12. Korsiak J, Lavigne E, You H, Pollitt K, Kulka R, Hatzopoulou M, *et al.* Air pollution and pediatric respiratory hospitalizations: effect modification by particle constituents and oxidative potential. *Am J Respir Crit Care Med* 2022;206:1370–1378.
13. Wu Y, Li S, Guo Y. Space-time-stratified case-crossover design in environmental epidemiology study. *Health Data Science* 2021;2021:1–3.
14. Bozigar M, Lawson AB, Pearce JL, Svendsen ER, Vena JA. Using Bayesian time-stratified case-crossover models to examine associations between air pollution and "asthma seasons" in a low air pollution environment. *PLoS One* 2021;16:e0260264.
15. Lu Y, Symons JM, Geyh AS, Zeger SL. An approach to checking case-crossover analyses based on equivalence with time-series methods. *Epidemiology* 2008;19:169–175.

An Experimental Human Colonization Model with Pneumococcal Serotype 3 has the Potential to be Used for Vaccine Studies

Pneumococcal infections are major contributors to morbidity and mortality worldwide, while being major causes of respiratory tract infections such as otitis, sinusitis, and community-acquired pneumonia with or without septicemia, and of meningitis (1, 2). *Streptococcus pneumoniae* (the pneumococcus) is a human specific pathogen, and a prerequisite for disease is pneumococcal colonization of the upper respiratory tract. Pneumococci can be divided into at least 100 different capsular serotypes (3). Pneumococcal conjugate vaccines (PCVs), targeting up to 13 of these capsules, have been introduced in the childhood vaccination program in many countries, leading to a dramatic decrease of invasive pneumococcal disease (IPD) in vaccinated children (4–6). Most serotypes covered by the PCVs have decreased postvaccine introduction, but concurrently non-PCV13 types have increased also in nonvaccinated age groups such as the elderly, and non-PCV13 serotypes now dominate in both invasive disease and carriage in several studies (5–8). However, no large reductions have been observed for the vaccine serotype 3, targeted by PCV13 (7, 9). Serotype 3 is an important prominent serotype that has been associated with high mortality (2, 10). Interestingly, pneumococcal capsules of serotypes 3 and 37 are anchored to the cell wall differently than other serotypes, and as a consequence, serotype 3 bacteria are fully covered by its capsular polysaccharide 3 (CPS3) that prevents phagocytosis (11). At the same time, the bacteria release large quantities of CPS3 antigen, potentially binding to available CPS3 antibodies (12). It is likely, but not proven, that this could be one explanation for the inability of PCV13 to give the required protection against IPD caused by serotype 3. In the current study, the authors have set up a pneumococcal human carriage model for studies of serotype 3 using a similar set up as they previously published for serotype 6B (13). This model will be important for increasing our knowledge on serotype 3 in humans, but also for future vaccine studies, especially considering that carriage may be used as a substitute for IPD.

In this issue of the *Journal* (pp. 1379–1392), 96 healthy volunteers with a median age of 21 years, without risk factors, were challenged with pneumococci of serotype 3 in escalating doses (14). Since most serotype 3 pneumococci belong to the clonal cluster CC180, the authors used three different serotype 3 strains, belonging to clades 1a, II, and no clade, of CC180. This seems appropriate, since prior to vaccine introduction clade Ia and 1b dominated, but following PCV introduction, whole genome sequencing has demonstrated that a clade II lineage expanded in carriage and IPD. Underlying mechanisms for the expansion of clade II is unknown. However, a sequence variant in the *galU* gene, encoding a key enzyme for CPS3 biosynthesis, was found among all clade II isolates (15). The participants were inoculated with four different doses with a maximum of 20,000 CFU/100 μl per nostril for the no clade strain, and up to 160,000 CFU/100 μl per nostril for the other two strains. Nasal washes were taken from the participants at Day 2, 7, and 14 postinfection and they identified carriage using culture and molecular methods. Carriage was successfully obtained in 33 (34.4%) participants that were culture positive at Day 2, of which 7 (21. 2%) were administered antibiotics early and were terminated from the study. A total of 88.5% (23/26) of those that were colonized on Day 2 remained culture positive on Day 14. The recovered bacterial densities were comparable between the three strains. These data are promising and suggest that the challenge strains can be used to study colonization in the same way as in the serotype 6B model. The immune responses were also analyzed and compared with the serotype 6B strain used in the previously published human model (13). The cytokine expression was found to vary depending on the serotype of the challenge strain and the inoculum dose. No differences were seen in IgG fold changes between the three serotype 3 stains and the levels were comparable with those induced by serotype 6B. IgA and IgM were not measured and would be interesting to study in future studies.

