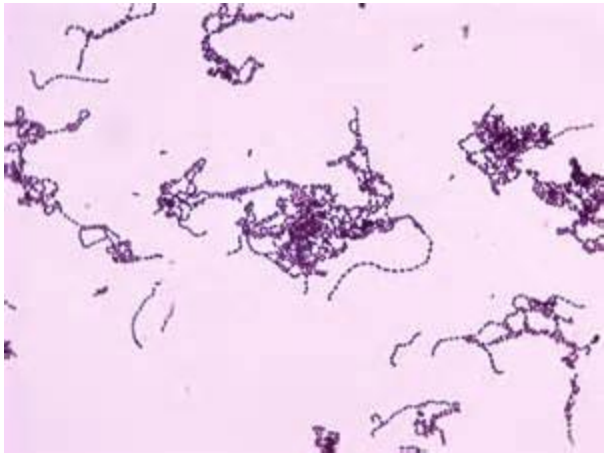
Medical Bacteriology- Lecture: 6

Gram Positive Cocci

Streptococcal Disease

Streptococcus pyogenes



Classification of Streptococci based on (1- Hemolysis reactions on blood agar) (Brown in 1903)

- The type of hemolytic reaction on blood agar has long been used to classify the streptococci.
- **Beta -hemolysis** is a complete lysis of red cells surrounding the colony, appearance as Clear zone

  Pyogenic Streptococci *(Strep. Pyogenes)* are always beta-hemolytic

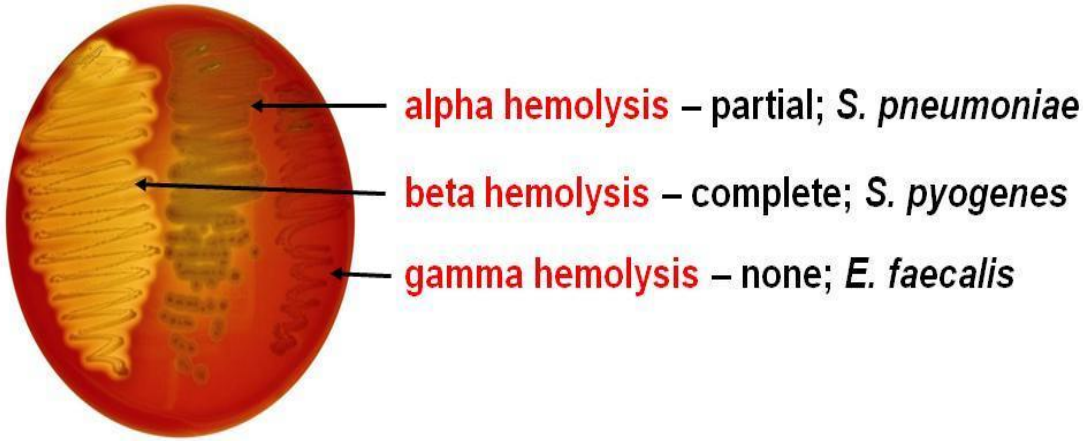
- **alpha-hemolysis** is a partial hemolysis (Greenish Discoloration) associated with reduction of red cell hemoglobin.

