

**PART II**  
**THE CUTTING EDGE**



# HAEMOPHILUS INFLUENZAE TYPE B: THE BURDEN IN ASIA

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Prior to the availability of a vaccine, *Haemophilus influenzae* type B (Hib) meningitis was the most common cause of bacterial meningitis in the United States. An estimated 20,000–25,000 cases of invasive Hib disease occurred annually in the country during this period (1). Even with the appropriate use of antibiotics and optimal clinical care, an estimated 5% of cases of Hib meningitis were fatal (2), and many children who survived the disease were left with lifelong neurological disabilities (3). For this reason, public health officials accorded high priority to the development of safe and effective vaccines against Hib, particularly vaccines that could be administered early in infancy.

The development of potent polysaccharide-protein conjugate vaccines against Hib and the demonstration that these vaccines could confer high-grade protection against invasive Hib infections represented a major landmark for vaccinology during the twentieth century. Moreover, the ability of these vaccines to reduce carriage of Hib organisms allowed the vaccines to confer unexpectedly high levels of herd immunity to Hib in vaccinated populations, which in turn enabled control of invasive Hib disease even with incomplete levels of vaccine coverage (4, 5).

Another major development in the evolution of these vaccines was the successful incorporation of Hib conjugates into multivalent, combination vaccines with DTP and other routine vaccines for infants (6). This meant that delivery of Hib conjugates in routine immunization schedules for this age group could be accomplished without requiring additional injections, a factor of major importance in augmenting provider and parental compliance with, and demand for, Hib vaccines.

The attractiveness of the vaccines led rapidly to their widespread use in Australia, Europe, and later, through the efforts of PAHO, to their introduction in Latin America. Yet, despite the demonstration of the importance of Hib as a major pathogen in certain other areas of the developing world, especially sub-Saharan Africa, movement of these vaccines into public health programs for the poor in Africa and Asia, was, until recently, almost nonexistent.

A major force to remedy this disparity was the recent creation of the Vaccine Fund, provided by the Bill and Melinda Gates Foundation and by the governments of several industrialized countries, for use by the Global Alliance for Vaccines and Immunization (GAVI). This fund currently supports the introduction of Hib conjugate, as well as various other vaccines, into infant immunization programs for the world's poorest countries and provides support for the improvement of pub-

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lic health infrastructure for vaccine delivery. It is noteworthy, however, that to date the Vaccine Fund has been used to purchase Hib conjugate vaccines for the developing countries of Africa, but not those of Asia.

While there are many possible reasons why Hib conjugate vaccines have not penetrated public health programs for the poor in Asia, one major contributor to this situation is the widespread perception among clinicians and public health policy professionals in the countries of this region that the burden of invasive Hib disease is low in infants and children. Thus, it remains for policymakers in Asia to be convinced of a high disease burden, since even if Hib conjugate vaccines are made available free of charge in the short run via the Vaccine Fund, it seems likely that procurement of these moderately expensive vaccines will have to be sustained partly by scarce local financial resources in the long run. Therefore, the economic argument for using Hib conjugate vaccines in Asia depends largely on the resources to be saved by the prevention of Hib disease, and the economic justification for the vaccines' use hinges on the existence of a high disease burden.

In support of prevailing perceptions of a low disease burden of Hib in Asia, past population-based studies have found rates of Hib meningitis to vary widely (7), in contrast to the consistently high rates observed in the United States during the pre-vaccine era. Yet, as shown in Table 1, recent reviews of case series of bacterial meningitis in infants and children in Asia have regularly found Hib to be a major cause of this syndrome (8, 9).

Several years ago the First International Conference on *Haemophilus influenzae* type b infection in Asia addressed this apparent paradox. The Conference concluded that past studies were too flawed to provide guidance about the true Hib disease burden in Asia, and that prospective, population-based studies using appropriate microbiological techniques were needed (9).

To address this issue, during the past three years several prospective, population-based

**TABLE 1. Importance of *Haemophilus influenzae* type B (Hib) as a cause of bacterial meningitis in Asian children.**

Country	Bacterial meningitis cases due to Hib (%)
Bangladesh	43–47 <sup>a</sup>
China (Mainland)	32–52
China (Taiwan)	29–39
China (Hong Kong)	21–29
India	0–51
Indonesia	0–11
Iran	10
Iraq	25
Israel	42
Japan	35–59
Jordan	50
Kuwait	45
Malaysia	16–50
Nepal	65
Pakistan	50
Philippines	5–34
Republic of Korea	6–42
Saudi Arabia	30–66
Singapore	19
Thailand	37–48
United Arab Emirates	63
Vietnam	30–53

<sup>a</sup> Ranges derive from countries with multiple studies.

studies of the burden of Hib meningitis in children under the age of 5 have been launched in Asia. One such effort was organized by investigators at the International Vaccine Institute (IVI), in collaboration with scientists at the Center for Vaccine Research at the UCLA School of Medicine. This project set up two-year, prospective surveillance studies that comprehensively tracked meningitis in defined populations of under age 5 in three areas of the Far East: Nanning, China; Jeonbuk, South Korea; and Hanoi, Vietnam. Aggressive efforts were made to establish surveillance at all treatment sites where children in the target populations with meningitis were being seen, as well as to ensure proper collection and laboratory evaluation of diagnostic specimens from patients with suspected cases. Despite these measures, annual rates of culture-confirmed Hib meningitis were found to be below 10 cases per 100,000 children under age 5 in each site (10). These rates contrast with the annual rates

of 40–60 cases per 100,000 children under 5 generally observed in the United States prior to the use of modern Hib vaccines (6).

For several reasons, however, we believe it is still premature to conclude that Hib is not a problem of sufficient magnitude to warrant introduction of modern Hib conjugate vaccines into public health programs for Asian children. Firstly, Asia is a heterogeneous continent, and there remains the possibility that Hib is an important problem in some areas of Asia, while not in others. One review (8), for example, has suggested that the data on Hib disease burden reveal a pattern of a greater burden in the Middle East and in South/Southeast Asia than in East Asia. Secondly, the sites selected for the IVI study were areas in which populations were well served by accessible medical facilities in which appropriate diagnostic tests could be undertaken. Many parts of developing countries in Asia are not as well served as these three study sites, and it is unknown whether the epidemiology of Hib in poorer areas is similar to that for areas that are better served. Thirdly, although descriptive epidemiological studies attempting to quantify the disease burden of Hib meningitis are useful, since this syndrome is amenable to clinical detection and microbiological diagnosis, in some areas of the developing world Hib pneumonia constitutes an even greater share of the invasive Hib disease burden. Unfortunately, because of the difficulty in isolating Hib from routine cultures of normally sterile body fluids in children with Hib pneumonia, the magnitude of the burden of Hib pneumonia is not readily discernable from descriptive epidemiological studies. Since prevention of Hib pneumonia can provide a compelling justification for the use of Hib vaccines, failure to consider the disease burden of Hib pneumonia as well as other Hib invasive syndromes may be a serious omission in disease burden assessments that are undertaken to guide vaccine policy development.

For these reasons, just as cross-sectional case series showing that Hib is a common cause of bacterial meningitis are not sufficient to in-

dicate that the population incidence of this syndrome is high enough to warrant the use of vaccines. The low incidence rates of Hib meningitis observed in recent longitudinal descriptive studies of young children in Asia do not provide sufficient evidence to close the door on the use of Hib vaccines in public health programs for the poor in Asia. A controlled field trial of a Hib-conjugate vaccine in infants in the Gambia demonstrated the utility of using the vaccine-prevented incidence of culture-negative syndromes clinically compatible with invasive Hib to infer the magnitude of the “iceberg” of the culture-negative Hib disease burden (11). This has given rise to the concept that Hib vaccines can be used as “probes” to more completely identify the burden of invasive Hib disease, especially Hib pneumonia. One such probe study is currently being undertaken in Lombok, Indonesia. If this important study finds a substantial disease burden attributable to Hib pneumonia, it may motivate additional probe studies elsewhere in Asia to inform judgments about the need for introducing Hib vaccines into public health programs for infants in this region.

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# DEVELOPMENT OF A LIVE VARICELLA VACCINE: CURRENT STATUS AND PROSPECTS

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## INTRODUCTION

Varicella is a highly contagious disease in children that causes fever and an average of 250 to 500 vesicles. A varicella patient poses a threat to hospital pediatric wards, necessitating the transfer of other patients to other wards. Complications of varicella in immunocompromised cases are occasionally life-threatening. A live varicella vaccine (Oka strain) was developed in the early 1970s by a classical method: 11 passages in human lung cells at 34°C, then 12 passages in guinea pig embryo cells, followed by propagation in human diploid (MRC-5) cells. Tolerability of the vaccine is excellent in healthy children and it is highly effective, with 85%–87% efficacy against clinical varicella and 97% against severe varicella. Recently, a genetic difference was found between vaccine Oka virus (V-Oka virus) and its parental virus (P-Oka virus). Major base and amino acid substitutions are accumulated in gene 62 (immediately early gene). Evidence suggests that a mutation in gene 62 is related to the attenuation of Oka-varicella-zoster virus (VZV).

A sequela of varicella infection may be the later occurrence of herpes zoster, particularly for the elderly. The incidence of herpes zoster

is estimated at approximately 15% among the elderly population worldwide, if average life expectancy is assumed to be 70 years. Postherpetic neuralgia is another sequela that mainly affects the elderly. The pathogenesis of herpes zoster has been elucidated. The main route of VZV to the dorsal ganglia is via the peripheral nerves from vesicles in the skin. In follow-up studies of vaccinated leukemic children, the incidence of herpes zoster is several times higher in the group with rashes after vaccination, as compared with those without rashes after vaccination. Since no or few rashes appear after vaccination of normal children, the incidence of the vaccine virus becoming latent in dorsal ganglia may be far lower than that of natural varicella infection. Thus, most vaccinated children are expected to be free from the risk of herpes zoster in future. For adults and elderly persons with a history of varicella, varicella vaccine has been given in an attempt to boost immunity against VZV. Enhancement of cell-mediated immunity is observed in most of them. Although questions regarding the duration of elevated immunity remain, severe postherpetic neuralgia in the elderly is expected to be prevented by administering varicella vaccine.

Several overviews of a live varicella vaccine (Oka strain) have been published (1–9). The following sections discuss the main points regarding the development, clinical use, and prospects of this vaccine.