Symptoms were reported daily, and 30.2% (29/96) of the participants experienced mild symptoms from the respiratory tract, with higher frequencies in colonized (52.6% [20/38]) versus noncolonized (15.5% [9/58]) individuals. A total of 24 out of 29 (82.8%) had a sore throat, which might be a bit unexpected and warrants further investigation in future studies. One participant got an otitis media already on Day 1 and was administered antibiotics. Most symptoms were reported within 7 days and were mild or

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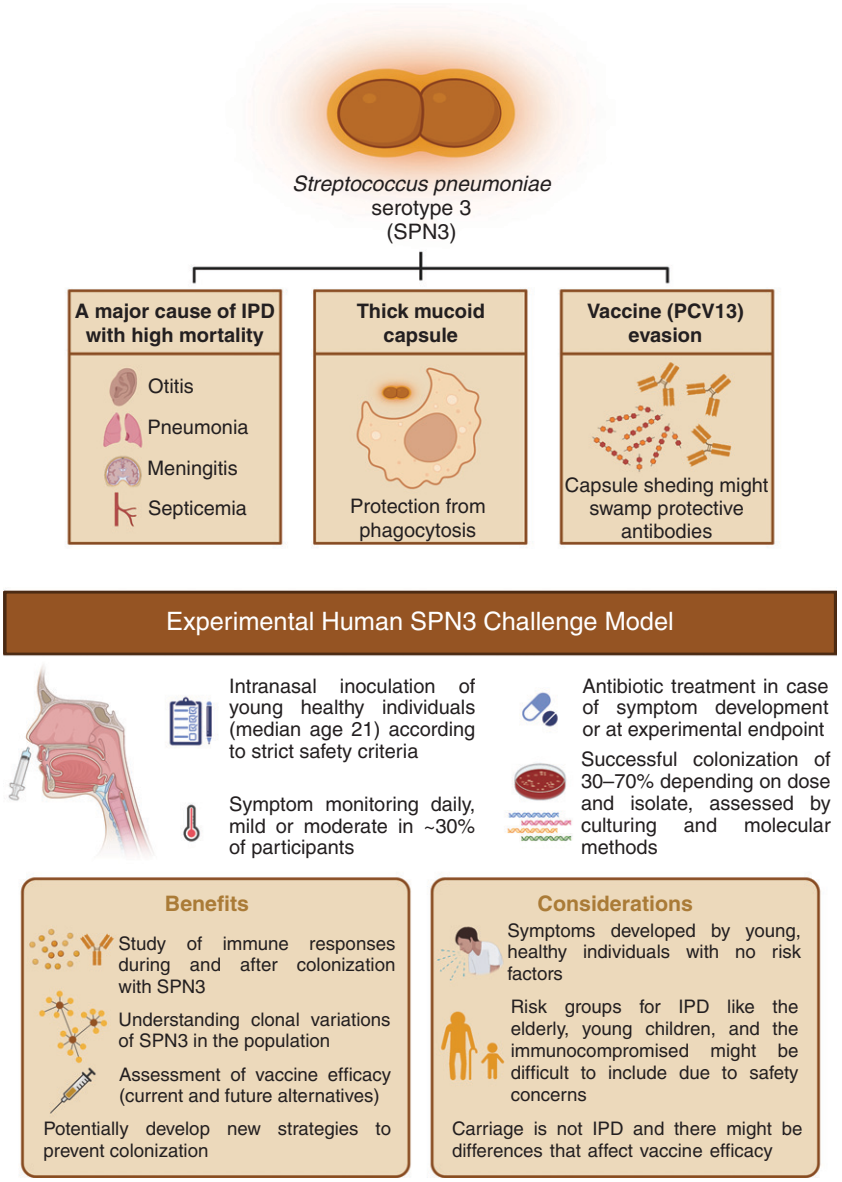