  ***S. pneumoniae* are alpha-hemolytic (**but can cause ß-hemolysis during anaerobic incubation).

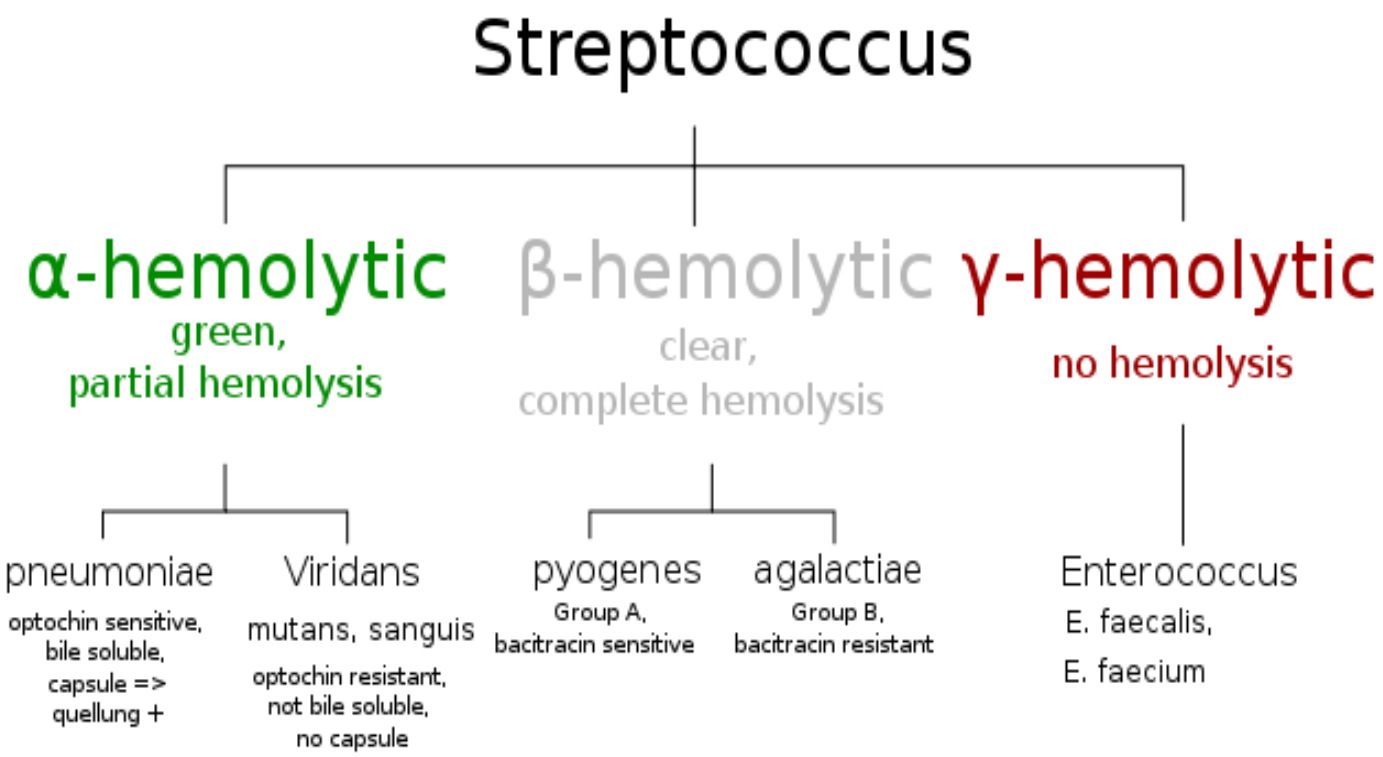
- **gamma-hemolytic** Nonhemolytic colonies.

  Most of the oral streptococci and enterococci are non hemolytic.

- The property of hemolysis is not very reliable for the absolute identification of streptococci, but it is widely used in rapid screens for identification of *S. pyogenes* & *S. pneumoniae.*



Groups of Streptococci
(1- **Hemolysis on blood agar)**



Classification of Streptococci
(2- Antigenic types) (Serology)

- The cell wall of Streptococci is composed of repeating units of N-acetylglucosamine and N-acetylmuramic acid, (standard peptidoglycan).

- The identification of streptococci based on the serologic reactivity of "cell wall" polysaccharide antigens described by **Lancefield classification**.

- Lancefield developed serotyping system for classification of **beta-hemolytic streptococci or gamma** based on the antigenic composition of **specific cell wall carbohydrates (C- Carbohydrates) or C-Substrate**

- **18 group-specific antigens (Lancefield groups) were known**.

- **The clinically important streptococci are grouped under A, B, C, D, F and G.**

The main species and groups of medical importance

– **Group A Streptococci (GAS): *Streptococcus pyogenes***

The cell surface structure *Strep. pyogenes* is the most studied of any streptococci bacteria.
Group A polysaccharide (*Strep. pyogenes*) is a polymer of N-acetylglucosamine and rhamnose.
This polysaccharide is called the **C substance** or **group carbohydrate antigen**.