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## PRIMARY ISOLATION OF THE VACCINE VIRUS

Fluid was taken from the vesicles of a 3-year-old boy who had typical chickenpox, but was otherwise healthy. The fluid was stored at  $-70^{\circ}\text{C}$  until it was inoculated onto primary cultures of human embryo lung (HEL) cells. Characteristic foci appeared after 7–10 days at  $34^{\circ}\text{C}$ . The virus strain was named Oka, after the boy from whom the vesicular fluid was derived (10).

## DIFFICULTIES IN PREPARING "CELL-FREE" VARICELLA-ZOSTER VIRUS

Since the earliest studies of *in vitro* propagation of varicella-zoster virus (VZV), it has been recognized that virus produced in cell cultures remains strongly cell associated; the inability to obtain cell-free infectious virus has hampered biological and immunological studies of VZV. Attempts were made to identify a suitable method for isolating cell-free virus from infected cultures and the composition of a suspension medium that would keep the infectivity of the virus as stable as possible.

Because VZV is highly heat-labile, particular caution was required in the selection of a suspension medium that would preserve its infectivity. After comparing several media, simple phosphate-buffered saline (Ca, Mg free) was selected as the most suitable, with sucrose (final concentration, 5%), sodium glutamate (0.1%), and other constituents (11).

## RATIONALE FOR AND DESIGN OF A LIVE VARICELLA VACCINE

VZV spreads from cell to cell, forming distinct foci that are visible by microscopy, even in unstained cell cultures, and that are clearly visible after methylene blue or fluorescent antibody staining. Cell-mediated immunity seems essential, or at least as important as humoral immunity in preventing the spread of VZV *in vivo*. Since inactivated or subunit viral antigens are usually weak inducers of cell-mediated immu-

nity, it was reasoned that a live vaccine might be the most useful for the prevention of varicella.

It had been very difficult to demonstrate the pathogenicity of VZV in laboratory animals. It was anticipated that attenuation would be proven only by extensive clinical trials, and that testing of only a limited number of candidate strains would be feasible. The classical (empirical) method of attenuation using passage in foreign cells was used. Of the various kinds of nonprimate cultured cells tested for susceptibility to infection with VZV (Oka strain), only guinea pig embryo fibroblasts (GPEF) were found to be somewhat susceptible.

VZV (Oka strain) was passaged 11 times in HEL cells at  $34^{\circ}\text{C}$  and 12 times in GPEF cells at  $37^{\circ}\text{C}$ , and then propagated in human diploid cells (W1-38) (10). The virus thus obtained exhibited better capacity for growth in GPEF than the original or other wild-type strains, which suggests that the vaccine virus is a variant with host dependency.

## BIOLOGICAL AND BIOPHYSICAL PROPERTIES OF THE VACCINE VIRUS

The Oka vaccine virus is temperature sensitive and has an enhanced capacity for growth in guinea pig embryo cells (3). Oka strain has been differentiated from other wild-type viruses by restriction-endonuclease digestion of extracted purified viral DNA, followed by agarose gel electrophoresis. In a comparison of the vaccine type DNA and wild-type virus DNAs, significantly different cleavage patterns were seen using HpaI, BamHI, BglI, and PstI enzymes (12–14). A more practical approach utilizing polymerase chain reaction (PCR) and restriction endonuclease digestion of the resulting DNA fragments was developed. Analysis of five variable regions with repeat elements (termed RI–R5) in the VZV genome—a cutting site of PstI in the PstI siteless region—was described (14). Later, we described a novel laboratory method for distinguishing the Oka strain from other isolates by combination analysis with the single strand-conformational polymorphism of repeating re-

gion 2 and with PstI cleavage of the PstI siteless region (15). Although Oka strain can be distinguished from other isolates of VZV using the methods described above, vaccine virus cannot be reliably distinguished from its parental virus by those methods.

### DIFFERENCE OF DNA SEQUENCES OF OKA VARICELLA VACCINE AND ITS PARENTAL VIRUS

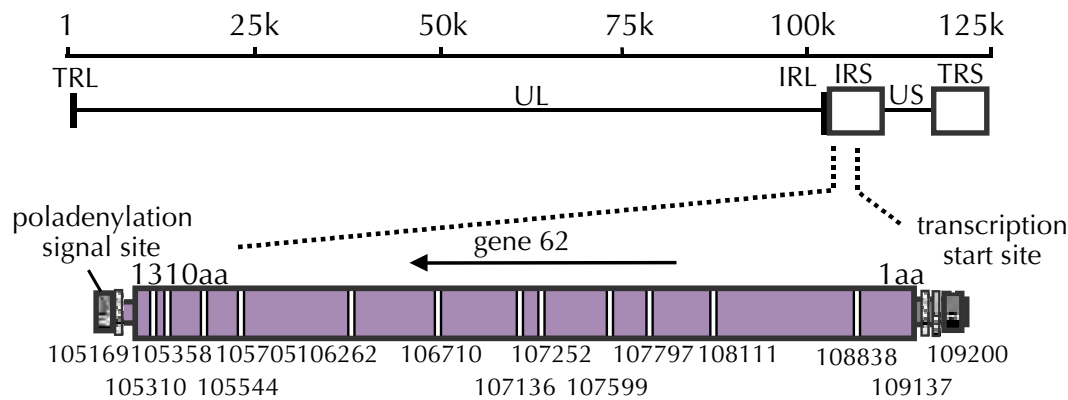
VZV is composed of 71 genes, classified as immediately early (IE), early (E), and late (L), which are known to function in cascading fashion in infected cells. Thus, IE genes have been regarded as the most important genes in initiating VZV growth in infected cells (16).

Genes 4, 10, 61, 62, and 63 have been reported as IE genes. When sequences were compared between V-Oka and P-Oka virus, no difference was found in the nucleotide sequences of genes 4, 10, 61, or 63, though as many as 15 nucleotide replacements and eight amino acid

changes were identified in gene 62 of the Oka vaccine virus (Figure 1) (17, 18). When the entire sequence of gene 62 was amplified by PCR, the reaction products from the vaccine virus were composed of a mixture of at least eight different clones that had a variety of mutations in that gene. On the other hand, the sequence analysis of nine clones derived from the Oka parental virus demonstrated that the parental virus consisted of a single sequence (18). It was further demonstrated that 15 base substitutions are specific for V-Oka and are not present in nine clinical isolates: three are from varicella patients around the same period of isolation of P-Oka (1971–1972), three are from varicella patients in the same clinic in 1995–1996, and three are zoster cases in different areas of Japan in 1995–1996 (19).

It was also demonstrated that S7-01 virus, a clone vaccine virus which had mutations in all eight amino acids (as found in the vaccine virus in IE62), spread more slowly in HEL cells (19). Thus, the substitutions that have accumu-

FIGURE 1. Structure of gene 62 and sequence analysis of the OKA parental and vaccine viruses.



position (100000+)	5169	5310	5356	5544	5705	6262	6710	7136	7252	7599	7797	8111	8838	9137	9200
Oka parental	A	A	T	A	T	T	A	T	T	A	A	T	A	A	A
Oka vaccine	A/G	A/G	C	G	C	C	A/G	C	C	A/G	A/G	C	A/G	A/G	A/G
	-	L/S	V	A	A	G	A	A	G	V/A	L/P	P	M/T	-	-

Source: Gomi Y, Imagawa T, Takahashi M, Yamanishi K. Oka varicella vaccine is distinguishable from its parental virus in DNA sequence of open reading frame 62 and its transactivation activity. *J Med Virol* 2000;61:497–503.

lated in gene 62 are likely to be important for the differences in the replication and spreading from infected to uninfected cells. Because V-Oka had been passaged in guinea pig cells and in human fibroblast cells at a low temperature, mutant viruses have been selected and grown under selective pressure. The reason why so many amino acid substitutions were accumulated in gene 62 of V-Oka is still unclear, but it is possible that the mutants IE62 contained in V-Oka may have a competitive advantage over P-Oka IE62 in interacting with some cellular transcription factor in guinea pig cells (19).

### EARLY CLINICAL TRIALS: VACCINATION OF HEALTHY AND HOSPITALIZED CHILDREN

With the informed consent of the parents, healthy children who were living at home and had no history of varicella received various doses of Oka strain varicella vaccine virus. A dose of 500 PFU elicited seroconversion in 19 of 20 children. Even at a dose of 200 PFU, an antibody response was detected in 11 of 12 children. No symptoms due to vaccination were detected in these children (10).

The first clinical trial of the vaccine in hospitalized children was undertaken in an effort to terminate the spread of varicella among children with no history of the disease (10). In the hospital where the trial was conducted, chickenpox had frequently spread in the children's ward, with severe cases seen on some occasions. In this protocol, children with no history of varicella were vaccinated immediately after the occurrence of a case of varicella. These children were suffering from conditions including nephritic syndrome, nephritis, purulent meningitis, and hepatitis. Twelve children had been receiving corticosteroid therapy. An antibody response was documented in all of the vaccinated children; within 10–14 days after vaccination, six children developed a mild fever, and two of the six developed a mild rash. It was uncertain whether these reactions were due to vaccination or to naturally acquired in-

fection modified by vaccination. No other clinical reactions or abnormalities of the blood or the urine were detected. Thus, in this ward, the spread of varicella infection was prevented except in one case: a child who was not vaccinated because his mother mistakenly believed that he already had varicella became severely ill. This study offered the first proof that the Oka strain varicella vaccine was well tolerated by patients receiving immunosuppressive therapy and stirred hopes that this vaccine would prove practical for the prevention of varicella.

### PROTECTIVE EFFICACY OF VACCINATION IN EARLY CLINICAL TRIALS

In an examination of its protective efficacy, the vaccine was given to susceptible household contacts immediately after exposure to varicella (20). Twenty-six contacts (all children) from 21 families were vaccinated, mostly within three days after exposure to the index cases. None of the vaccinated children developed symptoms of varicella. In contrast, all 19 unvaccinated contacts (from 15 families), exhibited typical varicella symptoms 10–20 days after the onset of the index cases. In three families, one sibling contact received the vaccine and the other did not; none of the vaccinated children developed symptoms, whereas all unvaccinated controls exhibited typical symptoms. In general, the antibody titers after clinical varicella were 8–10 times higher than those after immunization. This study clearly demonstrated that vaccination soon after exposure was protective against clinical varicella.

