The Prospect of Vaccination of Haemophilus influenzae type b

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Abstract

Any type of infection that is caused by the gram-negative coccobacillus called *Haemophilus influenzae* is called a Haemophilus influenzae disease.[1,2] Haemophilus influenzae type b (Hib), which is the most common Haemophilus influenzae disease, is a severe disease that is susceptible to anyone: but especially to infants and children as more than 10 million children younger than 5 years of age die annually.[1,3] The main concern about this disease is that even though the vaccines have started to be widely used, there is not a well-developed vaccine that can detect and treat nontypeable strains for Hib.[4,5] This should be very concerning to all as nontypeable H.influenzae (NTHi) causes invasive infections on children like pneumonia.[4,6] This paper examines the current use of FDA approved Hib vaccines, predictions for vaccines that effectively treat nontypeable strains, and thoughts on protein and antigen candidates for the conjugate vaccines.

Introduction

As the usage of vaccines develop immunological memory, prevent co-infections, have far-reaching effects for the society's health, vaccinations have been considered as an effective means of immunization to prevent future diseases. That said, our lives depend tremendously on our ability to control bacterial and viral populations that cause disease. Loss of control of bacterial and viral infections can lead to fatal diseases, which is one of the major reasons that vaccines are implemented.[7,8] How crucial the implementation of the vaccines are can be found in Figure 1, where you can see the immediate decreasing trend with the vaccine program implemented. Hib, a bacterial pathogen that leads to subsequent diseases, is transmitted by the inhalation of direct airborne respiratory droplets.[1,9] Regarding the origins, Haemophilus influenzae was originally known as the Pfeiffer's bacillus as Richard Pfeiffer first discovered it during an influenza pandemic in 1892. This influenzae caused jeopardy to global health at the beginning of the 20th century, as there were many casualties from the diseases.[10,11] In fact, the rise of Hib is especially unsettling to many as infants and children under the age of 5 are exclusively more susceptible to infection and subsequent secondary infections by 95%, even though Hib usually affects the upper respiratory tract of both children and adults. It is also known that adults above the age of 65 are susceptible to Hib.[1,3] American Indians and Alaska natives, and people with certain medical conditions such as sickle cell disease, human immunodeficiency virus (HIV) infection, asplenia, antibody and complement deficiency states, patients with cancer receiving chemotherapy, radiotherapy, and in post bone marrow transplant states are also susceptible to the disease as well. Hib is categorized as encapsulated and non-encapsulated strains (non-typeable), meaning that they are non reactive with typing antisera – Antisera are prepared to combat specific diseases and provide passive immunity. There are six encapsulated serotypes which are from 'a' to 'f', which all contain distinct capsular polysaccharides. It is known that the difference between an encapsulated and non-encapsulated strains are in the pathogenic mechanism.[12,13] Among the six capsular types from type a to type f, type b is more invasive than others as Hib may cause secondary diseases that are life threatening like meningitis (an infection of the lining of the brain and spinal cord), epiglottitis (an infection along the air passage from the throat to the lungs), and pneumonia (an infection of the

lungs).[12,14] These are highly morbid diseases as they lead on to hearing loss, seizures, loss of limbs, and learning disabilities.[15–17] The incubation period, the duration between the infection and the manifestation of the disease, is unknown. However, it is suspected to take a few days for the symptoms such as the diseases mentioned in the previous sentence. [1,18]

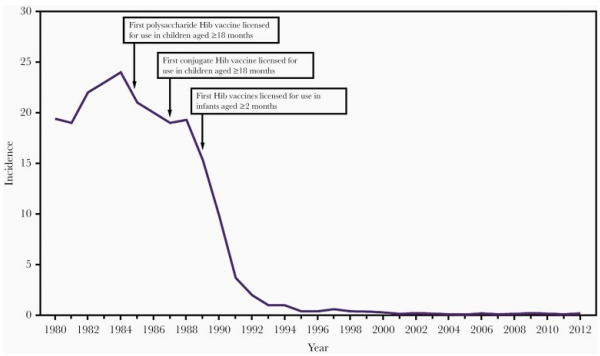

Figure 1. Impact of *Haemophilus influenzae* type b (Hib) vaccines on incidence per 100 000 children <5 years old in the United States, 1980–2012 (Gilsdorf, 2021)[19]

