STREPTOCOCCI

October 29, 2013

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Lecture Objectives

- Appreciation of Two Important Pathogens (Group A and B Streptococci) -and why you should respect, fear and hate them!

- To Use Streptococci to Provide a Paradigm for Understanding the Microbiology of a Pathogen's:
  - *Physiology and Structure*
  - *Pathogenesis and Immunity*
  - *Epidemiology*
  - *Clinical Diseases*
  - *Laboratory Diagnosis*
  - *Treatment, Prevention and Control*

Streptococci are major causes of human morbidity and mortality (SLIDE 2). Major diseases that you have likely heard of but may not know are caused by various streptococci are:

Strep throat (well, this is obvious!)
Rheumatic fever
Scarlet Fever
Necrotizing fasciitis (so-called flesh-eating bacteria)
Streptococcal toxic shock syndrome

Classification of medically important streptococci.

Streptococci are a diverse collection of gram positive bacterial strains, and typically are found in pairs or chains.

There are three ways in which they can be classified (SLIDE 4).
First, they can be classified based on whether they cause red blood cell hemolysis on plates:

β-hemolytic strains cause complete hemolysis
α-hemolytic strains cause 'incomplete' hemolysis. Hydrogen peroxide produced by the bacterium, oxidizes hemoglobin to green methemoglobin
γ-hemolytic strains cause no hemolysis

Second, β-hemolytic strains can be further subdivided into **groups** based on serologic properties (Lancefield groupings), with these differences being due to carbohydrates in the cell wall. These are designated Group A, B, etc. Of these, Groups A and B are the most important (SLIDE 3).

S. pyogenes -**Group A Streptococcus**-pharyngitis, cutaneous and invasive infections

S. agalactiae-**Group B Streptococcus**- neonatal and maternal infection. Urinary tract infection, Invasive infection (bacteremia, meningitis)-(Note the marked differences from GAS in niche and disease manifestations despite relatedness)

Other β-hemolytic streptococci-may also cause pharyngitis and bacteremia

Third, most non-hemolytic strep do not possess group specific wall antigens, and so cannot be placed into groups based on serologic properties. These strains must be identified based on various physiological properties. Examples include the Viridans group of streptococci, which are a cause of bacteremia and complications such as abscess formation and endocarditis.

Strep are catalase negative. In contrast, Staphylococcus, another important type of gram positive cocci (to be covered in a separate lecture), is catalase positive.

The most important Streptococci are:

Group A Strep - covered in this lecture
Group B Strep - covered in this lecture
S. pneumoniae-the pneumococcus. A major cause of bacterial pneumonia, this is covered in the **lecture on respiratory pathogens**

Physiology and Structure (SLIDE 4):

A. Facultative anaerobe (or aerotolerant). So, streptococci can use oxygen to make ATP, or they can use fermentation to make energy.

B. Autotrophic-complex growth requirements. As a result, blood or serum-enriched media is needed for isolation (blood agar plates, for example).

C. Many strains are β-hemolytic due to expression of Streptolysin S, including Groups A and B. Remember: all of the Groups (Group A, B, etc) are β-hemolytic.

