

Bacterial polysaccharide–protein conjugate vaccines

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Following demonstration that chemical conjugation of polysaccharide antigens to proteins could enhance their immunogenicity in the 1920s, interest in this approach to primary prevention of bacterial infections waned with the development and widespread use of antibiotics. Emergence of resistant bacteria rekindled interest in the late 20th century, which saw extremely rapid development and implementation of several vaccines which are already rapidly changing the epidemiology of childhood infections with *Haemophilus influenzae* type b, *Streptococcus pneumoniae* and *Neisseria meningitidis*. Others such as Group B streptococcus and *Salmonella typhi* infections may soon follow. However, several important questions about the immunology of these antigens remain unanswered and the long-term implications of reducing or eliminating the circulation of organisms which are more commonly nasopharyngeal commensals than pathogenic invaders are uncertain.

Introduction

Young children are prone to infections. There are now abundant data that this reflects not only their immunological naïvety, but also a degree of immunoincompetence relative to older children and adults. No doubt, to some extent structural immaturity, such as fragility of integument and mucosae, also contributes. There is a large published literature comparing, in infants and older individuals, the size or function of almost every element of immunity, cellular and humoral, innate and adaptive; studies which often demonstrate the former to be the weaker¹. It is hard to know what to make of much of this observational information, but in some instances the implications are clear. Adaptive cell mediated immune responses to a wide variety of foreign microbial antigens in newborns are both weaker and slower than in older children. Since all the necessary components for functional responses appear to be in place well before term², perhaps this

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incapacity reflects a post natal lag period in immunological regulation, still set to tolerate foreign (maternal) antigens while living in the (normally) sterile environment of the uterus.

If this supposition is true, it remains puzzling that a more rapid post natal immunological 'reboot' has not evolved, as this relative refractoriness often appears to persist for months, at least for some aspects of specific antimicrobial immunity. Interference by transplacentally acquired IgG—a vital element of immune protection against infection during early life, which disappears with a half-life of approximately 28 days after delivery—can to some extent account for the persistent suppression of antibody responses—particularly to protein antigens—but does not cause the down regulation of T cell function³. Nor does maternal antibody explain the profound and long lasting failure of infants to make antibodies to the polysaccharide (PS) capsular antigens which decorate many bacterial pathogens⁴, a failure which is presumably at least partly responsible for the high incidence of invasive infections due to these organisms in this age group.

Antibody responses to PS antigens are the subject of considerable modern myth. Since they are not peptides, PSs should not, by definition, be processed and presented by MHC class II antigens, so that the recruitment of T cell help by this route for B cell function is not expected. Nevertheless, isotype switching does occur—not only IgM but also abundant IgG and particularly IgA responses to these antigens are seen^{5,6}. It appears that a distinct signalling pathway may regulate this important antibody response pathway⁷ whose size and character vary not only between individuals⁸ and with age, but also with previous exposure⁹ and between antigens.

Complete and permanent tolerance to some bacterial capsular PS antigens is the rule. For example, Lancefield Group A streptococci (*Streptococcus pyogenes*) colonizing the nasopharynx, an important cause of both mucosal and invasive disease in children, are consistently encapsulated, although to a variable extent. However, the PS in question is hyaluronic acid—an antigen against which antibody responses would be unwanted as it is a major component of human connective tissues¹⁰. The capsule of *Neisseria meningitidis* group B is likewise poorly immunogenic as a vaccine¹¹, an observation which may be explained, at least in part, by the structural similarity of the capsular PS with polysialic acid moieties which decorate components of mammalian tissues including the central nervous system¹².

Nevertheless, the PS capsular antigens of many other pathogenic bacteria do induce substantial protective serum antibody responses when used as vaccines. However, for most of these antigens, this is not true in young children. Although the age of 2 years is often cited as that at which such responses start to be seen, in fact the doses of these antigens needed to

elicit protective responses and the average age at which such responses become active vary quite substantially and predictably between antigens. For example pneumococcus type 3 capsular PS is relatively immunogenic in infants whereas types 6A and 6B are extremely poor immunogens, with other types ranging in between¹³.

One is left wondering exactly why this is so. Presumably it is no accident. This implies some relative survival advantage of this temporary anergy, perhaps less marked than for the fully 'invisible' antigens described above, but operative nonetheless. On the other hand, it is also possible that the encapsulated bacteria that infect human children today may not have been around long enough in evolutionary time for the survival advantage of vigorous early immune responses to their capsules yet to have taken effect.

