Combination Vaccines

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ABSTRACT

The combination of diphtheria, tetanus, and pertussis vaccines into a single product has been central to the protection of the pediatric population over the past 50 years. The addition of inactivated polio, *Haemophilus influenzae*, and hepatitis B vaccines into the combination has facilitated the introduction of these vaccines into recommended immunization schedules by reducing the number of injections required and has therefore increased immunization compliance. However, the development of these combinations encountered numerous challenges, including the reduced response to *Haemophilus influenzae* vaccine when given in combination; the need to consolidate the differences in the immunization schedule (hepatitis B); and the need to improve the safety profile of the diphtheria, tetanus, and pertussis combination. Here, we review these challenges and also discuss future prospects for combination vaccines.

Key words: Adjuvant, Combination vaccine, Diphtheria, *Haemophilus influenza*, Hepatitis B, *Neisseria meningitidis*, Pertussis, Poliovirus, *Streptococcus pneumoniae,* Tetanus

INTRODUCTION

The development of combination vaccines for protection against multiple diseases began with the combination of individual diphtheria, tetanus, and pertussis (DTP) vaccines into a single product; this combined vaccine was first used to vaccinate infants and children in 1948.[1] It has become the cornerstone of pediatric and adult immunization programs, and over the years we have seen the addition of other vaccines to the combination and the replacement of components to improve its reactogenicity profile. An important advancement was the replacement of whole-cell pertussis antigens (wP) with less reactogenic acellular antigens (aP) in the early 1990s. This paved the way for the combination of diphtheria, tetanus, and acellular pertussis antigens (DTaP) with other routine vaccines such as inactivated polio vaccine (IPV), *Haemophilus influenzae* vaccine (Hib), and hepatitis B vaccine (HepB). In this article, we will describe in detail the composition of DTaP-based vaccines and provide an overview of the different variations of this combination that are licensed and in use. Furthermore, we will extensively review the technical challenges that have been faced as additional vaccines have been added to these combinations and speculate on the future developments for this group of vaccines.

Another significant combination vaccine that protects against more than one disease is the measles, mumps, and rubella vaccine (MMR). However, this vaccine will not be discussed extensively here as, unlike the DTaP combination, MMR has not been built upon with the inclusion of additional vaccines. For almost 40 years, the MMR vaccine has remained a trivalent vaccine that is given as a single product.

THE NEED FOR COMBINATION VACCINES

The number of immunizations recommended for children in the first 2 years of life has dramatically increased over time. In the United States the recommended immunization schedules for 2010 indicate that in the first 2 years children are expected to receive vaccines against 14 diseases [Figure 1]. Even with the use of the available pentavalent combination vaccine DTaP-IPV/Hib we have calculated that this recommendation can be achieved through a minimum of 17 injections.[2] In a single visit to the pediatrician, infants may need to receive as many as six injections to comply with these recommendations.[2] With such a complex immunization schedule, it becomes increasingly more challenging to incorporate new vaccines into the schedule.

Access this article online

Quick Response Code:

Website: www.jgid.org

DOI: 10.4103/0974-777X.77298

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Vaccine	Age										
	Birth	1 month	2 months	4 months	6 months	12 months	15 months	18 months	19-23 months	2-3 years	4-6 years
Hepatitis B	HepB	HepB			HepB						
Rotavirus			RV	RV	RV						
Diptheria, Tetanus, Pertussis			DTaP	DTaP	DTaP		DTaP				DTaP
Haemophilus influenza type b			Hib	Hib	Hib	Hib					
Pneumococcal			PCV	PCV	PCV						
Inactivated Poliovirus			IPV	IPV	IPV						IPV
Influenza					Influenza (Yearly)						
Measles, Mumps, Rubella						MMR					MMR
Varicella						Varicella					Varicella
Hepatitis A						HepA (2 doses)					

Figure 1: Recommended immunization schedule for children aged 0 through 6 years. This schedule has been adapted from the recommended immunization schedule from the Centers for Disease Control and Prevention.[2] Recommendations are for all children except certain high-risk age-groups. For more information please consult original source.[2]

Simplifying immunization schedules by combining multiple vaccines into a single syringe has been reported to have numerous positive effects [Table 1]. Reducing injections by combining vaccines reduces trauma to the infant and has been found to lead to higher rates of compliance with complex vaccination schedules.[3,4] A number of studies have also reported increased vaccine coverage with the use of combination vaccines. An example of this is a US study reporting increased coverage rates with a pentavalent DTaP-HepB-IPV (Pediarix™) vaccine than with multiple lower-valent vaccines containing the same antigens.[3]