In an institution for children under 2 years old, prompt vaccination had a similar protective effect (21). Varicella developed in an 11-month-old infant in a ward for 86 children. A total of 33 children over 11 months of age were not vaccinated, partly because they were expected to still possess maternal antibody. A small viral dose (80 PFU) was used for immunization. Of the vaccinated group, eight developed a mild rash and one of these eight had a mild fever (under 38°C) two to four weeks after vaccination. In contrast, typical varicella

developed in all 43 unvaccinated children during the 10 weeks after the onset of the index case. Symptoms were severe in 16 cases, with confluent vesicles and high fever; after recovery, scars remained in 13 of these 16 cases. These results suggested that vaccination with as little as 80 PFU frequently stopped the spread of varicella among children in close contact with one another.

#### ISOLATION OF VZV FROM THE BLOOD OF NATURALLY INFECTED AND VACCINATED CHILDREN

VZV could be recovered from blood mononuclear cells of immunocompetent patients for several days before and after onset of the disease (Table 1) (22). In contrast, no VZV could be recovered from a total of 27 children, 4 to 14 days after vaccination at a dose of 5,000 PFU (Table 2). It is generally believed that at the time of primary VZV infection, the virus multiplies in the respiratory mucosa and the regional lymph nodes, and that this multi-

plication leads to a primary viremia, during which the virus is delivered to the viscera, where further multiplication ensues. A secondary viremia, greater in magnitude than the first, then occurs and delivers virus to the skin, leading to the appearance of a rash. The above results suggest that the magnitude of replication of the vaccine virus in the susceptible viscera is far less than that of wild-type VZV, but sufficient to induce an immune response. Although the route of infection with the virus was not the same, it seems that viremia may be a marker of the virulence of VZV for the host, and the vaccine virus may be attenuated to the degree that it lacks the capacity to cause a viremia, except, possibly, in rare instances.

#### VACCINATION OF CHILDREN WITH MALIGNANT DISEASES

In the first vaccination trials in children with malignant diseases with virus doses of 200, 500, or 1,500 PFU, chemotherapy was suspended for one week before and one week

**TABLE 1. Viral isolation from mononuclear cells and antibody responses after close contact with varicella patients.**

Day of testing after onset of varicella	Viral isolation from mononuclear cells		Detectable antibodies <sup>a</sup>	
	Positive subjects/No. tested	%	Positive subjects/No. tested	%
-11	0/3	0	ND <sup>b</sup>	
-7	0/4	0	ND	
-6	0/1	0	0/1	0
-5	1/2	50	ND	
-4	1/3	33	0/2	0
-3	ND		0/1	0
-2	4/4	100	0/4	0
-1	4/5	80	0/5	0
0	4/17	24	0/13	0
1	7/32	22	0/28	0
2	0/14	0	0/12	0
3	0/3	0	4/12	33
4	0/1	0	9/18	50
5	0/3	0	14/14	100

<sup>a</sup> Measure by the assay for fluorescent antibody to membrane antigen.

<sup>b</sup> ND = Not done

**Source:** Asano Y, Itakura N, Hiroishi Y, Hirose S, Ozaki T, Kuno T, *et al.* Viral replication and immunologic responses in children naturally infected with varicella-zoster virus and in varicella vaccine recipients. *J Infect Dis* 1985;152:863-868.

**TABLE 2. Isolation of varicella-zoster virus from children inoculated with live virus vaccine (Oka strain).**

Day of testing after vaccination	Virus isolation source							
	Mononuclear cells		Throat		Detectable antibodies <sup>a</sup>		Positive skin reaction	
	Positive vaccinees/ No. tested	%	Positive vaccinees/ No. tested	%	Positive vaccinees/ No. tested	%	Positive vaccinees/ No. tested	%
0	ND <sup>b</sup>		0/3	0	0/28	0	0/22	0
3	0/11	0	0/8	0	0/8	0	0/10	0
4-5	0/14	0	0/13	0	0/11	0	1/11	9
6-7	0/17	0	0/16	0	2/8	25	8/11	73
8-9	0/6	0	0/6	0	2/5	40	4/5	80
10-14	0/11	0	ND		8/8	100	6/7	86
30-60	ND		ND		28/28	100	17/20	85

<sup>a</sup> Measure by the assay for fluorescent antibody to membrane antigen.

<sup>b</sup> ND = Not done

**Source:** Asano Y, Itakura N, Hiroishi Y, Hirose S, Ozaki T, Kuno T, *et al.* Viral replication and immunologic responses in children naturally infected with varicella-zoster virus and in varicella vaccine recipients. *J Infect Dis* 1985;152:863-868.

after vaccination (23). Of 12 immunized children with acute lymphocytic leukemia, 10 had been in remission for six months or less, one for nine months, and one for forty-eight months. Of these children, four had fewer than 3,000 white blood cells/mm<sup>3</sup>, but most had positive skin-test reactions with dinitrochlorobenzene, purified protein derivative, or phytohemagglutinin. Three of twelve children developed a mild rash; 13 papulae or incomplete vesicles developed in one of three children who received 1,500 PFU; 30 and 25 papulae, respectively, developed in two of five children who received 200 PFU; four children who received 500 PFU did not develop a rash; and one child had a fever (39°C) for one day about three weeks after vaccination. These results offered hope that a live varicella vaccine could be administered with some precautions to high-risk children (1, 7, 24).

#### CLINICAL VACCINE TRIALS IN THE U.S. AND EUROPE AND LICENSURE OF THE VACCINE

In the U.S., the National Institutes of Health (NIH) Collaborative Study Group was organized, and clinical trials were started with live varicella vaccine (Oka strain) produced by

Merck Research Laboratories (West Point, PA, U.S.A.). Many investigations were conducted by that group, including clinical reactogenicity, the frequency of household transmission from vaccinated acute leukemic children with rash, and the persistence of immunity. Other study groups also conducted clinical trials, most of which yielded favorable results. In Europe, clinical trials were conducted with varicella vaccine (Oka strain) prepared by SmithKline RIT (Rixensart, Belgium). In 1983, the Expert Committee was held at the World Health Organization in Geneva to prepare a manuscript entitled "Requirements for the Live Varicella Vaccine." The resulting document was circulated for review by authorities around the world and was finally published in 1985 (25, 26). Meanwhile, in 1984, the live varicella vaccine (Oka strain) produced by SmithKline RIT was licensed for administration to high-risk children in several European countries.

In 1986, live varicella vaccine produced by the Research Foundation for Microbial Diseases of Osaka University (BIKEN) was licensed in Japan for use in high-risk children and for optional use in children at normal risk. In South Korea, live varicella vaccine (Oka strain) was licensed for uses similar to those in Japan. In 1988. In 1995, live varicella vaccines

(Oka strain), produced by Merck Research Laboratories, were licensed for the universal immunization of healthy children in the U.S.

### VACCINE EFFICACY

Several follow-up studies, conducted after licensing of the vaccine in Japan, indicated that breakthrough cases occur in 15%–20% of the vaccine recipients. However, approximately 60% of such cases are extremely mild (a few vesicles) and 20% are mild (several to 50 vesicles). Thus, it is estimated that clinically significant breakthrough cases are no more than 5% of the varicella vaccine recipients. A quantitative comparison of the severity of symptoms of natural varicella and of breakthrough cases in vaccine recipients found that the symptoms of breakthrough cases are far milder than those of natural varicella (27).

In the U.S., several reports of breakthrough cases—approximately 15% of the vaccine recipients—manifested clinical symptoms. Conclusive data appeared in 2001 in the U.S.; a case-control study was conducted from March 1997 through November 2000 for 330 potential cases, of which 243 (74%) were in children who had positive PCR tests for VZV. Of the 202 children with PCR-confirmed VZV and their 389 matched controls, 23% of the former and 61% of the latter had received the vaccine (vaccine effectiveness, 85%). The vaccine was 97% effective against moderately severe and severe disease. Thus, it was concluded that varicella vaccine is highly effective as used in clinical practice (28).

### TOLERABILITY OF THE VACCINE

The varicella vaccine (Oka strain) has been shown to be safe and very well tolerated. Adverse clinical reactions (rash, fever, redness, and swelling) due to the vaccine are rare and generally mild, if at all, in normal children (29).

The risk of clinical reactions following the administration of Oka strain varicella vaccine was higher among high-risk individuals. A large study of 663 children attending a pedi-

atric clinic during a seven-year period showed that vaccination produced adverse reactions in 32.4% of children with malignant disease when administered with chemotherapy, compared with only 0.3% of those with other conditions, including congenital heart disease, neuromuscular disease, and immunological diseases (2). However, all the reactions were mild and resolved spontaneously. Importantly, administration of the Oka strain vaccine had no significant impact on relapse rates in children with acute leukemia (2). Likewise, children with other underlying diseases have also been effectively vaccinated with no adverse effect on their medical condition (2).

### HERPES ZOSTER AND THE LIVE VARICELLA VACCINE

It has generally been believed that VZV in the skin vesicles travels up the sensory nerves to the posterior ganglia, where it persists; this seems to be the main route of virus migration. A major question about live varicella vaccine had been whether the vaccine virus becomes latent, resulting in the later development of zoster. Since zoster is relatively uncommon in healthy children, long-term follow-up of vaccinated healthy children was required to answer this question definitively. However, children with acute leukemia tend to develop zoster soon after natural infection. Therefore, it was assumed that careful observation of the incidence of zoster in vaccinated children with acute lymphocytic leukemia would yield valuable insight.

A retrospective follow-up study of children with acute leukemia found that zoster occurred far more frequently in the group that developed a rash after vaccination (17.1% or 3.13 cases per 100 person-years;  $n = 70$ ) than in the group without rash (2.4%, or 0.46 cases per 100 person-years;  $n = 250$ ) (1, 2). These figures suggested that an absence of rash after vaccination is closely correlated with a low incidence of zoster, indicating that the incidence of zoster would be lower among vaccine recipients than among children who had natural varicella.

Studies by U.S. National Institute of Allergy and Infectious Diseases Collaborative Study Group showed clearly that an absence of rash is correlated with a low incidence of zoster. Of 268 vaccinated children with VZV rashes, 11 (4.1%) had zoster. In contrast, there were only two cases of zoster (0.7%) among the 280 vaccinated children with no VZV rash. The relative risk of zoster in the children who had had a VZV rash was 5.75 (30).

