Challenges for the development of vaccines against *Haemophilus influenzae* and *Neisseria meningitidis*

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Abbreviations:

NTHi nontypeable *Haemophilus influenzae*

Hib *Haemophilus influenzae* type b

OMP Outer membrane protein

PRP Polyribosyl ribitol phosphate

Introduction

The development and success of the *Haemophilus influenzae* type b (Hib) vaccine, introduced in the 1990s, seemed to mark a turning point in vaccine development. While the concept of polysaccharide-based vaccination in itself was not novel, the success of the polyribosyl ribitol phosphate [PRP]-conjugates provided an answer to the poor efficacy of the polysaccharide antigen. Like the discovery of the benefits of toxoid-based vaccines for diseases such as tetanus, the introduction of conjugation technologies seemed to be the answer for delivering polysaccharides from encapsulated microorganisms. Conjugate technology is being successfully applied to vaccines for *N. meningitidis* with the exception of serogroup B strains. The development of effective vaccines for nontypeable *Haemophilus influenzae* NTHi and *N. meningitidis* serogroup B present a major challenge.

The Burden and Profile of Disease

Prior to the introduction of the Hib vaccine, developed countries reported varied incidence rates for invasive disease. In the United States, the CDC reports between 40-100/100,000 children per year in the under 5 years age group [1]. Some countries in South America, Asia and the Middle East were reporting rates lower than 20/100,000 and in countries such as Alaska, northern Canada and Australia native populations had incidence rates exceeding 150/100,000 [2]. In 1991 Australia reported 515 cases and, since the introduction of the vaccine, this has dropped to only 27 cases reported in 2001 (Fig 1). The decade 1991-2001 has seen a remarkable drop in disease incidence in those countries that have introduced the vaccine with the USA now reporting less than 2 cases/100,000. These figures are very supportive of the

conjugated polysaccharide vaccine approach for managing serious disease caused by similar microorganisms.

Hib is not the only strain of *H. influenzae* to cause disease and while the Hib vaccine has been highly successful in controlling infections caused by this specific serotype, it has never had the potential to impact on the significant incidence rate of respiratory infections caused by nontypeable *H. influenzae* (NTHi). NTHi does not express a polysaccharide capsule and while invasive disease is not as big a problem as it was for Hib, it is known to occur. A common commensal of the upper respiratory tract it has carriage rates of about 40% in adults and between 80-100% in children and gives rise to a significant disease burden being a cause of acute and chronic infections such as otitis media, sinusitis, conjunctivitis, chronic bronchitis exacerbations, pneumonia and others in often otherwise healthy subjects. The morbidity and mortality in affected individuals causes a significant economic burden to health systems and the community. The strains associated with both Hib and NTHi disease derive from a variety of genotypes [3] and biotypes [4] and therefore targeting a vaccine against all *H. influenzae* infections is more complex than was the type B polysaccharide for Hib. A decade after the introduction of the successful Hib vaccine, and despite extensive research, no vaccine yet exists for NTHi, highlighting the difficulties still confronting vaccine development.

Meningitis, due to *Neisseria meningitidis*, is caused by multiple serogroups with the serious sequelae and rapid progression of the disease making it a highly feared infection. 10-15% of infections are fatal and approximately 10% who recover are left with permanent serious sequelae. The incidence rate can be up to 2% of the

population during an epidemic, particularly in sub-Saharan Africa in the region known as the "meningitis belt" [5]. Outbreaks of disease are usually associated with a particular clone being introduced and transmission occurring through direct contact with respiratory secretions. In general, infants and teenagers are most at risk, but during an epidemic, the risk extends to the general population, in particular, those residing in crowded communities. There are 12 serogroups based on their capsular polysaccharides but virtually all disease is caused by an isolate from either A, B, C, W135 or Y serogroups, with A, B and C accounting for approximately 90% of the disease burden. Countries such as Australia and New Zealand have seen rises in the incidence rates during the last decade. Australia's rate has doubled during this time (Fig 1), whereas New Zealand reports a 10-fold increase [6]. The multiple meningococcal serogroups associated with disease make the design of an effective vaccine more complex than was the case for the Hib vaccine.

It is often the "fear factor" that drives the demand for a vaccine. If we compare the incidence rates for disease associated with NTHi and *N. meningitidis*, then as far as economic burden is concerned, NTHi is responsible for greater costs. However, it is the unpredictable susceptibility, fear of death and rapid onset severe sequelae associated with *N. meningitidis* that drives the demand by the public for a vaccine.

Currently available *H. influenzae* and *N. meningitidis* vaccines and their limitations

The use of purified polysaccharide vaccines has been limited due to their poor immunogenicity in infants and young children as well as the relatively short duration of immunity induced. A number of licensed and highly efficacious conjugate

vaccines are now available for Hib. Each vaccine is comprised of the purified b capsular PRP, conjugated to one of four protein carriers (tetanus toxoid, diphtheria toxoid, a nontoxic mutant diphtheria toxin or an outer membrane protein (OMP) complex of *N. meningitidis*). As indicated above, the introduction of Hib conjugate vaccines has seen a dramatic decline in Hib disease, but access to the vaccine remains a significant problem for developing countries.