COMPOSITION OF DTAP VACCINES

DTaP vaccines from different manufactures are very similar in their composition, with the main differences being related to the number, amount, and detoxification method of the pertussis components.[5] Furthermore, some combinations contain additional vaccines such as HepB, Hib, and IPV in addition to the DTaP base. Here, we will use GlaxoSmithKline's (GSK) hexavalent vaccine Infanrix™-hexa as a model to describe the different components of DTaP-based combination vaccines, as this represents the latest advance in licensed combination vaccine development [Table 2]. There is no other licensed combination vaccine on the market that protects against as many indications as Infanrix™-hexa, which combines diphtheria, tetanus, acellular pertussis, hepatitis B, *Haemophilus influenzae*, and inactivated poliovirus vaccines (DTaP-HepB-Hib/IPV).[6] Thus, this is composed of diphtheria, tetanus, and acellular pertussis antigens; hepatitis B surface antigen; inactivated poliovirus; and *Haemophilus influenzae* polyribosylribitol phosphate antigen conjugated to tetanus toxoid. Detailed descriptions of these antigens together with common abbreviations are summarized in Table 2. Infanrix™-hexa is adjuvanted with both aluminum hydroxide and aluminum phosphate adjuvants, which is due to the combination of

Table 1: Potential advantages of combination vaccines

1. Fewer injections
2. Reduced trauma to the infant
3. Higher rates of compliance with complex vaccination schedules[3,4]
4. Better vaccine coverage[5]
5. Timely vaccination – vaccination schedule completed on time[5]
6. Reduced administration costs
7. Lower storage space requirements
8. Allows incorporation of new vaccines into immunization schedules[7]

DTaP vaccine adsorbed onto aluminum hydroxide and HepB vaccine adsorbed onto aluminum phosphate.

Overview of licensed DTP-based pediatric combinations in the US and Europe

In the US, three diphtheria, tetanus, and acellular pertussis (DTaP) combinations are licensed for use in the pediatric population: Tripedia™ and Daptacel™ from Sanofi Pasteur and Infanrix™ from GSK. Two larger licensed combinations currently in use are pentavalent Pediarix™, a DTaP-HepB-IPV vaccine from GSK and pentavalent Pentacel™, a DTaP-IPV/Hib combination from Sanofi Pasteur. In addition, there is the tetravalent booster DTaP-IPV vaccine Kinrix™ for use in 4- to 6-year-old children and adult booster DTaP vaccines Adacel™ and Boostrix™. Further details of these vaccines are outlined in Table 3. DTaP-based combinations currently in use outside the US include several other vaccines built upon Sanofi's five-pertussis antigen DTaP vaccine Daptacel™ and GSK's three-pertussis antigen DTaP vaccine Infanrix™. Infanrix™ is the leading DTaP vaccine used worldwide[7] and provides the backbone of the hexavalent vaccine Infanrix™-hexa which is licensed in Europe and protects against six different diseases and, as mentioned previously, defines the latest advance in combination vaccine development. Hexavalent DTaP combination vaccines are yet to gain licensure in the US, the authorities' reluctance stemming from reported reductions in antibody titers to Hib.[8]

TECHNICAL CHALLENGES FACED WHEN COMBINING VACCINES

Although there are clear benefits with combination vaccines, the main challenge in their development is the risk that the efficacy or safety of the combination would be less than that seen with the administration of the vaccines separately. New combinations cannot be less immunogenic, less efficacious, or more reactogenic than the previously licensed uncombined vaccines. Immunological, physical, and/or chemical interactions between the combined components have the potential to alter the immune response to specific components. Furthermore, if the vaccines to be combined have differing immunization schedules, consolidation of these should also not negatively affect immunogenicity, efficacy, or safety. Finally, and ideally, the many advantages of combination vaccines should not be achieved at the cost of reduced product stability. From a practical standpoint, uncommon transport and storage conditions and complicated bedside mixing could hamper the development of a combination vaccine.

In this section, we discuss in depth some of the key technical challenges that have faced the development and implementation of DTaP-based combination vaccines. With the use of specific examples, we highlight cases where putting together vaccines in DTaP-based combinations has affected the immunogenicity or safety of the final product.

