group B streptococci isolated in perinatal infections, *Antimicrob Agents Chemother* 48:677-680, 2004.
490. Swingle HM, Bucciarelli RL, Ayoub EM: Synergy between penicillins and low concentrations of gentamicin in the killing of group B streptococci, *J Infect Dis* 152:515-520, 1985.
491. Maduri-Traczewski M, Szymczak EG, Goldmann DA: In vitro activity of penicillin and rifampin against group B streptococci, *Rev Infect Dis* 5:S586-S592, 1983.
492. Hall MA, Ducker DA, Lowes JA, et al: A randomized prospective comparison of cefotaxime versus netilmicin/penicillin for treatment of suspected neonatal sepsis, *Drugs* 35:169-188, 1988.
493. Bradley JS, Ching DL, Wilson TA, et al: Once-daily ceftriaxone to complete therapy of uncomplicated group B streptococcal infection in neonates, *Clin Pediatr* 31:274-278, 1992.
494. Feldman WE: Concentrations of bacteria in cerebrospinal fluid of patients with bacterial meningitis, *J Pediatr* 88:549-552, 1976.
495. Kim KS: Clinical perspectives on penicillin tolerance, *J Pediatr* 112:509-514, 1988.
496. Poschl JM, Hellstern G, Dertlioglou N, et al: Six day antimicrobial therapy for early-onset group B streptococcal infection in near-term and term neonates, *Scand J Infect Dis* 35:302-305, 2003.
497. Fernandez M, Rench MA, Albanyan EA, et al: Failure of rifampin to eradicate group B streptococcal colonization in infants, *Pediatr Infect Dis J* 20:371-376, 2001.
498. Lannering B, Larsson LE, Rojas J, et al: Early onset group B streptococcal disease: seven year experience and clinical scoring system, *Acta Paediatr Scand* 72:597-602, 1983.
499. Fluegge K, Siedler A, Heinrich B, et al: Incidence and clinical presentation of invasive neonatal group B streptococcal infections in Germany, *Pediatrics* 117:e1139-e1145, 2006.
500. de Louvois J, Halket S, Harvey D: Neonatal meningitis in England and Wales: sequelae at 5 years of age, *Eur J Pediatr* 164:730-734, 2005.
501. Stoll BJ, Hansen NI, Adams-Chapman I, et al: Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection, *JAMA* 292:2357-2365, 2004.
502. Libster R, Edwards KM, Levent F, et al: Long-term outcomes of group B streptococcal meningitis, *Pediatrics* 130:e8-e15, 2012.
503. Centers for Disease Control and Prevention: Prevention of perinatal group B streptococcal disease: revised guidelines from CDC, *MMWR Morb Mortal Wkly Rep* 51:1-22, 2002.
504. Baker CJ: Immunization to prevent group B streptococcal disease: victories and vexations, *J Infect Dis* 161:917-921, 1990.
505. Gardner SE, Yow MD, Leeds LJ, et al: Failure of penicillin to eradicate group B streptococcal colonization in the pregnant woman, *Am J Obstet Gynecol* 135:1062-1065, 1979.
506. Gordon JS, Sbara AJ: Incidence, technique of isolation, and treatment of group B streptococci in obstetric patients, *Am J Obstet Gynecol* 126:1023-1026, 1976.
507. Hall RT, Barnes W, Krishnan L, et al: Antibiotic treatment of parturient women colonized with group B streptococci, *Am J Obstet Gynecol* 124:630-634, 1976.
508. Lewin EB, Amstey MS: Natural history of group B *Streptococcus* colonization and its therapy during pregnancy, *Am J Obstet Gynecol* 139:512-515, 1981.
509. Yow MD, Mason EO, Leeds LJ, et al: Ampicillin prevents intrapartum transmission of group B *Streptococcus*, *JAMA* 241:1245-1247, 1979.
510. Boyer KM, Gotoff SP: Prevention of early-onset neonatal group B streptococcal disease with selective intrapartum chemopraxis, *N Engl J Med* 314:1665-1669, 1986.