Whilst it is without question that Hib infection, a major cause of childhood mortality and morbidity, can now be effectively eliminated by vaccination, infection due to NTHi remains a significant problem. A great deal of effort is now being focussed on developing a vaccine against NTHi, but the antigenic components have not been as easy to define as was the case for Hib. Success will depend on the identification of an OMP antigen or more likely a group of antigens which are not only conserved across this genetically diverse species, but are capable of inducing an appropriate immunogenic response as a vaccine component.

Most cases of disease caused by *N. meningitidis* belong to five serogroups; A, B, C, W135, and Y. Whilst there is geographical and temporal variation of serogroups, disease due to serogroups B and C predominate. Several vaccines are currently licensed for *N. meningitidis* serogroup C, where the C polysaccharide is de-O-acetylated and conjugated to tetanus toxoid or a nontoxic mutant of diphtheria toxin, as for Hib. Similarly, conjugate vaccines against serogroups Y and W135 are also now available in some countries. The development of a vaccine against serogroup B is problematic. The B capsular polysaccharide has been shown to be poorly immunogenic, even when conjugated to a carrier protein. The most probable reason

for this poor immunogenicity is immune tolerance due to similarity between the serogroup B capsular polysaccharide and cell surface glycoproteins of neural tissue [7]. Hence, as for NTHi, non-polysaccharide antigens need to be identified and tested.

Both NTHi and *N. meningitidis* are obligate human parasites that exist as part of the normal upper airway microflora. Vaccine development against what are normally commensal microbes brings with it new and previously unexplored challenges. Significant debate has occurred as to whether reduction of bacterial load or sterile immunity should be the outcome of such vaccines, given that there is a good association between high bacterial load and incidence of bacterial infections in the airways.

It is perhaps more useful to consider what is the purpose of these vaccines in the first instance. Hib and *N. meningitidis* vaccines need to provide protection against invasive disease. A vaccine against NTHi, on the other hand, would be essentially therapeutic. It must also be recognised that sterile immunity following vaccination against commensal bacteria may not only be unachievable but is most likely undesirable. In either case, replacement of airway microflora with non-vaccine strains and genera is likely. This has certainly been reported following vaccination with some of the current conjugate vaccines for *Streptococcus pneumoniae* [8]. If the *S. pneumoniae* scenario holds true, then it is likely that replacement strains, following vaccination with *N. meningitidis* polysaccharides, may be less pathogenic. Of course, this assumes that the final formulation of the *N. meningitidis* vaccine will contain the five predominant disease-associated serogroups, which is currently not the case. Current vaccine practice for *N. meningitidis*, using mono-, bi- and tetra-valent formulations, may result

in replacement with other disease causing serogroups. Indeed, a recent study suggests this to be the case – following vaccination with a bi-valent A/C polysaccharide vaccine, an increase in serogroup W135 disease was reported [9].

The use of non-polysaccharide antigens for NTHi and serogroup B *N. meningitidis* raises other issues. Will the development of a vaccine containing protein antigens which are capable of protecting against the antigenic diverse repertoire of NTHi or *N. meningitidis* modify the composition of the upper airway microflora and if so, what will be the outcomes of such a change? Whilst answers are unknown at this stage, it will be fundamental that following the introduction of such vaccines, long term longitudinal studies of disease and bacterial colonisation profiles of the airways will need to be conducted.

In response to public opinion and to maintain immunisation rates, particularly in developed countries, it is necessary to reduce the number of injections received by young children. This will need to be achieved through the development of new combination vaccines which bring with them a range of issues and concerns, many of which are unfounded[10]. What will be important in new combination vaccines is a demonstration that immunogenicity and efficacy of the new combined vaccine is appropriate and achieves an acceptable level of protection against disease but not less than that of the components as individual vaccines. Re-examination of currently accepted immune correlates of protection would probably be required for the development of these vaccines.

What are the major issues for vaccine development?

Phenotypic and genotypic investigations of *H. influenzae* strains have demonstrated that type b strains and other non group B capsulated strains have restricted genetic diversity and may be clonal, whereas NTHi are heterogeneous and appear to lack clonality[11,12]. This diversity also occurs with *N. meningitidis* where there are rapid phenotypic changes allowed for by random genetic change and gene regulation [13].

Carriage of multiple phenotypic and genetic types of *H. influenzae* can occur in children less than 5 years of age with up to seven different strains colonising the upper respiratory tract at one time[14,15]. While carriage of more than one genetic type of *H. influenzae* correlates with otitis media susceptibility factors such as allergies and exposure to smoking, a direct correlation between carriage of multiple genetic types and otitis media is not known[15].

