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# Promises and pitfalls of live attenuated pneumococcal vaccines

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**The pneumococcus is a remarkably adaptable pathogen whose disease manifestations range from mucosal surface infections such as acute otitis media and pneumonia to invasive infections such as sepsis and meningitis. Currently approved vaccines target the polysaccharide capsule, of which there are over 90 distinct serotypes, leading to rapid serotype replacement in vaccinated populations. Substantial progress has been made in the development of a universal pneumococcal vaccine, with efforts focused on broadly conserved and protective protein antigens. An area attracting considerable attention is the potential application of live attenuated vaccines to confer serotype-independent protection against mucosal and systemic infection. On the basis of recent work to understand the mucosal and systemic responses to nasal administration of pneumococci and to develop novel attenuation strategies, the prospect of a practical and protective live vaccine remains promising.**

## Global Burden of Pneumococcal Disease and Current Vaccine Coverage

The pneumococcus is an amazingly adept human pathogen. Normally found as a commensal organism in the nasopharynx, the pneumococcus is a leading cause of otitis media, pneumonia, sepsis, and meningitis worldwide. Infections caused by pneumococci are particularly acute in the very young, who bear a disproportionate burden of disease, with approximately 14.5 million incidents of severe pneumococcal disease each year leading to more than 800,000 deaths in children younger than 5 y[1] This incidence ranks the pneumococcus as a significant cause of morbidity and mortality worldwide and has driven extensive efforts at disease prevention.

Current pneumococcal vaccines such as Pneumovax and Prevnar rely upon antibodies generated against the polysaccharide capsule, of which over 90 types have been described. Induction of antibodies against the capsule correlates with protection against invasive disease. Almost immediately after introduction, a shift in prevalence from vaccine-type strains to non-vaccine type strains was observed in populations in which the vaccine was introduced.[2] This outcome is partially due to serotype replacement from non-vaccine strains becoming more prevalent and to serotype switching, driven by recombination at the capsule locus. Introducing additional polysaccharide capsule types in the conjugate may not result in retained immunogenicity of the multiple capsule types included and may raise the production costs. The rise of non-vaccine serotypes that are competent for invasive disease is of particular concern.[3] Therefore, there is tremendous interest in developing serotype-independent pneumococcal vaccine that would confer protection against both mucosal and invasive disease across age groups (recently reviewed in [4]).

The decline in incidence of invasive pneumococcal disease as children age does not strongly correlate with antibody levels against the polysaccharide capsule, indicating that additional mechanisms of protection apart from anticapsular antibodies are important.[5] Numerous research efforts have demonstrated that various pneumococcal proteins can be protective antigens against invasive disease in murine models and in convalescent serum. This information led to the basis for the development of a protein-based vaccine that would

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confer serotype-independent protection against invasive disease. These efforts have mainly been focused on developing combinations of highly conserved protein antigens or protein fusions that have proven to be successful in multiple murine models of invasive pneumococcal disease.[4,6] Even in highly conserved antigens, recombination events can occur whereby strains retain invasive capacity in high-risk hosts or restricted tissue tropisms.[7,8] Such genetic plasticity makes the development of universal pneumococcal vaccines a major scientific challenge, though significant progress has been made in recent years.

## Strategies for Attenuation

Because the main portal of entry for the pneumococcus is the mucosa, there is significant interest in a vaccine that could engender mucosal and systemic protection against the pneumococcus. One potential means by which this could be accomplished is via the development of a live attenuated vaccine administered to the nasal passages. Live vaccines provide the advantage of a level of antigen exposure higher than that of traditional vaccines. Live vaccines have been successfully used against several bacterial and viral pathogens, typically conferring effective protective capacity, particularly at the mucosal surface. The most critical aspect of developing live attenuated vaccines remains obtaining the appropriate balance between attenuation and vaccine immunogenicity. Many of these efforts have focused on expressing pneumococcal antigens in other bacterial strains having specific auxotrophic phenotypes rendering them unable to replicate in the host or on expressing recombinant protein in innocuous species such as *Lactococcus lactis*. Such strategies provide effective protection at the mucosal surface and during invasive disease.[9,10] Still, expression of individual recombinant proteins severely limits the repertoire of potential antigens; hence, other strategies have been explored.