511. Matorras R, García-Perea A, Madero R, et al: Maternal colonization by group B streptococci and puerperal infection; analysis of intrapartum chemoprophylaxis, *Eur J Obstet Gynecol Reprod Biol* 38:203-207, 1990.

512. Mohle-Boetani JC, Schuchat A, Plikaytis BD, et al: Comparison of prevention strategies for neonatal group B streptococcal infection: an economic analysis, *JAMA* 270:1442-1448, 1993.
513. ACOG technical bulletin: Group B streptococcal infections in pregnancy, *ACOG Tech Bull* 170:1-5, 1992.
514. American Academy of Pediatrics Committee on Infectious Diseases: Committee on Fetus and Newborn: Guidelines for prevention of group B streptococcal (GBS) infection by chemoprophylaxis, *Pediatrics* 90:775-778, 1992.
515. Bergeron MG, Ke D, Ménard C, et al: Rapid detection of group B streptococci in pregnant women at delivery, *N Engl J Med* 343:175-179, 2000.
516. Edwards RK, Novak-Weekley SM, Koty PP, et al: Rapid group B streptococci screening using a real-time polymerase chain reaction assay, *Obstet Gynecol* 111:1335-1341, 2008.
517. Davies HD, Miller MA, Faro S, et al: Multicenter study of a rapid molecular-based assay for the diagnosis of group B *Streptococcus* colonization in pregnant women, *Clin Infect Dis* 39:1129-1135, 2004.
518. Gavino M, Wang E: A comparison of a new rapid real-time polymerase chain reaction system to traditional culture in determining group B streptococcus colonization, *Am J Obstet Gynecol* 197:388, 2007.
519. Honest H, Sharma S, Khan KS: Rapid tests for group B *Streptococcus* colonization in laboring women: a systematic review, *Pediatrics* 117:1055-1066, 2006.
520. Poncelet-Jasserand E, Forges F, Varlet MN, et al: Reduction of the use of antimicrobial drugs following the rapid detection of *Streptococcus agalactiae* in the vagina at delivery by real-ltime PCR assay, *Brit J Obstet Gynaec* 120:1098-1109, 2013.
521. El Helali N, Giovangrandi Y, Guyot K, et al: Cost and effectiveness of intrapartum group B *Streptococcus* polymerase chain reaction screening for term deliveries, *Obstet Gynec* 119:822-829, 2012.
522. Riedlinger J, Beqaj SH, Milish MA, et al: Multicenter evaluation of the BD Max GBS assay for detection of group B streptococci in prenatal vaginal and rectal screening swab specimens from pregnant women, *J Clin Microbiol* 48:4239-4241, 2010.
523. Martinez de Tejada B, Pfister RE, Renzi G, et al: Intrapartum group B streptococcus detection by rapid polymerase chain reaction assay for the prevention of neonatal sepsis, *Clin Micro Infect* 17:1786-1791, 2011.
524. Berg B, Houseman JL, Garrasi MA, et al: Culture-based method with performance comparable to that of PCR-based methods for detection of group B *Streptococcus* in screening samples from pregnant women, *J Clin Microbiol* 51:1253-1255, 2013.
525. Centers for Disease Control and Prevention: Prevention of perinatal group B streptococcal disease: a public health perspective, *MMWR Morb Mortal Wkly Rep* 45:1-24, 1996.
526. American College of Obstetricians and Gynecologists Committee on Obstetric Practice: Prevention of early-onset group B streptococcal disease in newborns, Washington, DC, 1996, American College of Obstetricians and Gynecologists.
527. Schuchat A: Group B streptococcal disease: from trials and tribulations to triumph and trepidation, *Clin Infect Dis* 33:751-756, 2001.
528. Schrag SJ, Whitney CG, Schuchat A: Neonatal group B streptococcal disease: how infection control teams can contribute to prevention efforts, *Infect Control Hosp Epidemiol* 21:473-483, 2000.