Figure 1. The figure has been created with BioRender.com. IPD = invasive pneumococcal disease.

moderate and when needed resolved by antibiotics. Seven participants were administered antibiotics early (27.6%); all were experimental carriers. Antibiotics were given to participants with symptoms above a threshold. Thus, in this young age group, no severe adverse effects were reported using serotype 3 strains, but this needs to be monitored carefully in future studies, including also other age groups.

This human challenge study using serotype 3 strains is promising and shows that it is possible to obtain carriage using serotype 3 strains in an experimental human model. The safety aspect of using a disease-causing invasive pneumococcal strain with high mortality is important and was addressed by the authors. Several of the participants that were colonized experienced mild or moderate symptoms that were resolved—some after taking antibiotics.

Risk groups for pneumococcal diseases such as the elderly were not studied, and whether this model can be used for risk groups needs to be explored in future studies taking in consideration the safety aspect. This model will be very useful for explaining mechanisms for carriage of serotype 3 and differences between different age groups. Unvaccinated children have been shown to carry high levels of CPS3 antibodies, suggesting frequent colonization events during childhood, but these colonization events have been suggested to be short because the incidence of nasopharyngeal colonization in children, in most studies, is quite low (16). Importantly, this model has the possibility to be used to analyze the efficacy of vaccines—especially against serotype 3—where the required protection needs to be strengthened, as a complement to large clinical vaccine studies of IPD (Figure 1). ■