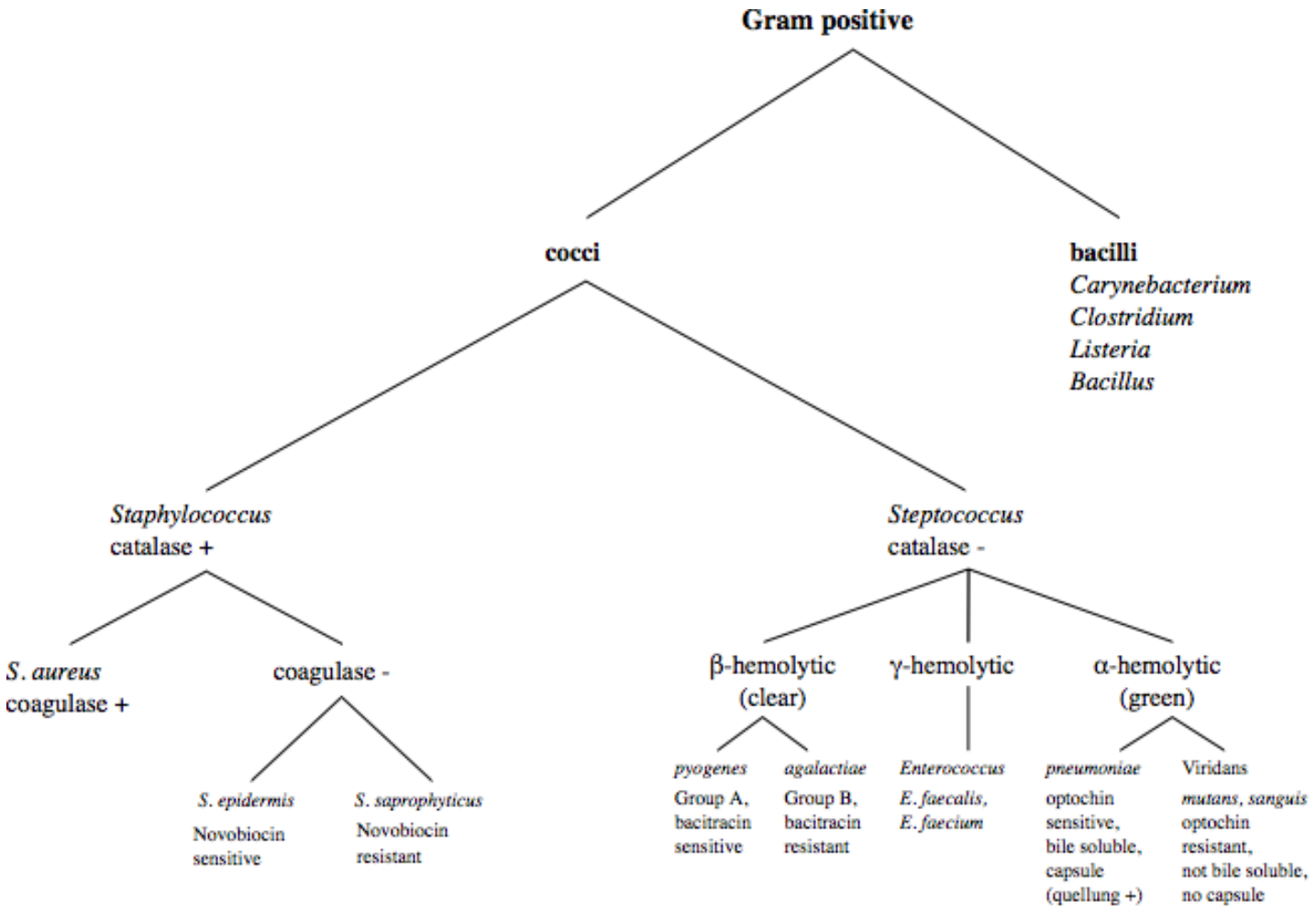
D. Detection of PYR (L-pyrrolidonly arylamidase) can be used for identification

E. Found in nature only within human host (no environmental reservoir)

Gram Positive Cocci Flow Chart (SLIDE 5)

If gram positive cocci are observed, they are either strep or staph. A catalase test distinguishes between these two major groups: strep are catalase negative. Strep are then distinguished based on their hemolytic properties, and then by serologic properties (for ß hemolytic strains), sensitivity to bacitracin (readily distinguishes Group A from Group B) or by physiological properties for the non ß hemolytic strains.

Key Points: Physiology and Structure
1) GAS is a Gram-positive coccus that grows in chains 2) GAS is β-hemolytic on blood agar 3) Streptococci are catalase negative



Group A Strep (*Streptococcus pyogenes*)

Pathogenesis and Immunity

There are many strains of Group A Strep. As a result, infections can be recurrent because immunity to one strain often fails to produce immunity to other strains. In addition, strains can vary in their virulence - their ability to cause disease. Together its virulence factors allow GAS to get through tissue barriers, adhere to sites of inflammation and inhibit otherwise effective clearance mechanisms. This may increase its persistence in a host and increase its chances for transmission to a new susceptible host.