Besides the main virus migration route (i.e., via the sensory nerves), there may be a minor hematogenous migration route to the ganglia. However, no viremia could be detected in healthy vaccine recipients, while viremia could be detected in cases of natural varicella for several days before and just after appearance of the rash (21). Therefore, whatever the route, it seems far less likely for the vaccine virus than for wild-type virus to become latent in the ganglia and cause subsequent zoster.

#### IMMUNIZATION OF THE ELDERLY TO ENHANCE IMMUNITY TO VZV ASSESSED BY THE VZV SKIN TEST FOR CELL-MEDIATED IMMUNITY AND HUMORAL ANTIBODY

The VZV skin test has been shown to be useful for assessing the susceptibility of individuals to clinical varicella (31). The skin test was negative or weakly positive during the early stage of herpes zoster infection, and strongly positive during recovery (32, 34). In a small-scale clinical trial, elderly individuals were immunized in order to prevent herpes zoster, and, hopefully, severe postherpetic neuralgia (35). Sixty individuals ( $\geq 50$  years old) were screened for VZV antibodies and were given a VZV skin test for cell-mediated immunity. All were seropositive, but eight were skin-test negative. Thirty-seven individuals, including the eight with negative skin tests, were immunized with varicella vaccine ( $3.0 \times 10^4$  PFU/dose). After five to seven weeks, the skin test reaction showed increased positivity, with a change in score from (-) to (+, ++) in seven of eight subjects, from (+) to (++,+++) in three of

five subjects, and from (++) to (+++) in six of ten subjects. Enhancement of the VZV antibody titer (twofold or greater) was observed in all 15 vaccine recipients with a prevaccination titer of  $\leq 1:16$ , and in 19 of 24 subjects with a prevaccination titer of  $\geq 1:32$ .

These results indicate that giving live varicella vaccine with a high viral titer can induce a good boost to immunity, particularly cell-mediated immunity, to VZV in the elderly, as assessed by the VZV skin test.

Immunity to VZV in 35 elderly subjects who were vaccinated previously was followed up for four years. All were positive by the VZV skin test after the previous vaccination. After four years, 31 (88.6%) were positive by the skin test, and four were negative and became positive after revaccination (36). These results suggest that administering live varicella vaccine to the elderly is effective for enhancing immunity, particularly cell-mediated immunity to VZV, and that enhanced cell-mediated immunity lasts four years in most vaccine recipients.

The duration of immunity enhanced by vaccination is a crucial matter for the application of vaccination to the prevention of zoster, particularly for postherpetic neuralgia. It is expected that vaccination of elderly persons around and older than 60 years of age at four- to five-year intervals will significantly reduce their risk of severe herpes zoster and, particularly, of severe postherpetic neuralgia. A large scale clinical trial is under way in the U.S. for the prevention of herpes zoster, particularly postherpetic neuralgia, by giving live varicella vaccine (produced by Merck Research Laboratories) to elderly subjects.

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# HEPATITIS A VACCINES

*Stanley M. Lemon*<sup>1</sup>

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## INTRODUCTION

Despite the recent successful development and international marketing of inactivated hepatitis A vaccines, hepatitis A remains a common infectious disease in many regions of the world. Transmission occurs largely by the fecal-oral route, although in recent years a rise in parenteral transmission has been noted in economically developed countries where infections have been related to illicit injection drug use. In such nations, point-source outbreaks due to ingestion of contaminated food also continue to occur sporadically, as well as less dramatic outbreaks that are associated with preschool day care centers and maintained via person-to-person transmission. But in less developed countries, infections are much more prevalent. Transmission occurs in the early years of life and is related in general to inadequate water supplies and poor public health sanitation.

Hepatitis A causes significant morbidity, but only rarely leads to death (1). The incubation period averages around one month, and onset of the illness may be sudden in nature. Most cases of fulminant hepatitis are reported in older individuals or in the very young. Relapsing hepatitis and cholestatic hepatitis are also recognized complications of infection with hepatitis A virus (HAV), but there are no

chronic sequelae of hepatitis A such as those which occur with hepatitis B or hepatitis C. There is no association with cirrhosis, no persistence of the virus (except perhaps rarely, and only for a matter of months, in infected premature infants), and certainly no association with hepatocellular carcinoma.

In the United States, prior to the licensure of inactivated hepatitis A vaccine in 1995, hepatitis A accounted for approximately 50% of the cases of acute hepatitis that precipitate visits to the emergency room or to personal physicians. That picture is not much different today (2). The most recent summaries from the U.S. Centers for Disease Control and Prevention (CDC) indicate that there are approximately 30,000 cases of hepatitis A reported to public health authorities annually. The incidence has decreased somewhat since the licensure of the vaccine, but the proportion of cases of hepatitis due to HAV infection is similar to what it was prior to licensure. This reflects, no doubt, the relatively high cost of this vaccine, and the fact that it generally has been administered only to individuals in special, high-risk populations.

Thus, while the vaccine is extremely efficacious in preventing disease in immunized persons, as pointed out below, economic considerations have limited its ability to control the spread of HAV within the U.S. population. Overseas, in regions where hepatitis A is considerably more prevalent than in the United States, the vaccine has had even less impact on public health.

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## INACTIVATED HEPATITIS A VACCINES

The chronology of the hepatitis A vaccine begins with the first description of the syndrome of infectious hepatitis as a disease distinct from other causes of infectious jaundice. This occurred early in the last century, at which time the disease was known as "catarrhal jaundice" (3). By the end of World War II, hepatitis A was clearly distinguished both clinically and epidemiologically from hepatitis B. These two infections were shown to be due to agents that were immunologically distinct (4), although the alphabetic system for classification of the hepatitis viruses did not follow until several years later. By that time, pooled human immune globulin was known to be protective against infectious hepatitis when administered parenterally, either prior to or as long as two weeks after exposure (5). This important finding indicated early on that circulating antibodies are highly protective against symptomatic hepatitis A, and that neither secretory immunity nor cytotoxic T-cell activity is required for protection against the disease.

These early observations were followed by the classic clinical studies of the natural history of hepatitis A that were carried out by Krugman beginning in the 1950s and extending into the 1970s (6, 7). However, the modern era of hepatitis A virology began in 1973, when HAV particles, the causative agent of hepatitis A, were identified in human fecal material by Feinstone, Kapikian, and Purcell working at the National Institutes of Health (8). To accomplish this, these investigators used the then relatively new technique of immune electron microscopy, demonstrating the aggregation of viral particles by convalescent sera containing specific antibodies to the virus. These pioneering studies paved the way for development of sensitive and specific serologic tests for hepatitis A, and shortly thereafter, in large part because of these tests, to the recognition of the third major type of viral hepatitis in humans, then called "non-A, non-B hepatitis," and now known as hepatitis C.

The breakthrough that led directly to the hepatitis A vaccines available today was the isolation and propagation of HAV in cultured cells by Provost working with Hilleman at Merck in the latter part of the 1970s (9). In 1986, a team led by Binn at Walter Reed Army Medical Center described the successful immunization of small primates with a prototype vaccine produced by formalin-inactivation of virus particles harvested from infected cell cultures (10). This seminal work demonstrated that cell culture infections could produce sufficient amounts of viral antigen for vaccine production, and it was followed shortly afterwards by advanced vaccine development efforts within the industry. In 1992, the first demonstration of clinical efficacy in humans was reported by Werzberger and colleagues in a now classic study carried out in Monroe, New York, using an inactivated vaccine (Vaqta) produced by Merck (11). Comparable efficacy was subsequently shown to exist for a similar vaccine (Havrix) produced by SmithKline-Beecham (now GlaxoSmithKline, or GSK) in a study carried out in Thailand (12). This vaccine was the first to be licensed by the U.S. Food and Drug Administration, receiving approval in 1995. Both the Merck and GSK vaccines are now registered in many countries, and they have been joined on the market by other inactivated hepatitis A vaccines produced in Europe and Japan. These vaccines as a group are marked more by their similarities than by their differences. A more complete description of the Merck and GSK vaccines that are licensed within the United States can be found elsewhere (13).

By and large, all of these vaccines have been produced using "old" technologies (14). Although in some cases the vaccine antigen is highly purified from accompanying cellular materials prior to inactivation, the basic principles underlying the inactivated hepatitis A vaccines are those employed for production of the Salk inactivated poliovirus vaccine. This is somewhat ironic for an infectious agent that has only been discovered in the past few

decades, but it is consistent with what we know about the infectious agent, which, like the polioviruses, is a member of the family *Picornaviridae*. A brief review of HAV virology makes it clear why this type of vaccine is prevalent among hepatitis A vaccines today, although an attenuated vaccine has been used extensively in China.

## THE VIROLOGY OF HEPATITIS A

The HAV particle contains three large capsid polypeptides (VP1, VP2, and VP3) that contribute to a very tightly assembled, non-enveloped viral capsid that protects the positive-strand viral RNA packaged within from nuclei present in the external environment (15). This capsid possesses receptor-binding activities that direct the virus to its cellular site of replication. Sixty copies of each of the capsid polypeptides are presumed to be present in each particle, given what is known about the structure of this and related viruses. They fold in a way that conformationally determines the neutralizing antigenic epitopes of the virus (16). Thus, when the capsid proteins are individually expressed from recombinant cDNA, the proteins have very poor immunogenicity and elicit only very low levels of neutralizing antibodies in animals. The generation of a protective antibody response thus requires immunization with the complete viral capsid in its assembled form. While it is possible to assemble such a particle from capsid polypeptides expressed in bacteria (17), the production of virus particles in infected cell cultures has thus far proven to be the only practical pathway to vaccine manufacture on a commercial scale.

A second important point concerning the antigenicity of the virus is that there is only a single serotype of HAV (18), despite the existence of multiple viral genotypes that are defined by differences in the nucleotide sequence of the RNA genome. Thus, infection (or immunization) with any one strain of HAV confers protection against all other strains of the virus. This cross-strain protection extends even to

several simian genotypes, despite the fact that these particular strains of HAV do demonstrate differences in the amino acid sequences of some critical neutralization epitopes. From a practical point of view, the fact that there is only one serotype makes it possible for a single hepatitis A vaccine antigen to protect against the disease anywhere in the world. From a theoretical perspective, the lack of significant antigenic diversity suggests that the capsid antigens may play a critical role in the viral life cycle, perhaps in recognition of the cellular receptor for the virus.

