How Bacterial Pathogens Avoid Phagocyte Killing

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Bacterial evasion of phagocyte killing

- Important step in the pathogenesis of bacterial disease
- Numerous bacterial mechanisms to avoid phagocyte killing
- Studies of bacterial immune evasion mechanisms are not only important for our understanding of the molecular pathogenesis of bacterial disease, but may also reveal which part(s) of the host immune system are of importance for host protection against bacteria

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Aim of presentation

- Background on phagocytic cells – macrophages and neutrophils
- Different types of phagocytosis and phagocytic killing mechanisms
- Examples of bacterial mechanisms to evade phagocytosis/phagocytic killing

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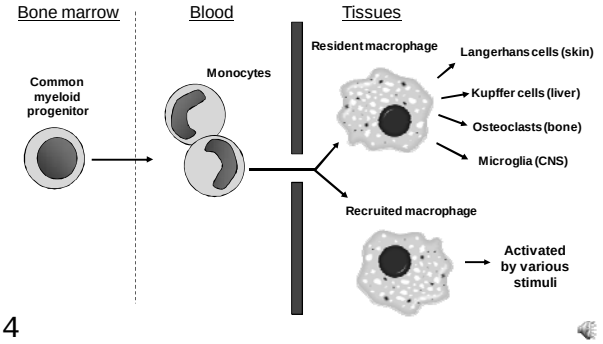
The macrophage



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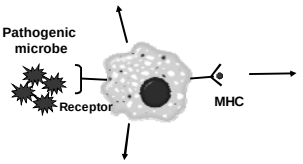
The macrophage – a central part of the immune system

1. Inflammatory response:

- Production of cytokines / chemokines – recruitment of neutrophils
- Secretion of antibacterial molecules, *e.g.* AMPs, phospholipases A_2



3. Antigen presentation:

- Presentation of antigens on MHC
- Activation of T- and B-cells
- Production of antibodies

2. Phagocytosis

- Killing of microbes
- Processing of microbial antigens

Innate immunity

Aquired immunity

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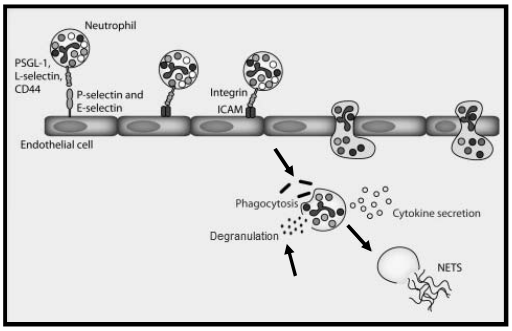
The neutrophil – effective killer of bacteria



6 Adapted from: Borregaard, *Immunity*, 2010, **33** (5) 657-670

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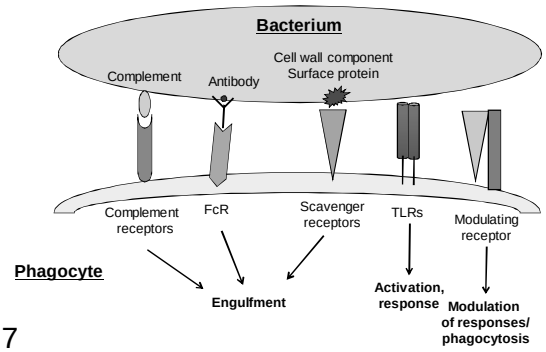
Phagocytosis – receptor-ligand interactions



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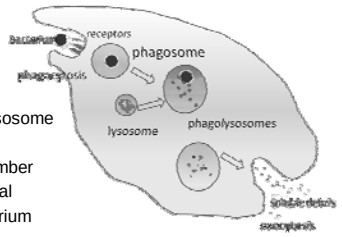
Phagocytosis – phagosome maturation and intracellular killing

- Immediately after uptake – phagosome maturation through fusion with:
  - Early endosomes
  - Multivesicular bodies
  - Late endosomes
- Final step – fusion with a lysosome to form the phagolysosome; the lysosome contains a number of enzymes and anti-bacterial molecules that kill the bacterium
- The pH in the phagosome decreases during maturation from 7.4 to 4.5 when the phagolysosome is formed