Characteristics that promote Group A Strep virulence include (SLIDE 7):

- The ability to adhere to the surface of epithelial cells
  - The ability to invade into and hide in epithelial cells (internalization thought to be important for maintenance of persistent infections)
- Ability to avoid opsonization (antibody and complement binding) and phagocytosis

- Ability to cause tissue damage and inhibit clearance by producing toxins (toxins akin to snake venom)

Binding to the cell surface is influenced/mediated by:
- lipoteichoic acid
- M protein
- F protein

The ability to invade host cells is mediated by:
- M protein
- F protein

Mechanisms to avoid opsonization and phagocytosis:
- M protein can bind the serum ß-globulin factor H, which regulates alternative complement pathway.
- Complement component C3b is destabilized by factor H. So, when C3b clings to the cell surface in the region of M protein, it is degraded by the associated factor H, and phagocytosis is prevented
- This is overcome if a patient has antibodies to the strain's M protein
- *S. pyogenes* (Group A Strep) can produce a protease that inactivates C5a, which in turn blocks chemotaxis of neutrophils

Ability to produce toxins and enzymes
- Different strains express different toxins (described below).

Common virulence factors of Group A Strep (SLIDE 8)

Exotoxins - The 'leukocydins' Streptolysins O (a pore-forming toxin) and S (the β-hemolysin) damage tissue and inhibit clearance. Pyrogenic exotoxins (SpeA, SpeB and SpeC) are also produced, and as the name suggest lead to fever by inducing the release of numerous cytokines. This can lead to fever and shock and organ failure in patients with streptococcal toxic shock syndrome (see below). They are also responsible for the rash observed in patients with scarlet fever.

M protein – provides antigenic variation; blocks opsonization by complement alternate pathway, thus evading phagocytosis. It also plays a role in mediating adherence to host cells, and is described in more detail in the next slide.

Capsule – A thick coat of hyaluronic acid (also found in connective tissues) that confers resistance to phagocytosis

Hyaluronidase – Degrades hyaluronic acid in connective tissue.

C5a Peptidase – Destroys C5a as a chemotactic signal to neutrophils (PMNs)

Streptokinase – Catalyzes activation of plasmin to lyse blood clots, which may lead to spread of S. pyogenes (Group A strep) in infected tissues.

Streptodornase (DNase) – Helps solubilize pus, thus facilitating spread of the organism.

Lipoteichoic acid, & F-protein – Mediates attachment to epithelium, fibronectin

Streptococcal M protein (SLIDE 9)

The M protein is the major, immunodominant protein expressed by virulent streptococci. It consists of two polypeptide chains that are anchored in the bacterial membrane and that extend through the cell wall. The C-terminal portion of the protein, which is inside the cell, is highly conserved. However, the external portion is highly variable, and there are >80 known M-serotypes. Development of immunity to one type leaves an individual still susceptible to all remaining types! This accounts for why streptococcal pharyngitis is often recurrent.

Antiphagocytic activity of M protein

As described two slides ago, the M protein helps prevent strep from being phagocytosed and destroyed by neutrophils:
You can show this in a phagocytosis assay (SLIDE 10). If a strain does not have M protein (you can knock this out genetically), it is phagocytosed. However, if M protein is expressed, it is not. But, if antibodies bind to the variable region of M protein (because the patient was previously exposed and made antibodies), the Fc region of the bound antibody binds to Fc receptor on the surface of neutrophils, and the bacteria is phagocytosed. Fc can also bind complement via the classical pathway of complement activation allowing for binding via complement receptors. When targets have bound antibodies/complement allowing recognition by host phagocytes, the process is opsonization.

Key Points: Pathogenesis and Immunity

1) Numerous virulence factors allow it to cause tissue damage, avoid clearance and invade its host
2) M-protein is immunodominant but variable so prior infection is not protective
3) M-protein is anti-phagocytic and effective immunity requires type-specific antibody
4) Toxins contribute to diseases caused by GAS

Epidemiology (SLIDE 11)

Streptococci have a predilection for the upper respiratory tract or skin; Strains that primarily colonize the skin are usually distinct "antigenic types" from those that colonize the throat.

Group A streptococci (*S. pyogenes*) commonly colonize the oropharynx of healthy children and young adults or the skin. Colonization is transient due to development of M protein-specific immune response requiring 1-4 weeks. Asymptomatic colonization is common. Disease is usually caused by a recently acquired strain that can establish an infection prior to the induction of antibodies to the M protein.

Responsible for an estimated >10,000,000 cases/yr in US of non-invasive GAS and 4500 cases of invasive GAS.

