VRBPAC Meeting

Staphylococcus aureus – a dangerous and difficult-to-tackle pathogen

November 7\textsuperscript{th}, 2017
S. aureus causes a multitude of infections

- Lung infections (Pneumonia, influenza-associated)
- Skin infections (mostly moderately severe, rarely necrotizing fasciitis)
- Bone infections (e.g., osteomyelitis)
- Blood infections (bacteremia, sepsis)
- Indwelling medical device associated infections (e.g., endocarditis)
- Specific toxin-related diseases (toxic shock syndrome, food-borne illness)
S. aureus is a leading cause of death in hospitalized patients

- “Staphylococcus aureus bacteremia (SAB) is an important infection with an incidence rate ranging from 20 to 50 cases/100,000 population per year. Between 10% and 30% of these patients will die from SAB. Comparatively, this accounts for a greater number of deaths than for AIDS, tuberculosis, and viral hepatitis combined.” (van Hal et al. Clin Microbiol Rev 2012)

- In the U.S., invasive (serious) MRSA infections occur in ~ 94,000 people each year causing ~ 19,000 deaths. (Kleven et al., JAMA 2007)

  85% of those are hospital-associated.
Antibiotic resistance in *S. aureus*: MRSA

- Penicillin, 1941
- Penicillin-resistant *S. aureus*
- Pandemic *S. aureus*
- Methicillin, 1959
- Methicillin-resistant *S. aureus* (MRSA)
- 1st MRSA outbreak in USA
- Worldwide spread of MRSA
- Community MRSA

Community-associated MRSA

- “Resistance and virulence converge” (H. F. Chambers)
- Characteristically as virulent as many MSSA and more virulent than hospital-associated MRSA
- 15% of invasive MRSA infections
- Pulsed-field type USA300 in the U.S.
- Increasing on a global scale with a multitude of genetically divergent strains
- USA300 now also frequent in hospitals
Strategies to overcome the problem of AMR in *S. aureus*

- Novel antibiotics
- Anti-virulence drugs
- Probiotics
- mAbs (toxin-directed)
- Vaccines
Who could benefit from an *S. aureus* vaccine?

<table>
<thead>
<tr>
<th>Active immunisation</th>
<th>Passive immunisation</th>
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<tbody>
<tr>
<td>Haemodialysis patients</td>
<td>Patients undergoing emergency surgery</td>
</tr>
<tr>
<td>Residents of nursing homes and other</td>
<td>Patients implanted with intravascular or</td>
</tr>
<tr>
<td>long-term care facilities</td>
<td>prosthetic devices</td>
</tr>
<tr>
<td>Men who have sex with men</td>
<td>Trauma victims</td>
</tr>
<tr>
<td>Military personnel</td>
<td>Immunocompromised individuals</td>
</tr>
<tr>
<td>Prisoners</td>
<td>Low birthweight neonates</td>
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<tr>
<td>Patients undergoing elective surgery</td>
<td>Patients in intensive care units</td>
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<tr>
<td>Individuals with diabetes</td>
<td></td>
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<tr>
<td>Patients with HIV</td>
<td></td>
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<tr>
<td>Intravenous drug users</td>
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<tr>
<td>Healthcare providers</td>
<td></td>
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<tr>
<td>Athletes</td>
<td></td>
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<tr>
<td>School children</td>
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</tbody>
</table>

HIV, human immunodeficiency virus.

Schaffer & Lee *Int J Antimicrob Agents* 2008
S. aureus infections are mainly controlled by innate host defense

- Previous S. aureus infection does not protect from re-infection.
- S. aureus infection is controlled by innate host defense (mostly neutrophils).
- Patients with defects in innate host defenses (e.g., CGD patients) are much more prone to develop S. aureus infection.
- The role of protective immunity in staphylococcal infections, if any, is poorly understood.
S. aureus has multiple factors to subvert innate host defenses

- Many proteins that block pathogen recognition receptors (PRRs) and complement cascade
- Toxins that attack white blood cells: Leukotoxins [AB (GH), ED, SF (PVL)], PSMalpha
- Toxins that attack a wide variety of cell types including leukocytes: alpha-toxin, PSMalpha, gamma-toxin, delta-toxin
- Toxins that destroy leukocytes after phagocytosis: LukAB (GH), PSMalpha: a particular problem for drug and vaccine development
- Systems to sense and evade the activity of antimicrobial peptides (AMPs): Dlt, MprF, surface polysaccharides, sensor Aps (Gra)
The role of adaptive immunity in *S. aureus* infections

- Humans produce antibodies to staphylococcal antigens, but they do not seem to offer significant protection.
- Protective immunity seen in animal infection models is not completely understood mechanistically, but appears to be mediated by T-cells.
- Role of T-cell mediated protection is being unraveled.

Broeker et al. *Pathogens* 2016
Dual mechanisms of Protein A to subvert antibody-mediated opsonophagocytosis

- SpA present on the surface of *S. aureus* binds the Fc region of antibody, thereby inhibiting opsonophagocytosis.
- Alternatively, SpA binds the Fab regions of the B-cell receptor, which induces B-cell death and prevents the production of antibody specific for *S. aureus*.

O. Schneewind
http://www.who.int/immunization/research/forums_and_initiatives/4_OSchneewind_Staphylococcal_Vaccines_gvirf16.pdf?ua=1
S. aureus circumvents killing after phagocytosis

S. aureus is opsonized by bacteria-specific antibody and serum complement, which promote rapid binding and uptake of the bacteria by neutrophils. After uptake, S. aureus uses multiple mechanisms to survive and cause the death of the cell, allowing the escape of sequestered bacteria.