As indicated above, the major scientific advance that made the hepatitis A vaccine possible, given that recombinant approaches proved to be impractical, was the development of cell culture systems allowing the propagation of the virus (9). Either primary or continuous African green monkey kidney cells are permissive for replication of the virus and are usually used for primary isolation of the virus. MRC-5 cells generally are used for production of the viral antigen for vaccine manufacture. The infection in both of these cell types is typically noncytopathic. It is also not very robust, with the titer of virus produced at least 10- to 100-fold less than what would be expected with poliovirus. Some variants of the virus that have been highly adapted to growth in cell culture are cytopathic, at least in part through induction of apoptosis in infected cells (19, 20). Such viruses can be used in conventional plaque-reduction neutralization assays. On the other hand, much more has been learned about the neutralizing antibody response to the virus using radioimmunofocus inhibition assays, which depend upon the use of a radiolabelled antibody for detection of cell foci infected with HAV under an agarose overlay (21).

Although the hepatitis vaccines that are licensed today are, by and large, cell-culture-propagated, whole virus, inactivated vaccines, a live attenuated vaccine has enjoyed extensive use in China (22, 23). This vaccine utilizes a strain of HAV that has been propagated and adapted to growth in cell culture. Studies done

by Provost and Hilleman and their colleagues at Merck during the late 1970s and early 1980s demonstrated clearly that passage of the virus in cell culture leads to its attenuation for primates, including humans (24, 25). This was subsequently confirmed in studies done with a second viral isolate at the National Institutes of Health (26). However, neither of these vaccine development programs led to a virus that had an acceptable balance of attenuation and immunogenicity, and these efforts were eclipsed by the subsequent success of the inactivated vaccine. There is not much in the literature concerning the attenuation properties of the Chinese hepatitis A vaccine, even though it has been used quite extensively in that country.

The licensed inactivated vaccines generally are formulated with an alum adjuvant and used in a two-dose regimen (13). They have low reactogenicity and, although they have been associated with a low incidence of anaphylaxis and central nervous system adverse events, they appear to be among the safest vaccines in the infectious disease armamentarium. As mentioned above and described in greater detail below, they have excellent efficacy in the prevention of disease.

## HEPATITIS A VACCINE EFFICACY

Table 1 summarizes the two pivotal efficacy studies that supported the licensure of Vaqta, the Merck vaccine, and Havrix, the vaccine licensed by GSK in the mid-1990s. The Vaqta trial

was carried out in Monroe, New York, within an orthodox Jewish community in which most families were large and which had extensive day care arrangements for very young children (11). Historically, prior to the vaccine study, this community had experienced high rates of hepatitis A in children and young adults with almost annual summertime epidemics. The vaccine efficacy trial was begun with the intent to deliver a two-dose regimen, but a typical seasonal epidemic of hepatitis A broke out within the community shortly after the study was started, and efficacy was proven before a second dose could be administered. Hence, it was shown that one dose of vaccine was sufficiently immunogenic to provide protective immunity. In fact, no individual developed hepatitis in that trial who had been immunized more than 16 days previously. The overall vaccine efficacy was 100%, with 95% confidence intervals.

The Havrix trial in Thailand was quite different, although its conclusions were not. It involved almost 40,000 children aged 1 to 16 who were immunized with either hepatitis A vaccine or, as a control, a hepatitis B vaccine rather than a placebo (12). Although this clinical efficacy trial monitored the ability of the vaccine to prevent endemic rather than epidemic disease, it gave a very similar result (94% protective efficacy), one that is statistically identical to the result obtained with the Merck vaccine in Monroe, New York. Both studies are indicative of nearly complete protection against the disease following immunization.

**TABLE 1. Hepatitis A vaccine efficacy.**

Vaccine	Study site (Subject ages)	No. of subjects	Vaccine efficacy (95% CI)
Vaqta™ (Merck) 1 dose; 25 units	Monroe, N.Y. (2–16 years)	1,037	100% (85%–100%)
HAVRIX® (SKB) 2 doses; 360 EL.U.	Thailand (1–16 years)	38,157	94% (79%–99%)

**Sources:** Werzberger A, Mensch B, Kuter B, Brown L, Lewis J, Sitrin R, *et al.* A controlled trial of a formalin-inactivated hepatitis A vaccine in healthy children. *N Engl J Med* 1992;327(7): 453–457. Innis BL, Snitbhan R, Kunasol P, Laorakpongse T, Poopatanakool W, Kozik CA, *et al.* Protection against hepatitis A by an inactivated vaccine. *JAMA* 1994;271(17):1328–1334.

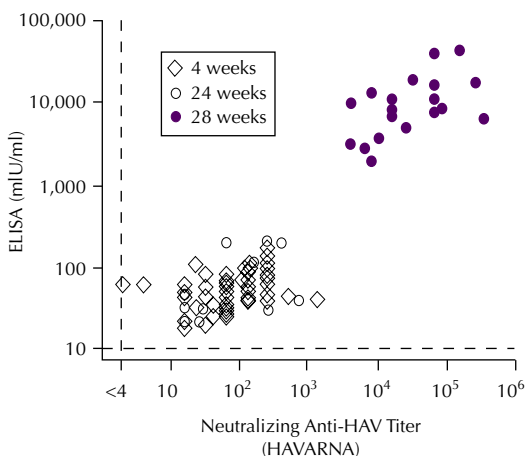
Following the completion of the Vaqta efficacy study in Monroe, we had the opportunity to study the anti-HAV antibody response in study participants and to compare it with that present in persons receiving immune serum globulin at a dose known to be protective. We determined antibody titers using the radioimmunoassay inhibition viral neutralization assay alluded to above, as well as a hepatitis A virus antigen reduction neutralization assay, and compared these titers with the level of antibody determined in an ELISA assay (27). As shown in Figure 1, there was a very close correlation between the results of these different assays. This indicates that the ELISA assay, which is commonly available in the clinical setting, can be used as a measure of the protective antibody response.

Virtually every immunized individual had antibody within four weeks of receiving the first dose of Vaqta. Most had antibody levels that equaled or exceeded those present seven days after the administration of immune globulin. There was a substantial booster effect when a second dose of vaccine was given six months after the first. The neutralizing antibody titers were substantially elevated in both

assays (27). This booster effect most likely extends the duration of protection. Prior to the clinical vaccine efficacy trials, it was recognized that the efficacy of these vaccines could be predicted from the measurement of neutralizing antibody levels in the blood (21). These studies confirmed that notion, in addition to providing formal proof of vaccine efficacy.

Despite the fact that the neutralizing antibody titer is an excellent correlate of protection, antibodies that are induced within the first few weeks after active immunization are qualitatively dissimilar from those present in immune serum globulin (27). Figure 2 shows antibody titers in persons who had received a single dose of the Merck vaccine 24 days previously, plotted along with antibody titers in persons who had received immune globulin one week before being bled. When titers obtained in the ELISA assay were compared with those detected in a viral immunoprecipitation assay (one employing HAV particles that were endogenously labeled during their production in cell culture), the relative activities were strikingly different in the vaccine vs. immune globulin recipients. Although formal measurements of the affinity of these antibodies for HAV have yet to be done, the data suggest that antibody is of low avidity in the early weeks after immunization with vaccine (27). In contrast, the recipient of immune globulin, while similarly protected, appears to have a much lower abundance of high avidity antibody. However, these differences, while interesting, are not likely to be of significance clinically, much as borne out by the results of the clinical trials.

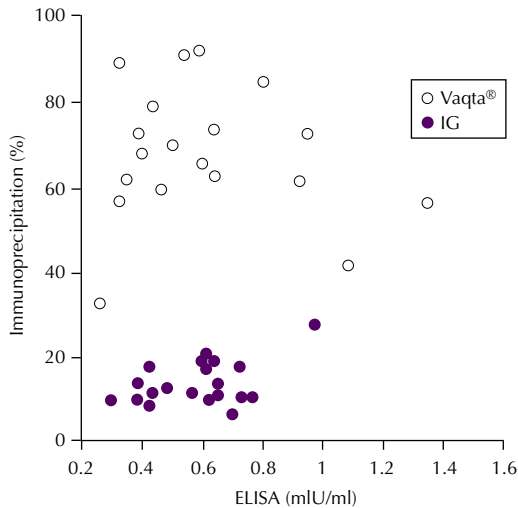
**FIGURE 1. Anti-HAV antibody titers in recipients of Vaqta vaccine who participated in the Monroe, New York, efficacy study, and in recipients of immune globulin.**



## PATHOGENESIS AND MECHANISMS OF PROTECTION

It is very likely that the protection afforded by vaccines is due to the ability of antibody to limit the spread of virus within the liver during the early stages of infection. Current views of the pathogenesis of this infection hold that the virus usually enters via the gastrointestinal tract and establishes a primary infection in epithelial cells within the crypts of the small

**FIGURE 2. Antibody titers determined by an enzyme-linked solid-phase immunoassay (ELISA) and by a viral neutralization test (HAVARNA) 4 and 24 weeks after a first dose of vaccine, and 4 weeks after a second, booster dose of vaccine given at 24 weeks.**



**Note:** There is an excellent correlation between the immunoassay and viral neutralization results. Antibody titers were determined by an immunoprecipitation assay employing labeled virus particles and by ELISA approximately four weeks after a first dose of vaccine, or one week after a dose of immune globulin. Sera with similar ELISA reactivities have levels of reactivity in the immunoprecipitation assay that are many-fold higher among the immune globulin recipients compared with the vaccine recipients. Greater reactivity in the immunoprecipitation assay, which employs very small amounts of viral antigen, is likely due to the presence of high affinity antibody. Figures modified from Lemon et al. (27).

**Source:** Figures modified from Lemon SM, Murphy PC, Provost PJ, Chalikonda I, Davide JP, Schofield TL, et al. Immunoprecipitation and virus neutralization assays demonstrate qualitative differences between protective antibody responses to inactivated hepatitis A vaccine and passive immunization with immune globulin. *J Infect Dis* 1997;176(1):9-19.

intestine (28). Whether by release of virus into the intestine and reentry via specialized M cells in the terminal ileum, or by direct invasion of the virus through the epithelial cells of the small intestine, there is spread of the virus via the bloodstream to the liver. The production of virus by infected hepatocytes leads to a secondary viremia of much greater magnitude (29, 30), and this results in the further spread of the virus within the liver, with growing

numbers of hepatocytes being infected over a period of several weeks. When this noncytotoxic infection of the liver is finally recognized by the immune system, there is a variable degree of collateral damage to the liver that occurs during the process of viral elimination. It is unclear exactly how the immune system accomplishes the elimination of the infection, but it is probably through a combination of innate antiviral host defenses involving the expression of interferons and cytokines, and the induction of an adaptive, cytotoxic T-cell response (31).