Pharyngitis (scarlet fever and other complication of streptococcal pharyngitis):
Transmitted person-to-person by droplets from respiratory secretions and via fomites.
Crowding increases risk (e.g., classrooms, military, day care facilities),
Hospital spread such as post-surgical infection from carriers (and inadequate handwashing!) Especially common in children 5-15 years. Less common in adults due to acquired immunity?
Cold months
Invasive and deep cutaneous diseases are complications of spread from pharynx
Acute rheumatic fever (explained below) and glomerulonephritis are the post-infectious sequelae of pharyngitis

Cutaneous & soft tissue infections:
Transmitted by bacteremic spread or through breaks in skin after direct contact with oral secretions from infected person, fomite, or arthropod vector.
Impetigo more common in warm months and occurs following minor trauma especially when hygiene is suboptimal.
Glomerulonephritis (explained below) is the only post-infectious sequelae

Key Points: Epidemiology

1) Humans are the exclusive host for GAS and so transmission is person to person
2) The niche for GAS includes the throat and skin but throat strains may cause skin infection
3) Bacterial and host factors resulting in colonization v. disease not well understood
4) Infection (pharyngitis, impetigo) is common, especially among school-aged children

Clinical Diseases (SLIDE 12)

Group A Streptococcus (*S. pyogenes*) is one of the most important human pathogens; Commonly associated with a diverse group of human diseases, including both suppurative (pus-forming) and nonsuppurative diseases.

Suppurative streptococcal diseases (Acute streptococcal infection)
Pharyngitis (& tonsilitis): "strep throat" --- Suffix "-itis" refers to inflammation of (in this case, inflammation of the pharynx or tonsils). (SLIDE 13) Occurs within several days of acquisition of organism.

Chief Symptoms: Sore Throat, Fever, Malaise, Headache, Abdominal Pain

Physical Signs: Erythematous Posterior pharynx +/- exudate (acute inflammatory response with neutrophils), Cervical lymphadenopathy. It can be difficult to distinguish strep throat from viral pharyngitis: some children with viral pharyngitis have exudates, while some with strep throat do not. A culture or rapid diagnostic test from a throat swab is needed to distinguish the two.

Other suppurative diseases

Puerperal sepsis (associated with childbirth); Lymphangitis (inflammation of lymphatic vessels); Pneumonia, Bacteremia(bacteria in the blood)

Septicemia (sepsis): systemic disease associated with persistent presence of bacterial cells, bacterial toxins or other bacterial products in the blood causing multi-system failure and mortality approaching 40%;

Cutaneous & soft tissue infections (SLIDE 14):

1. Pyoderma (Impetigo: contagious pyoderma with superficial yellow weeping-crusty lesions)(SLIDE 17): Generally caused by strains of different M-type from pharyngitis and other infections, and is generally seen in young children.

2. Erysipelas (SLIDE 18): Acute superficial cellulitis of skin with lymphatic involvement; face and lower extremities, skin and subcutaneous tissues. Associated with local pain; involved area is typically raised and distinct from adjoining, uninvolved areas. Most common in young children and older adults.

3. Cellulitis (SLIDE 18): Involvement of deeper subcutaneous tissues; Deeper invasion with systemic symptoms. Involved areas not as well demarcated. Many other organisms can cause cellulitis, so you can't assume it is caused by Group A Strep.

4. Necrotizing fasciitis (a.k.a., "flesh-eating bacteria")(SLIDE 16): Infection deep in subcutaneous tissues that spreads along fascial planes, destroying muscle and fat; Initially cellulitis followed by bullae (fluid filled blisters), gangrene, systemic toxicity, multiorgan failure and mortality in more than 50% of patients. You can't just treat this with antibiotics - you need to surgically debride the affected area.

5. Scarlet fever (SLIDE 15): complication of streptococcal pharyngitis with 'scarletina' rash on upper chest spreading to extremities. Scarlet fever results from infection with a strain that is lysogenized with a temperate bacteriophage that genetically encodes for pyrogenic exotoxin (formerly known as erythrotoxin or erythrogenic toxins)

Acute nonsuppurative sequelae (non-pus-forming complications) of Group A streptococcal disease: These are post-infectious sequelae.

Rheumatic fever (SLIDE 20) is an inflammatory disease that may develop after an infection with GAS but not other bacteria and can involve the heart, joints, skin, and brain. Clinical diagnosis by a combination of features referred to as the modified Jones criteria- there are only supporting rather than diagnostic laboratory tests. These document recent GAS infection but do not distinguish cases of rheumatic fever.

