Cystic Fibrosis and anaerobes

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Outline

• Background
  – CF: the disease
  – Recent developments in CF

• Detection of anaerobes in sputum from Cystic Fibrosis patients
  – Methods
    • Detection and isolation of anaerobes
    • Identification of aerobes and anaerobes
  – Results to Date
  – Future Work
CF: the disease

- Most common genetic disease in UK
  - 1 in 2500 live births
  - ~ 7500 patients

- Abnormal cystic fibrosis transmembrane regulator (CFTR) protein
  - Secretion of thickened mucus
  - Pulmonary infection
Pathogenic events in CF lung disease

- CF gene mutations
  - CFTR dysfunction
    - Ion transport abnormalities
      - Altered airway secretion

- Vicious Cycle:
  - Inflammation
  - Infection

- Tissue damage
Do CF Lungs Contain Anoxic Zones?

- Worlitzsch et al. (2002)
  - steep oxygen gradients in the mucus of CF patients
  - Proliferation of \textit{P. aeruginosa} → fully anoxic conditions

- Yoon et al. (2002)
  - \textit{P. aeruginosa} formed robust biofilms under anaerobic conditions
Significance of Anoxic Regions in the CF Lung

- Efficacy of antibiotics reduced under anaerobic conditions
- Susceptibility of *P. aeruginosa* may be altered under anaerobic conditions
- Anaerobic bacteria may be present and contributing to inflammatory process
Evidence of Anaerobes in Lung Infections

- 90% of the mucosal surface of the oropharynx is colonized with anaerobic bacteria
- Anaerobes have been identified in other pulmonary infections
- Small studies: culture detection
- Molecular detection
  - Terminal-restriction fragment length polymorphism profiling (T-RFLP)
Culture detection

- **Brook & Fink, 1983**
  - Transtrachael aspiration samples
  - Anaerobes present in 4/6 samples
- **Thomassen et al. 1984**
  - Sputa and thoractomy samples
  - Anaerobes present in 2/10 patients
  - Quantitative culture: $1 \times 10^5$-$4 \times 10^8$ cfu/g
- **Jewes & Spencer, 1990**
  - Sputa samples
  - Anaerobes present in 9/21 samples
Culture detection

- *Prevotella* spp.
- *Veillonellae* spp.
- *Porphyromonas* spp.
- *Propionibacterium acnes*
- *Bacteroides* spp.
- *Peptostreptococcus anaerobius*
Detection of anaerobes in sputum from Cystic Fibrosis patients
Objectives

- To determine if the use of strict anaerobic bacteriological practice can improve the detection of anaerobic bacteria in CF pulmonary infection
Methods

• Sputum collection
• Samples processed under strict anaerobic conditions
• Strains isolated by aerobic and anaerobic culture
  – Aerobic: nutrient agar
  – Anaerobic: anaerobic blood agar, kanamycin-vancomycin laked blood agar & phenyl ethyl alcohol agar
SAMPLE PROCESSING

Sputum sample

Sputolysin treatment, serial dilution & plating

Anaerobic incubation

Different Colonies

Re-plate in duplicate

Growth

Aerobic Incubation

Store for further characterization

No growth

Obligate anaerobe

Aerobe or microaerophile

Aerobic incubation

Different Colonies

Re-plate

Growth

Aerobic incubation
Methods

• Anaerobes identified by colony PCR and sequencing of the 16S rRNA gene using universal primers (LiPuma et al. 1999)

• Aerobes first screened for *P. aeruginosa* using oprL PCR (Xu et al. 2004)

• Aerobes negative for oprL identified in the same manner as anaerobes
RESULTS
Sputum Sample Collection

- 64 sputum samples from 49 patients
  - 25 male, 24 female
  - mean age: 26.65 years
  - Range: 18-50 years

- 60 of these samples, representing 45 patients have been processed

- Anaerobes have been detected in
  - 42/60 samples (70%)
  - 33/45 patients (73%)
### Sequential processing of samples

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Number of samples</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>4/4 same</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>2/3 same</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>2/2 same</td>
</tr>
</tbody>
</table>
Summary of anaerobes detected

- 60 samples have yielded 105 potential anaerobes
- 45 have been identified via 16S rRNA sequencing

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Prevotella</em> species</td>
<td>15</td>
</tr>
<tr>
<td><em>Veillonella</em> species</td>
<td>9</td>
</tr>
<tr>
<td><em>Bifidobacterium</em> species</td>
<td>2</td>
</tr>
<tr>
<td><em>Propionibacterium acnes</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Actinomyces</em> species</td>
<td>5</td>
</tr>
<tr>
<td><em>Streptococcus</em> species</td>
<td>10</td>
</tr>
<tr>
<td><em>Gemella sanguinis</em></td>
<td>2</td>
</tr>
<tr>
<td><em>Lactobacillus casei</em></td>
<td>1</td>
</tr>
</tbody>
</table>
# Comparison of Viable Counts

