INFECTION AND IMMUNITY, June 2007, p. 2650–2654 Vol. 75, No. 6
0019-9567/07/$08.00+0 doi:10.1128/IAI.01774-06

MINIREVIEW

Haemophilus influenzae Type a Infection and Its Prevention▽

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Haemophilus influenzae was first described by Pfeiffer in 1892 (30). Forty years later Pittman identified six capsular polysaccharides (types) of *H. influenzae* (a, b, c, d, e, and f) whose structures were later elucidated (Table 1) (12, 31, 40). Systemic infections in otherwise healthy children caused by this bacterial species occur throughout the world and are due mostly to *H. influenzae* type b (Hib). Pittman also showed that a small fraction of *H. influenzae* infections was caused by type a (Hia) and by type f (32). In a longitudinal study of 104 children with *H. influenzae* infections from June 1964 through October 1965, Sell et al. found Hia among carrier and disease isolates. The peak Hia carriage was in children up to 1 year of age (38).

Epidemiological surveys for systemic *H. influenzae* infections were originally reported as "type b" or "non-type b." The non-type b reports lacked epidemiological information such as patients' ages, predisposing illnesses, and mortality. A critical level of serum antibodies against Hib capsular polysaccharide, mostly immunoglobulin G, confers immunity to Hib by complement-mediated bacteriolysis (34, 35, 37). Routine immunization with Hib conjugates in developed countries has led to significant reductions in the incidence of disease and carriage of this pathogen in the past decade (8, 9, 44). In the United States, following 10 years of Hib conjugate vaccine usage, the annual incidence of Hib meningitis among children less than 5 years of age decreased from >10,000 to <200 cases (4). "Herd" immunity followed widespread usage of the Hib conjugate vaccine as unvaccinated children benefited from the reduction in both the incidence of disease and the virtual elimination of carriage. The near-elimination of Hib disease in some populations led to the speculation that other *H. influenzae* serotypes and nontypeable strains may emerge as causes of invasive disease (3, 7, 33, 45). For example, in Brazil, the incidence of Hib meningitis decreased by 69% during the first year following initiation of Hib conjugate immunization, while the incidence of Hia meningitis increased eightfold (33). The annual report (2005) of The Netherlands Reference Laboratory for Bacterial Meningitis showed that among patients older than 50 years of age, most isolates were Hib and *H. influenzae* type f (12.5% and 7.8%, respectively), while type a was observed only in children less than 4 years of age (27).

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▽ Published ahead of print on 12 March 2007.

PROPERTIES OF Hib AND Hia CAPSULAR POLYSACCHARIDES

The capsular polysaccharide structures of the six types (Table 1) (6, 12) are likely related to the virulence properties of *H. influenzae*. Hib capsular polysaccharide confers virulence by "shielding" the deeper bacterial structures such as the lipopolysaccharide from the lytic activity of complement (40, 41, 46). *H. influenzae* can be divided into three groups of two based upon the structures of their capsular polysaccharides and resistance to antibody-free complement: types a and b are the most virulent and are composed of a neutral sugar, an alcohol (ribitol), and a phosphodiester; types c and f, with lesser complement resistance and low virulence, are composed of an N-acetylated amino sugar, another saccharide, and a phosphodiester; types d and e, which are rapidly lysed by complement alone, have a repeat unit of an *N*-acetylglucosamine and *N*-acetylmannosamine uronic acid (40).

Sutton et al., showed that in intraperitoneal challenge of 6-day-old rats, the 50% effective dose for bacteremia of both Hib and Hia was ~1 to 1×10^2, which is several logs lower than for the other types. Intranasal challenge of infant rats with type b or a strains isolated from patients resulted in 55 to 90% bacteremia with type b and 35% with type a strains. Intranasal instillation of types c, d, e, and f was not attempted because the values for 50% effective doses after intraperitoneal challenge were high (40). These results were confirmed by Zwahlen et al. (41, 46) using a series of capsular transformants (a to f) constructed using DNA from clinical isolates of all six serotypes and a genetically defined capsule-deficient recipient strain Rb^-:02. The virulence of the capsular transformants of types a to f were compared, using intranasal inoculation of 21-day-old rats. All strains colonized the nasopharynx, persisting up to 71 h in most animals, but bacteremia was detected only in animals challenged with serotypes a, b, and f. The type b transformant gave the highest bacteremia (geometric mean $\log_{10}$ bacteremia, 3.89) in more animals (16/18), followed by type a (geometric mean $\log_{10}$ bacteremia, 2.03) for 9/18 animals, while the type f transformant caused bacteremia in only a single animal (46). The results showed that the changes at the capsulation locus *cap* alone are sufficient to alter the virulence of *H. influenzae*. The resulting type a and type b transformants had the same phenotypic and *cap* genotypic characteristics as those generated with chromosomal DNA and showed the same relative virulence in the infant rat bacteremia and meningitis assay (40, 41, 46).