In any case, faced with the real problem of the serious morbidity of these infections in young children and evidence that serum antibodies to capsular antigens protect, such theoretical considerations have taken second place to efforts to render these antigens immunogenic in this high risk group. The principle that made this possible was established over 70 years ago with the first description of an effective bacterial PS protein conjugate vaccine¹⁴. Covalent conjugation of PS haptens to protein carriers renders them capable of inducing humoral immune responses with the characteristics of T cell dependent antigens: responses with memory, affinity maturation and, critically, immunogenicity in young children. A conceptual schema for this, as most usually expounded, is depicted in Figure 1, which combines the afferent functions of antigen recognition and presentation and the efferent function of antibody production and release into a single B cell for simplicity's sake.

There is a tendency to focus on the effects of age and antigen on immune responses and ignore the heterogeneity that exists between individuals. Every geometric mean titre disguises a population whose antibody responses to a standard vaccine dose regimen vary over at least one, and often more than two orders of magnitude and among whom there are always some who make no detectable response at all. The genetic allotypes which doubtless underlie this heterogeneity between individuals (and racial groups) remain incompletely understood¹⁵ and may be distinct for T-independent and T-dependent regulation of antibody responses in some cases¹⁶ but not others⁸. The current 'one size fits all' approach to immunization may be the only workable one at present but perhaps, one day, we will designate immunization regimens not by national boundaries, but also design them for each individual recipient. Meantime, it is worth remembering that vaccine immunogenicity and efficacy findings in one group cannot necessarily be extrapolated to others whose genetic characteristics are distinct.

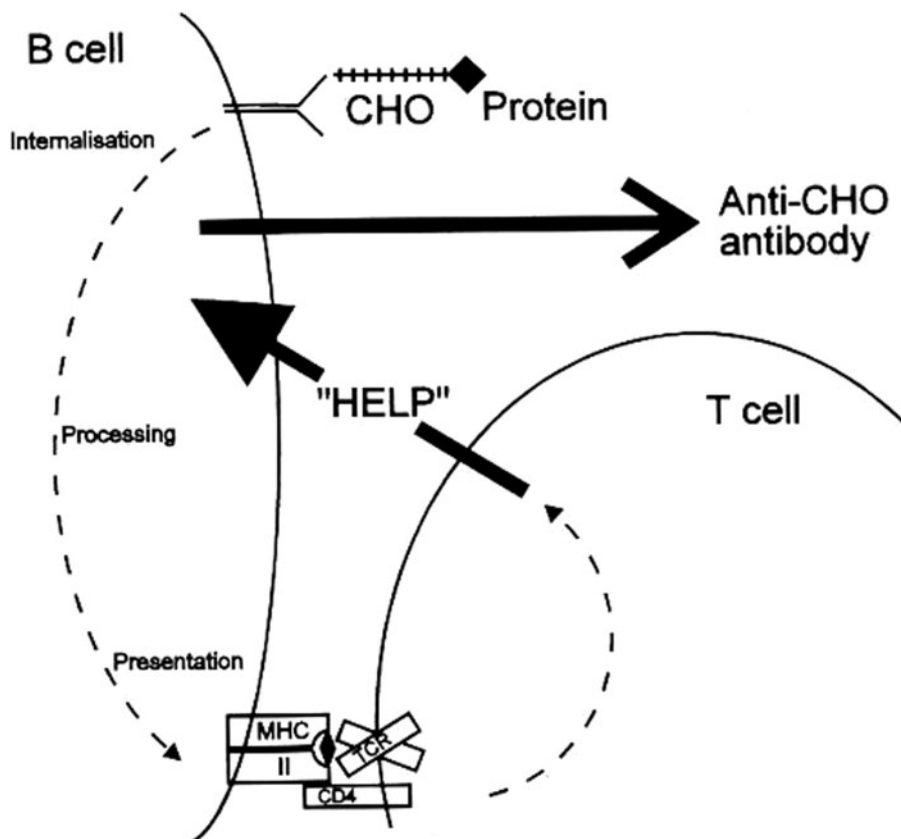


Fig. 1 Schematic diagram of mechanism of action of PS-protein conjugate vaccines.

