VACCINE INSIGHTS

RESPIRATORY DISEASES

SPOTLIGHT

Whole-cell pneumococcal vaccines: a future-proof approach to overcoming pneumococcal serotype replacement

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VIEWPOINT

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"[PPV and PCV] advances are being offset by the steady rise in the incidence of disease caused by non-vaccine-covered serotypes..."

Serotype replacement is an issue associated with currently licensed pneumococcal vaccines, all of which target the serotype-specific capsular polysaccharide. The use of pneumococcal polysaccharide vaccines and pneumococcal conjugate vaccines in particular, have profoundly reduced the burden of invasive pneumococcal disease. However, these advances are being offset by the steady rise in the incidence of disease caused by non-vaccine-covered serotypes, which may be observed soon after the introduction of capsule-based vaccines in a given region [1–3].

To address changes in serotype prevalence, there has been a need to expand the valency of pneumococcal conjugate vaccines (PCVs) in order to maintain coverage against a reasonable proportion of circulating disease-causing strains. However, these vaccines impose serotype-specific selective pressure and are inevitably poised to drive further replacement disease. The steady trend toward ever-higher valency PCVs may also come at the cost of immunogenicity, with evidence of expanded valency PCVs displaying dampened responses. Numerically lower immunoglobulin G (IgG) geometric mean concentrations were observed recently for PCV20 for those serotypes shared with PCV13 after doses three and four, suggesting that PCV serotype expansion may be approaching its limit [4]. The selection of serotypes to include in a PCV also represents a challenge. Even if a vaccine is reformulated to include emerging serotypes, these proportions may differ at the time of their licensure. Geographical regions also display widely varied serotype distributions and selection of capsular antigens is a challenge in the absence of clear dominating serotypes common across regions [5, 6]. These are major shortcomings that will continue to plague further expanded or alternative serotype PCVs.

A potential solution to these challenges may be found by drawing insights from naturally acquired immunity to the pneumococcus, where proteins have been shown to play an important role. In fact, studies of natural exposure to the pneumococcus suggest that capsular polysaccharide is not the dominant target of naturally acquired immunity [7]. Rates of invasive pneumococcal disease decline by age 5 years, at which time antibody responses to pneumococcal proteins have been reported to increase [8]. The increase in disease incidence in older adulthood (over 65 years) also coincides with a decline in anti-protein antibodies, whereas the decline in anti-capsular IgG is far less pronounced [9]. As protein antigens are generally well conserved between serotypes, vaccines that bring forward acquisition in the young, and/or boost immunity to pneumococcal proteins in the elderly, are expected to provide effective broad-spectrum immunity.

Several protein-based vaccines have progressed to clinical evaluation and have been reviewed extensively elsewhere [10–12]. However, there are limitations associated with this approach. Purified protein vaccines rely on the selection of a small number of target antigens relative to the 270 surface proteins expressed on the pneumococcus [13], and these may be susceptible to immune evasion through small changes to their protein structure or by dampening their expression [14]. There is also potential for large-scale manufacture of protein antigens to cause changes in protein conformation, which may impact epitopes important for vaccine efficacy. In addition, adjuvants are required to enhance the immunogenicity of protein-based vaccines [15] and there are few adjuvants (such as alum, AS04, MF59, AS03, CpG, and AS01b)

approved by regulatory authorities for human use. Commonly used alum adjuvants induce T helper cell 2-based responses; however, antibody isotypes associated with T helper cell 1 responses (IgG2a in mice and IgG1 in humans) are known to have high affinity to Fc receptors involved in inducing functional antibody responses with opsonophagocytic activity [17]. Despite protein-based pneumococcal vaccines having undergone significant evaluation in preclinical and clinical trials, to our knowledge, no protein vaccine has outperformed or even matched a licensed PCV using the gold standard correlate of protection, the opsonophagocytic assay (OPA). The capacity to elicit an OPA response that is not deemed inferior to existing PCVs is a key consideration for licensure of new expanded formulations, and candidate vaccines that can meet this bar will be well positioned for further progression and licensure [18].

Another promising approach that targets non-capsular antigens is whole-cell vaccines (WCVs), comprised of inactivated and/or attenuated bacteria [19–26]. Such vaccines have been utilized effectively for many years for the control of pertussis and tuberculosis in children. In addition to presenting antigens in a manner most likely to resemble the natural conformation of proteins, WCVs have the potential to elicit responses to a breadth of antigens surpassing all polysaccharide- or purified protein-based vaccine approaches. Such vaccines could provide a future-proof approach to effectively target emerging and entirely new pneumococcal serotypes that may arise. To date, however, there are few reports of WCVs eliciting robust OPA responses. It is important to note that chemical inactivation using formalin or beta-propiolactone is associated with cross-linking between proteins and reduced immunogenicity, while live attenuated vaccines pose a significant biological and health risk. The use of γ-irradiation has been reported as an effective alternative inactivation method for the development of highly immunogenic and safe WCVs [27, 28], because of reduced protein damage and maintenance of the structural integrity of inactivated pathogens [29]. γ-irradiated vaccines have been found to mimic live pathogens in terms of stimulating both innate and adaptive responses [28].