Genome plasticity appears essential for persistence in the human host. Microsatellites in *Haemophilus* and *Neisseria* containing repetitive DNA tracts increase or decrease in length by gain or loss in repeat units in the genome allowing translational shifts in the open reading frame. Modelling has shown that this is one mechanism for rapid increases in diversity [16], particularly in association with changes to adhesion factors, lipooligosaccharides and iron acquisition.

The ability of *Neisseria* to undergo phase variation and recombination between species is far greater than for *Haemophilus* [17,18]. Of more concern, though, is the ability of *N. meningitidis* to undergo clonal diversification during the course of an

epidemic[19]. This ability would yield a mono- or even multi-valent vaccine of little use in the control of the outbreak.

Vaccine candidates for diseases caused by *N. meningitidis* and *Haemophilus sp.* must avoid induction of autoimmune disease or initiation of inappropriate inflammatory responses. The potential for molecular mimicry by these pathogens must be kept in mind. Lipooligosaccharides and other *Neisseria* structures can mimic human glycolipids and glycosphingolipids and NTHi lipooligosaccharides can act as a platelet activating factor (PAF), binding to the PAF receptor on airway epithelial cells. Due to such mimicry, infection sometimes elicits the production of autoantibodies, for example, antibodies to embryonic neural cells following infection by *N. meningitidis* serogroup B[20] and such adverse responses must be avoided in any vaccine.

Approach to selection of antigens for nontypeable *H.influenzae* and *N.*

***meningitidis* serogroup B**

The challenge for any vaccine still remains with identifying antigens for consideration in human trials. There are strict key performance criteria that the antigens must meet. The antigen or key antigenic component must be conserved across the genus, induce protective immune mechanisms against infection, not enhance tissue damage at the site of infection and finally, the immune response induced must overcome the immuno-modulatory and immune evasion capabilities of the bacteria. Where infection can be caused by multiple strains, and recurrent infections are possible, finding antigens that meet these criteria has been a major challenge and no single approach has proven better than another in arriving at an answer.

The *H. influenzae* genome was the first whole genome available [21]. The information contained in the genome has been of great assistance, but seven years later, its information has not resulted in identifying new or better vaccine candidates. Most antigens on the list as potential vaccine candidates were identified by means other than genomic mining. The list includes proteins such as P6 [22], epitopes of the P5 fimbriae [23], protein D [24], segments of P2, the major porin protein [25], a group of high molecular weight proteins ranging in mass from 120-125 kDa [26] and OMP26, a 26 kDa protein [27]. Modified lipooligosaccharide [28] appears to be the only significant toxin-like antigen on the list. To date, no human trial data on any of these antigens has been presented in the literature.

While the capsular polysaccharide appears to be a reasonable candidate for some *N. meningitidis* serogroups, for *N. meningitidis* serogroup B this is not the case. For this serogroup, high throughput genomic analysis may speed up the identification of potential vaccine antigens by the selection of conserved open reading frames. However, this approach does not allow prediction of surface expression or which sequence the microbe is using. Testing the plethora of proteins produced in this way in the animal systems will then be the rate-limiting step to further advancement.

Outer membrane vesicle vaccines for *N. meningitidis* group B appeared to show promise[29], but the vaccines were inefficient in adults, due to the heterogeneous antibody response, and did not protect children under 2 years of age[30]. The major proteins targeted by the immune response were class 1 (PorA) and Opc. The PorA protein appears an important *N. meningitidis* group B vaccine candidate due to its

ability to induce high levels of bactericidal antibody, but PorA types show no geographical grouping and extensive variability [31]. If a polyvalent PorA antigen is to be a good vaccine target, then characterisation of the variability of the 2 variable regions, VR1 and VR2, is crucial [19].

Other lead vaccine antigens have included Opc and TbpB. However, both demonstrate significant variability between strains and antibodies to these proteins lack cross-strain bactericidal activity [32,33].

Correlates of protection/efficacy

There is an increasing acceptance that measuring total antibody levels *per se* does not provide sufficient information to draw definitive conclusions with respect to vaccine efficacy. This is particularly so for commensal bacteria where there is already an established immune response prior to the infection episode. For Hib and *N. meningitidis* serogroup B there is good evidence that protection correlates with specific bactericidal antibody titres, whereas this is not necessarily the case for NTHi. Therefore, for some vaccines it will be necessary to develop new correlates based on a range of immune assays that reflect functional activity of antibodies as well as specific cellular responses.

Conclusion

The development and successful implementation of polysaccharide-protein conjugate vaccines for Hib have been the most significant advance in vaccinology in recent time. The application of this approach to *N. meningitidis* has been highly successful. The development of non-polysaccharide vaccines for *N. meningitidis* serogroup B and

NTHi remain a significant challenge, largely due to the enormous antigenic diversity of these organisms. It is hoped that characterisation of this diversity at the genomic level will assist the development of future vaccines against infections caused by these bacteria.

Figure Legend

Figure 1. Incidence of Hib and *N. meningitidis* in Australia between 1991 and 2001.

Figure 1.

^aCommunicable Diseases Network Australia - National Notifiable Diseases Surveillance Systems, personal communication

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