TABLE 1. Structures of *H. influenzae* capsular polysaccharide[a]

Type	Structure[b]
a	4)-β-D-Glc-(1 → 4)-D-ribitol-5-(PO_4 →
b	3)-β-D-Ri*b*-(1 → 1)-D-ribitol-5-(PO_4 →
c	4)-β-D-GlcNAc-(1 → 3)-α-D-Gal-1-(PO_4 → 3 ↑ | R = OAc (0.8) | H (0.8) R
d	4)-β-D-GlcNAc-(1 → 3)-β-D-ManANAc-(1 → 6 ↑ | R = L-serine (0.41) | L-threonine (0.14) R L-alanine (0.41)
e	3)-β-D-GlcNAc-(1 → 4)-β-D-ManANAc-(1 →
e′	3)-β-D-GlcNAc-(1 → 4)-β-D-ManANAc-(1 → 3 ↑ 2 β-D-fructose
f	3)-β-D-GalNAc-(1 → 4)-α-D-GalNAc-1-(PO_4 → 3 ↑ OAc

[a] Reprinted from reference 40.
[b] Ribose and fructose are in the furanose ring form; Glc, Gal, GlcNAc, GalNAc, and ManANAc are in the pyranose ring form.

INVASIVE DISEASES CAUSED BY Hia

Similar to Hib, most cases of systemic Hia infection are in young children (11, 17, 20, 25, 28, 29, 36, 45). Rutherford et al. in a review of cases from 1933 to 1984 (36) showed that meningitis was the most common disease caused by Hia. Holdaway and Turk studied 106 *H. influenzae* capsulated strains among 2,171 *Haemophilus* isolates (recovered from more than 30,000 clinical upper and lower respiratory tract specimens in Newcastle upon Tyne hospitals, May 1962 to April 1966). They found that 41 isolates were type b (39%) and 12 were type a (11%) (19). A review of systemic infections caused by Hia is shown in Table 2. The near elimination of Hib disease in countries that instituted the routine use of the Hib conjugate vaccine raised the question of replacement of Hib with other *H. influenzae* types as disease isolates.

Rates of Hia disease have been constant in the United States regardless of the use of Hib vaccination (10). From 1998 to 2002, the Active Bacterial Core surveillance program, part of the Emerging Infections Program operated by the CDC to conduct active laboratory- and population-based surveillance for *H. influenzae* disease in eight states in the United States, analyzed microbiologic data from nine participating sites with an approximate population of 35 million. Seventeen of 1,743 invasive isolates were Hia (10, 20). However, Hia organisms are an important cause of meningitis in certain populations.

TABLE 2. Review of systemic infections caused by Hia

Country of origin (population) and year(s)	Use of Hib vaccine	Sample source	No. of Hia cases/ total no. of *H. influenzae* cases	*H. influenzae* type(s) (no. of cases)[a]	Identification method(s) (source of antisera)	Reference
England (Newcastle upon Tyne), 1962–1966	No	Upper and lower respiratory tract	12/106	Hib (41), Hic (2), Hid (4), Hie (32), Hif (15)	Slide agglutination (Hyland Laboratory, California)	19
United States (Vanderbilt University Hospital), 1964–1965	No	Nasopharynx, blood	13/128[b]	Hib (56), Hic (3), Hid (23), Hie (22), Hif (11)	Slide agglutination (Margaret Pittman)	38
United States (White Mountain Apache), 1973–1982	No	CSF, blood	3/18	Hib (15)	Slide agglutination (Biologic Products Division, CDC, Atlanta, GA), biotyping	24
Papua New Guinea	No	CSF	9/73	Hib (60), Hif (1), NT (3)	Slide agglutination (Wellcome Reagents Ltd., Beckenham, United Kingdom), biotyping	26
Senegal	No	Pleural fluid, blood	10/504	Hib (488), Hic (3), NT (1)	Slide agglutination	
Gambia, 1980–1984	No	Pleural fluid, blood	2/13	Hib (7), NT (4)	Slide agglutination	
Brazil (16 regions), 1990–1999	No	CSF, blood, pleural fluid	16/3,204	Hib (3,110), Hic (1), Hid (2), Hif (2), NT (49)	Slide agglutination; biotyping	45
El Salvador; 2000	Yes	Blood, CSF	5/431 (8/52)[c]	Hib (467), Hif (1), NC (2)	Slide agglutination (Difco Laboratory), PCR	33
United States (9 sites), 1998–2002	Yes	"Sterile body site"	17/1743	NT (1,220), Hib (96), Hif (314), Hie (91)		20
United States (American Indian, Alaska), 2003	Yes	Blood, joint fluid	5[d]		Slide agglutination (Difco Laboratory)	17
South Africa, 1999–2004	Yes	CSF, blood	10/281	Hib (218), Hic (6), Hid (5), Hie (3), Hif (39)	Slide agglutination, PCR	14
Canada (University of Manitoba Health Sciences Centre), 2000–2004	Yes	CSF, blood	26/52	NT (20), Hib (3), Hic (1), Hid (1), Hif (1)	Slide agglutination (Difco Laboratory and Denka Seiken), biotyping, PCR	42