Haemophilus influenzae type b

Early vaccine studies using polyribosyl-ribitol phosphate (PRP), the capsular PS purified by detergent extraction from cultures of *Haemophilus influenzae* group b (Hib), showed good protection in older children, but limited immunogenicity or efficacy in children aged under 2 years¹⁷.

Development and implementation of conjugate Hib vaccines in those countries able to afford them have been a remarkable success¹⁸. Four different protein carriers have been used in vaccines with distinctly different structures and conformations¹⁹ (Table 1) and most countries have adopted regimens involving three doses in infancy followed by a fourth dose in the second year of life. The UK chose instead a three dose regimen given at 2, 3 and 4 months. Within 2–3 years of introduction, administration of Hib vaccines mixed with the combined diphtheria, tetanus, whole cell pertussis vaccines then in use became routine in the UK, based on a series of studies suggesting similar immunogenicity for the combination²⁰.

Table 1 Some PS-protein conjugate vaccines currently or previously used or approaching licensure

Saccharide(s)	Protein(s)	Manufacturer(s)	Trade name
<i>Haemophilus influenzae</i>			
Type b polysaccharide	Diphtheria toxoid ('PRP-D')	Aventis Pasteur (Connaught)	ProHIBIT®
Type b saccharide	Mutant non-toxic diphtheria toxin CRM197 ('HbOC')	Wyeth	HibTITER®
Type b saccharide	<i>Neisseria meningitidis</i> outer membrane protein in outer membrane vesicles ('PRP-OMP')	Merck	PedvaxHIB®
Type b polysaccharide	Tetanus toxoid ('PRP-T')	Aventis Pasteur and Glaxo SmithKline	ActHIB®; Hiberix®
<i>Neisseria meningitidis</i>			
Group C saccharide	Mutant non-toxic diphtheria toxin CRM197	Wyeth	Meningitec®
Group C saccharide O-acetylated	Mutant non-toxic diphtheria toxin CRM197	Chiron	Menjugate®
Group C polysaccharide deO-acetylated	Tetanus toxoid	Baxter	Neis-Vac-C®
<i>Streptococcus pneumoniae</i>			
Serotypes 4, 9V, 14, 19F and 23F polysaccharides; 6B saccharide	Mutant non-toxic diphtheria toxin CRM197	Wyeth	Prevenar®
As above plus types 1 and 5 polysaccharides	Mutant non-toxic diphtheria toxin CRM197	Wyeth	Not-licensed
1, 4, 5, 7F, 9V, 19F and 23F polysaccharides*; types 3, 14 18C and 6B polysaccharides**	Tetanus toxoid*; Diphtheria toxoid**	Aventis Pasteur	Not-licensed
1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F polysaccharides	Non-typeable <i>H. influenzae</i> outer membrane protein	Glaxo SmithKline	Not-licensed

Note: Hib vaccines are now commonly given in combined formulations with other primary schedule antigens.

The different Hib vaccines are distinct in their immunogenicity. The PRP-OMP (meningococcal outer membrane protein) conjugate vaccine, only two priming doses of which are recommended in the USA, appears to induce good T cell dependent antibody responses after only one dose in infancy, unlike the other conjugates. Recent evidence suggests this may be because this protein carrier complex has direct adjuvant effects²¹. PRP-D, while comparable to other conjugates in older children, elicits much lower antibody responses in infants aged less than 6 months²². Although highly efficacious in an infant study conducted in Finland²³, it showed no efficacy in a study conducted in native Alaskan infants under conditions of intense exposure²⁴.

Serological surrogates of protection of 0.15 and 1 µg/ml, based largely on passive protection studies and a study using unconjugated PRP in older children¹⁷ gained much currency in the early years of conjugate vaccine use. The widespread acceptance of these thresholds meant that there was considerable concern when a series of studies involving each of the available Hib conjugate vaccines showed that, when combined

with various diphtheria, tetanus, and acellular pertussis vaccines (DTaP), there was a marked reduction in immunogenicity so that many infants had serum IgG below the accepted levels for protection following the first three doses of the vaccine²⁵. However, further consideration of the facts that children immunized in this way appeared to display features of good immunological memory including affinity maturation and substantial booster responses both to further conjugate doses and to plain PS PRP and that children immunized with unmixed Hib conjugates commonly have serum IgG to PRP well below these thresholds by the end of the first year of life without developing Hib disease led to a re-evaluation of the significance of these findings²⁶. All seemed well until an upturn in Hib disease in England and Wales was recently noted which led to the demonstration of worryingly lowered efficacy rates²⁷, due in part to the effects of the lowered immunogenicity against Hib when these vaccines had been combined with DTaP²⁸. A catch up programme to provide pre-school children with a fourth Hib dose was rapidly instituted. However, it now seems clear that our understanding of the relative importance of circulating antibody and of immunological memory in the prediction of protection provided not only for Hib but also other conjugate vaccines is very incomplete.

