Enhancing mucosal immunity to \textit{Streptococcus pneumoniae} by nasal administration of live attenuated strains

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In 2017, the WHO included *S. pneumoniae* as one of 12 priority pathogens

**Children**

826,000 deaths annually in children <5 years old

**Adults**

*S. pneumoniae* is a dominant pathogen for:
- pneumonia: 40-50% cases
- exacerbations of COPD: 25%

UK numbers per annum:
189,000 admissions to hospital with pneumonia
117,000 with exacerbations of COPD

**Carriage and disease**

Ferreira 2011, Trends Microbiol

**Immunizing effect of exposure**
Life attenuated vaccination with *S. pneumoniae*

MRC Experimental Medicine grant (coPI Gordon + Ferreira)

**Challenge:** Prevention of adult *S. pneumoniae* lung infections

**Overall aim:** Does intranasal administration of live *S. pneumoniae* strains attenuated in virulence effectively boost adaptive immunity and prevent *S. pneumoniae* lung infections
FIRST STEP - MAKE SUITABLE MUTANT STRAINS AND TEST IN LABORATORY pre-EHPC TRIAL

MUTANT REQUIREMENTS:
1. Reduced virulence in systemic infection and pneumonia
2. Still able to colonize the nasopharynx
3. Induce protective immunity after nasopharynx colonization
4. Stable mutations
5. No unexpected effects on *S. pneumoniæae* biology

PREVENTION OF COLONIZATION AND SUBSEQUENT DISEASE + LUNG INFECTION?
- Antibody to protein antigens and capsular antigens

CD4+ Th17 responses to protein antigens and antibody capsular antigens

ARTIFICIAL Nasopharynx Colonization

New vaccines strategies LIVE ATTENUATED STRAINS

UCL/UCLH LSTM EHPC model

PREVENTION OF SYSTEMIC INFECTION
POTENTIAL CANDIDATES FOR EHPC MODEL
MUTANT S. pneumoniae 6B STRAINS

Known roles in virulence for other strains
• Required for immune evasion (capsule, pspA, pneumolysin)
• Required for bacterial replication in the host: (ABC transporters, biosynthesis)

Novel targets
• Not described in detail before
• Identified from published virulence screens and transcriptome data (colonisation versus lung / sepsis)

Mutants constructed by overlap extension PCR (gene replacement with antibiotic resistance cassette)
VIRULENCE OF SINGLE MUTANT STRAINS (PNEUMONIA)

1x10^7 CFU intranasal inoculation, cull at 28 hrs, 14 mutants tested in total

Of the single mutants tested the 'new' mutants have a marked degree of attenuation in a mouse model of pneumonia compared to the wild type strain 6B.
Nasopharyngeal colonization was maintained with bacterial levels around $10^4$ CFU/ml in most of the single mutant strains. Levels were reduced in mutant carrying the *cps* locus deletions.

Four double mutants chosen and constructed: Δ*cps*/IV and Δ*cps*/V (highly attenuated but potentially less immunogenic?) ΔV/II and ΔVIII/II (less attenuated but more immunostimulatory?)
CHARACTERIZATION OF DOUBLE MUTANTS

- Mutant stability test
- WGS/RNAseq
- Virulence and immunogenicity
- Colonizing ability at 7 days
- Protective effect of colonisation against pneumonia challenge
- Protective effect of colonisation against recolonization challenge

**COLONIZATION MODEL**

- Nasal washes to determine bacterial levels in the NP
- IN inoculation of *S. pneumoniae* 6B or mutant in a small volume (10ul)

**PNEUMONIA/SEPSIS MODEL**

- Sera collection for serology and immunology tests
- IN inoculation of homologous *S. pneumoniae* in a big volume (50ul)
- Bacterial CFU from target organs at 28 h

**PROTECTION MODEL**

- IN inoculation of homologous *S. pneumoniae* in a big or small volume
- Bacterial CFU from target organs at 28 h or 7 days

**TIMELINE**

- 1st carriage episode
- 2nd carriage episode

**WEEKS**

- Week 0
- Week 1
- Week 2
- Week 3
- Week 4
- Week 5
- Week 6
Double mutants do not cause systemic infection and are completely attenuated on a sepsis model of infection.
COLONIZING ABILITY OF THE ATTENUATED MUTANT STRAINS AT 7 DAYS