Nontypeable *Haemophilus influenzae* (NTHi) is an emerging pathogen that is derived from the change of the serotype of Hib. NTHi is the main cause of otitis media in infants and children, sinusitis in children and adults, and non-bacteremic pneumonia in the elderly.[1,20] Respiratory pathogens like NTHi continue to affect the host and exacerbate other respiratory problems, and depend on the host to get the necessary elements they need to maintain their growth and to be resistant to traditional antibiotic treatments. The rising amount of children and adults getting infection and diseases due to NTHi is rising, and it is an invariable problem in both developed and non industrialized nations as it causes respiratory tract infections. Even after the host has been treated with specific vaccines to counter the capsular *H.influenzae,* NTHi has been causing infections. The encapsulated *H. influenzae* utilizes proteins like protein H and Haemophilus surface fibrils (Hsf), but these features do not exist in NTHi subtypes.There are a few ways in which *H. influenzae* attach to the host cell and cause damage to the host. NTHi uses mechanisms such as attaching to the surface of the epithelial cells, accessing the underlying extracellular matrix layer, and invading certain serum factors to affect a group of proteins. This allows NTHi to be connected to each other and enter to the host cells with ease.

The vaccines that are used to counter Hib is known as a conjugate vaccine, which is a polysaccharide antigen conjugated to a carrier molecule. The development of the vaccine started with the discovery of a polysaccharide of a pathogen polyribosylribitol phosphate (PRP) in the 1970s in Finland.[21,22] Then, it was found out that conjugate vaccines are more capable of inducing an immune response. Polysaccharide vaccines are important as they help activate B cells, a type of cell that creates antibodies. Children under 18 months of age have poorly developed B cells, therefore have poor immunogenicity as well. To that end, people utilized the conjugate vaccine more. Hib conjugate vaccine was in fact the first licensed conjugate vaccine in the USA in 1987.[23,24] In the 1980s, Hib protein conjugate vaccines were developed, and this improved the immunogenicity of the PRP polysaccharide.

Despite advancements in the development of a reliable vaccine for Hib, there have been no vaccines developed for nontypeable strains of *H. influenzae* to date.[25,26] The reason that it is still not yet developed is because the NTHi strains differ phenotypically and also genotypically, meaning they have different traits. Moreover, NTHi is known for its heterogeneity, and this has been the major hindrance when developing a vaccine.[26,27] Today there is still a significant disease burden of Hib for pediatric populations, and current research has focused on understanding the structure of Hib (Fig 1), with a focus on the Hib pili as it may contribute to the onset of disease. Overall, additional research into Hib prevention is crucial in order to decrease the significant global disease burden.[1,28] This paper will explore currently used vaccines and the development strategies for the prevention of Hib.

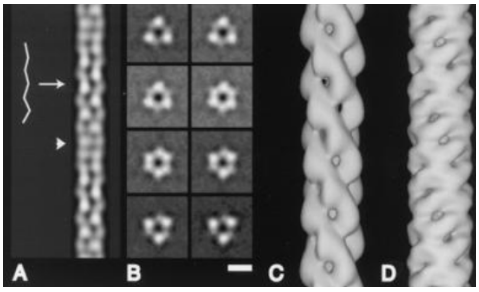

Figure 2. The picture on the left shows the (A) helical axis in a zigzag appearance of a negatively charged pili, (B) cross sections, (C) Surface area, and (D) 3D shape. Regarding the structure of Hib pili, it is concluded that Hib pili is similar to pili class 1, and is strongly associated with p-pili. There is no association between Hib pili and E.coli or the Type IV virus. (X.Mu, 2002)[28]

Vaccine development

As it has to be very clear that the vaccines can provoke an immune response, the vaccine approbation process is composed of methodological and precise steps to be approved by the Food and Drug Administration (FDA). After the vaccine shows promising results, there will be clinical trials for further determinations regarding the efficacy. First, the researchers advance an Investigational New Drug (IND) application to the FDA.[29,30] This consists of the information of animal studies data, information on manufacturing technology, and the quality of the vaccine, which is important as it displays the efficacy in long and short term protection from the disease. The clinical development stage consists of the following three phases. Phase 1 is when a group of people from 20 to 100 receive the trial vaccine, and the researchers gather information on the safety of the vaccine in people. This phase facilitates the researchers' knowledge about the side effects, and how successful the vaccine is to create an immune response. In phase 2, the group of participants increases from 100 to 300 of them and are characterized by their common characteristics (age, physical health, etc). This phase also takes into consideration the representativeness of the participants, and brings in groups of people from many diverse backgrounds. This phase enables the researchers to know about the side effects, risks, and its ability to trigger an immune response. Phase 3 is when the clinical trial expands to 1000 to 3000 people. This is when the researchers corroborate how well the vaccines function and the availability of side effects to ensure the use of it. After the FDA

approves a vaccine, it leads on to phase 4. Phase 4 is the continuation of study to test the vaccine's safety over a long period of time.