<table>
<thead>
<tr>
<th>Patient</th>
<th>Aerobe/microaerophile (CFU/g)</th>
<th>Anaerobe (CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC-1</td>
<td><em>P. aeruginosa</em> 1.15x10^5</td>
<td><em>Prevotella</em> spps 9x10^7</td>
</tr>
<tr>
<td>TH-3</td>
<td><em>P. aeruginosa</em> 1x10^4</td>
<td><em>Prevotella</em> spps 1x10^7</td>
</tr>
<tr>
<td></td>
<td><em>Rothia denticariosa</em> 1.1x10^5</td>
<td></td>
</tr>
<tr>
<td>DM-11</td>
<td><em>P. aeruginosa</em> 2x10^4</td>
<td><em>Prevotella</em> spps 1.2x10^5</td>
</tr>
<tr>
<td></td>
<td><em>Rothia denticariosa</em> 6.25x10^4</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Streptococcus oralis</em> 1x10^7</td>
<td></td>
</tr>
<tr>
<td>RC-17</td>
<td><em>P. aeruginosa</em> 1.44x10^5</td>
<td><em>Bifidobacterium longum</em> 1.2x10^7</td>
</tr>
<tr>
<td></td>
<td><em>Streptococcus salivarius</em> 1x10^6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Bifidobacterium spps</em> 3x10^6</td>
</tr>
<tr>
<td>LM-22</td>
<td><em>P. aeruginosa</em> 7x10^5</td>
<td><em>Veilonella atypica</em> 5x10^6</td>
</tr>
<tr>
<td></td>
<td><em>S. hominis</em> 2x10^6</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Streptococcus</em> spp 3x10^7</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Gemella sanguinis</em> 2x10^7</td>
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<th>Anaerobe (CFU/g)</th>
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</thead>
<tbody>
<tr>
<td>RM 1st</td>
<td><strong>P. aeruginosa</strong> 2.42x10⁵</td>
<td><strong>Prevotella spps/salivae</strong> 1.06x10⁶</td>
</tr>
<tr>
<td></td>
<td><strong>S. aureus</strong> 4.2x10⁴</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Streptococcus spp</strong> 7.2x10⁵</td>
<td></td>
</tr>
<tr>
<td>RM 2nd</td>
<td><strong>P. aeruginosa</strong> 2.8x10⁴</td>
<td><strong>Prevotella spps</strong> 1.06x10⁶</td>
</tr>
<tr>
<td></td>
<td><strong>Micrococcus luteus</strong> 1.1x10⁴</td>
<td><strong>Veillonella spps</strong> 2x10⁵</td>
</tr>
<tr>
<td>GD 1st</td>
<td><strong>P. aeruginosa</strong> 1.2x10⁵</td>
<td><strong>Prevotella melaninogenica</strong> 2x10⁵</td>
</tr>
<tr>
<td>GD 2nd</td>
<td><strong>P. aeruginosa</strong> 4x10⁷</td>
<td><strong>Prevotella spps</strong> 6x10⁶</td>
</tr>
<tr>
<td>CC 1st</td>
<td><strong>P. aeruginosa</strong> 8.3x10³</td>
<td><strong>Prevotella spps/salivae</strong> 1.06x10⁵</td>
</tr>
<tr>
<td></td>
<td><strong>Rothia denticaariosa</strong> 2.1x10⁶</td>
<td></td>
</tr>
<tr>
<td>CC 2nd</td>
<td><strong>P. aeruginosa</strong> 1.3x10⁵</td>
<td><strong>Veillonella spps</strong> 1x10⁴</td>
</tr>
<tr>
<td></td>
<td><strong>Rothia denticaariosa</strong> 4x10⁶</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Gemella sanguinis</strong> 2x10⁵</td>
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</table>
Molecular detection

• Rogers et al 2004
  – Terminal-restriction fragment length polymorphism profiling (T-RFLP)
  – T-RF bands for
    • *P. melaninogenica*
    • *Prevotella sp. oral clone*
    • *V. atypica*
    • *S. hominis*
    • *Rothia spp.*
    • *Streptococcus spp.*

• Rogers et al 2005
  – Reverse Transcription (RT) T-RFLP
  – Metabolically active
Anaerobic isolates: Are they Significant?

- Are associated with the oropharynx
  - Oral cavity may be reservoir
  - Oral contaminants?????
- *Prevotella* spps have been shown to produce
  - β-lactamases
  - proteases
- Many have been implicated in other disease processes, particularly dental caries
Future Work: CF Trust Funded Study

- Anaerobe detection in patients with acute exacerbation of CF pulmonary infection
- 50 CF patients
  - Sample prior to and at end of antibiotic treatment
  - Culture detection, direct molecular screening
  - Examination of antibiotic susceptibility of anaerobic strains isolated
- 25 healthy volunteers
Acknowledgements

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