529. Centers for Disease Control and Prevention: Hospital-based policies for prevention of perinatal group B streptococcal disease—United States, 1999, *MMWR Morb Mortal Wkly Rep* 49:936-940, 2000.
530. Fairlie T, Zell E, Schrag S: Effectiveness of intrapartum antibiotic prophylaxis for prevention of early-onset group B streptococcal disease, *Obstet Gynec* 121:570-577, 2013.
531. Blaschke A, Pulver LS, Korgenski EK, et al: Clindamycin-resistant group B *Streptococcus* and failure of intrapartum prophylaxis to prevent early-onset disease, *J Pediatr* 156:501-503, 2010.
532. Dunn AB, Blomquist J, Khousami V: Anaphylaxis in labor secondary to prophylaxis against group B streptococcus: a case report, *J Reprod Med* 44:381-384, 1999.
533. Heim K, Alge A, Marth C: Anaphylactic reaction to ampicillin and severe complication in the fetus, *Lancet* 337:859-860, 1991.
534. Philipson EH, Lang DM, Gordon SJ, et al: Management of group B *Streptococcus* in pregnant women with penicillin allergy, *J Reprod Med* 52:480-484, 2007.
535. Sheikh J: Intrapartum anaphylaxis to penicillin in a woman with rheumatoid arthritis who had no prior penicillin allergy, *Ann Allergy Asthma Immunol* 99:287-289, 2007.
536. Paccione K, Wiesenfeld H: Guideline adherence for intrapartum group B streptococci prophylaxis in penicillin-allergic patients, *Infect Dis Obstet Gynec* 917304, 2013.
537. Pinto NM, Soskolne EI, Pearlman MD, et al: Neonatal early-onset group B streptococcal disease in the era of intrapartum chemoprophylaxis: residual problems, *J Perinatol* 23:265-271, 2003.
538. Cárdenas V, Davis RL, Hasselquist MB, et al: Barriers to implementing the group B streptococcal prevention guidelines, *Birth* 29: 285-290, 2002.
539. Puopolo K, Eichenwald E: No change in the incidence of ampicillin-resistant, neonatal early-onset sepsis over 18 years, *Pediatrics* 125:e1031-e1038, 2010.
540. Baker C, Byington C, Polin R: Policy statement—recommendations for the prevention of perinatal group B streptococcal disease, *Pediatrics* 128:611-616, 2011.
541. Brady M, Polin R: Prevention and management of infants with suspected or proven neonatal sepsis, *Pediatrics* 132:166-168, 2013.
542. Balter S, Zell ER, O'Brien KL, et al: Impact of intrapartum antibiotics on the care and evaluation of the neonate, *Pediatr Infect Dis J* 22: 853-857, 2003.
543. Bromberger P, Lawrence JM, Braun D, et al: The influence of intrapartum antibiotics on the clinical spectrum of early-onset group B streptococcal infection in term infants, *Pediatrics* 106:244-250, 2000.
544. Davis RL, Hasselquist MB, Cardenas V, et al: Introduction of the new Centers for Disease Control and Prevention group B streptococcal prevention guideline at a large West Coast health maintenance organization, *Am J Obstet Gynecol* 184:603-610, 2001.
545. Rubin EE, McDonald JC: Group B streptococcal disease in twins: failure of empiric therapy to prevent late onset disease in the second twin, *Pediatr Infect Dis J* 10:921-923, 1991.
546. Schuchat A: Epidemiology of group B streptococcal disease in the United States: shifting paradigms, *Clin Microbiol Rev* 11:497-513, 1998.
547. Madoff LC, Michel JL, Gong EW, et al: Protection of neonatal mice from group B streptococcal infection by maternal immunization with beta C protein, *Infect Immun* 60:4989-4994, 1992.
548. Wessels MR, Paoletti LC, Kasper DL, et al: Immunogenicity in animals of a polysaccharide-protein conjugate vaccine against type III group B *Streptococcus*, *J Clin Invest* 86:1428-1433, 1990.