Reduced Hib response when combined with DTaP

The most commonly reported example of immune interference in DTaP-based combination vaccines is the reduction in antibody titers to the Hib component of the vaccine polyribosylribitol phosphate antigen.[9–11] This has been reported for many DTaP-based vaccines, including the hexavalent vaccine DTaP-HBV-IPV/Hib.[12,13] The interference has not been reported to the same extent for DTwP-based combination vaccines, mainly, it has been suggested, due to the adjuvant effect of the whole-cell pertussis (wP) component.[10,14,15] However, the adjuvant

Table 2: Antigen components of Infanrix™-hexa

Vaccine abbreviation	Indication	Infectious agent	Antigens	Antigen abbreviation	Clinical correlate of protection: surrogate markers for seroprotection	Production
IPV	Poliomyelitis*	Poliovirus types 1, 2 and 3	Trivalent formaldehyde-inactivated poliovirus types 1, 2, and 3	IPV	Serum titer level ≥1:8 (WHO microneutralization assay)	Continuous Vero cell line
D	Diphtheria*	*Corynebacterium diphtheriae*	Formaldehyde-inactivated toxoid	DT	Serum antibody level ≥0.1 IU/mL	*Corynebacterium diphtheriae* culture
T	Tetanus*	*Clostridium tetani*	Formaldehyde-detoxified tetanus toxoid	TT	Serum antibody level ≥0.1 IU/mL	*Clostridium tetani* culture
Hib	*H influenzae* disease*	*H influenzae*	*H influenzae* type b polysaccharide (polyribosylribitol phosphate) conjugated to TT	PRP	Serum anti-PRP antibody levels: 0.15 µg/mL for short-term protection; 1.0 µg/mL for long-term protection	Purified *H. influenzae* type b polysaccharide
aP	Whooping cough	*Bordetella pertussis*	Formaldehyde- or glutaraldehyde-detoxified pertussis toxoid and surface adhesins (filamentous hemagglutinin and pertactin)	PT, FHA, FIM types 2 and 3, PRN	Serological correlates are not well defined	*Bordetella pertussis* culture
HepB	Hepatitis B	Hepatitis B virus	Hepatitis B surface antigen	HBsAg	Serum antibody levels ≥10 mIU/mL	Produced in yeast cells (*Saccharomyces cerevisiae*) by recombinant DNA technology

*Data from[8]

Table 3: Avaliable DTaP-based vaccines in the US*

Vaccine	Trade name	Manufacturer	Adjuvant	No. of doses in series	Schedule	Antigen doses†
Childhood vaccines						
DTaP	Tripedia™	Sanofi Pasteur Inc.	Alum	5	2, 4, 6, 15–18 months, 4–6 years	6.7 Lf DT 5 Lf TT 23.4 µg PT 23.4 FHA
	Infanrix™	GlaxoSmithKline Biologicals	Aluminum hydroxide	5	2, 4, 6, 15–20 months, 4–6 years	25 Lf DT 10 Lf TT 25 µg PT 25 µg FHA 8 µg PRN
	Daptacel™	Sanofi Pasteur, Ltd.	Aluminum phosphate	5	2, 4, 6, 15–20 months, 4–6 years	15 Lf DT 5 Lf TT 10 µg PT 5 µg FHA 3 µg Pertactin 5 µg Fimbriae types 2 and 3
DTaP-HepB-IPV	Pediarix™	GlaxoSmithKline Biologicals	Aluminum hydroxide and aluminum phosphate	3	2, 4, 6 months	25 Lf DT 10 Lf TT 25 µg PT 25 µg FHA 8 µg PRN 10 µg HBsAg 40 D-antigen units type 1 poliovirus (Mahoney strain) 8 D-antigen units type 2 poliovirus (MEF-1 strain) 32 D-antigen units type 3 poliovirus (Saukett strain)
DTaP- IPV	Kinrix™	GlaxoSmithKline Biologicals	Aluminum hydroxide	1	1 dose: 4–6 years (to be used as a booster)	25 Lf DT 10 Lf TT 25 mcg PT 25 µg FHA 8 µg PRN 40 D-antigen units type 1 poliovirus (Mahoney strain) 8 D-antigen units type 2 poliovirus (MEF-1 strain) 32 D-antigen units type 3 poliovirus (Saukett strain)
DTaP-IPV-Hib	Pentacel™	Sanofi Pasteur Ltd.	Aluminum phosphate	4	2, 4, 6, 15–18 months	15 Lf DT 5 Lf TT 20 µg PT 20 µg FHA 3 µg Pertactin 5 µg Fimbriae types 2 and 3 40 D-antigen units type 1 poliovirus (Mahoney strain) 8 D-antigen units type 2 poliovirus (MEF-1 strain) 32 D-antigen units type 3 poliovirus (Saukett strain) 10 µg PRP conjugated to 24 mcg TT
Adult						
DTaP	Adacel™	Sanofi Pasteur, Ltd.	Aluminum phosphate	1	1 dose: at ages 11–64 years (to be used as a booster)	2 Lf DT 5 Lf TT 2.5 µg PT 5 µg FHA 3 µg PRN 5 µg Fimbriae types 2 and 3
	Boostrix™	GlaxoSmithKline Biologicals	Aluminum hydroxide	1	1 dose: at ages 10–64 years (to be used as a booster)	2.5 Lf DT 5 Lf TT 8 µg PT 8 µg FHA 2.5 µg PRN