Using the pneumococcus itself rather than another bacterial species as a platform for a live attenuated vaccine has several potential advantages and challenges. One challenge is in choosing the strategy with which to modify the pneumococcus to have a self-limiting replication capacity at the mucosal surface to eliminate the risk of invasive disease or undesired inflammatory damage to the site of vaccination. This risk is of particular concern in the pneumococcus, whose natural competence and genetic plasticity leads to rapid recombination events that could readily revert such a modified strain to fully virulent status if such risks are not sufficiently mitigated. Another advantage is that more potential antigens will be produced if using such a delivery platform than if expressing the individual recombinant proteins in various species of bacterial vectors. Furthermore, if an encapsulated strain is used, capsule-specific antibodies could be generated in addition to the expected protein-based antibodies. However, even with the deletion of the polysaccharide capsule to attenuate pneumococcus, effective serotype-independent protection against colonization and during invasive disease can be conferred, indicating the potential application of this strategy to generate a universal vaccine.[11] The immune response at the mucosal surface in response to live versus heat-killed bacteria is likely to be distinct, indicating that more effective protection may be mediated by replication-competent bacterial strains, though the strategy of properly adjuvanted whole-cell vaccines has shown substantial promise.[12-14]

Crippling the capacity of the pneumococcus to cause invasive disease while retaining colonization via selective deletion of virulence genes has proven an effective means in developing a live-attenuated vaccine. Deleting either the major surface adhesion protein PspA or the cholesterol-dependent toxin pneumolysin resulted in pneumococci with significantly attenuated virulence in murine models.[11,15] However, if the mutant strains are encapsulated, then the ability to colonize the nasopharynx was retained.[11] Even with the lack of an antibody response against these 2 important antigens, excellent serotype-independent antibody responses and protection against invasive disease was conferred in response to intranasal administration of these strains as vaccines. This outcome indicates that even with the loss of major antigenic virulence genes, such attenuation strategies are a viable option in vaccine development.

Another potential strategy for generating live vaccines is targeting atypical virulence determinants that are unlikely to be highly immunogenic in the host, such as microbial transporters and signaling molecules that are required for infection yet do not result in significant antibody recognition. This strategy has the advantage of retaining expression of all the antigenic virulence genes and the polysaccharide capsule. This approach has resulted in considerable success. Deletion of *pep27*, involved in pneumococcal lysis, dramatically reduces pneumococcal virulence in murine models, being cleared in less than 24 hours after inoculation.[16] When administered as a live vaccine via intranasal inoculation, this mutant strain induced a potent antibody response that conferred protection at the mucosal surface and during invasive disease.[16,17] Another strategy involved vaccination with strains containing deletions of other key proteins. Deleting *caxP/mgtA,* a calcium/magnesium transporter, resulted in a vaccine strain that was cleared from the nasal passages within 24 hours. Deleting *ftsY*, a central component of the signal recognition particle (SRP) protein secretion pathway, resulted in a strain that does not cause invasive disease or long-term colonization.[18] These deletions were both generated on the D39 (serotype 2) and BHN97 (serotype 19F) backgrounds and used to vaccinate mice. All 4 of these live attenuated vaccines were able to confer a serotype-independent antibody response, though the BHN97Δ*ftsY* vaccination induced the most potent antibody response, potentially due to it having the longest colonization time of the vaccine strains. The BHN97Δ*ftsY* vaccination was also able to confer serotype-independent protection against acute otitis media, sinusitis, and bacteremia.[18] The BHN97Δ*ftsY* vaccine conferred effective protection against both otitis media and pneumonia in the context of viral co-infection in murine infection models. This live vaccine also proved effective in the chinchilla model of otitis media, further substantiating protective capacity.

These examples highlight the intriguing possibilities of a live attenuated universal pneumococcal vaccine engendering both mucosal and systemic protection.

