Pathogenesis of Gram-positive bacteria: clues for therapy

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NF - 2 days ceftriaxone

Bacterial count

<table>
<thead>
<tr>
<th>Day 0</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 × 10^3</td>
<td>1 × 10^5</td>
</tr>
</tbody>
</table>

Necrotic tissue

Healthy tissue

Group A Streptococcus

Streptococcus pyogenes

Ceftrixone
Group A Streptococcus

*Streptococcus pyogenes*

GAS carriage rates: 15-20% in children, 1-2% adults

Commensal Misbehaving:
Global burden of disease / year:
- 600 million pharyngitis (strep throat)
- 663,000 invasive infections
- 163,000 deaths

Lethality 35%

Streptococcal toxic shock syndrome
Necrotizing fasciitis: Rapidly-progressive, destructive infection of the soft tissues.
Commensals- Pathogens

HOST: Innate Resistance

Commensals:
- Digestion
- Vitamin production
- Space holder
- Immune functions

Pathogens:
- Virulence Factors

BACTERIA:
Commensal in the right place
Pathogen in the wrong place
Virulence factors
Commensals misbehaving
Severe invasive GAS infections

Acquisition of additional virulence factors

Know your enemy

Group A Streptococci before 1980

Group A Streptococci after 1980
# Invasive GAS disease increasing

## Table

Number of cases of invasive group A streptococcal infections and incidence per 100,000 inhabitants, Sweden, 2006–2012

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>2006 (incidence)</th>
<th>2007 (incidence)</th>
<th>2008 (incidence)</th>
<th>2009 (incidence)</th>
<th>2010 (incidence)</th>
<th>2011 (incidence)</th>
<th>2012 (incidence)</th>
<th>Mean incidence 2006-2011</th>
<th>Difference in incidence (95% CI)</th>
<th>Incidence rate ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-19</td>
<td>25 (1.2)</td>
<td>40 (1.9)</td>
<td>31 (1.4)</td>
<td>30 (1.4)</td>
<td>17 (0.8)</td>
<td>33 (1.5)</td>
<td>45 (2.1)</td>
<td>1.4</td>
<td>0.73 (0.35-1.11)</td>
<td>1.65 (1.09-2.22)</td>
</tr>
<tr>
<td>20-39</td>
<td>42 (1.8)</td>
<td>58 (2.5)</td>
<td>60 (2.5)</td>
<td>60 (2.5)</td>
<td>38 (1.6)</td>
<td>58 (2.4)</td>
<td>69 (2.8)</td>
<td>2.2</td>
<td>0.57 (0.12-1.01)</td>
<td>1.30 (1.00-1.60)</td>
</tr>
<tr>
<td>40-59</td>
<td>79 (3.2)</td>
<td>85 (3.5)</td>
<td>96 (3.9)</td>
<td>91 (3.7)</td>
<td>78 (3.2)</td>
<td>77 (3.1)</td>
<td>116 (4.7)</td>
<td>3.4</td>
<td>1.23 (0.90-1.56)</td>
<td>1.37 (1.24-1.49)</td>
</tr>
<tr>
<td>60-79</td>
<td>110 (6.5)</td>
<td>137 (7.9)</td>
<td>157 (8.8)</td>
<td>156 (8.5)</td>
<td>129 (6.9)</td>
<td>118 (6.2)</td>
<td>218 (11.4)</td>
<td>7.5</td>
<td>3.89 (2.76-5.02)</td>
<td>1.55 (1.31-1.78)</td>
</tr>
<tr>
<td>≥80</td>
<td>67 (13.4)</td>
<td>89 (17.8)</td>
<td>118 (23.5)</td>
<td>101 (20.1)</td>
<td>98 (19.3)</td>
<td>116 (22.9)</td>
<td>136 (26.8)</td>
<td>19.5</td>
<td>7.30 (3.45-11.15)</td>
<td>1.42 (1.09-1.75)</td>
</tr>
</tbody>
</table>

Cl: confidence interval.
Pathogenesis

*S. aureus* and Streptococci

- **Phages** – virulence factors
- **Adhesion & Invasion**
  - M protein, protein A
- **Immunmodulation**
  - Cytolysis (PVL, α-toxin, PSM, SLO, SLS)
  - Proteases (IL-8)
  - DNases
- **Immunstimulation** Superantigens
- **Extracellular bacteria** – Intracellular persistence
Disarm the pathogens:
Inhibition of bacterial virulence factors