*Information taken from 'Complete List of Vaccines Licensed for Immunization and Distribution in the US' and supporting documents at www.fda.com; †Lf: Limits of flocculation

effect simply masks the underlying interference, and the mechanism by which the Hib response is reduced after combination with DTaP is complex and is still not completely understood.

Consistent with clinical data, this reduction in Hib immunogenicity has also been demonstrated in preclinical animal models. Studies in a rat model looking at the interference of TT and different aP antigens with Hib reported reduced anti-PRP response with combined administration of Hib and TT, or Hib and FHA (a component of aP vaccine).[16] The level of this reduction was reported to be comparable to that observed for combined administration of DTaP and Hib.[16] The interference of TT with Hib is of particular interest, as TT is also present in the Hib vaccine as a carrier protein conjugated to the capsular polysaccharide PRP. Possible mechanisms for the effect of free unconjugated TT on the Hib PRP-TT conjugate includes competition between TT-specific and PRP-specific B cells for the Hib

conjugate antigen, suppression of PRP response by clonal expansion of TT-specific B cells, and physical prevention of binding of the conjugate antigen to PRP-specific B cells by the TT carrier protein.[17] The reduced anti-PRP response with FHA is in line with the finding that it is a potent suppressor of IL-12 and IFN-γ production *in vivo* and *in vitro*, suppressing immune responses to co-injected antigens.[16,18] Another explanation for the reduced Hib response when combined with DTaP vaccines is incompatibility with the alum adjuvant. Experiments in the rat model with Hib alone have reported 5- to 11-fold lower levels of anti-PRP antibodies when adsorbed to aluminum hydroxide adjuvant.[16] Taken together, the reduced Hib response in DTaP/Hib combinations is at least in part a result of the interaction of Hib with TT, FHA, and aluminum hydroxide adjuvant.

The reduced immunogenicity of Hib in DTaP-based combination vaccines *vs* DTwP-based combination vaccines has been suggested to be a contributory factor in the increase in Hib disease incidence observed in the UK between 1999 and 2002 and a similar increase observed in Ireland.[19–21] It must be stated however that other factors have been suggested to explain the increased incidence, including decreasing herd immunity, the short UK immunization schedule, and the lack of a booster immunization in the second year of life.[21] Despite multiple reports of lowered Hib antibody responses with DTaP combination vaccines containing Hib, the effect of changing from whole-cell to acellular pertussis was actively monitored in Canada and this shows that among children 1–4 years of age incidence rates of pertussis disease has been reduced by 85% in the acellular-pertussis vaccine era.[22] Furthermore, extensive data generated by Eskola and coworkers[10] indicate that the current serological correlate of efficacy for Hib vaccines, which is set at anti-PRP levels of 1.0 μg/mL, is too high and that antibody responses below this threshold are reported which are not associated with impaired antibody function and loss of immune memory. These findings led to the approval of many DTaP-based Hib combinations in Europe.[7]