– **Group B Streptococci: *Strep. agalactiae***

– **Group D Streptococci*: Enterococcus faecalis***

- Viridians streptococci and anaerobic streptococci are not grouped under Lancefield Classification

- **(viridians streptococci & *Strep. pneumonia* have no group-specific antigen)**

- **Common pathogens**
  - **Group C Streptococci: *S. equismilis* (pharyngitis); *S. anginosus* (abscess formation)**
  - **Group F Streptococci: *S. anginosus* (abscess formation)**
  - **Group G Streptococci: *S. anginosus* (abscess formation)**

- **Uncommon pathogens**
  - **Group D Streptococci: *S. bovis, S. durans, S. avium* (foodborne disease)**
  - **Groups E, H, and K**

Classification of Streptococci
(3- Biochemical /Physiological properties)

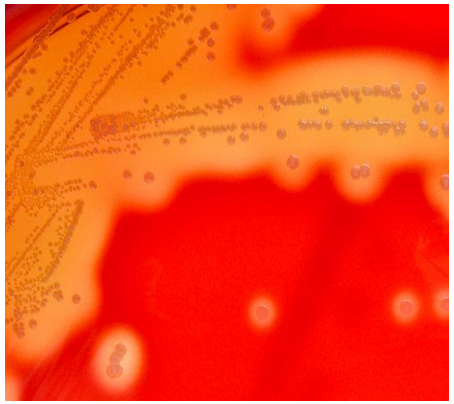
- **Bergeys manual of bacteriology, based on growth at 6.5 % NaCl, 10 and 45 degree centigrade**

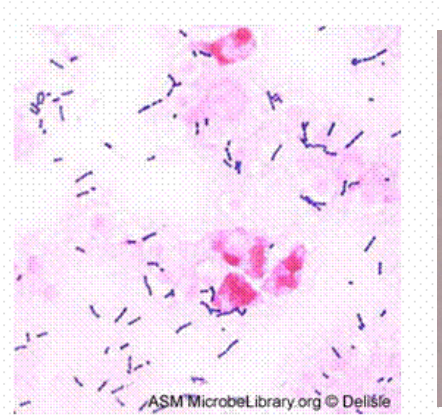
Growth in	Temperature		Group
6.5 % NaCl	45 C	10 C	
-	-	-	*Strep. pyogenes*
-	+	-	*Strep. Viridans*
+	+	+	Enterococcus
-	-	+	Lactic acid bacteria

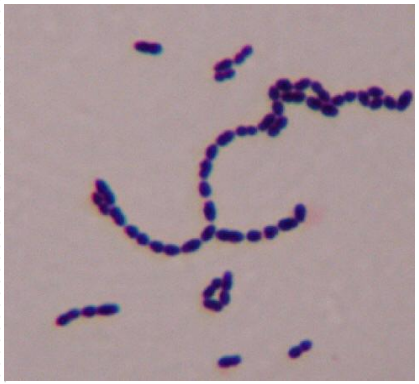
Streptococcus pyogenes (Group A streptococcus)

- **General characteristics:**
- *Lactobacllaceae (Streptococcus)*
- Gram-positive
- **Catalase negative,** Oxidase negative, Nitrate negative
- Nonmotile
- Non sporeforming
- Coccus, occurs in chains or in pairs of cells (divide in one plane and occur in pairs or (especially in liquid media or clinical material) in chains of varying lengths.

- The metabolism of *S. pyogenes* is fermentative; the organism is facultative anaerobic
- requires enriched medium containing blood to grow.
- Group A streptococci typically have a capsule composed of **hyaluronic acid**
- Beta hemolysis on blood agar







Strep. pyogenes. **Right. Colonies of *Strep. pyogenes* on blood agar exhibiting beta (clear) hemolysis.**

Left. Gram stain of *Strep. pyogenes* in a clinical specimen and from pure culture.

Pathogenesis

Strep. pyogenes is one of the most frequent pathogens of humans.

Its major success pathogen to its ability to colonize, rapidly multiply and spread in host while evading phagocytosis and confusing the immune system.