A recent WCV that has advanced to the clinical stages of evaluation is a γ-irradiated vaccine developed by GPN Vaccines Ltd (Gamma-PN) [21, 23]. Gamma-PN is differentiated from other WCV approaches in both design and formulation, which ultimately are associated with improved immune responses. A modification was introduced to remove a manganese import gene from the Gamma-PN vaccine strain, which was associated with enhanced survival after experimental challenge in immunized mice [23, 30]. Restriction of manganese availability is a key feature of the host innate response to an infection [31] and the improved protective efficacy may be attributable to changes in the antigenic profile of the vaccine strain, which better reflects that of pneumococci during an infection. A subsequent study in rabbits demonstrated that vaccination with Gamma-PN induced antibodies against a broad range of pneumococcal proteins known to be associated with natural immunity; with significant reactivity to 50 antigens reported [32], although the total number of antigens that reacted with the immune sera exceeded this number. Most critically, this study also reported positive functional antibody responses. For most serotypes tested, Gamma-PN administered with an adjuvant elicited higher OPA titers than PCV13. However, without adjuvant, Gamma-PN performed even better, eliciting OPA titers that were either comparable to or far superior to those elicited by PCV13 or pneumococcal polysaccharide vaccine (PPV)23 for various vaccine-included serotypes (6A, 23F, 11A, 22F, and 33F). Further, this vaccine also induced high OPA titers against serotypes not covered by any current licensed vaccines (9N, 15A, 23B, and 35B) [32]. This data indicated that a shift toward T helper cell 2 responses in animals vaccinated with

adjuvanted Gamma-PN was associated with reduced OPA responses, highlighting the important role of T helper cell 1 responses in the ability of Gamma-PN (without adjuvant) to induce high OPA responses.

While Gamma-PN is still undergoing clinical evaluation in a Phase 1/2a trial, this broad-spectrum approach could eliminate the need to reformulate vaccines to address new serotypes. Indeed, any new serotypes that may emerge could be immediately tested for OPA responses using existing clinical trial serum samples. This future-proof approach could offer significant advantages, circumventing the need for further vaccine reformulation and extensive clinical trials. With current vaccines suffering from diminished usefulness over time and serotype replacement a growing concern, Gamma-PN may offer a solution to provide enduring and broad-spectrum protection against pneumococcal disease.

REFERENCES

1. Lewnard JA, Hanage WP. Making sense of differences in pneumococcal serotype replacement. *Lancet Infect. Dis.* 2019; 19(6), e213–e220.
2. Feikin DR, Kagucia EW, Loo JD, *et al.* Serotype-specific changes in invasive pneumococcal disease after pneumococcal conjugate vaccine introduction: a pooled analysis of multiple surveillance sites. *PLoS Med.* 2013; 10(9), e1001517.
3. Weinberger DM, Malley R, Lipsitch M. Serotype replacement in disease after pneumococcal vaccination. *Lancet* 2011; 378(9807), 1962–1973.
4. Senders S, Klein NP, Lamberth E, *et al.* Safety and immunogenicity of a 20-valent pneumococcal conjugate vaccine in healthy infants in the United States. *Pediatr. Infect. Dis. J.* 2021; 40(10), 944–951.
5. Cui YA, Patel H, O'Neil WM, Li S, Saddier P. Pneumococcal serotype distribution: A snapshot of recent data in pediatric and adult populations around the world. *Hum. Vaccin. Immunother.* 2017; 13(6), 1–13.
6. Lochen A, Croucher NJ, Anderson RM. Divergent serotype replacement trends and increasing diversity in pneumococcal disease in high income settings reduce the benefit of expanding vaccine valency. *Sci. Rep.* 2020; 10(1), 18977.
7. Turner P, Turner C, Green N, *et al.* Serum antibody responses to pneumococcal colonization in the first 2 years of life: results from an SE Asian longitudinal cohort study. *Clin. Microbiol. Infect.* 2013; 19(12), E551–E558.
8. Wilson R, Cohen JM, Reglinski M, *et al.* Naturally acquired human immunity to pneumococcus is dependent on antibody to protein antigens. *PLoS Pathog.* 2017; 13(1), e1006137.
9. Simell B, Lahdenkari M, Reunanen A, Kayhty H, Vakevainen M. Effects of ageing and gender on naturally acquired antibodies to pneumococcal capsular polysaccharides and virulence-associated proteins. *Clin. Vaccine Immunol.* 2008; 15(9), 1391–1397.
10. Pichichero ME, Khan MN, Xu Q. Next generation protein based Streptococcus pneumoniae vaccines. *Hum. Vaccin. Immunother.* 2016; 12(1), 194–205.
11. Briles DE, Paton JC, Mukerji R, Swiatlo E, Crain MJ. Pneumococcal vaccines. *Microbiol. Spectr.* 2019; 7(6).
12. Li S, Liang H, Zhao SH, Yang XY, Guo Z. Recent progress in pneumococcal protein vaccines. *Front. Immunol.* 2023; 14, 1278346.
13. Morsczeck C, Prokhorova T, Sigh J, *et al.* Streptococcus pneumoniae: proteomics of surface proteins for vaccine development. *Clin. Microbiol. Infect.* 2008; 14(1), 74–81.
14. Sempere J, Llamosi M, Del Rio Menendez I, *et al.* Pneumococcal choline-binding proteins involved in virulence as vaccine candidates. *Vaccines (Basel)* 2021; 9(2), 181.
15. Lagousi T, Basdeki P, Routsias J, Spoulou V. Novel protein-based pneumococcal vaccines: assessing the use of distinct protein fragments instead of full-length proteins as vaccine antigens. *Vaccines (Basel)* 2019; 7(1), 9.
16. Kim H, Yu J, Bai D, Nahm MH, Wang P. Potentiating pneumococcal glycoconjugate vaccine PCV13 with saponin adjuvant VSA-1. *Front. Immunol.* 2022; 13, 1079047.
17. Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. *Front. Immunol.* 2014; 5, 520.