Combining the differing HepB and DTaP immunization schedules

Another significant technical issue in the implementation of combination vaccines is consolidation of the different immunization schedules of the vaccines to be combined. For example, hepatitis B is often transmitted from mothers to their newborns at the time of birth.[23] Therefore, in countries where a high percentage of HBV infections are acquired, the WHO recommends that the first HepB vaccine dose be administered <24 hours after birth. Furthermore, the Advisory Committee on Immunization Practices (ACIP) recommends a birth dose of hepatitis B vaccine for all US infants. This is to be followed by second and third doses of the HepB vaccine at 1 and 6 months. The primary immunization series of DTaP, on the other hand, requires doses at 2, 4, and 6 months. Therefore, combination of HepB and DTaP still requires administration of monovalent HepB at birth followed by doses in combination with DTaP at 2, 4, and 6 months, resulting in an unnecessary fourth dose of HepB at the 6th month. A study comparing DTaP-HepB combination administered a 2, 4, and 6 months against separate administration of HepB at birth, 1 and 6 months and DTaP at 2, 4, and 6 months showed significantly lower HepB antibody titers with the combined vaccine.[24] However, antibody levels were still above serologically recognized levels of protection in 99% of the subjects.[25] Furthermore, administration of a DTaP-HBV-IPV/Hib vaccine at 2, 4, and 6 months after a dose of HepB vaccine shortly after birth did not impact protective anti-HBs titers and was not more reactogenic than the same combination given without the birth dose of HepB.[26] Nevertheless, reduced efficacy for hepatitis B antigen was the reason for the suspension of another hexavalent combination vaccine, Hexavac™, by the EMEA in 2005.[27] Of particular concern was the reduction in long-term protection, which is important in hepatitis B, as individuals immunized as infants need to have protective levels of antibody when they reach adolescence and adulthood.[28,29]

Improving the safety and tolerability profile of DTP-based vaccines

Safety and tolerability is another important consideration and a potential technical hurdle to overcome when combining vaccines. An improvement for DTP-based vaccines in this respect has been the substitution of wP with aP antigens. Acellular pertussis combinations were first licensed in 1992 and eventually replaced wP vaccines in the USA, primarily due to their improved reactogenicity profile.[30] Significantly higher incidence of localized redness and swelling was reported with wP in studies comparing DTwP-IPV/Hib and DTaP-HBV-IPV/Hib vaccines.[31] Also, increased frequency of adverse events with whole-cell pertussis vaccine was observed in a study comparing DTwP-IPV/Hib and DTaP-IPV/Hib.[8]

When compared to DTwP vaccines, DTaP vaccines are considered to have a good safety profile with low incidence of systemic adverse events such as fever, fatigue, or appetite loss.[32,33] However, reactions are still seen with all

DTaP vaccines, notably increased frequency and severity of swelling with the booster immunizations given as the fourth or fifth dose.[34] These were first reported in 1997[35] and despite several hypotheses regarding the cause of these reactions[34,36–39] the immunological mechanisms responsible remain unexplained. For example, incidences of local symptoms were higher after administration of a booster dose of hexavalent DTaP-HBV-IPV/Hib in the second year of life than following the primary doses.[40] Other examples include redness (erythema) at the site of injection (reported in up to 37% of subjects) and swelling (seen in up to 27% of individuals) following a fifth dose of DTaP.[34,36,41] Also, more local responses were observed with a fourth dose of DTaP-IPV/Hib vaccine than following the primary series given in the first 6 months of age.[8,42] Although most countries continue to use full-dose DTaP vaccines for booster doses, there is a growing body of reactogenicity and immunogenicity data to support the use of reduced antigen content in the 4–6 year age-group.[43–45] This has already been implemented in the UK and Germany, where reduced-dose DTaP or DTaP-IPV booster vaccines are administered in the 3–6 year age-group.[46,47]

FUTURE PERSPECTIVES ON DTAP-BASED VACCINES

Until recently there has been reluctance in the US to license pentavalent DTaP combination vaccines and, at the time of writing, hexavalent vaccines still have not gained licensure despite approval for use in Europe. This reluctance stems from reported decreases in antibody titers to Hib when combined with other components in a single syringe.[8] However, clinical data generated with hexavalent vaccines combining DTaP-HepB-Hib/IPV is consistent, with efficacy and safety profiles comparable to that with separate administration of these antigens.[6]

The ACIP recommends that combination vaccines be used whenever possible. As new vaccines that target previously unaddressed diseases are added to the vaccination calendar, the use and improvement of currently available combination vaccines will be paramount if high vaccine coverage is to be maintained.

In the final section below we will discuss future developments in this area, speculating on the possibility of addition of more vaccines to DTaP-based combinations as well as improvements to current combinations by increasing the compatibility of antigens, the addition of more potent adjuvants, or development of new methods for monitoring vaccine production.