- It is found usually in the respiratory tract, without signs of disease. As normal flora, *Strep. pyogenes* can infect when defenses are compromised or when its able to penetrate the constitutive defenses. When the bacteria are transmitted to vulnerable tissues, a variety of types of **suppurative infections** can occur.

- Streptococcal diseaces is most often associated in the **respiratory tract (pharyngitis or tonsillitis)**, **bloodstream**, or the **skin (pyoderma)**.

- *S. pyogenes* is usually an **exogenous secondary invader**, following viral disease or disturbances the normal flora

- Generally, streptococcal isolates from the pharynx and respiratory tract do not cause skin infections.

- Some strains of streptococci show a predilection for the respiratory tract; others, for the skin.

Pathogenesis - Summary of diseases caused by *Strep. pyogenes*

- **Suppurative infections** (active infections associated with pus occur in the throat, skin & systemically).
- **Acute diseases: Respiratory infections: Throat:** *S. pyogenes* is the leading cause of **pharyngitis** (**strep throat).** It is acquired by inhaling aerosols by infected individuals. The symptoms reflect the inflammatory events at the site of infection. (1-3%) people develop rheumatic fever.
- Other respiratory infections include **sinusitis**, **otitis**, and **pneumonia**.
- **Skin: Impetigo** (superficial) infection of epidermal layers of skin.
- **Cellulitis** (deep) occurs when the infection spreads subcutaneous tissues.
- **Erysipelas** infection of the dermis (a form of cellulitis accompanied by fever & systemic toxicity) is less common today.
- **Necrotizing fasciitis** (destructive wound infections) infection of the fascia & may proceed rapidly to underlying muscle. It is **severe invasive infections**, prompting descriptions of "**flesh eating bacteria**" in the news media.
- **puerperal fever** (sepsis after childbirth).
- **Systemic** : **Scarlet fever** (rash) is a severe complication of streptococcal infection, but now, because of antibiotic therapy, it is little more than streptococcal **pharyngitis** accompanied by rash. It is caused by erythrogenic toxin.
- **Toxic shock** is caused by a few strains that produce a toxic shock toxin. Scarlet fever & streptococcal toxic shock syndrome are systemic responses to circulating bacterial toxins.
- Invasive, toxigenic streptococci cause **joint or bone infections**, and **myositis**, **meningitis** and **endocarditis, Bacteremia**
- **Non-suppurative Sequelae: Two post streptococcal sequelae rheumatic fever** & **glomerulonephritis**, may follow streptococcal disease, and occur in 1-3% of untreated infections. Due to immunological reactions to *Strep. pyogenes* antigens. Some of the antibodies produced during the above infections cross-react with certain host tissues. These can indirectly damage host tissues
- after the organisms cause non suppurative complications.
- **Rheumatic fever. M protein** cross reacts with sarcolemma.- Antibodies cross-react with heart tissue, fix complement, and cause damage.
- **Glomerulonephritis.** Antigen-antibody complexes may be deposited in kidney, fix complement, and damage glomeruli- Only a few M-types are nephritogenic.

Virulence Factors

- *Strep. pyogenes* produces a wide array of **virulence factors** and a very large number of diseases, **include:**
- (1) **M protein**, fibronectin-binding protein (**Protein F**) and **lipoteichoic acid** for adherence (inhibit phagocytosis)
- (2) **hyaluronic acid capsule** (non antigenic) as an immunological disguise and to inhibit or prevent opzonized phagocytosis by neutrophils or macrophages
- (3) **invasins** such as Haemolysins, **streptokinase**, **hyaluronidase** and **streptolysin (**streptolysin O, streptolysin S) (ASO test)

Haemolysin	Streptolycin O	Streptolysin S
stability of oxygen	**No**	**Yes**
Antigenic	**yes**	**No (small)**