## Colonization Versus Vaccination

If the protection conferred by a live attenuated pneumococcal vaccine is so effective, why is repeated colonization by different serotypes not so? Although this question is far from being answered, recent studies may provide partial explanations for this discrepancy. Colonization confers protection against subsequent homologous re-challenge in murine models and in humans.[19] The duration of the colonization during inoculation with live vaccines may be critically important in conferring effective protection, though prolonged carriage is not required for a robust mucosal and serum antibody response.[20] However, some evidence shows that the degree of protection at various host sites may differ based on the longevity of the initial vaccine strain. Strains of the same genetic background and serotype confer vastly different protective capacity at the mucosal surface, with strains that are more rapidly cleared being ineffective and strains colonizing for approximately 7 d being more effective despite both types inducing potent serotype-independent antibody responses.[18] Interestingly, a greater antibody response has been observed upon repeated administration of a live vaccine than upon inoculation with the parental strain having prolonged carriage.[18] This observation indicates that the lifestyle of the pneumococcus during prolonged carriage in the nasopharynx may not be as conducive to immune recognition than is repeated inoculation and subsequent rapid clearance. One possibility is that the strains in the nasopharynx exist primarily as a biofilm community that limits immune recognition of various pneumococcal epitopes. One line of evidence to support this hypothesis is that convalescent sera have distinct immunoreactivity to pneumococci that are grown either planktonically or in biofilm communities.[21] How these growth patterns affect the generation of protection at mucosal sites remains unknown.

## Practical Considerations

One significant barrier to a live pneumococcal vaccine is the rapid exchange of genetic material between strains as a result of the natural competence of this bacterium. Hence there is considerable concern of a reversion to pathogenicity in the case of a live attenuated vaccine. Several approaches can be used to mitigate this risk, including the deletion of the competence machinery to render the vaccine strain unable to uptake foreign DNA to prevent potential recombination events with the resident nasal flora. Because genes in the competence loci have also been implicated in colonization and invasive disease, the retention of immunogenicity and protective capacity in the final, stabilized form of competence-machinery–deleted vaccines would require evaluation. Another approach to consider is the replacement of the pneumolysin toxin with a toxoid version that would have greatly reduced capacity to damage host cells yet retain immunogenic properties.[6,22] Given the understanding of pneumococcal competence and the ease of manipulating these factors, producing tailored strains to achieve these objectives should be feasible and readily obtained.

Another consideration is the stability and administration of a live vaccine. Culturing the bacterium is clearly not feasible, as pneumococcus undergoes autolysis once it reaches stationary phase, making it nonviable. Pneumococcus is tolerant to desiccation and remains infectious upon reconstitution.[23] Hence, one promising approach would be to generate desiccated vaccine strains that could be stored and reconstituted for inoculation. Whether the approaches used to attenuate the strains would have detrimental effects on either storage or effectiveness of protection following reconstitution are questions that will need to be addressed.

Additional care will have to be taken to understand how administration of a live pneumococcal vaccine would alter the microbiota of the nasopharynx. Vaccination with the PCV7 vaccine was shown to result in a temporary increase in colonization by *Staphylococcus aureus* in young children,[24] and live attenuated influenza vaccines can dramatically alter the relative bacterial burden in the nasal passages in murine systems.[25] Hence, the impact of this strategy on the normal nasal flora should also be considered in the development of these vaccines. Despite these potential hurdles, the protective efficacy of these vaccines in murine systems warrants their further investigation.

## Future Prospects

Numerous studies have demonstrated robust efficacy of live attenuated pneumococcal vaccines created via various attenuation strategies, though the precise mechanisms underlying activity remain to be elucidated. Although the serotype-independent antibody response is clearly important, discerning the cellular properties that play roles in mucosal immunity will be a critical factor in understanding how to optimize these vaccines to provide the most comprehensive protection. Defining the cellular factors underlying the observed protective capacities of such vaccines will provide insight into the factors involved in inducing potent immunity at the mucosal surface. Recent work has defined many of these factors and has led to novel strategies to enhance these responses.[12,26] Such insights into both the strategies utilized by pneumococcus to survive in the host and optimizing the immune response to confer more effective protection shows great promise in strategies to prevent a major cause of childhood mortality worldwide.


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No potential conflicts of interest were disclosed.

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