- **Diphtheria**: caused by a polypeptide exotoxin of *Corynebacterium diphtheriae*
  - Diphtheria antitoxin 1890s: in horses hyperimmunised with diphtheria toxoid
  - diphtheria toxoid containing vaccines
  - Endemic countries: Afghanistan, Bangladesh, Cambodia, China, India, Indonesia, Malaysia, Nepal, Pakistan, Papua New Guinea, the Philippines, Thailand, Vietnam and the Pacific Islands
Disarm

• **Disadvantages:** Pathogen is not killed

• **Advantages:**
  – Very specific, no collateral damage, i.e. diversity, microbe–microbe interactions
    • Skin/Mucosa: human skin/nasal commensals affect *S. aureus* behavior and fitness
      – Corynebacteria control *S. aureus*, pneumococci
        » Ramsey MM et al, 2016, Frontiers in Microbiology
    • Gastrointestinal: *Clostridium difficile*-associated diarrhea
      – pathology induced by two exotoxins: toxin A and toxin B, antibodies specific for TcdA and TcdB
BULLETIN OF
THE NEW YORK ACADEMY
OF MEDICINE

AUGUST 1938

THE TREATMENT OF HEMOLYTIC STREPTOCOCCUS INFECTIONS AND THE NEWER APPLICATIONS OF SULPHANILAMIDE*

REUBEN OTTENBERG
Rate of bacterial killing by beta-lactams is proportional to the bacterial growth rate.

Tuomanen *et al*, J Gen Microbiology, 1986
Antibiotics

• **Cell wall active antibiotic:**
  – Penicillin: 100% susceptibility

• **Protein synthesis inhibitor:**
  – Clindamycin
    • clindamycin is not affected by the inoculum size or stage of bacterial growth
Antibiotics: Eliminate + Disarm

- **Cell wall active antibiotic:**
  - Penicillin: 100% susceptibility

- **Protein synthesis inhibitor:**
  - Clindamycin
    - clindamycin is not affected by the inoculum size or stage of bacterial growth
    - suppressor of bacterial toxin synthesis
      - Sriskandar et al., J Antimicrob Chemother. 1997
      - Mascini et al., Int J Antimicrob Agents 2001
      - Goscinski G et al., Scand J Infect Dis 2006

IDSA Guidelines, CID 2005
# Reality


## Table 2. Clinical Manifestations and Treatment of 84 Patients With Severe Invasive Group A Streptococcal Disease

<table>
<thead>
<tr>
<th>Manifestation</th>
<th>No. of Patients</th>
<th>No. (%) Treated With Clindamycin</th>
<th>No (%) Treated With IVIG$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF + STSS</td>
<td>20</td>
<td>20 (100)</td>
<td>7 (35)</td>
</tr>
<tr>
<td>NF + septic shock</td>
<td>1</td>
<td>1 (100)</td>
<td>0</td>
</tr>
<tr>
<td>NF alone</td>
<td>8</td>
<td>7 (88)</td>
<td>1 (13)</td>
</tr>
<tr>
<td>STSS alone</td>
<td>29</td>
<td>17 (59)</td>
<td>6 (21)</td>
</tr>
<tr>
<td>Septic shock alone</td>
<td>16</td>
<td>6 (38)</td>
<td>0</td>
</tr>
<tr>
<td>Severe cellulitis</td>
<td>10</td>
<td>2 (20)</td>
<td>0</td>
</tr>
<tr>
<td>Total severe iGAS</td>
<td>84</td>
<td>53 (63)</td>
<td>14 (17)</td>
</tr>
</tbody>
</table>
Antibiotics: Eliminate + Disarm

- **Cell wall active antibiotic:**
  - Penicillin: 100% susceptibility

- **Protein synthesis inhibitor:**
  - Clindamycin
  - clindamycin is not affected by the inoculum size or stage of bacterial growth
  - suppressor of bacterial toxin synthesis
  - **Clindamycin-resistant** GAS, NF VF?
    - China Emerg Infect Dis 2012
    - France Plainvert Med Mal Infect. 2015
    - Germany clindamycin (0.7%) Imöhl M et al Plos One 2015
    - Israel Chazan Microb Drug Resist. 2015