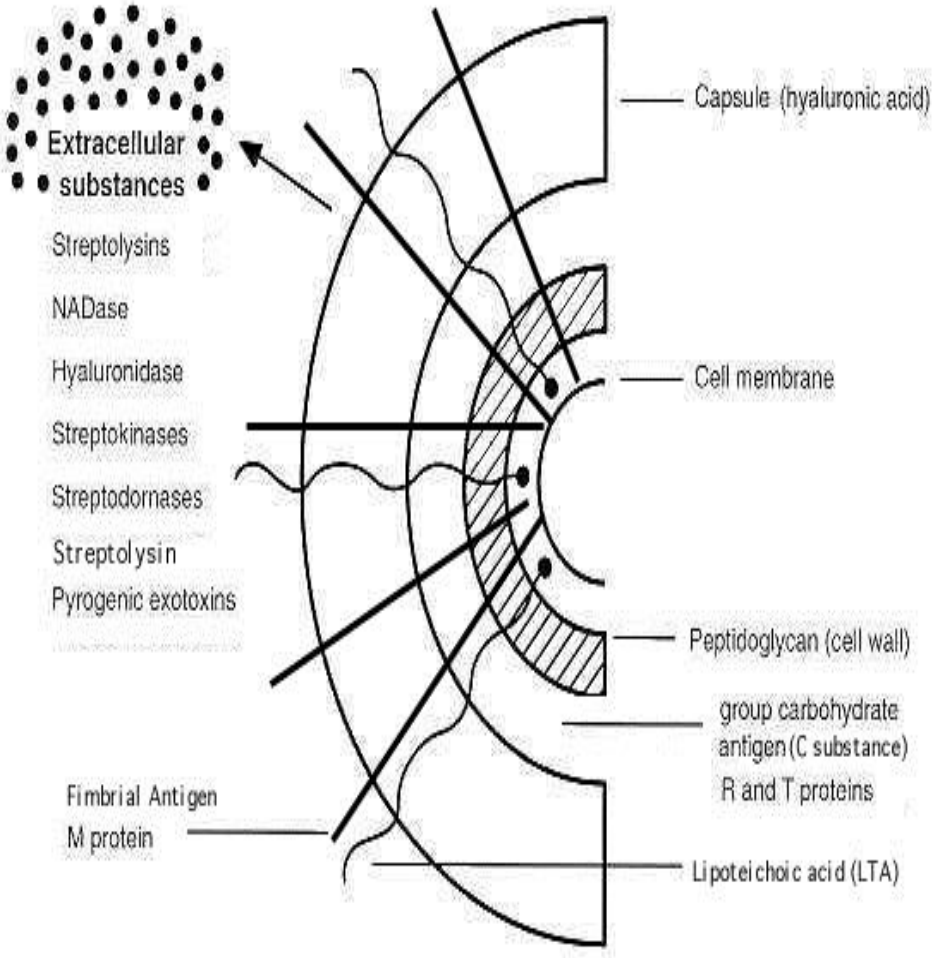
- (4) exotoxins, such as **pyrogenic (erythrogenic) toxin** which causes the rash in **scarlet fever** and systemic **toxic shock syndrome**.
- **5-DNAase (streptodornase- 4 types)**
- 6-Protease
- 7- Amylase
- 8- **C5a peptidase (evasion of pahgocytosis)- C5a enhances chemotaxis of phagocytes .**

The **cell surface** of *Strep. pyogenes* contains many of virulence determinants, especially with colonization and evasion of phagocytosis and the host immune responses.

The surface of *Strep. pyogenes* is complex & chemically-diverse.

Antigenic components include
Specific carbohydrate layer (C-substance), cell wall **peptidoglycan** and **lipoteichoic acid (LTA)**, a variety of surface proteins, including **M protein**, **fimbrial proteins**, **fibronectin-binding proteins**, (e.g. **Protein F**) and cell-bound **streptokinase**.