A somewhat unexpected bonus of the Hib programme has been the extent to which use of these parenteral vaccines has induced nasopharyngeal mucosal immune responses and the elimination of asymptomatic carriage, the natural reservoir for this pathogen. The consequential herd immunity resulted in a more rapid decline in disease than expected following introduction of the vaccines and its existence was confirmed by elimination of disease in age groups who had not been immunized²⁹. Antibodies to PRP demonstrated in nasopharyngeal secretions are presumably responsible for this, although their mechanism of action in this context is unknown.

Neisseria meningitidis

For the UK, the epidemiology of bacterial meningitis in children made *Neisseria meningitidis* the logical second target after Hib, a mission rendered more urgent by a steady rise in incidence of invasive disease during the latter part of the 1990s and the steady emergence of a group of 'hypervirulent strains' known as the ET-37 complex³⁰. For reasons explained above, prevention of group B, the most prevalent cause of invasive disease, with capsular PS conjugate vaccines has not been pursued with much vigour, despite that fact that antibodies to this antigen are found in healthy people³¹. Most efforts are currently focused on identifying candidate group B protein or lipopolysaccharide-based vaccine

candidates³². In contrast, group C disease is now in steep decline in the UK following the introduction of an immunization programme starting in late 1999³³ (Fig. 2). Three conjugate vaccines have been used to date, two conjugates to CRM197 (a non-toxic mutant diphtheria toxin) and one to tetanus toxoid (Table 1). The latter consistently induces bactericidal antibody ($\geq 1:8$) after only a single dose at 2 months³⁴. The vaccines have been given to infants at 2, 3 and 4 months and an extensive catch up programme has involved immunization of all children up to school leaving age. Following introduction of the vaccines in the UK, they have been licensed and introduced in several other European countries and, most recently, in Canada.

Carriage rates in young children are very low, making studies difficult to undertake. Several large cohorts of teenagers and young adults are being followed in UK centres and the first report of these has showed a significant reduction in carriage rates of group C coincident with introduction of immunization³⁵. We have studied mucosal immune responses to these vaccines and it is clear that both IgG and secretory IgA to the PS-antigen are induced in nasopharyngeal secretions in teenagers^{5,36}. IgA responses to the CRM197 conjugates in infants following the primary three dose course are poor but salivary IgG is induced³⁷. As with Hib, the relative importance of these two antibody isotypes and their mode of action, if any, on carriage, remain unclear.

Development of conjugate vaccines to other meningococcal serogroups is progressing. A conjugate against serogroup A might be a valuable tool in the control of epidemic disease in sub-Saharan Africa. A combination vaccine including serogroup Y will be potentially attractive for use in the

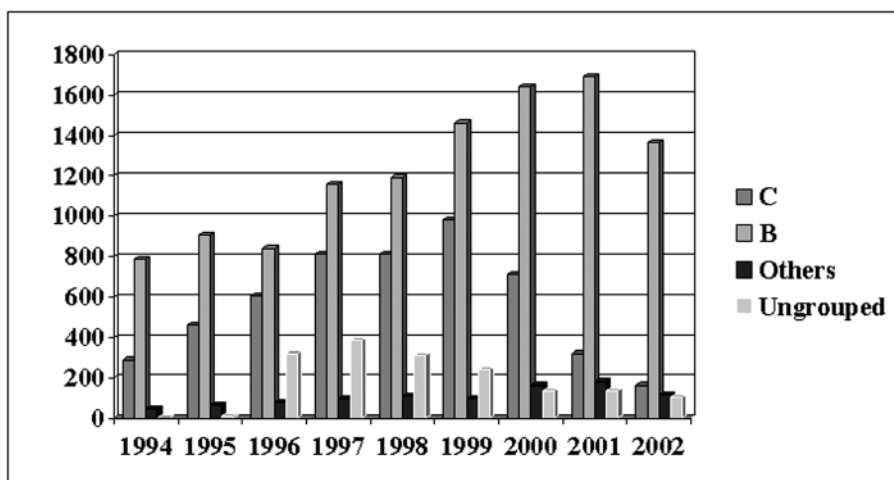


Fig. 2 Laboratory isolates (culture and PCR) of *N. meningitidis* by calendar year, England and Wales (data from HPA website: <http://www.hpa.org.uk>).