549. Wessels MR, Paoletti LC, Pinel J, et al: Immunogenicity and protective activity in animals of a type V group B streptococcal polysaccharide-tetanus toxoid conjugate vaccine, *J Infect Dis* 171:879-884, 1995.
550. Paoletti LC, Wessels MR, Rodewald AK, et al: Neonatal mouse protection against infection with multiple group B streptococcal (GBS) serotypes by maternal immunization with a tetravalent GBS polysaccharide-tetanus toxoid conjugate vaccine, *Infect Immun* 62:3236-3243, 1994.
551. Madoff LC, Paoletti LC, Tai JY, et al: Maternal immunization of mice with group B streptococcal type III polysaccharide-beta C protein conjugate elicits protective antibody to multiple serotypes, *J Clin Invest* 94:286-292, 1994.
552. Paoletti LC, Madoff LC: Vaccines to prevent neonatal GBS infection, *Semin Neonatol* 7:315-323, 2002.
553. Kasper DL, Paoletti LC, Wessels MR, et al: Immune response to type III group B streptococcal polysaccharide-tetanus toxoid conjugate vaccine, *J Clin Invest* 98:2308-2314, 1996.
554. Baker CJ, Paoletti LC, Wessels MR, et al: Safety and immunogenicity of capsular polysaccharide-tetanus toxoid conjugate vaccines for group B streptococcal types Ia and Ib, *J Infect Dis* 179:142-150, 1999.
555. Baker CJ, Paoletti LC, Rench MA, et al: Use of capsular polysaccharide-tetanus toxoid conjugate vaccine for type II group B *Streptococcus* in healthy women, *J Infect Dis* 182:1129-1138, 2000.
556. Baker CJ, Paoletti LC, Rench MA, et al: Immune response of healthy women to 2 different group B streptococcal type V capsular polysaccharide-protein conjugate vaccines, *J Infect Dis* 189:1103-1112, 2004.
557. Baker CJ, Rench MA, Fernandez M, et al: Safety and immunogenicity of a bivalent group B streptococcal conjugate vaccine for serotypes II and III, *J Infect Dis* 188:66-73, 2003.
558. Baker CJ, Rench MA, McInnes P: Immunization of pregnant women with group B streptococcal type III capsular polysaccharide-tetanus toxoid conjugate vaccine, *Vaccine* 21:3468-3472, 2003.

559. Michel JL, Madoff LC, Kling DE, et al: Cloned alpha and beta C-protein antigens of group B streptococci elicit protective immunity, *Infect Immun* 59:2023-2028, 1991.
560. Pannaraj PS, Kelly JK, Madoff LC, et al: Group B *Streptococcus* bacteremia elicits beta C protein-specific IgM and IgG in humans, *J Infect Dis* 195:353-356, 2007.
561. Pannaraj PS, Kelly JK, Rench MA, et al: Alpha C protein-specific immunity in humans with group B streptococcal colonization and invasive disease, *Vaccine* 26:502-508, 2008.
562. Yang HH, Madoff LC, Guttormsen HK, et al: Recombinant group B streptococcus beta C protein and a variant with the deletion of its immunoglobulin A-binding site are protective mouse maternal vaccines and effective carriers in conjugate vaccines, *Infect Immun* 75:3455-3461, 2007.
563. Larsson C, Holmgren J, Lindahl G, et al: Intranasal immunization of mice with group B streptococcal protein Rib and cholera toxin B subunit confers protection against lethal infection, *Infect Immun* 72:1184-1187, 2004.
564. Martin D, Rioux S, Gagnon E, et al: Protection from group B streptococcal infection in neonatal mice by maternal immunization with recombinant Sip protein, *Infect Immun* 70:4897-4901, 2002.
565. Cheng Q, Debol S, Lam H, et al: Immunization with C5a peptidase or peptidase-type III polysaccharide conjugate vaccines enhances clearance of group B streptococci from lungs of infected mice, *Infect Immun* 70:6409-6415, 2002.