It seems likely that very small amounts of neutralizing antibody, either from passive administration of immune globulin or from prior immunization, act by limiting both the primary and secondary viremia. This would reduce the number of infected hepatocytes within the liver at the time of recognition by the immune system, resulting in minimal, if any, inflammation and necrosis within the liver as the infection is eliminated. Such a series of events was termed "passive-active immunity" by Krugman and was recognized as leading to long-term protection against HAV following the use of immune globulin in epidemic settings (7). With respect to vaccine-induced immunity, events are less well understood. It is possible that small amounts of vaccine-induced antibody may actually prevent the spread of the virus to the liver through the bloodstream and thus may block infection at its earliest stages.

## RECOMMENDATIONS FOR VACCINE USE

Recommendations for the use of hepatitis A vaccines within the United States have largely targeted persons at increased risk of the disease, based on risk factors associated with acquisition of hepatitis A in this country (2). These include travel to developing regions where the infection is more prevalent, close association with children under the age of 2 who are attending preschool day care centers, multiple sexual partners (particularly among male homosexuals), and illicit injection drug

use, which is increasingly recognized as a risk factor for parenteral transmission of HAV (32). Although HAV generally has not been considered to be parenterally transmitted, the high titer secondary viremia that marks the prodromal phase of the infection provides an excellent opportunity for transmission by contaminated needles or other drug paraphernalia. Despite the identification of these specific risk factors, however, a source of infection cannot be ascertained in a large proportion of persons presenting with hepatitis A.

As of this writing, there are three categories of individuals for whom this vaccine is recommended in the United States (2). The first consists of individuals who are at increased risk of acquiring hepatitis A, as described above. A second category includes those who are at increased risk of fulminant liver disease if they become infected with HAV, even though they may be at no more risk for infection than the general population. Leading that list are individuals with chronic liver disease due to hepatitis C virus infection. Finally, it has been recommended that children who live in areas with a high historic prevalence of hepatitis A infection be immunized uniformly after the age of 2 years. The vaccine is not approved for use in children under age 2, since there is not enough information available concerning the immune response to the vaccine in this age group to make such a recommendation. Furthermore, maternally-acquired antibodies to HAV can lead to reduced immunogenicity of inactivated hepatitis A vaccines.

Areas of historically high prevalence are defined in the United States as those in which the incidence of infection exceeds 20 cases per 100,000 persons per year, or about twice the national average (2). The recommendation to immunize children in these regions is based on recognition that children play an important role in the transmission of this virus, given the fact that it is largely spread by the fecal-oral route. Several projects have demonstrated that universal immunization of children will essentially eliminate, if not eradicate, the virus from a community, causing very significant re-

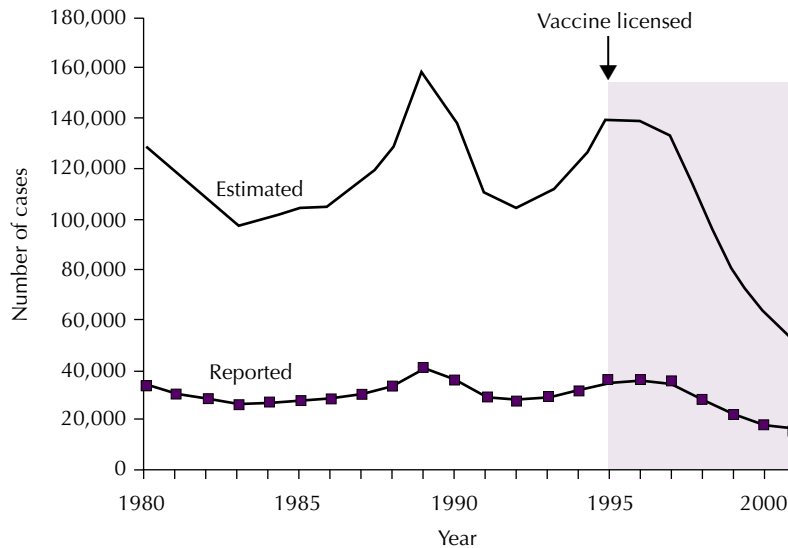
ductions in the number of hepatitis A cases. A case in point is Monroe, New York. Over the six years that followed the vaccine efficacy study, children continued to be immunized against hepatitis A. There have been virtually no cases of hepatitis A recognized in the community, despite a long prior history of annual hepatitis A outbreaks (33). Similar results have been obtained by the CDC in demonstration projects carried out with the GSK vaccine in California.

## VACCINE EFFECTIVENESS

The single largest remaining difficulty with hepatitis A vaccines is that they remain relatively expensive. In general, their high cost continues to restrict their use and thus their overall benefit within the public health context. Unquestionably, the vaccine has prevented morbidity in individuals who have been immunized. However, it is difficult—outside of the context of particular situations in certain communities—to say with assurance that the vaccine has reduced the overall public health burden related to hepatitis A. It is interesting to look at the reported incidence of hepatitis A since the vaccine's introduction in 1995. As shown in Figure 3, incidence has been declining generally over the past several decades in the United States and no longer shows the large cyclic swings that occurred up to the middle of the last century. This almost certainly reflects disruption of prior, long-standing transmission patterns through improved public health sanitation. There has been an acceleration of the rates of decline in disease incidence since 1995, but it is difficult to know whether this is related to the vaccine and its availability, or to continued nationwide improvements in living conditions and sanitation infrastructure.

Historically, the United States is a country with at most a low or intermediate incidence and prevalence of HAV infection. However, it is important to note that hepatitis A vaccines have had essentially no impact on the global disease burden due to hepatitis A. Outside

**FIGURE 3. Reported and estimated incidence of hepatitis A cases, United States, prior to and following the introduction of the hepatitis A vaccine into clinical practice in 1995.**



**Source:** Figure modified from United States of America, Department of Health and Human Services, Centers for Disease Control and Prevention. Prevention of hepatitis A through active or passive immunization: Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 1999;48(RR-12):1-37.

of the few economically developed countries that have been able to afford them, the global effectiveness of these vaccines has been negligible.

In 2003, the cost of the vaccine at public tender within the United States was approximately US\$ 11 for a pediatric dose and US\$ 18 for an adult dose. It is very clear that a vaccine of this price is not going to be available in those regions of the world where it is most needed; i.e., developing areas with improving sanitation in which hepatitis A is becoming more apparent as infection is increasingly delayed from early childhood to adolescence and beyond, when disease accompanies infection more regularly. Public health policy-makers must consider the vaccine-preventable morbidity and mortality of hepatitis A within the context of other preventable diseases that are prevalent in their regions. They must reach a

decision regarding where to commit very limited public health resources. It is unlikely that the answer for many would be the hepatitis A vaccination.

## SUMMARY

Hepatitis A vaccines have proven highly successful from a scientific point of view. They are exceptionally efficacious when given to individuals prior to exposure to HAV and may even provide some protection if given a week or more after exposure. They probably provide very long-term protection and are relatively safe. However, despite these very positive and desirable attributes, these vaccines have had relatively little impact on the health of the public outside of the relatively few populations residing in highly developed areas of the world.

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# CONJUGATE MENINGOCOCCAL VACCINES FOR AFRICA

*F. Marc LaForce*<sup>1</sup>

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## INTRODUCTION

Over the last 100 years, sub-Saharan Africa has suffered repeated epidemics of meningococcal meningitis. The human toll has been enormous; the 1996–1997 outbreak resulted in more than 188,000 reported cases and over 20,000 deaths. The first part of this paper, therefore, will provide background information on epidemic meningitis in sub-Saharan Africa. The second part will describe the general characteristics of meningococcal polysaccharide (PS) vaccines, which have been traditionally employed to control epidemics in this corner of the world; and meningococcal conjugate vaccines, whose development and widespread use offer an attractive alternative, principally due to their greater potency, among other factors. The final section highlights the activities of the Meningitis Vaccine Project, a partnership created in 2001 between the World Health Organization (WHO) and the Program for Appropriate Technology for Health (PATH) with the goal of eliminating epidemic meningitis as a public health problem in sub-Saharan Africa.

## EPIDEMIC MENINGITIS IN AFRICA

Epidemic meningitis in Africa has been a significant problem for at least 100 years (1). Fig-

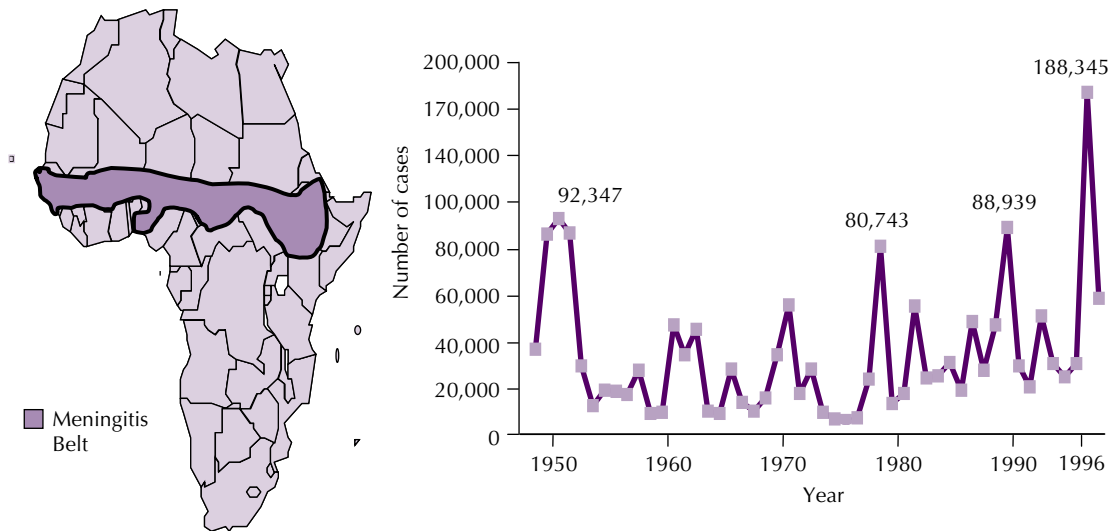
ure 1 shows the cases of meningitis between 1950 and 1996 in Africa's infamous meningitis belt that was first well characterized by Lapeysonnie (2).<sup>2</sup> Over the last 10 years, the belt has extended southward, and epidemic meningococcal meningitis has been reported in Angola, Democratic Republic of Congo, Rwanda, and Uganda. Approximately every 10–12 years, sizeable epidemics of meningitis occur, and over the last 10–15 years baseline rates of meningitis have been increasing as well. During 1996–1997, Africa suffered a massive outbreak of Group A meningococcal meningitis that was responsible for close to 200,000 reported cases and 20,000 deaths. Because these numbers reflect only those cases which were officially reported to health authorities, the true magnitude of the problem is most likely underestimated. The year 2002, the latest for which figures are available, was considered a "non-epidemic" year, yet more than 44,000 cases and 3,000 deaths were reported from African countries.