Addition of pneumococcal or meningococcal vaccines to DTaP-based vaccines

Although described as hexavalent on the basis of the number of diseases the vaccine targets, the currently available vaccine in this class, Infanrix™-hexa, carries nine antigens plus inactivated polio virus. Other vaccines which protect solely against pneumococcal infections carry more antigens: for example, the 23-valent pneumococcal polysaccharide vaccine Pneumovax 23™; the updated conjugate vaccine Prevenar™ will contain 13 valences. It is likely that new combination vaccines will reach the market in the coming years, targeting additional diseases such as pneumococcal or meningococcal infections. Although there is no clinical data for DTaP-based combinations containing pneumococcal or meningococcal vaccine, there is considerable experience with the co-administeration of these vaccines.

Multiple clinical studies evaluating concurrent administration of DTaP-based combination vaccines with a seven-valent pneumococcal conjugate vaccine found no significant immunological interference between the vaccines.[8,48,49]

For larger combinations, a study looking at co-administration of DTaP-HepB-Hib/IPV with a seven-valent pneumococcal conjugate vaccine found that after three primary immunizations and a fourth booster dose, titers for several of the antigens were reduced compared to administration of DTaP-HepB-Hib/IPV or pneumococcal vaccine separately according to their respective immunization schedules.[40] However, there was minimal effect on seroprotection/vaccine response rates, with these ranging from 96.8%–100%.[40]

As seen with DTaP vaccines, increased local reactions are also observed with pneumococcal polysaccharide vaccines, which may pose another hurdle to combining these vaccines.[50] A couple of studies with seven-valent pneumococcal conjugate vaccine co-administered with hexavalent DTaP-HepB-Hib/IPV reported increased incidence of systemic reactions, specifically fever, compared to administration of the two vaccines separately.[40,51] Encouragingly, a review of the data available on this topic by Tozzi and coworkers[52] identified no negative effects of particular note in the few published studies that have examined the co-administration of pneumococcal or meningococcal conjugate vaccines with hexavalent DTaP-HepB-Hib/IPV.

Studies looking at co-administration of DTaP-HepB-Hib/IPV with meningococcal serogroup C vaccines have found that the combination is well tolerated and

remains immunogenic with both CRM197-conjugated vaccines (Meningitec™ and Menjugate™) and the tetanus toxoid conjugate vaccine (NeisVac-C™).[53–56] A significant increase in antibody titers to the meningococcal antigens was observed in one study when the vaccines were administered on separate schedules. This was attributed to the 'priming' effect of the diphtheria toxoid in the DTaP-HepB-Hib/IPV vaccine, enhancing the response to subsequent immunizations with the CRM197-conjugated meningococcal vaccine.[53] Another study, monitoring co-administration of DTaP-IPV/Hib vaccines with meningococcal conjugate (MCC) vaccine showed no negative effects on antibody responses.[57] A study in adolescents and young adults receiving Novartis's MenACWY polysaccharide conjugate vaccine (Menveo™) found the response to be the same whether administered alone or in combination with DTaP.[58]

New carrier protein strategies for conjugate vaccines

It is generally the case that each of the vaccines contained in DTP-based combination vaccines have usually been previously licensed either as a stand-alone vaccine or as part of a simpler combination. As well as the addition of more vaccines to existing combinations, the future could also see reformulation of existing combinations by changing either the antigens or the carrier proteins used for conjugates. The problems associated with combining conjugate vaccines with carrier proteins which are also present as antigens in the combination have been discussed above. To address this, the future could see new approaches to carrier protein technology, with novel carrier proteins replacing TT. One strategy described for a candidate 11-valent pneumococcal conjugate vaccine has been to use two carrier proteins, TT and DT, and to further reduce the amount of TT by conjugating the polysaccharides that required the largest carrier amounts to DT.[59]

Another approach has been to replace full-length protein carriers such as DT and TT with peptides containing T helper cell epitopes and lacking B cell epitopes. Universal CD4+ T cell epitopes could enable a strong helper effect to the conjugated antigen without inducing an antibody response to the carrier itself. One example of this approach is the N19 recombinant polyepitope made up of CD4+ T cell epitopes from *Clostridium tetani*, *Plasmodium falciparum*, HBV, and influenza virus.[60] When evaluated in the mouse model as a carrier protein for a combination of capsular polysaccharides from *Neisseria meningitides* serogroups A, C, W, and Y, good antibody responses to all four polysaccharides were obtained after a single immunization. Importantly N19-specific antibodies do not cross-react with TT or influenza virus hemagglutinin, the parent proteins from which N19 is obtained.[61]