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Bacterial mechanisms to evade phagocytosis/phagocytic killing

1. Evasion of complement-mediated phagocytosis
2. Evasion of non-opsonic phagocytosis
3. Targeting of inhibitory receptors
4. Evasion *via* T3SS
5. Increased intracellular survival

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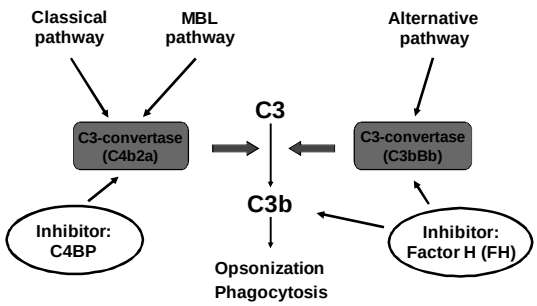
The complement system



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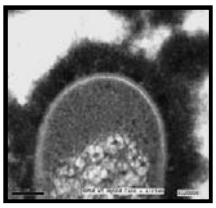
Evasion of complement – expression of a polysaccharide capsule

- Expression of polysaccharide capsule common among bacterial pathogens
  - *Neisseria meningitidis*
  - *Haemophilus influenzae*
  - *Streptococcus pneumoniae*
  - Group B streptococcus (GBS)
- Often important virulence factors with antiphagocytic properties affecting complement deposition/activation at the bacterial surface



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Group B streptococcus (GBS)

Neonatal infections

- The most common cause of life-threatening bacterial infections in newborns
- Pneumonia, septicaemia and meningitis

Infections in adults

- Growing clinical problem
- Skin infections, urinary tract infections and meningitis in adults with underlying illness

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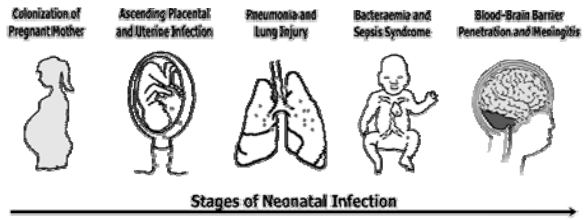
Pathogenesis of neonatal GBS infections



Doran and Nizet (2004) *Mol Microbiol* **54**: 23-31

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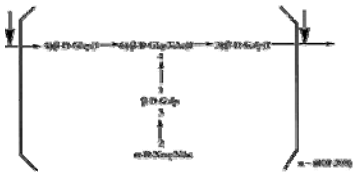
Polysaccharide capsule – structure and function

- Serotype III strains clinically most important
- Virulence factor
- Prevents complement-mediated phagocytosis by neutrophils
- The terminal sialic acid moiety is crucial for its anti-phagocytic properties

Structure of serotype III cps



Deng *et al.*, (2000) *J Biol Chem* **275**: 7497

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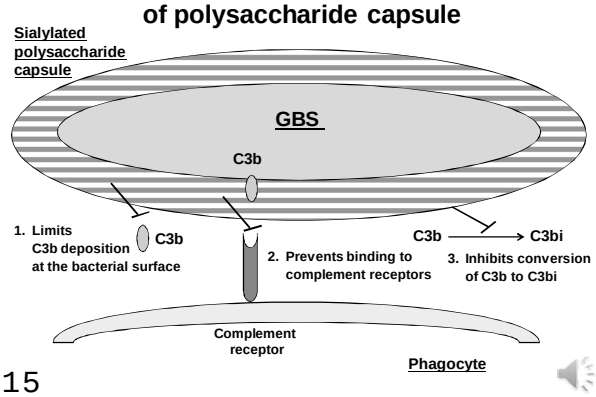
Antiphagocytic properties of polysaccharide capsule