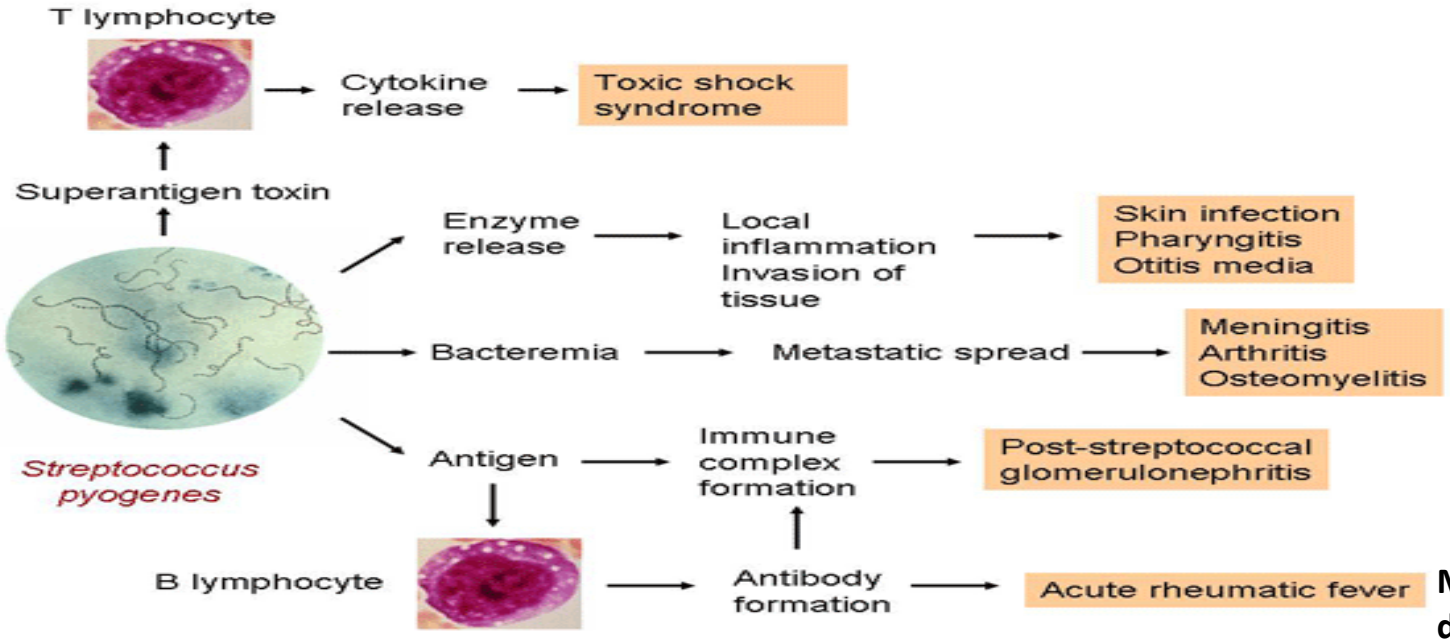
The cytoplasmic membrane of *Strep. pyogenes* contains some antigens similar to those of human cardiac, skeletal, and smooth muscle, heart valve fibroblasts, resulting in **molecular mimicry** and a tolerant or suppressed immune response by the host.



Cell surface structure of *Strep. pyogenes* secreted products involved in virulence.

Summary of virulence determinants of *S. pyogenes* *(further information)*

- **Adherence (colonization) surface macromolecules** M protein
  Lipoteichoic acid (LTA)
  Protein F (fibronectin-binding proteins)
- **Enhancement of spread in tissues** Hyaluronidase hydrolyses hyaluronic acid
  Proteases
  Streptokinase lyses fibrin
- **Evasion of phagocytosis** Capsule: hyaluronic acid.
  C5a peptidase: C5a enhances chemotaxis of phagocytes .
  M protein is a fibrillar surface protein. It is interferes with phagocytosis. It also blocks complement deposition on the cell surface. Mutations during the course of infection alter the structure of M proteins, rendering some antibodies ineffective. Strains that persist in carriers frequently exhibit altered M proteins.
  Leukocidins, including streptolysin S and streptolysin O, are proteins secreted by the streptococci to kill phagocytes (and probably to release nutrients for their growth)



Mechanisms of disease due to *Strep. pyogenes*

Host defenses

. In the normal human the **skin** is an effective barrier against invasive streptococci, and **nonspecific defense** mechanisms prevent the bacteria from penetrating the superficial epithelium of the **upper respiratory tract** (cilia movement, coughing, sneezing and epiglottal reflexes).