Diagnosis of rheumatic fever: There are five major criteria: carditis, polyarthritis, chorea, erythema marginatum, and subcutaneous nodules; minor criteria include fever, arthralgia, evidence of systemic inflammation (elevated erythrocyte sedimentation rate or C-reactive protein), and prolonged PR interval on ECG. Diagnosis requires laboratory evidence of recent GAS infection, plus two major and one minor criteria, or one major and two minor criteria; revised Jones criteria allow the diagnosis when indolent carditis or chorea exists with no other cause, or in patients with a previous history of rheumatic fever who have one major or two minor criteria in association with a recent streptococcal infection.

Pathogenesis: An Autoimmune Disease (Due to induction of immune response to certain M-protein sequences resembling those in cardiac and other tissues???)

1. Inflammatory reaction characterized by arthritis, carditis, chorea (disorder of nervous system with involuntary spastic movements), erythema marginatum (skin redness with defined margin), or subcutaneous nodules
2. Morbidity and mortality linked to subsequent valvular heart disease
3. Poorly understood pathogenesis with several proposed theories including cross-reactivity of heart tissues and streptococcal antigens, exotoxins, or direct invasion
4. Rheumatic heart disease: Refers to chronic, progressive heart valve damage.

Causes, incidence, and risk factors

Rheumatic fever is common worldwide and remains a major factor in damaged heart valves. While it is far less common in the U.S. since the beginning of the 20th century, there have been a few outbreaks since the 1980s.

Rheumatic fever primarily affects children between ages 6 and 15 and occurs approximately 20 days after strep throat or scarlet fever. In up to a third of cases, the underlying strep infection may not have caused any symptoms.

The rate of development of rheumatic fever in individuals with untreated strep infection is estimated to be 0.1% following sporadic pharyngitis but may be higher in epidemic situations. Persons who have suffered a case of rheumatic fever have a tendency to develop flare-ups with repeated strep infections and progressive cardiac damage(rheumatic heart disease).

The recurrence of rheumatic fever is relatively common in the absence of maintenance of low dose antibiotics, especially during the first 3 - 5 years after the first episode of rheumatic fever. Heart complications (damage to heart valves, in particular, mitral stenosis and aortic stenosis) may be long-term and severe.

Acute glomerulonephritis (SLIDE 21-22)*:* Acute inflammation of renal glomeruli

1. Signs include dark, smoky (coke) urine with red and white blood cells, depressed serum complement, decreased glomerular filtration rate, hypertension
2. Granular accumulations of immunoglobulin due to deposition of immune complexes within the kidney

Renal failure due to acute glomerulonephritis (acute inflammation of renal glomeruli) generally follows infection with nephritogenic streptococcal strains. Autoimmune disease with deposition of antigen-antibody complexes with complement activation triggering inflammation.

Latent period: 1-2 weeks after skin infection and 2-3 weeks after pharyngitis
Associated with specific, well-defined group of serotypes
Incidence following infection varies from less than 1% to 10-15%
Most often seen in children in the developing world
Morbidity due to renal failure

Streptococcal toxic shock syndrome (SLIDE 22-23)

Multisystem 'systemic' toxicity following soft tissue infection progressing to shock (low blood pressure) and organ failure (not to be confused with Staphylococcal Toxic Shock Syndrome where noninvasive infection, ex.hyperabsorbent tampons, have been identified as an important risk factor);

Pathogenesis will be explained in more detail in the lecture on Staphylococci.

Key Points: Clinical Diseases

1) Suppurative (pus-forming) and nonsuppurative diseases
2) Post-infectious sequelae include rheumatic fever and acute glomerulonephritis
3) May cause toxic shock syndrome like staphylococci
4) Responsible for a variety of cutaneous manifestations including: impetigo, erysipelas, cellulitis, scarlet fever, necrotizing fasciitis
5) Rheumatic fever, especially recurrent bouts, leads to valvular damage (rheumatic heart disease)

6) Laboratory Diagnosis (SLIDE 24)

Gram stain of tissue sample can provide rapid initial diagnosis

Throat swabs from the posterior oropharynx (back of throat, e.g., tonsils) or from skin lesion followed by:

1) Culture: Bacitracin sensitivity test for presumptively distinguishing between beta-hemolytic streptococci isolated from pharyngeal swabs (95% accuracy of Grp A strep sensitivity) Negative catalase used to differentiate from Staphylococcus

or

2) Rapid identification tests: Based on extraction of Grp A carbohydrate (antigen) directly from throat swabs and agglutination in the presence of antibody

ASO Test: Detection of antibodies against the conserved toxin streptolysin O confirms a recent Group A streptococcal infection (retrospective diagnosis only-used in suspected acute rheumatic fever) Similarly detection of anti-DNase B antibodies. Unlike the ASO test, anti-DNase B is useful for diagnosing recent GAS infection in cases of rheumatic fever as well as glomerulonephritis.