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References

1. Henriques-Normark B, Tuomanen EI. The pneumococcus: epidemiology, microbiology, and pathogenesis. *Cold Spring Harb Perspect Med* 2013; 3:a010215.
2. Harboe ZB, Thomsen RW, Riis A, Valentiner-Branth P, Christensen JJ, Lambertsen L, *et al.* Pneumococcal serotypes and mortality following invasive pneumococcal disease: a population-based cohort study. *PLoS Med* 2009;6:e1000081.
3. Ganaie F, Saad JS, McGee L, van Tonder AJ, Bentley SD, Lo SW, *et al.* A new pneumococcal capsule type, 10D, is the 100th serotype and has a large *cps* fragment from an oral streptococcus. *MBio* 2020;11: e00937-20.
4. Moore MR, Link-Gelles R, Schaffner W, Lynfield R, Lexau C, Bennett NM, *et al.* Effect of use of 13-valent pneumococcal conjugate vaccine in children on invasive pneumococcal disease in children and adults in the USA: analysis of multisite, population-based surveillance. *Lancet Infect Dis* 2015;15:301–309.
5. Savulescu C, Krizova P, Lepoutre A, Mereckiene J, Vestrheim DF, Ciruela P, *et al.*; SpIDnet group. Effect of high-valency pneumococcal conjugate vaccines on invasive pneumococcal disease in children in SpIDnet countries: an observational multicentre study. *Lancet Respir Med* 2017;5: 648–656.
6. Galanis I, Lindstrand A, Darenberg J, Browall S, Nannapaneni P, Sjöström K, *et al.* Effects of PCV7 and PCV13 on invasive pneumococcal disease and carriage in Stockholm, Sweden. *Eur Respir J* 2016;47:1208–1218.
7. Naucler P, Galanis I, Morfeldt E, Darenberg J, Örtqvist Å, Henriques-Normark B. Comparison of the impact of pneumococcal conjugate vaccine 10 or pneumococcal conjugate vaccine 13 on invasive pneumococcal disease in equivalent populations. *Clin Infect Dis* 2017; 65:1780–1789.
8. Lindstrand A, Galanis I, Darenberg J, Morfeldt E, Naucler P, Blennow M, *et al.* Unaltered pneumococcal carriage prevalence due to expansion of non-vaccine types of low invasive potential 8 years after vaccine introduction in Stockholm, Sweden. *Vaccine* 2016;34:4565–4571.
9. Horácio AN, Silva-Costa C, Lopes JP, Ramirez M, Melo-Cristino J; Portuguese Group for the Study of Streptococcal Infections. Serotype 3 remains the leading cause of invasive pneumococcal disease in adults in Portugal (2012-2014) despite continued reductions in other 13-valent conjugate vaccine serotypes. *Front Microbiol* 2016;7:1616.
10. Sjöström K, Spindler C, Ortqvist A, Kalin M, Sandgren A, Kühlmann-Berenzon S, *et al.* Clonal and capsular types decide whether pneumococci will act as a primary or opportunistic pathogen. *Clin Infect Dis* 2006;42:451–459.
11. Luck JN, Tettelin H, Orihuela CJ. Sugar-coated killer: serotype 3 pneumococcal disease. *Front Cell Infect Microbiol* 2020;10: 613287.
12. Choi EH, Zhang F, Lu YJ, Malley R. Capsular polysaccharide (CPS) release by serotype 3 pneumococcal strains reduces the protective effect of anti-type 3 CPS antibodies. *Clin Vaccine Immunol* 2015;23: 162–167.
13. Ferreira DM, Neill DR, Bangert M, Gritzfeld JF, Green N, Wright AK, *et al.* Controlled human infection and rechallenge with *Streptococcus pneumoniae* reveals the protective efficacy of carriage in healthy adults. *Am J Respir Crit Care Med* 2013;187:855–864.
14. Robinson RE, Mitsi E, Nikolaou E, Pojar S, Chen T, Reine J, *et al.* Human infection challenge with serotype 3 pneumococcus. *Am J Respir Crit Care Med* 2022;206:1379–1392.
15. Groves N, Sheppard CL, Litt D, Rose S, Silva A, Njoku N, *et al.* Evolution of *Streptococcus pneumoniae* serotype 3 in England and Wales: a major vaccine evader. *Genes (Basel)* 2019;10:845.
16. Andrews N, Kent A, Amin-Chowdhury Z, Sheppard C, Fry N, Ramsay M, *et al.* Effectiveness of the seven-valent and thirteen-valent pneumococcal conjugate vaccines in England: the indirect cohort design, 2006-2018. *Vaccine* 2019;37:4491–4498.

Using Real-World Data to Understand Who Has Cardiovascular Benefits from Continuous Positive Airway Pressure: The Importance of Male Sex, Excessive Sleepiness, and Primary Prevention

Although there is a wide consensus about the positive impact of continuous positive airway pressure (CPAP) on symptomatic patients with obstructive sleep apnea (OSA), there are conflicting results about the ability of CPAP to reduce cardiovascular risk and mortality (1–3). Secondary prevention studies in patients diagnosed with OSA after a prior cardiovascular event, such as SAVE (Sleep Apnea Cardiovascular Endpoints study) (4), ISAACC (the Impact of Sleep Apnea syndrome in the evolution of Acute Coronary syndrome - effect of intervention with CPAP) (5) or RICCADSA (Randomized Intervention with Continuous Positive Airway Pressure in CAD and OSA) (6), were neutral or negative with respect to the benefits of CPAP, probably because of poor CPAP adherence, the less symptomatic patients enrolled, and the difficulty to reverse an altered vascular structure in patients with established cardiovascular disease (3, 7–9). It is reasonable to suspect that primary prevention studies with better CPAP adherence and inclusion of more "real-world" patients (e.g., more symptomatic patients with more severe hypoxemia, who reflect individuals seen in sleep clinics) would find cardiovascular benefits of treatment. To demonstrate the positive impact of CPAP on cardiovascular risk and

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