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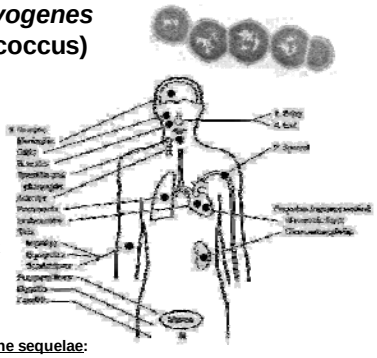
Streptococcus pyogenes (Group A streptococcus)



Self-limiting infections:

- Acute pharyngitis
- Skin infections (impetigo)
- Scarlet fever

Severe invasive disease:

- Necrotizing fasciitis
- Streptococcal toxic shock syndrome (STSS)
- Puerperal sepsis (childbed fever)

Post-infectious autoimmune sequelae:

- Acute glomerulonephritis (AGN)
- Acute rheumatic fever (ARF)

- **Globally ~500,000 mortality cases per year, of which ~50% are due to ARF** (Carapetis, *Lancet Infect Dis*, 20

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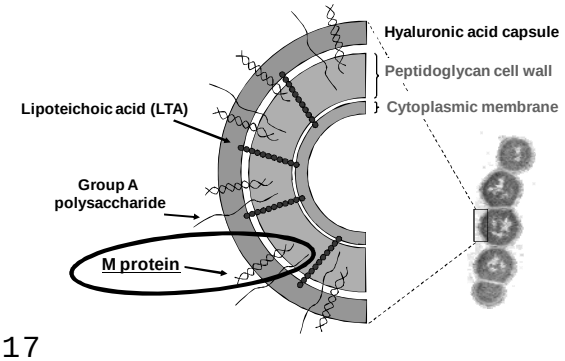
Schematic surface structure of *S. pyogenes*



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M protein – an antiphagocytic surface protein

Mix *S. pyogenes* with whole human blood

↓

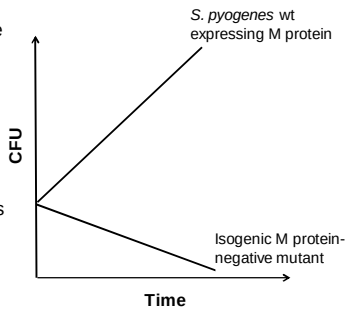
Incubate

↓

Plate onto blood agar plates

↓

Viable counting



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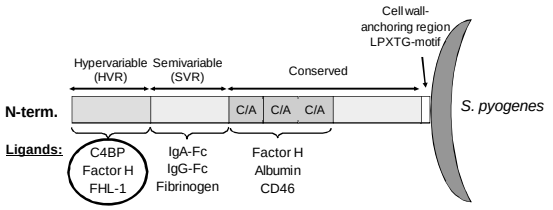
M protein – an antiphagocytic surface protein (2)



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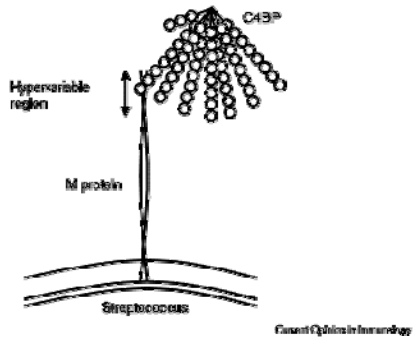
M protein – an antiphagocytic surface protein (3)



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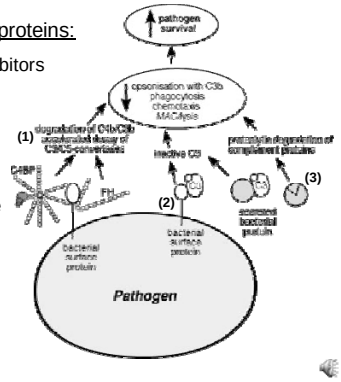
Evasion of the complement system

Bacterial surface/secreted proteins:

1. Binding of complement inhibitors (FH, C4BP) – inhibition of complement activation
2. Direct binding to C3 – prevents participation in the complement cascade
3. Proteolytic cleavage of complement proteins – inactivation