IDSA Guidelines, CID 2005
Severe invasive GAS infections

Acquisition of additional virulence factors
Know your enemy

Group A Streptococci
bevor 1980

Group A Streptococci
after 1980

Global dissemination of clone with severe invasive disease capability

Minor transcriptional alterations

36-kb chromosomal replacement (circa mid-1980s)

Ancestral 36-kb region

\( \text{speA} \text{ sdaD2} \)

Ancestral 36-kb region

\( \text{speA} \text{ sdaD2} \)

M12 36-kb region

\( \text{speA} \text{ sdaD2} \)

Breakdown of NETs

\( \text{sdaA} \text{ DNAse} \)
Neutrophil Extracellular Traps

- Released from neutrophils
- Trap and kill pathogens
- DNA backbone with embedded cationic histones, antimicrobial peptides and granule proteases with antibacterial properties

Katrin Schilcher
Bacterial nucleases degrade NETs

Released from neutrophils
Trap and kill pathogens

DNA backbone with embedded cationic histones, antimicrobial peptides and granule proteases with antibacterial properties

GAS and S. aureus

DNase ko  WT
Protein synthesis inhibitor reduces DNA degradation in CLI R clinical isolates
Reduced virulence ... in mouse

Reduced virulence ... in mouse

Reduced virulence ... in mouse

Reduced virulence ... and man

<table>
<thead>
<tr>
<th>bacterial count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 × 10^00</td>
</tr>
<tr>
<td>1 × 10^02</td>
</tr>
<tr>
<td>1 × 10^04</td>
</tr>
<tr>
<td>1 × 10^06</td>
</tr>
<tr>
<td>1 × 10^08</td>
</tr>
</tbody>
</table>

Ceftriaxone
Clindamycin


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Inhibition of *S. aureus* Nuc1 activity CLI and by IVIG.
Enhanced bacterial clearance in presence of CLI and IVIGs
PVL - Necrotizing Pneumonia

Genetic approach

WT vs \( \Delta pvl \) S. aureus endotracheal

Diep, PNAS 2010
PVL - Necrotizing Pneumonia

Genetic approach

WT vs Δpvl S.aureus endotracheal

Pharmacological approach

Protein synthesis inhibitor

Diep, PNAS 2010

Diep, JID 2013
Disarming the pathogen
Proteinsynthesis inhibition

MRSA Pneumonia
- Vanco or Linezolid or Clindamycin
  IDSA Guidelines, CID 2011

MSSA Pneumonia
- BetaLactam plus Clindamycin
Colonisation vs invasive infections

Sumby et al. PLOS Pathogens 2006
Paucity of neutrophils relative to the large number of invading bacteria.

Hidalgo-Grass et al. Lancet 2004

IL-8 Degradation Slows Neutrophil Endothelial Transmigration Towards Bacteria in vitro & in vivo

Zinkernagel et al. Cell Host Microbe 2008
Colonisation vs invasive infections

Sumby et al. PLOS Pathogens 2006

Log_{10}-fold differential transcription (ITP relative to PTP)
SpyCEP hinders adherence

Eukaryotic cells

Andreoni et al. 2014
SpyCEP hinders adherence

Eukaryotic cells

Biofilm

Confocal fluorescence microscopy

Biofilm formation

Andreoni et al. 2014
Atopic Dermatitis

- Why are atopic individuals more prone to infections with Gram positive bacteria?

Ref: Adachi et al., JDS 1998;
• Allergic ‘type-2’ inflammation (IL4) -> hampers neutrophil expansion and migration
• Does IL-4 modulate neutrophils during streptococcal skin infections?
• IL4-complex = fewer neutrophils, anti-IL4 = more neutrophils
Staphylococcus aureus: a commensal misbehaving

- Gram positive extracellular bacterium
- 30% colonization
  - Protection from Staphylococcal Shock Syndrome
- Increased risk for subsequent infections
  - Superficial infections (surgery – decolonization)
  - Difficult to treat infections:
    - Endocarditis, osteomyelitis

(Fowler et al. 2005 JAMA; Cosgrove and Fowler. 2008 CID; Chang et al. 2003 Medicine)
Relapses: Weeks - years after apparent cure

Daptomycin versus Standard Therapy for Bacteremia and Endocarditis Caused by *Staphylococcus aureus* - open-label, randomized trial

Table 3. Reasons for Treatment Failure According to the Adjudication Committee.