USA where it is relatively more prevalent³⁸. The production of combinations of conjugate vaccines against meningococcal, pneumococcal and Hib disease would clearly simplify administration of primary schedules of immunization in the future, if they prove to be immunogenic.

Re-immunization with plain PS meningococcal group C vaccine produces smaller seroresponses than primary dosing. This observation, made in the 1970s³⁹ has attracted attention recently⁹ and it appears that similar hyporesponsiveness may occur following administration of conjugate vaccines to recipients of PS vaccines as well⁴⁰. The clinical importance of this, if any, is uncertain, but it tells us something about the way these antigens are handled. More work exploring different PSs, doses and dosing schedules given at different ages needs to be done in order to define it more clearly.

Streptococcus pneumoniae

For the USA and Finland, the epidemiology of bacterial meningitis in children, along with the large morbidity of other pneumococcal infections in children, made *Streptococcus pneumoniae* the logical second target after Hib. The emergence of antibiotic resistance in pneumococcus has been an added spur to the development of effective conjugate vaccines. Large field trials in these two countries have demonstrated that a combination vaccine, containing capsular PSs of seven of the most prevalent disease causing serotypes each conjugated to CRM197 (Table 1), is efficacious in the prevention of invasive disease, lower respiratory tract infection and otitis media^{41,42}. Three additional efficacy studies of conjugate pneumococcal vaccines have recently been published^{43–45}. The seven-valent vaccine was licensed and introduced into general use in 2000 in the USA and received a European licence in 2001. However, its high cost will preclude its use in many areas for the time being.

Pneumococcus presents a more challenging problem for future control by immunization. Many of the 90 odd serotypes, each with a unique PS capsular antigen, cause disease. Several other combined PS-conjugates containing up to 11 serotypes are under development (Table 1)⁴⁶ but there must be some limit to the number that can practicably be included. Concerns about possible interference or inhibitory effects of using large quantities of the same carrier protein for all the different conjugates have already led to one formulation with two distinct protein carriers (Table 1). Vaccine recipients elaborate anti-capsular IgG and IgA antibodies in nasopharyngeal secretions^{47,48} and immunization in infancy reduces nasopharyngeal carriage rates of the serotypes included in the vaccine, at least for a period of up to 2 years^{49,50}, although these effects may cease to be evident by the age of 3–4 years in unvaccinated populations⁵¹.

However, this process is accompanied by compensatory rises in the rates of nasopharyngeal colonization with serotypes not covered by the vaccine⁵⁰. The first Finnish efficacy study also showed a modest increase in cases of otitis media (a mucosal infection) caused by non-vaccine serotypes among vaccinees to some extent cancelling out the observed reduction in vaccine type cases⁴². Pneumococci are capable of capsular switching⁵², a process that could be selected for by immunization. Although the current conjugate vaccines will undoubtedly be valuable in preventing significant morbidity where they are used, it is clear that monitoring of epidemiological trends will be especially important. That said, trends so far from the USA are very encouraging indicating significant herd immunity effects in addition to consistent effectiveness in vaccine recipients⁵³. Identification of alternative or adjunctive immunization strategies will also be important⁵⁴.

***Streptococcus agalactiae* (Group B streptococcus)**

Although judicious and systematic use of maternal intra-partum antibiotics has been shown effective in dramatically reducing the incidence of neonatal infections due to Group B streptococcus in the USA⁵⁵, primary prevention by immunization could have added advantages of more complete control, avoidance of antibiotic use (and thus emergence of resistance) and, if the vaccine were so used, prevention of disease in older age groups. To prevent neonatal disease, because the infection is acquired from the mother at the time of birth and onset is thus early in life, conjugate vaccines have been developed with immunization of the mother in mind. The main strategic aim has been to induce significant protective IgG titres in the mother to provide passive protection of the infant during the neonatal period by transplacental transfer of antibody. However, by analogy to the effects that conjugate vaccines can have on upper respiratory carriage by induction of nasopharyngeal antibodies, as discussed above, Group B streptococcal conjugate vaccines could also theoretically reduce or eliminate genital carriage in the mothers themselves by inducing mucosal immune responses.