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Bacterial mechanisms to evade phagocytosis/phagocytic killing

Evasion of non-opsonic phagocytosis

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Pattern recognition receptors (PRR)

1. Signalling

   Toll-like receptors (TLR) – trigger an intracellular signalling pathway which culminates in induction of proinflammatory cytokines, chemokines, type I interferon and subsequent activation of phagocytes

2. Phagocytosis

   Scavenger receptor A (SR-A) – recognition/binding and non-opsonic phagocytosis of pathogenic microbes

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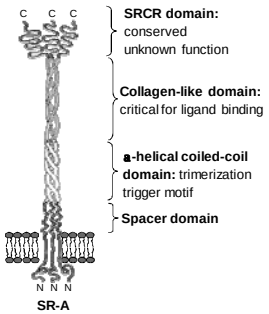
Scavenger receptor A (SR-A)

- Expressed by most MØ populations
- Endocytosis of mLDL contributes to foam cell formation in atherosclerosis

Role in immunity

- Acts as a PRR
- Phagocytic receptor: mediates direct non-opsonic phagocytosis of several bacterial species
- Contributes to resistance to experimental infection with Gram-positive bacteria (*L. monocytogenes, S. aureus, S. pneumoniae*)



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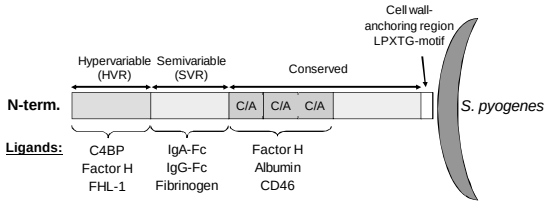
M protein – an antiphagocytic surface protein



- The M protein can mask other surface stuctures at the bacterial surface
- Can the M protein mask the ligands for PRRs such as SR-A?

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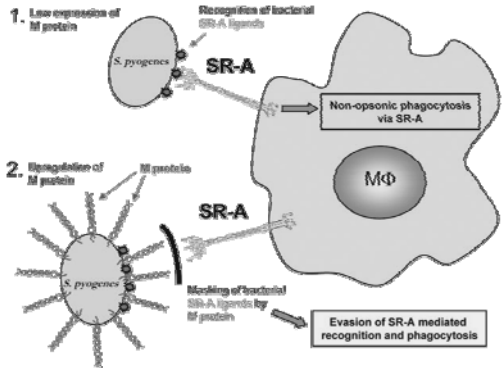
Evasion of SR-A mediated phagocytosis



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Areschoug and Gordon, *Cell Microbiol*, 2009; **11**: 1160-9

Bacterial mechanisms to evade phagocytosis/phagocytic killing

Targeting of inhibitory receptors

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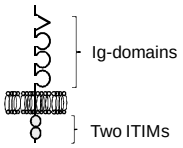
Human Siglec-5



- Expressed by neutrophils, monocytes, macrophages and basophils
- Four extracellular Ig-like domains
- Two cytosolic ITIMs (immunoreceptor tyrosine-based inhibitory motifs)

Plays a role in:

- Cell-cell interactions
- Inhibition of inflammatory signals

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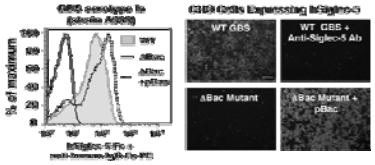
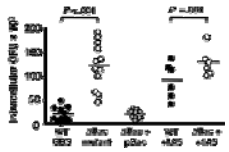
The b protein of GBS binds to Human Siglec-5 and inhibits phagocytosis

b-protein / bac expressing GBS

- Binds rec. Siglec-5 and surface anchored Siglec-5



- Inhibits phagocytosis



Carlin A F *et al.*, *J Exp Med* 2009

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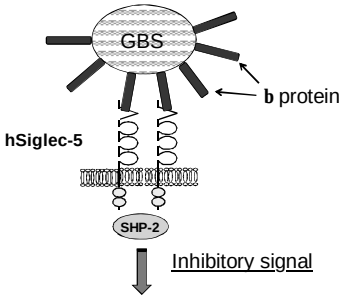
The b protein of GBS binds to Human Siglec-5 and inhibits phagocytosis (2)