18. Jodar L, Butler J, Carlone G, *et al.* Serological criteria for evaluation and licensure of new pneumococcal conjugate vaccine formulations for use in infants. *Vaccine* 2003; 21(23), 3265–3272.

19. Malley R, Lipsitch M, Stack A, *et al.* Intranasal immunization with killed unencapsulated whole cells prevents colonization and invasive disease by capsulated pneumococci. *Infect. Immun.* 2001; 69(8), 4870–4873.

20. Malley R, Morse SC, Leite LC, *et al.* Multiserotype protection of mice against pneumococcal colonization of the nasopharynx and middle ear by killed nonencapsulated cells given intranasally with a nontoxic adjuvant. *Infect. Immun.* 2004; 72(7), 4290–4292.

21. Babb R, Chen A, Hirst TR, *et al.* Intranasal vaccination with gamma-irradiated Streptococcus pneumoniae whole-cell vaccine provides serotype-independent protection mediated by B-cells and innate IL-17 responses. *Clin. Sci. (Lond.)* 2016; 130(9), 697–710.

22. Jwa MY, Jeong S, Ko EB, *et al.* Gamma-irradiation of Streptococcus pneumoniae for the use as an immunogenic whole cell vaccine. *J. Microbiol.* 2018; 56(8), 579–585.

23. David SC, Laan Z, Minhas V, *et al.* Enhanced safety and immunogenicity of a pneumococcal surface antigen A mutant whole-cell inactivated pneumococcal vaccine. *Immunol. Cell Biol.* 2019; 97(8), 726–739.

24. Chan WY, Entwisle C, Ercoli G, *et al.* A novel, multiple-antigen pneumococcal vaccine protects against lethal Streptococcus pneumoniae challenge. *Infect. Immun.* 2019; 87(3), e00846-18.

25. Jang AY, Ahn KB, Zhi Y, *et al.* Serotype-independent protection against invasive pneumococcal infections conferred by live vaccine with *lgt* deletion. *Front. Immunol.* 2019; 10, 1212.

26. Ramos-Sevillano E, Ercoli G, Felgner P, *et al.* Preclinical development of virulence-attenuated Streptococcus pneumoniae strains able to enhance protective immunity against pneumococcal infection. *Am. J. Respir. Crit. Care Med.* 2021; 203(8), 1037–1041.

27. Alsharifi M, Mullbacher A. The gamma-irradiated influenza vaccine and the prospect of producing safe vaccines in general. *Immunol. Cell Biol.* 2010; 88(2), 103–104.

28. David SC, Lau J, Singleton EV, *et al.* The effect of gamma-irradiation conditions on the immunogenicity of whole-inactivated Influenza A virus vaccine. *Vaccine* 2017; 35(7), 1071–1079.

29. Singleton EV, Gates CJ, David SC, *et al.* Enhanced immunogenicity of a whole-inactivated influenza A virus vaccine using optimised irradiation conditions. *Front. Immunol.* 2021; 12, 761632.

30. McAllister LJ, Tseng HJ, Ogunniyi AD, *et al.* Molecular analysis of the PSA permease complex of Streptococcus pneumoniae. *Mol. Microbiol.* 2004; 53(3), 889–901.

31. Aggarwal S, Kumaraswami M. Managing manganese: the role of manganese homeostasis in streptococcal pathogenesis. *Front. Cell Dev. Biol.* 2022; 10, 921920.

32. David SC, Brazel EB, Singleton EV, *et al.* A nonadjuvanted whole-inactivated pneumococcal vaccine induces multiserotype opsonophagocytic responses mediated by noncapsule-specific antibodies. *mBio* 2022; 13(5), e0236722.

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