SIRS: the BUGS view
Prof Mark Schembri
School of Chemistry & Molecular Biosciences, UQ
m.schembri@uq.edu.au

Meningococcal septicaemia
Hemorrhagic purpura due to disseminated intravascular coagulation

Bacterial features associated with dissemination

• Serum is CIDAL
  • Some bacteria are resistant to bactericidal action of serum
    – Gram negatives
      Capsule
      O Antigen of LPS
    – Gram positives
      Capsule
      Thick peptidoglycan layer

Gram negative cell wall

Gram-negative bacterial cell wall
Lipopolysaccharide (LPS)

O ANTIGEN

Oligosaccharide (sugars vary from species to species within genus)
Oligosaccharide (sugars are constant for each genus)
Lipid A (toxic)
Peptidoglycan
Antigenic and toxic
**Lipopolysaccharide structure**

![Image of LPS structure]

Important part of LPS for pathogenicity

- Smooth: +
- Rough: -

**Serum resistance**

<table>
<thead>
<tr>
<th>E. coli strain</th>
<th>+ O Ag</th>
<th>Serum</th>
<th>Lysis</th>
<th>Resistant to lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rough</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Smooth</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

**Classical pathway**
- Antibody binds to specific antigen on the bacterial surface

**Alternative pathway**
- Pathogen surface allows complement activation (Ab independent)

**Lectin pathway**
- Mannose binding protein binds to mannose on the bacterial surface

**COMPLEMENT ACTIVATION**

Opsonization → Cytolysis → Inflammation

**The complement cascade**

- **Opsonization**: Coating with C3b, enhanced phagocytosis
- **Inflammation**: Increase in blood vessel permeability, chemotactic attraction of phagocytes
- **Cytolysis**: Membrane attack complex

**Assembly of the Membrane Attack Complex**

**How does complement kill Gram-negative bacteria?**

1. **Complement proteins form holes in the bacterial cell wall**
   - Damage to OM and IM

2. **Influx of fluids and salts**

3. **Bacterium expands and bursts**
Bacterial properties that provide serum resistance

- Certain OAg’s protect bacteria from phagocytosis & cidal action of serum
  - smooth E. coli more resistant in serum assays than rough
  - degree of resistance proportional to LPS content
  - E. coli serotypes (O7, O8, O18) associated with septicemia survive better in serum

- Long side chains project OAg away from bacterial surface
  - Antibody reactions occur away from cell surface
  - less likely to have lytic effect
  - damage may occur but thick supportive LPS coat prevents lethality

- OAg masks underlying bacterial surface molecules that activate complement

Sepsis and bacterial cell lysis

- Autolysis
- Rapid and massive release of endotoxin

N. meningitidis

ENDOTOXIN

In small amounts

- Kupffer cells
- Increase in IL-1, TNF, IL-6
- Fever
- Activation of alternative pathway
- Inflammation

In large amounts

- All of the above plus shock and intravascular coagulation

LPS (endotoxin) is a powerful immune stimulus

- Gram -ve LPS
- LPS lysis
- CD14
- TLR4
- MABC attack
- Cytokines
  - IL-1, IL-6, IL-8, TNF-a
- Blood clotting
  - Coagulation pathway
  - Complement pathway
  - Opsonization
  - Inflammation

Some clinical conditions in which endotoxin has been implicated

- Septic shock
- Liver disease
- Inflammatory bowel disease
- Acute renal failure
- Glomerulonephritis
- Adult respiratory distress syndrome
- Major abdominal trauma
- Neonatal necrotising enterocolitis
- Radiation injury
- Toxic shock syndrome
If LPS is so toxic, why can't we simply vaccinate against it?

- Different types
  - Salmonella: 1 core (2000 O types)
  - E. coli: 6 cores (150 O types)
- Endotoxin not directly exposed
- Normal flora
  - Gram negatives in the gut
- Keeps immune system alert to infection
  - 1 E. coli cell contains ~ 5 x 10^6 LPS molecules

Gram Positive Shock

- Approx 50% of cases of sepsis and septic shock are caused by G+ves
- G+ve virulence factors that induce shock
  - Lipoteichoic acid
  - Peptidoglycan
  - Exotoxins
- Purified components can induce sepsis and septic shock
- Synergism
  - LTA and PGN?
  - Endotoxin from G+ve?

Gram positive cell wall components stimulate the immune system

- Gram +ve
- LTA
- PGN
- CD14
- TLR2
- Induction
- Lysis
- Blood clotting
- Inflammation
- Opsonization
- Cytokines
  - IL-1, IL-6, IL-8, TNF-α
- Coagulation pathway
- Complement pathway
- CD14

Exotoxins

- Secreted bacterial toxins
- Bound to surface and released upon lysis
- Mechanisms of action
  - Spreading factors that facilitate dispersal
  - Active killing of host cells by destroying their membranes
  - Prevention of protein synthesis
  - Alteration of normal cell function
  - Blocking of nerve function
  - Superantigens

Microbial Superantigens

- Protein exotoxins
- Trigger a non-specific T cell response
- Damage by induction of hypersensitivity reactions
- Examples of Superantigens:
  - Staphylococcus aureus toxic shock syndrome toxin
  - Staphylococcal enterotoxins
  - Streptococcus pyogenes erythrogenic toxin
Activation of T cell by bacterial antigens

**Antigen-mediated T cell activation**
- Antigen specific T cell clone
- Activation (1 in 10⁵)
- TCR
- Antigen presentation cell

**Superantigen-mediated T cell activation**
- T cells of a specific TCR type
- Activation (1 in 5)
- TCR
- Superantigen
- Antigen presentation cell

Differences between G-ve and G+ve sepsis

<table>
<thead>
<tr>
<th>Gram negatives</th>
<th>Gram positives</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Triggers</strong></td>
<td>LPS (endotoxin)</td>
</tr>
<tr>
<td><strong>Signaling mechanism</strong></td>
<td>TLR4</td>
</tr>
<tr>
<td><strong>Evidence</strong></td>
<td>Purified LPS mimics</td>
</tr>
<tr>
<td><strong>Source of bacteria</strong></td>
<td>GI, genitourinary tract</td>
</tr>
<tr>
<td><strong>Bacterial killing</strong></td>
<td>Killed by Complement and Antibody</td>
</tr>
</tbody>
</table>

**Net host response is the same**

Potential sites of action in adjunctive therapies for septic shock

1. **Endotoxin and other bacterial products**
   - Responsive cells: Macrophages, Neutrophils, Endothelium
   - Release of secondary inflammatory mediators:
     - Cystokines, Prostanoids, Leukotrienes, PAFs, Kinins
     - Activation of coagulation
     - Complement activation
   - Hypotension
   - Vasodilation, Myocardial depression
   - Tissue damage:
     - Hypoxia, Neutrophil migration, reactive oxygen metabolites, Proteolytic enzymes

   **Further cellular activation**
   - 1. Prevent activation of host cells
   - 2. Inhibition of secondary mediators
   - 3. Limit organ damage