Cps+ double mutants colonisation level similar to wild-type

Cps- double mutants reduced colonisation density x2 log_{10} (may effect adaptive responses?)
ANTIBODY RESPONSE OF COLONIZED MICE

SERUM whole cell ELISAs 21 days after two episodes of colonization

- Good antibody response to two episodes of colonization
- No increase in 6B specific IgG
- Antibodies mainly to protein rather than capsular antigens
- Cross-reactive to other strains
- Stronger responses to wild type compared to mutant strains
• Multiple protein antigens recognized
• Largely similar pattern of bands for all the strains and the mutants
• Reduced for mice colonised with mutants v. wild type?
• Big question – what about protein responses in humans?
SPECIFIC PROTEIN ANTIGENS RECOGNIZED BY SERUM IgG RESPONSES TO COLONIZATION

Measured using David Goldblatt’s MSD

In collaboration with David Goldblatt

Not significant anti-capsular responses detected. Good responses only to PsaA and PspA F2
NASAL ADMINISTRATION x2 OF THE MUTANTS PROTECTS AGAINST INVASIVE INFECTION 6B

- Total protection against bacteraemia
- Partial protection against lung infection

Sera collection

CFU from target organs at 28h (homologous)

1x10^7 CFU 6B/mutant IN in 10ul inoculum

1x10^7 CFU 6B IN in 50ul inoculum

Blood

BALF

Lung

- CFU ml⁻¹ blood
- CFU ml⁻¹ BALF
- CFU ml⁻¹ lung

* 1x10^7 CFU 6B/mutant IN in 10ul inoculum

* 1x10^7 CFU 6B IN in 50ul inoculum

- Total protection against bacteraemia
- Partial protection against lung infection
CD4+ cells do not have a role in protection against sepsis or pneumonia.
ROLE OF ANTIBODIES IN LUNG PROTECTION

Prior colonisation with wild type or mutants failed to prevent sepsis in uMT mice
6B OR MUTANT COLONIZATION PROTECTS AGAINST HOMO / HETEROLOGOUS COLONIZATION

Sera collection

CFU from nasal washes 7 days later

Week 0 1 2 3 4 5 6 7

1x10^7 CFU 6B/mutant IN in 10ul inoculum

1x10^7 CFU 6B/TIGR4 IN in 10ul inoculum

Homologous protection 6B

Heterologous protection TIGR4

CD4- depleted

Reduced nasal wash CFU after homologous 6B or heterologous TIGR4 challenge
Objective 2. EHPC (LSTM): does nasal administration of live attenuated S. pneumoniae prevents wild-type re-colonisation?

Recruitment complete
Re-challenge with wild-type almost finished
Results available soon

1er endpoint: re-colonisation rate
2er endpoints: multiple immunological + microbiology parameters
Other data on double mutant strains

- No differences in cellular and inflammatory response on pneumonia rechallenge between mice previously colonised with wild-type or double mutant strains
- RNAseq in vitro broth culture
- Whole genome sequencing (minimal genetic differences outside of mutations)
- Interactions with host epithelium in vitro (Caroline Weight)
- Some mechanistic data on why novel mutations affect virulence
CONCLUSIONS

- Identified 8 mutations required for *S. pneumoniae* 6B virulence

  *Double mutant* cps+ strains had major effect on virulence (= loss of the capsule)

- Colonization density of cps+ strains was similar to wild-type

- Cps- strains reduced colonisation and reduced adaptive immune responses

- Mutant colonization generated an antibody response to conserved protein antigens similar pattern to wild-type colonisation (although probably weaker)

- Mutant colonization followed by 6B homologous strain challenge:
  - prevented bacteraemia
  - reduced bacterial lung and BALF CFU
  - reduced nasal wash CFU

- Mutant colonization prevented colonisation with the heterologous TIGR4 strain

- CD4+ cells were:
  - not necessary for protection against sepsis (required antibody)
  - were necessary for protection against re-colonisation

- Now waiting for the results of the clinical trial........
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