566. Martins E, Melo-Cristino J, Ramirez M: Distribution of pilus islands in *Streptococcus agalactiae* that cause human infections: insights into evolution and implication for vaccine development, *Clin Vaccine Immunol* 20:313-316, 2013.
567. Stratton KR, Durch JS, Lawrence RS, editors: Committee to Study Priorities for Vaccine Development, Division of Health Promotion and Disease Prevention, Institute of Medicine: Vaccines for the 21st century: a tool for decisionmaking, Washington, DC, 1999, National Academy of Sciences.
568. Kogan G, Uhrín Dusan, Brisson Jean-Robert, et al: Structure of the type VI group B *Streptococcus* capsular polysaccharide determined by high resolution NMR spectroscopy, *J Carbohydr Chem* 13: 1071-1078, 1994.
569. Adair CE, Kowalsky L, Quon H, et al: Risk factors for early-onset group B streptococcal disease in neonates: a population-based case-control study, *Can Med Assoc J* 169:198-203, 2003.
570. Baker CJ, Webb BJ, Kasper DL, et al: The natural history of group B streptococcal colonization in the pregnant woman and her offspring. II. Determination of serum antibody to capsular polysaccharide from type III group B *Streptococcus*, *Am J Obstet Gynecol* 137:39-42, 1980.
571. Boyer KM, Gadzala CA, Burd LI, et al: Selective intrapartum chemoprophylaxis of neonatal group B streptococcal early-onset disease. I. Epidemiologic rationale, *J Infect Dis* 148:795-801, 1983.
572. Cueva JP, Egel RT: Anterior fontanel herniation in group B *Streptococcus* meningitis in newborns, *Pediatr Neurol* 10:332-334, 1994.
573. Schimmel MS, Schlesinger Y, Berger I, et al: Transverse myelitis: unusual sequelae of neonatal group B streptococcus disease, *J Perinatol* 22:580-581, 2002.
574. Ellenbogen RG, Goldmann DA, Winston KR: Group B streptococcal infections of the central nervous system in infants with myelomeningocele, *Surg Neurol* 29:237-242, 1988.
575. Mukherjee S, Askwith JK: Transient isolated oculomotor nerve paralysis in neonatal group B streptococcal meningitis, *J Paediatr Child Health* 44:231-232, 2008.
576. McAdams RM, Simone S, Grant G, et al: Ventricular peritoneal shunt infection resulting from group B streptococcus, *Pediatr Crit Care Med* 7:586-588, 2006.
577. Ramsey PG, Zwerdling R: Asymptomatic neonatal bacteremia, *N Engl J Med* 295:225, 1977.
578. Roberts KB: Persistent group B *Streptococcus* bacteremia without clinical "sepsis" in infants, *J Pediatr* 88:1059-1060, 1976.
579. Poschl JM, Hellstern G, Ruef P, et al: Ophthalmia neonatorum caused by group B *Streptococcus*, *Scand J Infect Dis* 34:921-922, 2002.
580. Klusmann A, Engelbrecht V, Unsöld R, et al: Retrobulbar abscess in a neonate, *Neuropediatrics* 32:219-220, 2001.
581. Rench MA, Baker CJ: Group B streptococcal breast abscess in a mother and mastitis in her infant, *Obstet Gynecol* 73:875-877, 1989.
582. Brian MJ, O'Ryan M, Waagner D: Prepatellar bursitis in an infant caused by group B *Streptococcus*, *Pediatr Infect Dis J* 11:502-503, 1992.
583. Ruiz-Gomez D, Tarpay MM, Riley HD: Recurrent group B streptococcal infections: report of three cases, *Scand J Infect Dis* 11:35-38, 1979.
584. Frieden IJ: Blistering dactylitis caused by group B streptococci, *Pediatr Dermatol* 6:300-302, 1989.
585. Turner MC, Naumburg EG: Acute renal failure in the neonate: two fatal cases due to group B streptococci with rhabdomyolysis, *Clin Pediatr (Phila)* 26:189-190, 1987.