There are seven distinct serotypes, each characterized by a different PS capsular antigen, which are major virulence factors and against which serum antibody is protective. After work towards development of multivalent conjugate vaccine, recent efforts have focused on a monovalent type III vaccine as this predominates among late-onset perinatal infections, incidence of which has been unaffected by perinatal antibiotic treatment programmes⁵⁶. However, the main impediment to further development of this promising control strategy may be anxieties about possible litigation arising from immunizing pregnant women.

Salmonella typhi

Enteric fever due to *Salmonella typhi* remains a major problem particularly in school age children in many areas of the world where sanitation and clean water are not available. Major outbreaks occur in association with natural disasters and war. Development of vaccines to prevent typhoid dates back to the 1890s but, as with most of the pathogens discussed in this paper, has been held back by the widespread availability and use of antibiotics during the 20th century. Again, emergence of resistance has promoted interest in vaccine development. Many distinct vaccines have been developed and inactivated whole cell and live attenuated oral vaccines are available and in use. Purified PS vaccines consisting of the Vi capsular PS have been licensed and available for some time. Of course, this is a T-independent antigen so that development of an immunogenic conjugate vaccine which could induce immunological memory is a logical extension of this strategy. Just such a vaccine has recently been shown to be efficacious in a large study in children in Vietnam⁵⁷. The challenge now will be to make this vaccine widely and affordably available in the areas it is most needed.

Other conjugate vaccines and approaches

Infections due to several other bacterial pathogens are theoretically preventable by this type of vaccine. Conjugates based on the O-PS of the lipopolysaccharide of Gram-negative organisms such as *Pseudomonas aeruginosa* (an important pathogen in patients with cystic fibrosis) and *E.coli* have shown some promise in phase II studies^{58,59} and *Staphylococcus aureus* capsular PS protein vaccines are also in trials, although predominantly in veterinary practice⁶⁰. The mucosal administration of conjugate PS antigens either associated with or directly conjugated to protein biological mucosal adjuvants is also an intriguing possibility for the future^{61,62}.

The use of maternal immunization to protect infants, despite the fact that there is ample precedent in the prevention of tetanus dating back to the 1960s, has been hampered by general concerns about administration of pharmaceuticals during pregnancy. To date, no vaccine is licensed for prevention of Group B streptococcus in this way although phase 2 clinical trials of conjugate vaccines are in progress (see above). Since infection with *S. pneumoniae* is also a significant problem in the first 2 months of life, this approach may also be valuable for pneumococcal disease prevention. Immunization of lactating mothers with the unconjugated 23-valent pneumococcal PS vaccine, which produces robust if relatively

short lived serum IgG responses in adults, can also induce significant functional secretory IgA responses in breast milk⁶³. This vaccine, which is significantly cheaper than conjugate vaccine at present, might be effective in the prevention of pneumococcal infections in early infancy.

Key points for practice

Important advances in the primary prevention of invasive community-acquired bacterial infections in children have been made through the untargeted use of PS-protein conjugate vaccines in primary immunization schedules of wealthy countries.

It may prove possible to extend the application of this technology to the prevention of neonatal infections through administration of such vaccines to mothers. The relative value of conjugate vaccines and unconjugated PS vaccines in the prevention of, for example, pneumococcal disease in the elderly remains to be established.

Despite their rapid development and implementation, there remain significant gaps in our understanding of immunological responses to these compounds in humans, including why young children are tolerant to the PSs, how previous immunization with PS antigens modulates responses to subsequent doses, the genetic basis for the wide heterogeneity in size of antibody responses between individuals, how mucosal antibodies are induced and by what mechanism they influence mucosal colonization. Since nearly all children now receive these vaccines, it behoves us to clarify these issues.

Since there is no immediate prospect of eradication of any of the pathogens against which these vaccines protect, continuous careful surveillance of colonization and disease isolates must remain an essential component of immunization strategy in the foreseeable future.

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