Inhibition of: Phagocytic uptake, IL-8 secretion, oxidative burst and formation of NETs

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Bacterial mechanisms to evade phagocytosis/phagocytic killing

Type III secretion system

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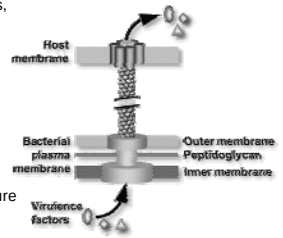
Type III secretion system (T3SS)

T3SS

- Found in many Gram-negative pathogens, including *Salmonella, Shigella, Yersinia, E. coli, Pseudomonas, Bordetella* and *Chlamydia*
- Often encoded by pathogenicity islands
- Highly conserved structure – structurally related to flagellum
- Direct translocation of effector proteins into host cell through a needle-like structure
- Main functions: promote bacterial uptake into non-phagocytic cells, inhibit phagocytic killing



The type III secretion system

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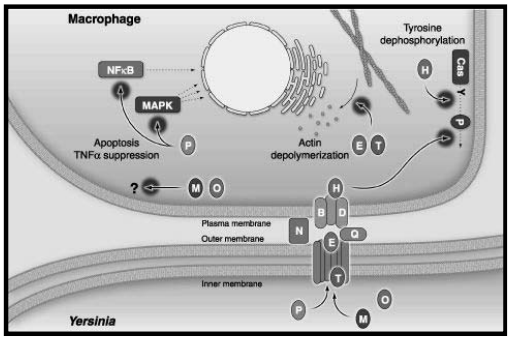
T3SS-mediated phagocytosis resistance in Yersinia



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Bacterial mechanisms to evade phagocytosis/phagocytic killing

Increased intracellular survival

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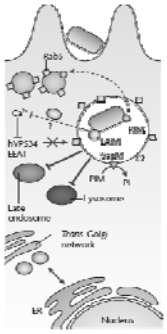
M. tuberculosis – preventing phagolysosomal maturation

- Uptake and intracellular survival of *Mtb* in macrophages is an important step in the pathogenesis of tuberculosis
- Phagocytosis through CR3 and other receptors
- The main strategy of intracellular survival is to prevent phagosomal maturation by preventing fusion with late endosomes and the lysosome
- Inhibition of PI(3)P signalling and Ca^{2+} influx the main mechanisms



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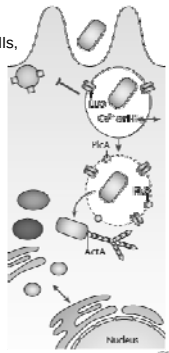
Flannagan RS, *et al.*, *Nat Rev Microbiol*, 2009, **7**: 355

L. monocytogenes – escaping the phagosome

- The cause of listeriosis
- Is internalized into both non-phagocytic and phagocytic cells, needed for its propagation and dissemination
- Uptake by macrophages occurs through scavenger receptors
- Early after uptake by macrophages, *L. monocytogenes* secretes listeriolysin O (LLO), which creates holes in the phagosome
- Inhibits the maturation of the phagosome because of loss of Ca^{2+} which is needed for fusion with endosomes/lysosomes
- Also express phospholipase C enzymes, which in concert with LLO cause the breakdown of the phagosome allowing the bacterium to escape into the cytosol



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Flannagan RS, *et al.*, *Nat Rev Microbiol*, 2009, **7**: 355

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Conclusions

- Bacterial pathogens have evolved diverse mechanisms to avoid phagocytosis
- Important steps in the molecular pathogenesis of bacterial disease
- Whereas most research has focused on evasion of complement-mediated phagocytosis or on how bacteria can survive inside phagocytes, less is known about mechanisms of how bacteria can evade non-opsonic receptors or how they target inhibitory receptors

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