Disease burden of this magnitude should be considered an unacceptable public health menace everywhere in the world. Nonetheless, these data do not adequately capture the chaos, confusion, and often misinformation that result whenever an outbreak of meningo-

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<sup>2</sup> Guinea Bissau, the Gambia, and portions of Guinea, Mali, Burkina Fasso, Benin, Nigeria, Niger, Chad, the Central African Republic, Sudan, Eritrea, Ethiopia, and Kenya.

**FIGURE 1. Epidemic meningitis in Africa's meningitis belt, 1950–1966.**

coccal meningitis occurs. Often, routine public health services such as childhood immunizations cease, and public health authorities and clinicians become overwhelmed attempting to respond to the clinical and preventive challenges these outbreaks pose. However, the epidemics themselves are very circumscribed temporally. They begin during the dry season, usually in December or January, and promptly cease with the first rains in May. Persons of age 6 months to 29 years make up >95% of cases. The highest rates of disease are in infants, but because of the wide age distribution, most cases occur in individuals >5 years of age (3).

## MENINGOCOCCAL VACCINES

Table 1 shows the general characteristics of polysaccharide (PS) and conjugate meningococcal vaccines. Control of epidemic meningococcal meningitis has largely depended upon use of the A/C polysaccharide vaccine. PS vaccines have been available for more than 30 years, and these vaccines are quite effective in individuals >age 2. However, PS vaccines are not reliably immunogenic in children 2 years

**TABLE 1. Properties of polysaccharide and conjugate meningococcal vaccines.**

Property	Polysaccharide vaccines	Conjugate vaccines
Immunogenicity:		
in 5-year-olds to adults	High	High
in young children	Poor	High
Response to booster	Poor	High
Quality of antibody in children		
Avidity	Low	High
Bacterial activity	Low	High
Induction of memory	+/-	Yes
Effect on colonization	+/-	Yes

of age and under, do not induce memory, and have had little effect on colonization in community-based studies. However, when polysaccharide antigens are linked to proteins such as diphtheria and tetanus toxoids, their immunogenic properties are dramatically increased. Conjugate vaccines stimulate T helper cells and provide good humoral antibody response and memory (4).

Given the dramatic success of conjugate Hib vaccine in eliminating *Haemophilus influenzae* meningitis, and the equally impressive data

from the United Kingdom after the introduction of a Group C conjugate meningococcal vaccine, there has been considerable interest in the development of conjugate meningococcal vaccines to combat African meningococcal outbreaks (5, 6). In fact, conjugate A/C meningococcal vaccines were tested in the Gambia and Niger in the early and mid-1990s, but the projects were discontinued because these vaccines were not considered commercially viable.

### **DEVELOPMENT OF THE MENINGITIS VACCINE PROJECT**

After the devastating 1996–1997 epidemic there was renewed interest in the development of conjugate meningococcal vaccines at WHO. The Epidemic Vaccines for Africa Project was created by the Organization, and a series of in-depth discussions with vaccine manufacturers were held in 1999 and 2000 to explore their interest in developing these vaccines. In addition, with the help of a dedicated group of consultants, a costing model for the vaccines' development was done. A collaboration aimed at exploring the possibility of developing conjugate meningococcal vaccines gradually evolved between WHO and the Children's Vaccine Project at PATH. A series of expert panels were convened during 2000 and 2001, and these groups concluded that the development of the vaccines held potentially important public health advantages. They cited the previously mentioned successes that followed the introduction of conjugate Hib and meningococcal C vaccines. A proposal was prepared and sent to the Bill and Melinda Gates Foundation, and in June 2001 the Meningitis Vaccine Project (MVP) was created with a US\$ 70 million grant. The project is a 10-year partnership between WHO and PATH with the goal of eliminating epidemic meningitis as a public health problem in sub-Saharan Africa through the development, testing, licensure, and widespread use of conjugate meningococcal vaccines.

Soon after the project was funded a series of discussions were held with African public health officials that focused on understanding

the limitations of introducing new vaccines in sub-Saharan Africa. Three overarching considerations emerged from these meetings: first, vaccine cost was cited as the most important limiting factor to the introduction of new vaccines; second, the African meningitis belt countries are among the poorest in the world; and third, wide use of a conjugate meningococcal vaccine would not be possible unless the vaccine were priced at less than \$0.50 per dose. These discussions were key in the sense that they forced the project partners to make affordability—i.e., a vaccine priced at less than \$0.50 per dose—an important criterion for the product's development.

Extensive discussions took place throughout the fall of 2001 about the makeup of the conjugate vaccines being developed by MVP. The project was committed to the testing of a polyvalent Expanded Program on Immunization (EPI) vaccine (DTPw, Hib, HepB, Men A/C) being developed by Glaxo Smith Kline (GSK). This product was being developed by GSK for markets outside of Africa but there was interest on the part of various African health ministries in having the product tested in this region because of the major simplification in their logistics with the availability of a polyvalent EPI vaccine with a conjugate A/C meningococcal component. Discussions were held between GSK, MVP, and the Ministry of Health of Ghana, and plans have been formulated to begin clinical trials of this polyvalent product in December 2003. The vaccine would be proposed for use in selected meningitis belt countries as a replacement for a pentavalent product (DTPw, Hib, HepB) that was being introduced as an EPI vaccine in several African countries as part of the Global Alliance for Vaccines and Immunization initiative.

For epidemiological and logistical reasons, a decision was also made to develop a monovalent A meningococcal conjugate vaccine. Historically, the majority of meningococcal isolates from Africa have been Group A, and developing a conjugate monovalent A vaccine offered the advantages of simplicity, less risk, affordability, and the potential for a solid pub-

lic health impact. The monovalent A conjugate vaccine was developed to be used as a single dose for mass vaccination campaigns throughout the meningitis belt for persons ages 1–29 years. In addition, the vaccine would be tested as an EPI antigen in infants <1 year old, so that it would be available as an EPI vaccine for those countries unable or unwilling to purchase the heptavalent (DTPw, Hib, HepB, Men A/C) product previously described.

Throughout the fall of 2001 and the spring of 2002, MVP negotiated with major vaccine manufacturers, but no satisfactory agreement could be reached. Consequently, beginning in February and March of 2002, discussions were initiated with a consortium of manufacturers to develop a conjugate A vaccine. This partnership evolved into a group of three companies. SynCo Bio Partners, an Amsterdam-based Dutch contract manufacturer, agreed to produce vaccine grade A PS. BiosYnth, a discovery company in Siena, Italy, agreed to develop a conjugation method for the product. Lastly, the Serum Institute of India, based in Pune, agreed to manufacture the A conjugate vaccine at a target price of \$0.40 per dose.

Clinical lots of the monovalent A conjugate vaccine will be available by the second quarter of 2004. Phase 1 studies in India could begin as early as the first quarter of 2004, and phase 2 studies could start in Africa in the second or third quarter of 2004. The project wishes to conduct a large demonstration study in 1–29-year-olds in one of four meningitis belt countries classified as hyper-endemic for meningococcal disease (Burkina Faso, Chad, Mali, and Niger). The vaccine could be licensed in India as early as 2007.

### AN INNOVATIVE APPROACH TO VACCINES DEVELOPMENT

The model that has been described for the introduction of conjugate meningococcal vaccines is quite different from the one commonly used to develop most licensed vaccines. In the traditional scenario, major vaccine companies choose the products to be developed and assume the financial risk associated with the de-

#### BOX 1. Challenges and opportunities inherent in the vaccine development model being pursued by the Meningococcal Vaccine Project.

##### Challenges:

- Higher risks.
- Technical and managerial complexity dealing with technology transfer and with clinical and regulatory issues.

##### Opportunities:

- Low cost of vaccine (target price, US\$ 0.40).
- Acceptable timelines (2006–2007).
- No opportunity costs.
- Tailor-made for Africa.
- Developing country vaccine capability is strengthened.
- Can serve as a model for other orphan vaccines.

velopment phase. For obvious reasons, vaccine manufacturers are most interested in products that are likely to bring financial return to that particular company. Vaccines for diseases that are almost exclusively seen in developing countries, such as Group A *Neisseria meningitidis*, are largely ignored unless the size of the travel market warrants the product's development. Group A *N. meningitidis* falls in this category of "not likely to be developed," because meningococcal polysaccharide vaccines currently service the travel market, and African countries are usually unable to purchase a conjugate meningococcal vaccine at a price that is attractive enough to interest major vaccine manufacturers.

Box 1 shows the challenges and opportunities in the model being developed by the Meningococcal Vaccine Project. The model carries higher risk for several reasons. There is greater technical and managerial complexity, and technology transfer must occur smoothly if timelines are to be met. Supporters and critics have all predicted that technology transfer of the conjugation method from BiosYnth to Serum Institute of India will be difficult. In addition, there are the regulatory hurdles of li-

censing the vaccine in India for use in Africa. On the other hand, there are important opportunities. A low-cost conjugate vaccine that is effective against a major African public health problem is of great interest to the region's ministries of health and of finance. The ability to use grant funds to cover development costs and thus minimize risk to the partners allows for the development of a vaccine that otherwise might not have been developed. Lastly, the model, if it is successful, might well prove to be a useful paradigm for the introduction of other vaccines (7).

Over the project's first year and a half, a number of important lessons have emerged. The first is that price is important. Second, altruism is not enough to get a needed vaccine produced. Third, developing a vaccine must make economic sense to all of the project's partners. Fourth, project members' travels over the past 18 months have enabled them to come into contact with a group of excellent vaccine manufacturers in developing countries—the so-called “emerging suppliers.” Fifth, working with these manufacturers might offer a useful model for providing additional needed vaccines in the future that today have only limited market potential as defined by major vaccine manufacturers.