Novel approaches to adjuvants

Another future challenge will be to extend the period of protection from pediatric vaccine combinations administered in infancy, beyond childhood into adolescence and adulthood. Improvements in this area may involve optimization of vaccine formulation and schedule and the use of adjuvants more potent than alum. Many studies have reported that antibody responses to Hib are reduced when delivered in combination with DTaP. One explanation for this is incompatibility with alum adjuvant, and experiments with Hib alone have reported 5- to 11-fold lower levels of anti-PRP antibodies when adsorbed to aluminum hydroxide adjuvant.[16] Furthermore, reduction in Hib response has come to the fore since the substitution of wP antigens with aP antigens and, with it, the removal of the adjuvant properties of the endotoxin-containing wP antigens. Use of alternative adjuvants may be able to improve immunogenicity and overcome the reduced responses observed with Hib and HepB in DTaP combinations. Novel adjuvants can potentially bring a range of other advantages to combination vaccines, such as antigen dose reduction and reduction in the number of immunizations in the schedule.[62]

Singh and coworkers[63] explored the use of alternative adjuvant formulations, including the oil-in-water emulsion MF59 and polylactide co-glycolide (PLG) microparticles, with established vaccine antigens such as DT, TT, HepB (HBsAg), *N meningitides* serotype C conjugate (MenC), and a *N meningitides* serotype B recombinant antigen (MenB). MF59 emulsion stood out as a good alternative to alum for TT, HBsAg, MenC, and MenB vaccines, with the indication that it may be possible to replace alum with MF59 and improve immune responses to these antigens. PLG microparticles also showed promise, with responses comparable to or better than alum with both MenC and MenB vaccines.

Improved *in vitro* assays for measuring batch-to-batch consistency

Another future trend, whilst not limited to combination vaccines, will be the development of improved *in vitro* assays for measuring batch-to-batch consistency of vaccine production. For combination vaccines in particular, where one or more components may affect immunogenicity, *in vivo* potency testing remains the gold standard for evaluation of the final vaccine product. However, efforts are on to reduce the number of animals used in *in vivo* potency testing (e.g.,

many laboratories rely on a single-point potency test) and this may lead to increased difficulty in monitoring trends.[64] *In vitro* approaches as a correlate to *in vivo* protection have already been applied for some components of combination vaccines, e.g., HepB.[65,66] For other components of DTP-based vaccines, a recent example is the development of an enzyme-linked immunosorbent assay (ELISA) to quantify diphtheria toxoid antigen in DTP-based combination vaccines.[64] Similar assays for tetanus toxoid have also been described.[67,68] Specific antibody-based assays such as these have multiple advantages over other biochemical and biophysical tests applied to these toxins, such as gel electrophoresis, size-exclusion chromatography, and circular dichroism.[69–72] These tests are often limited to characterization of unformulated bulk antigens due to interference from other components in the final vaccine product. This can be overcome in antibody-based assays that are specific for the target antigen. Furthermore antibody-based assays are very sensitive and can detect very low concentrations of target antigen as may be found in booster vaccines.[64] Finally, a significant advantage of antibody-based assays is that they have the potential to measure antigen that is adsorbed to alum, avoiding potentially destructive methods otherwise required for the elution of antigens prior to quantification. For monovalent vaccines, this has recently been reported by a direct alum formulation immunoassay (DAFIA), which was able to quantify alum-bound malaria antigen AMA1-C1 over a linear detection range of 0.16–10 μg/mL.[73]

CLOSING REMARKS

Disease prevention and eradication are the ultimate and immediate goals of immunization, and central to reaching these goals is achieving vaccine coverage. Combination vaccines can help overcome some of the key challenges to maintaining coverage through simplification of vaccine schedules and reduction of injections required. However, there are multiple technical challenges in maintaining immunogenicity and safety when combining vaccines. Continuing vaccine development will only increase the need for the use of combination vaccines, and the future development of larger combinations appears inevitable.

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How to cite this article: Skibinski DA, Baudner BC, Singh M, O'Hagan DT. Combination vaccines. J Global Infect Dis 2011;3:63-72.

Source of Support: Nil. **Conflict of Interest:** The authors David A G Skibinski, Barbara C Baudner, Manmohan Singh and Derek T O'Hagan are all employees of Novartis Vaccines and Diagnotics.