Key Points: Laboratory Diagnosis

1) Easily identified in the laboratory after culture on blood agar as bacitracin sensitive, β-hemolytic streptococcus
2) Rapid antigen test available for throat swab as alternative to culture
3) Antibodies to ASO or anti-DNase B useful to confirm recent GAS infection for diagnosis of post-infectious complications

Treatment, Prevention and Control (SLIDE 25)

Aimed at prevention of suppurative complications and the nonsuppurative sequelae of rheumatic fever and glomerulonephritis

Group A Pharyngitis

Drug of Choice: Oral penicillin or intramuscular benzathine penicillin. Although GAS in not resistant, treatment failure may be caused by neighboring oral flora that secrete b-lactamases and inactivate penicillin.

Alternatives: Erythromycin (but rate of resistance increasing), clindamycin, cephalexin (oral cephalosporin)

Acute Rheumatic Fever

Salicylates and corticosteroids for acute symptom reduction and control of long-term sequelae; Prevention of reoccurrences by preventing strep infection with long-term prophylactic antibiotics.

Acute Post-Streptococcal Glomerulonephritis

Therapy directed at secondary phenomenon of renal failure:volume excess, hypertension, and seizures; Sodium restriction, diuretics, anticonvulsants

Key Points: Treatment, Prevention and Control

1) Pharyngitis generally self-limited but antibiotics prevent rheumatic fever
2) Penicillin remains the drug of choice-there is no β-lactam resistance

3) Rheumatic fever treated to decrease inflammation and then to prevent future episodes
4) No vaccine

Group B Streptococcus

Physiology and structure (SLIDE 27)
A. Facultative Anaerobe or aerotolerant streptococcus-like GAS
B. Autotrophic-complex growth requirements-like GAS
C. Despite the many similarities to GAS this species have a completely different lifestyle and causes a different set of diseases. For example, its niche is the lower gastrointestinal and genitourinary tract. In addition it has an animal reservoir-and is a cause of bovine mastitis.
D. It can be distinguished from GAS by its inherent resistance to the antibiotic bacitracin.

Pathogenesis and Immunity (SLIDE 28):
GBS has capsules consisting of sialic acid –a polysaccharide (These are different from the GAS hyaluronic acid capsule.)
These thick layers are anti-phagoctyic (more on encapsulated pathogens later!) by inhibiting opsonization of underlying structures and minimally immunogenic 'self' antigens. Specific antibody to the capsular polysaccharide type is protective but there are multiple chemically and antigenically distinct capsules types.

Epidemiology (SLIDE 29):
The major clinical disease is infection of newborns, especially premies, who at risk because of lack of maternal fetal transfer of antibodies before 32 weeks gestation and delayed responses of fetal immune system to polysaccharide antigens. Also an important cause of puerperal sepsis in mothers immediate following delivery.

Clinical Diseases (SLIDE 30-31):
Following maternal-fetal transmission (vertical transmission) before at at delivery (early-onset disease), the infant is susceptible to invasive infections (pneumonia, sepsis, meningitis). About 1/3 of newborns cases occur days after delivery and appear to be from post-partum exposure (late onset).
Also, a cause of urinary tract infection in adults.

Prevention (SLIDE 32-33):
Because the idea of immunization during pregnancy to provide IgG by transplacental transfer is not well accepted, prevention is aimed at identifying mothers carrying GBS late in gestation and treating them with antibiotics to reduce the burden of organisms. Newborns at high risk are given prophylactic treatment.

Key Points: Group B Streptococcus

1) Niche is the lower gastrointestinal and genitourinary tract
2) Capsule allows GBS to evade clearance
3) Leading cause of neonatal infection due to maternal-fetal transmission
4) Neonatal infection decreased by screening and prophylactic antibiotic treatment late in pregnancy