Gene expression of candidate GAS vaccine antigens in natural vs controlled infection

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Why we need a Group A streptococcus vaccine

Asymptomatic carriage

Pharyngitis  Impetigo  Scarlet Fever  Rheumatic Heart Disease  Post-streptococcal GN  Necrotising fasciitis  Toxic shock syndrome  Sepsis

Mild  Severe

0.5 million deaths/year  10 million DALY/year

Exclusive human pathogen

Increasing burden & relevance

Neglected

0.5 million deaths/year  10 million DALY/year

0  200  400  600  800

PHE Notification Data

Macleod 2019
The global push for a GAS vaccine

The Path to Group A Streptococcus Vaccines: World Health Organization Research and Development Technology Roadmap and Preferred Product Characteristics

Clin Infect Dis 2019

Epidemiology & transmission

Antigen discovery

Immunology and correlates of protection
GAS vaccine development

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Target/s</th>
<th>Pre-clinical</th>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III</th>
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</thead>
<tbody>
<tr>
<td>SteptAnova</td>
<td>• M protein 30-valent (variable regions), Spa18 antigen</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>JMJ8VAX</td>
<td>• M protein (conserved – J8)</td>
<td>X</td>
<td></td>
<td>X</td>
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</tr>
<tr>
<td>StreptInCor</td>
<td>• M protein (conserved)</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Combo</td>
<td>• SLO, SpyCEp, Spy0269, GAS carbohydrate</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SpeAB</td>
<td>• SpeA, SpeB</td>
<td>X</td>
<td></td>
<td></td>
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<tr>
<td>Spy7</td>
<td>• C5a peptidase, oppA, pulA, nucleoside-binding protein, hypothetical</td>
<td>X</td>
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</tr>
<tr>
<td></td>
<td>membrane associated protein, cell surface protein, SpyAD</td>
<td></td>
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</tbody>
</table>

...... many more!

Davies et al, 2019
Project aim and objectives

Aim

To describe and compare the gene expression profile of GAS from a range of clinical samples to inform vaccine development strategies

Focus on genes related to candidate vaccine antigens

Focus on clinically relevant samples

Objectives

1. Describe mRNA expression of key vaccine targets during different GAS infection/disease states
2. Compare GAS gene expression in CHIM vs natural infection
3. Describe relationships with viral co-infection, host mucosal inflammatory response and GAS carriage density
Sample collection

Mild

Asymptomatic carriage

Pharyngitis

Impetigo

Scarlet Fever

Rheumatic Heart Disease

Post-streptococcal GN

Necrotising fasciitis

Toxic shock syndrome

Sepsis

Severe

Throat swab

Saliva

Nose swab

RNAalter

STGG
1) Asymptomatic carrier samples

<table>
<thead>
<tr>
<th>Study name</th>
<th>Location</th>
<th>Cohort</th>
<th>Number of participants</th>
<th>Samples</th>
<th>Estimated GAS carriage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transmission of Pneumonia (TOP)</td>
<td>Bristol, UK</td>
<td>2 year olds pre/post LAIV &amp; Family contacts</td>
<td>1230</td>
<td>NPS Saliva</td>
<td>~5-10%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>410 families</td>
<td></td>
<td>&lt;5%</td>
</tr>
<tr>
<td>SPIT</td>
<td>Bristol, UK</td>
<td>16-17 year olds given 4CMenB</td>
<td>300</td>
<td>OPS, Saliva</td>
<td>&gt;5%</td>
</tr>
<tr>
<td>Meningococcal carriage study</td>
<td>Coimbra, Portugal</td>
<td>18-25 year olds</td>
<td>1006</td>
<td>OPS, Saliva</td>
<td>1.3%</td>
</tr>
</tbody>
</table>
2) Pharyngitis samples

GASP: Group A Strep Project

- Children aged 2-15 presenting with sore throat
- Bristol Royal Children’s Hospital
- Throat swab (OPS), Saliva
- Clinical and demographic data

.... recruitment ongoing
Respiratory viral infection is associated with changes in bacterial population dynamics

**S. pneumoniae**

Thors et al, 2018, Pediatr Infect Dis J

**N. meningitidis**

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<thead>
<tr>
<th></th>
<th>N.m+</th>
<th>N.m-</th>
</tr>
</thead>
<tbody>
<tr>
<td>RhV+</td>
<td>12.9%</td>
<td>87.1%</td>
</tr>
<tr>
<td>RhV-</td>
<td>7.0%</td>
<td>93.0%</td>
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<tr>
<td>Total</td>
<td>102</td>
<td>1278</td>
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</table>

P=0.0354

Thors et al, 2018, Pediatr Infect Dis J

**GAS**

NPS %+ PCR by SNOT score

P = 0.0007
Chi-squared test
Exploring the GAS carriage state

- Association with viral infection
  - Influenza, VZV (necrotising fasciitis), EBV, measles

- How does viral infection affect GAS carriage?
  - Presence
  - Density
  - GAS gene expression profile
  - Host inflammatory profile

- Additional research questions:
  - Longevity of carriage?
  - Longitudinal density profile?
  - Can carriers transmit?
Methods

Gene expression profiling

Nanostring technology:
- ~53 GAS gene transcript targets
- Mainly vaccine antigens
- Host immune/ inflammatory profile?

Molecular microbiology

- Determine GAS presence/density using qPCR (+/- culture)
- Culture & sequence GAS strains
- Other bacterial species?
- Viral co-infection?
Why use NanoString?
Methods

Gene expression profiling

Nanostring technology:
- ~53 GAS gene transcript targets
- Mainly vaccine antigens
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Molecular microbiology
- Determine GAS density using qPCR (+/- culture)
- Culture & sequence GAS strains
- Viral co-infection
- Other bacterial species?
NanoString Codeset panel genes

53 Vaccine antigen targets:
emm, slo, spyAD, speB, SpyCEP, pulA, Spy0843, nbl (Spy1228), hmap (Spy1057), oppA fbp54, sagP, ropA (TF), shr, scpA, sib35, Sse, SpeC, SpeA, sof, r28, spa, sfb1, malE, sdaD, sls, fbaB, Mrp, dnaK, srtA, isp, soda, LysM, GAPDH, sibA, MtsA, GRAB, dacA,
gacl, gacA
Spy0488, Spy0872, cpsX, Spy1536, mraW, Spy1727, fba, enn, SpnA, FbaA
tee, ap1, ap2

5 Housekeeping genes:
ProS
Spy1258
recA
tufA
gyrA

3 Regulatory genes:
mga
rofA
Nra
NanoString Codeset has good strain coverage

<table>
<thead>
<tr>
<th>S. pyogenes Strain Coverage</th>
<th>M1</th>
<th>M28</th>
<th>M2</th>
<th>M1</th>
<th>M6</th>
<th>M75</th>
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Summary

• GAS infections are a globally relevant neglected disease associated with major morbidity/mortality

• Vaccine development hindered by poor understanding of epidemiology, antigen diversity & immunity

• We aim to address these main questions:
  • Vaccine antigen gene expression during infection? CHIM
  • The effect of viral infection on GAS carriage and gene expression?
  • The role of the GAS carrier state?

• GAS emm75 CHIM results in reproducible and convincing pharyngitis in healthy adults

• NanoString is a useful tool for probing RNA samples from patients
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