[a] Hic, Hid, Hie, and Hif, are *H. influenzae* types c, d, e, and f, respectively. NT, nontypeable; NC, noncapsulated.
[b] Through the fifth year of life.
[c] Prevaccine period (postvaccine period).
[d] Outbreak.

For example, in a prospective 15-month study (1981 to 1983) of the Apache Indians, 15 Hib and 3 Hia (17%) strains were isolated from children under 5 years of age (24). A retrospective analysis of the prior 10 years of the same population identified an annual incidence of 254 *H. influenzae* meningitis cases per 100,000 persons (24). Extrapolating from the incidence of 17%, a rate of 43 Hia cases per 100,000 annually would be derived. Between 1991 and 2003 a range of 6 to 43.8 cases per 100,000 with an overall rate of around 25 cases of Hia disease per 100,000 persons was found in Navajo and Apache children less than 5 years of age. No Hia isolates were reported during 1988 to 1990 (25). Hib vaccination was widespread in Alaska in 1992. Eleven years later, Hammitt et al. reported an outbreak of invasive Hia disease in Alaska among Native infants; Hia strains were isolated from the blood, cerebrospinal fluid (CSF), and joints of three patients, two infected twice at 3- and 4-month intervals during a 6-month period from two adjacent villages (17, 18).

Invasive diseases caused by *H. influenzae* were reported in Brazil from 1990 to 2003 (2, 33, 45). Hib conjugate vaccine was introduced into the National Immunization Program in Brazil in the second half of 1999. A retrospective analysis of serotypes, biotypes, and antimicrobial resistance of *H. influenzae* invasive strains was conducted to document the characteristics of *H. influenza* isolates during a decade prior the use of the Hib vaccine. Among the 3,204 isolates from 1990 to 1999, type b was the most common (97.8%), type a comprised 0.5%, and 1.5% were nontypeable (45). From 1999 to 2000, 233,516 doses of Hib conjugate were administered three times to a target population of 117,673 children less than 1 year of age, and 36,949 doses were administered once to a target population of 120,537 children aged 12 to 23 months. During the 1-year period after initiation of immunization, the incidence of Hib meningitis decreased by 69% (from 2.62 to 0.81 cases per 100,000 persons per year; $P < 0.001$). The incidence of *H. influenzae* type a meningitis, in contrast, increased eightfold (from 0.02 to 0.16 cases per 100,000 persons per year; $P = 0.008$) (33). Continued surveillance is needed in this population since the rates of Hia disease are based on a small number of cases.

Hia disease has been documented in other countries. In a study conducted from March 1980 to September 1985 of 155 highlands children in Papua New Guinea with culture-positive meningitis (Hib vaccination was not implemented), 84% were 12 months of age or younger, and 92% were infected with either *H. influenzae*, *Streptococcus pneumoniae*, or both. Of 73 strains of *H. influenzae*, 60 (82%) were type b, and 9 (12%) were type a (15, 16, 26). Gottberg et al. analyzed invasive *H. influenzae* disease in South Africa within the first 5 years of the introduction of the Hib conjugate vaccine. The absolute number of Hib cases among children less than 1 year of age decreased by 65%, from 55 cases in 1999 to 2000 to 19 cases in 2003 to 2004, while other typeable *H. influenzae* diseases increased by 68%, from 8 to 25 cases. Among the 63 cases of typeable non-type b *H. influenzae* infection from 1999 to 2004, 10 were caused by Hia (14). Tsang et al. studied 52 *H. influenzae* isolates from patients with invasive disease in the province of Manitoba, Canada, where Hib conjugate was used successfully during the previous 5 years (2000 to 2004). Half of the 52 isolates were Hia, and 20 were nontypeable (42).