Mechanisms of Resistance:
- Resistance to ROS-, AMP- mediated killing
- Neutrophil-lytic toxins:
  - PSMalpha
  - LukAB (GH)

DeLeo & Otto
S. aureus survives intracellularly

- Instead of killing leukocytes after phagocytosis, S. aureus may persist intracellularly.
- Intracellular S. aureus may provide for immune evasion and recurrent, persistent infection.
- Role of small colony variants (SCVs).
Biofilms broadly inhibit immune defenses
S. aureus forms large biofilm-like aggregates in synovial fluid

After 20 min in SF, fibers cover bacterial clumps and macroscopic aggregates develop.

Likely a common phenotype in all orthopedic infections

1. Initial binding to fibrin and fibronectin mediated by ClfA, ClfB, FnbA, FnbB

2. Low activity of Agr causes low PSM production, resulting in increased cell-cell attachment and surface attachment of the biofilm matrix molecule PIA

Dastgheyb et al. J Infect Dis 2015
Evidence for the role of immune mechanisms in \textit{S. aureus} systemic infection from knockout mice

B-cell-deficient: no difference

Hypersusceptibility:
T-cell-deficient
IFN-gamma-deficient
TNF-deficient
IL 17A/F-deficient

There is T-cell and possibly innate immunity-mediated protective memory against \textit{S. aureus} infections in mice. In humans?

Lin et al. \textit{PloS Pathog} 2009
Ishigame et al. \textit{Immunity} 2009
## Previous active vaccination attempts

<table>
<thead>
<tr>
<th>Drug</th>
<th>Company</th>
<th>Target</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>StaphVAX</td>
<td>NABI</td>
<td>CP5/CP8</td>
<td>Failed phase 3</td>
</tr>
<tr>
<td>Pentastaph</td>
<td>NABI (=&gt; GSK)</td>
<td>CP5/CP8/WTA/PVL/alpha-toxin</td>
<td>Phase 1 (PVL/alpha-toxin) ended</td>
</tr>
<tr>
<td>V710</td>
<td>Merck</td>
<td>IsdB</td>
<td>Failed phase 3</td>
</tr>
<tr>
<td>NVD3</td>
<td>Novadigm</td>
<td>Als3</td>
<td>Phase 1/2</td>
</tr>
<tr>
<td>STEBVax</td>
<td>IBT</td>
<td>SEB</td>
<td>Phase 1</td>
</tr>
<tr>
<td>SA3Ag</td>
<td>Pfizer</td>
<td>CP5/CP8/ClfA</td>
<td>Phase 1</td>
</tr>
<tr>
<td><strong>SA4Ag</strong></td>
<td>Pfizer</td>
<td><strong>CP5/CP8/ClfA/MntC</strong></td>
<td>Phase 2b</td>
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S. aureus vaccines: previous phase-III attempts

- **StaphVax (Nabi)**
  - Antigen: Capsular polysaccharides CP5 and CP8
  - Failed phase III (Hemodialysis patients)
  - Likely problems: poor protective efficacy, modest contribution to virulence
  - Not present in important clinical strains (e.g., USA300)

- **V710 (Merck)**
  - Antigen: IsdB (surface protein involved in iron uptake)
  - Failed phase II/III in patients undergoing cardiothoracic surgery with median sternotomy (no efficacy; safety concerns)
S. aureus vaccines: optimal antigen characteristics

- Immunogenicity in humans
- Surface location
- Essential for in-vivo survival (growth, virulence)
- Widespread in epidemiologically important strains
Pfizer SA4Ag vaccine targets

- Recombinant mature form of ClfA
- Conjugated CP5 & CP8
- Recombinant MntC
Pfizer vaccine targets: CP5/CP8

- No efficacy in StaphVax trial
- Only modest contribution to virulence O’Riordan & Lee *Clin Microbiol Rev* 2004
- Capsule-negative mutants are more virulent than the parental isolates in catheter-induced endocarditis model. Baddour et al. *J Infect Dis* 1992
Pfizer vaccine targets: ClfA

- Surface protein, fibrinogen/fibrin-binding
- Nasal colonization factor
- Virulence factor in bloodstream infection, septic arthritis, skin infection
- Anti-ClfA Abs efficacious in mouse models for prevention of sepsis, in combination with
  alpha-toxin Ab or thrombin inhibitors
  Tkaczyk et al. MBio 2016, McAdow PLoS Pathog 2011
- Passive immunization with human anti-ClfA-enriched intravenous (i.v.) immunoglobulin (Ig) (Veronate) did not result in significantly changed rates of late-onset sepsis. De Jonge et al. J Pediatr 2007; an anti-ClfA mAb (Aurexis) failed to treat S. aureus bacteremia
- Conflicting results of targeting ClfA alone by active immunization
  Li et al. MBio 2016 (mouse abscess, surgical wound, bacteremia); Joseffson et al. J Infect Dis 2001 (mouse arthritis); Narita et al. Infect Immun 2010 (mouse bacteremia)
Pfizer vaccine targets: MntC

- Manganese transporter; conserved in staphylococci
- Active vaccination with MntC reduced bacterial load (~ 1 log) in murine bacteremia model (S. aureus & S. epidermidis)
- Anti-MntC mAbs reduced CFUs in rat intraperitoneal infection model (~ 1 log).

  Anderson et al. J Infect Dis 2012

- Membrane-inserted protein (lipoprotein): accessibility?