The **host phagocytic system** is a **second line** of defense against streptococcal invasion. Organisms can be opsonized by activation of the **complement** pathway and by **anti-streptococcal antibodies** in the serum.

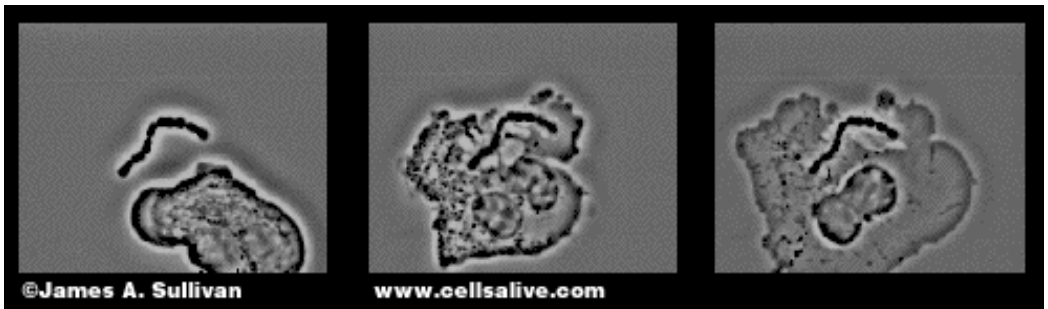
Strep. pyogenes is rapidly killed following phagocytosis enhanced by specific antibody.

The bacteria do not produce **catalase** or significant amounts of **superoxide dismutase** to inactivate the oxygen metabolites (hydrogen peroxide, superoxide) produced by the oxygen-dependent mechanisms of the phagocyte. Therefore, they are quickly killed after engulfment by phagocytes. The streptococcal defense must be one to stay out of phagocytes.

- In **immune individuals**, **IgG antibodies** reactive with **M protein** promote phagocytosis which results in killing of the organism. This is the major mechanism to terminate Group A streptococcal infections.
- **Treatment and prevention**

Penicillin is still effective in treatment of GAS disease. It is important to identify and treat GAS infections to prevent sequelae. No effective vaccine has been produced, but specific M-protein vaccines are being tested.

- **M protein vaccines** are a major candidate for use against rheumatic fever, but certain M protein types cross-react antigenically with the heart and themselves may be responsible for rheumatic carditis. This risk of autoimmunity has prevented the use of Group A streptococcal vaccines.



Phagocytosis of *Strep. pyogenes* by a macrophage.

Avoiding Host defenses

- **Defense against host immune responses**
- **Antigenic disguise** provided by **hyaluronic acid capsule**
- **Antigenic variation.** Antibody against M protein antigen is the only effective protective antibody, but there are more than 50 different M types, and subsequent infections may occur with a different M serotype.

- **Production of toxins and other systemic effects**

Toxic shock exotoxin is **superantigen** that binds abnormally to the T cell receptor of many (up to 20% of) T cells. Exaggerated production of cytokines causes the signs of shock: fever, rash, low blood pressure. aberrant interaction between toxin, macrophage, and T cells.

- **Induction of circulating, cross-reactive antibodies**
- Some of the antibodies produced during infection by certain strains of streptococci cross-react with certain host tissues. These antibodies can indirectly damage host tissues, even after the organisms have been cleared, and cause autoimmune complications.

Phagocytosis

Review Questions

- 1- What do you know about *Streptococcus pyogenes*
- 2- Give four examples of acute disease that causes by GAS, two exapmles of non supparative sequels?
- 3- What is the virulence factors of *S. pyogenes*?
- What is the effective antibiotic to treat CAS infections? And what is the ineffective antibiotic against *S. aureus* infections?
- How can GAS avoid Host defenses? (points only)
- You studied three categories to classify Streptococcal species, what they are, discus only haemolytic basis and serology classification with give examples?
- Why the alpha haemolytic Streptococci not included with Lancifield classification
- What do you know about Rheumatic fever?
- Group A Streptococci surface is antigenic complex and chemically diverse. Discus?