In summary, since Hia surveillance data prior to the introduction of Hib vaccination were incomplete, it is difficult to determine if the apparent increase in Hia cases is due to replacement of Hib disease, to better surveillance that followed the introduction of Hib vaccines, or to an unmasking of previously unrecognized cases due to the high rates of Hib disease in the prevaccination era. The number of reports of invasive diseases caused by Hia urges continued surveillance to monitor trends of this pathogen.

IS*1016-BEXA* DELETION, POSSIBLE ASSOCIATION WITH VIRULENCE

The majority of capsulated *H. influenzae* strains have a duplication of the *cap* locus flanked by an insertion sequence (IS*1016*). These strains have been grouped in a single electrophoretic group (division 1) (22). The *cap* locus contains the *bexA* to *bexD* genes (21). Recent clusters of invasive Hia disease have documented a partial deletion of IS*1016-bexA* (1, 20, 23). While most *H. influenzae* type a strains are of the a(T) genotype, which contains a complete duplication in the regions containing the *bex* capsular genes, the mutant strains are of the a(N) genotype that have a 1.2-kb partial deletion in one of the *bexA* capsular gene regions. In 2005 Kapogiannis et al. reported two cases of severe invasive disease due to Hia with the *bexA* partial deletion. The isolated strains showed a 300-bp amplicon by PCR analysis and a different pattern by Southern blotting (20). The apparent increase in cases in selected populations in the United States, such as the Navajo, White Mountain Apache, and Native Alaskans, has raised concerns about a niche previously filled by Hib that now accommodates Hia strains. The mutation of IS*1016-bexA* has been found in three invasive Hia strains isolated from Utah (10-month period from 1998 to 1999) (1). Thus far, the Hia strains from the North American Arctic (Alaska and Canadian northern territories) have not shown the *bexA* deletion (5, 17). Continued surveillance for this mutation is needed as the most recent Hia strains containing this mutation were isolated in Georgia (20).

A VACCINE FOR Hia

No cross-protection is afforded to type a by immunization with Hib conjugate vaccines. The number of Hia cases in any of the areas with a relatively high incidence is still too low to conduct a randomized, double-blinded and controlled trial to demonstrate efficacy. There is precedence for adding an additional capsular type within a species to a vaccine without evidence for its clinical efficacy. Several pneumococcal types and meningococcal groups Y and W135 (all capsular polysaccharides) were licensed based upon data for their safety and their ability to elicit biologically active antibodies to these pathogens (35). A similar approach was sufficient to license type 2 poliovirus vaccines (13, 35). Extensive clinical experience has validated this approach. We propose that demonstration of the ability of Hia polysaccharide conjugates to elicit bactericidal antibodies will be sufficient to license a vaccine, provided it is safe and standardized (35, 43). The development of new vaccine formulations (multivalent vaccines) requires the development of laboratory assays that can serve as correlates of vaccine-induced protection. These assays can also assist in the

serosurveillance of Hia in populations at risk such as the Native American communities and in countries where the incidence of disease caused by this pathogen is unknown. Hammitt et al. reported the poor antibody response when infants were infected with Hia at a very early age (18). At this time only natural immunity can offer protection to an otherwise naïve population, since no vaccination strategies have been pursued for non-type b *H. influenzae* disease (5, 39). The addition of Hia conjugate vaccine to routine childhood schedules has had considerable public health and cost benefits (4, 8, 9). Improved estimates of Hia disease burden, especially in countries that are now introducing Hia vaccination, will help determine the need for the introduction of a new vaccination strategy.

CONCLUSION

The structure, experimental, and clinical properties of Hia capsular polysaccharide closely resemble those of type b. Hia causes low rates of endemicity and outbreaks of meningitis and bacteremia in infants and children of certain populations. The apparent increasing numbers of cases of Hia invasive disease suggest that development of a vaccine comparable to the current Hib conjugate is reasonable. The methods for conjugating the type b capsule to a protein carrier are applicable to Hia. Increased *H. influenzae* surveillance, such as that provided through the current Active Bacterial Core surveillance system, is needed in the United States. The Meningitis Surveillance Network in other countries, including countries in Africa, should include non-type b *H. influenzae* surveillance in their routine schedule where Hib has not been recognized previously.

ACKNOWLEDGMENT

We thank Nancy Rosenstein-Messonnier, CDC, Atlanta, GA, for her helpful comments on the manuscript regarding the epidemiology of Hia disease.

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Editor: J. B. Kaper