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# THE EFFICACY AND EFFECTIVENESS OF PNEUMOCOCCAL CONJUGATE VACCINES

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## INTRODUCTION

Acute respiratory infections remain the leading cause of death in children and are also the leading infectious cause of death in adults (1). As *Streptococcus pneumoniae* (the pneumococcus) is the leading bacterial cause of these infections, the development of a conjugate vaccine has been an important public health goal, though this has been frustrated by the large number of vaccine serotypes of pneumococci causing invasive disease. The development of *Haemophilus influenzae* type b conjugate vaccine laid the groundwork for the development of multivalent pneumococcal conjugate vaccines. Two important experiences with *Haemophilus* conjugate vaccines led to the conclusion that pneumococcal vaccines may have efficacy beyond direct protection of immunized children from invasive pneumococcal disease. The first is the demonstration that communities in which children received *Haemophilus* conjugate vaccine experienced reductions in invasive disease greater than those expected by the level of immunization coverage in the community. One such example was the Navajo community in

the United States of America, where the burden of invasive disease was reduced by 57% and 73% respectively, in communities with vaccine coverage of only 22%–40% and 40%–60%, respectively (2). Furthermore, a study conducted in the Gambia showed that in addition to the significant impact on invasive *Haemophilus influenzae* type b disease, the vaccine reduced pneumonia—defined by consolidation on X-ray—by more than 20% (3).

## SEROTYPES IN THE VACCINE

Although the distribution of the leading pneumococcal serotypes causing invasive disease in children is similar in most countries, there is some global diversity with serotypes 1 and 5, which are common in South America and in developing countries, but not in the U.S. (4, 5). The first vaccine to reach phase 3 clinical trial and licensure, however, has been designed to cover the seven leading serotypes causing invasive disease in children in the U.S. (6). This pneumococcal conjugate vaccine contains oligosaccharide or polysaccharide capsular material of serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F, conjugated to the diphtheria cross-reacting molecule CRM<sub>197</sub>. The vaccine was developed by Wyeth-Lederle, in Pearl River, New York. A vaccine consisting of the same serotypes but conjugated to meningococcal outer membrane proteins, developed by Merck, in Philadelphia, Pennsylvania, was studied for its

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efficacy against otitis media in a population of Finnish children, but the development of that vaccine has not proceeded to an application for licensure (7). A nine-valent conjugate vaccine using the CRM<sub>197</sub> conjugate has recently been tested in a large phase 3 clinical trial in Africa (8). The same vaccine is under investigation in the Gambia. Trials are ongoing of 11-valent vaccines with the addition of serotypes 3 and 7 for the reduction of invasive disease in the Philippines (conjugated to tetanus and diphtheria toxoids, developed by Aventis Pasteur in Lyon, France) and for the reduction of otitis media in the Czech and Slovak Republics (conjugated to *Haemophilus* D protein, developed by GSK Biologicals in Brussels, Belgium).

### EFFICACY AGAINST INVASIVE DISEASE

To date, three large clinical trials have documented the efficacy of pneumococcal conjugate vaccines against invasive pneumococcal disease. In the first, a study conducted in the Kaiser Permanente Health Maintenance Organization in northern California (U.S.A.), the vaccine efficacy was 97% (9). The same vaccine in the Navajo nation in the U.S. had an efficacy of 86% in the intent-to-treat analysis (10), and the nine-valent vaccine in South Africa had an intent-to-treat efficacy of 83% against vaccine serotypes (8). These studies were underpowered to detect an increase in the number of nonvaccine serotypes causing invasive disease. The South African study also reveals efficacy against the cross-reacting serotype 6A, but not against serotype 19A (8). While most of the invasive disease in the U.S. studies was pneumococcal bacteremia without a source of infection, most of the pneumococcal disease prevented in the South African trial was due to pneumonia and meningitis.

### INVASIVE DISEASE IN HIV-INFECTED CHILDREN

The global HIV pandemic has had a major impact on the burden of pneumococcal disease in children (11). It is therefore essential to the

success of a vaccination strategy in countries where HIV is endemic that the pneumococcal conjugate vaccine reduce invasive pneumococcal disease among HIV-infected children. This issue was addressed in the South African study, and the nine-valent conjugate vaccine was shown to reduce invasive pneumococcal disease in HIV-infected children by 65% in the intent-to-treat analysis (8).

### EFFECTIVENESS STUDIES IN INVASIVE DISEASE

The U.S. is the only country to date to introduce pneumococcal conjugate vaccine into its routine immunization program. Two studies in that country on the effectiveness of the vaccine after its introduction have been reported. The first demonstrated significant reductions in vaccine serotypes and vaccine-related serotypes in children in northern California (12). The larger effectiveness study conducted in seven states by the U.S. Centers for Disease Control (CDC) revealed significant reductions in 2001 (after vaccine introduction) from 1998–1999 (prior to introduction), for each of the seven vaccine types, from 63% for type 9V to 83% for types 4, 14, and 19F (13). Vaccine effectiveness against all vaccine serotypes was 78%, and there was a 50% reduction against vaccine-related serotypes (significantly so for serotypes 6A and 9A). Protection against serotype 19A was not significant, although there was a reduction of 40%, which tended to significance ( $p = 0.09$ ). It is important to note that while vaccine serotypes were reduced from an average of 156 cases per 100,000 in 1998 and 1999 to 34 cases per 100,000 in 2001, nonvaccine serotypes increased from 12 to 16 per 100,000 over the same period. This increase in nonvaccine serotypes was not significant, but there was a trend in that direction ( $p = 0.014$ ). These data suggest that the vaccine has had a major effect on invasive disease due to vaccine serotypes and vaccine-related serotypes in children under 2 years of age, and that serotype replacement is likely to occur, but that the amount of replacement may be small compared to the

scale of the reduction of invasive disease due to vaccine serotypes. An important observation from the CDC effectiveness study was that there were significant reductions in invasive disease caused by vaccine serotypes among adults. It has been known for some time that children in the household, particularly those in day care, represent a risk for invasive pneumococcal disease in adults (14), and it has also been demonstrated that the proportion of invasive pneumococcal disease in adults in the U.S. due to pediatric serotypes has increased in recent years (15). These data suggest that there has been a significant herd immunity effect since the introduction of the pneumococcal conjugate vaccine and that the cost-effectiveness of this vaccine may be greatly enhanced by it. The CDC surveillance has also documented the effectiveness of the seven-valent vaccine in reducing pneumococcal meningitis by 59% (13).

#### **VACCINE EFFICACY AGAINST OTITIS MEDIA**

Vaccine efficacy against otitis media has been investigated in two large clinical trials. In the first, in Finland (16), vaccine efficacy against specific serotypes could be documented by the performance of tympanocentesis among vaccinated children with otitis media. The seven-valent CRM<sub>197</sub> conjugated vaccine reduced otitis media due to vaccine serotypes by 57% and all confirmed pneumococcal otitis media by 34%. There was a non-significant overall reduction in otitis media of only 6%, as the proportion of nonvaccine-type pneumococci increased by 33%. Similar results have been presented for the seven-valent vaccine conjugated to meningococcal outer membrane proteins (7). An analysis of otitis media episodes in vaccinated children in the Kaiser Permanente study (9) revealed a 7.8% reduction in otitis media visits in the intent-to-treat analysis, with increasing protection of up to 12.3% in children who had frequent otitis (defined as five episodes in six months or six episodes in a year). The vaccine also prevented 20% of ven-

tilatory tube placement. Pneumococcal conjugate vaccines therefore have been shown to significantly reduce otitis media when the infection is caused by vaccine serotypes, but the overall impact of the vaccine on otitis media has been reduced by the phenomenon of replacement by nonvaccine serotypes.

#### **VACCINE IMPACT ON CARRIAGE**

A number of studies have shown that children who have received pneumococcal conjugate vaccines have had about a 50% reduction in carriage of vaccine serotypes, but that serotype replacement occurs. The reduction in carriage of vaccine serotypes appears to be a vaccine-mediated inhibition of acquisition of carriage, rather than direct eradication of existing carried strains. The impact of the vaccine on carriage was recently reviewed (17).

#### **VACCINE IMPACT ON PNEUMONIA**

The seven- and nine-valent conjugate vaccines have been evaluated for their impact on pneumonia. In the Kaiser Permanente study (9), there was a reduction in pneumonia (with a positive chest radiograph) of 20.5% in fully immunized children and 17.7% in the intent-to-treat analysis. The nine-valent conjugate vaccine has been shown in the South African trial to have a similar level of efficacy in the prevention of first episodes of radiographically defined pneumonia. The reduction in first episodes among fully immunized children was 25% (8). In both studies, the Hib conjugate vaccine was given to both vaccinees and controls, so there is a reasonable inference that the combination of these vaccines may reduce radiologically confirmed pneumonia by approximately half. The efficacy of these vaccines in the prevention of pneumonia is possibly the most important public health aspect of their efficacy, and it will be important to monitor the effectiveness of these vaccines in preventing pneumonia when they are introduced in developing countries.

## PREVENTION OF ANTIBIOTIC RESISTANCE

The nine-valent conjugate vaccine has been shown to reduce invasive pneumococcal disease due to penicillin-resistant strains by 67% (8). These data, as well as data documenting the impact of the vaccine on the carriage of antibiotic-resistant pneumococci (18, 19), support the observation that the introduction of the vaccine has been associated with a decrease in antibiotic-resistant invasive disease in the U.S. (13).

## SAFETY

While there have been no significant associations of severe adverse events with the introduction of the conjugate vaccine in the U.S., an association of vaccination with an increased incidence of asthma was found in the South African study (8). This association has not been found in other studies, but the introduction of conjugate vaccines should be accompanied by careful surveillance for any unanticipated adverse events.

## CONCLUSIONS

The introduction of pneumococcal conjugate vaccine has been associated with a dramatic reduction in invasive disease due to vaccine serotypes and significant reductions in pneumonia and meningitis. The impact on otitis media has been reduced by the phenomenon of serotype replacement. Herd immunity induced by the vaccine has led to significant reductions in invasive disease in adults in the U.S. The vaccine has also reduced the burden of antibiotic-resistant pneumococcal disease and has reduced disease in HIV-infected children. These data suggest that this vaccine may be a very valuable public health intervention in developing countries. However, the largest factor limiting vaccine introduction is cost. A consortium of scientists, governments, non-governmental organizations, and industry is being developed under the auspices of the

World Health Organization to design strategies that may enable the rapid deployment of these effective vaccines.

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