<table>
<thead>
<tr>
<th>Reason for Failure</th>
<th>Daptomycin (N=120)</th>
<th>Standard Therapy (N=115)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>67 (55.6)</td>
<td>67 (58.3)</td>
<td>0.22</td>
</tr>
<tr>
<td>Microbiologic failure, clinical failure, or both</td>
<td>23 (19.2)</td>
<td>15 (13.0)</td>
<td></td>
</tr>
<tr>
<td>Microbiologic failure‡</td>
<td>19 (15.8)</td>
<td>11 (9.6)</td>
<td>0.17</td>
</tr>
<tr>
<td>Clinical failure without microbiologic failure‡</td>
<td>4 (3.3)</td>
<td>4 (3.5)</td>
<td>1.00</td>
</tr>
<tr>
<td>Adverse event</td>
<td>8 (6.7)</td>
<td>17 (14.8)</td>
<td>0.06</td>
</tr>
<tr>
<td>Receipt of nonstudy antibiotics that could have influenced outcome</td>
<td>20 (16.7)</td>
<td>16 (13.9)</td>
<td>0.59</td>
</tr>
<tr>
<td>Death</td>
<td>13 (10.8)</td>
<td>13 (11.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>No blood obtained for culture†‡</td>
<td>9 (7.5)</td>
<td>12 (10.4)</td>
<td>0.50</td>
</tr>
<tr>
<td>Patient could not be evaluated (e.g., withdrew consent, left hospital against medical advice)</td>
<td>9 (7.5)</td>
<td>14 (12.3)</td>
<td>0.27</td>
</tr>
</tbody>
</table>
Small colony variants

- Reduced metabolism and slow growth
- Increased tolerance to antibiotics
- *E. coli*, Tbc, *S. aureus*, Salmonella enterica serovar Typhi, *S. typhi*

Clinical isolates:
- Challenging detection – slow growth
- Phenotype switching, revert to normal colony phenotype
- Indispensable feature for recurrent infections
How do *S. aureus* withstand antibiotics?

**Resistance - MRSA**

**Susceptible**
- Located in ‘privilged’ sites
  - Abscess, intracellular
  - AB do not reach bacteria, milieux
Low pH selected for SCV

MSSA Cowan

MSSA 6850

MRSA JE2

% SCVs

pH 4.0

pH 5.5

pH 6.5

pH 7.4

time (days)

ESCMID Online Lecture Library © by author
Bacteria – growth

Peptidoglycan labeling with dsRed-CBDs

SCV  LCV

Control  pH 4.0  pH 7.4
Intracellular localization

Day 0

Day 3

Day 5

Day 7

Scale bars 20 and 5 µm, respectively


DAPI (DNA)

LAMP-2 (lysosome)

CFSE (bacteria)
Reduction of SCV frequency by phagolysosominal alkalinization

Baf A1

Ctrl

AC

CQ

Ctrl

bafilomycin A1


ammonium chloride

ESCMID Online Lecture Library © by author
Reduction of SCV frequency by phagolysosomal alkalinization in vivo

Living with the enemy

Drew Smith

There's another big problem with antibiotics: indiscriminately killing bugs is making us sick. It may be time to call a truce.
Pathogenesis of *Staphylococcus aureus* and *Streptococcus pyogenes*: clues for therapy

Invasive Gram positive infections remain a problem

**GAS:** Rise in invasive diseases
- NF: Rapid progressive disease, Lethality ~35%

**S. aureus:** Resistances – intracellular location

Know your enemy – IL-8 protease, DNase, SCV

Therapeutic options for bacterial infections

1. **Kill:** Antibiotics
2. **Disarm:** Inhibition of bacterial virulence factors
3. **Reveal the hiding places and get them out**
Many thanks

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Katrin Schilcher
Nadja Leimer
Fabio Ugolini