In this section, the current FDA licensed monovalent and combination vaccines, and conjugate vaccines are the main ideas that will be discussed. ActHIB (PRP-T) is one of the three monovalent vaccines that has been licensed by the FDA in the U.S. Monovalent vaccines for Hib are made so that infants from 6 weeks of age can use this vaccine.[23,31] Combination vaccines such as Vaxelis (DTaP-IPV-Hib-HepB) and Pentacel (DTaP-IPV/Hib) are designed so that a child can use more than one vaccine simultaneously. Among many combination vaccines, Vaxelis and Pentacel are the two approved vaccines that have the Hib vaccine. To continue, conjugate vaccines are designed to induce an acute immune response against bacterial capsular polysaccharides (CPCs), which are a group of high molecular weight polysaccharides (simple sugars) that cause viral infections of many pathogens such as the gut, respiratory tree urinary tract and other host tissues of humans by hiding cell-surface components that might trigger an immune response.[32,33]

1. **ActHIB (PRP-T)**

ActHIB is a Polyribosylribitol Phosphate-Tetanus Conjugate (Hib) Vaccine; tetanus is a deadly infectious disease that is caused by clostridium tetani, which is a group of anaerobic bacteria (they thrive in the environment without oxygen).[34–36] ActHIB, a vaccine developed by Sanofi Pasteur Inc. in France, was approved by the FDA on September 27, 1996.[36–38] This vaccine is designed so that it prevents the invasive diseases caused by Hib, and also for the prevention of secondary infection. As this vaccine is a monovalent vaccine, it is designed to combat only Hib and the invasive diseases from Hib; it won't protect against other types of bacteria.[34,39] The way vaccines function is by injecting an antigen of a disease and mimicking it, stimulating the immune system.[30,40] Therefore, the vaccine can likely bring side effects, and it is a signal from your body that it is starting to build immunity against a disease. Some of the side effects are irritability, sleepiness, loss of appetite, and swelling.[41,42] Some individuals with severe allergies should not use this vaccine, and convulsions are listed as a side effect. ActHIB is designed for 3 doses: 1 dose at 2 months of age, 1 dose at 4 months of age, 1 dose at 6 months of age. The Centers for Disease Control and Prevention (CDC) states that "A child 7 to 11 months of age should receive 2 doses of ActHIB Vaccine at 8-week intervals and a booster dose at 15 to 18 months of age. A child 12 to 14 months of age should receive 1 dose of ActHIB Vaccine followed by a booster 2 months later. [2,28]

There were over 110,000 infants and children in Canada, the United States, Finland, France, Chile, Israel, and the United Kingdom for the clinical trials. In Sanofi's product monograph, it was said that "In clinical trials where 921 infants were given the vaccine at 2, 4 and 6 months, a titre of at least 0.15 µg/mL was achieved after dose 3 in 99% and a titre of at least 1.00 µg/mL in 93%. The weighted GMT achieved was 7.0 µg/mL (95% confidence limits are 3.4 - 14.2 µg/mL). Protective levels of anti-PRP developed after the second dose in 92.8% of these infants." These insightful aspects of this clinical trials is that as the number of doses rise, the vaccine efficacy rises. However, it is known that not a lot of children and adults do not have access to vaccines. This is proven by a study in India, which showed that nationwide usage of vaccines is needed to cover the diffusion of Hib. The important note to take away from

this study was that the role of private sector is undefined, and that the universal spread of Hib could wane if the vaccinations were prevalent.[43,44] Therefore, it is unlikely for one to get all three vaccines, and many individuals do not have the immunity to this.[43,45] To add, the protective level of getting until the second dose is 92.8%, which means that 7920 individuals will not get the protection.

One of the most prevalent studies regarding the vaccine that can combat Hib by detecting NTHi strains is by Akkoyunlu Et al. It is about how LPD (lipoprotein D) and PDm(The non acylated form of protein D) are conjugated to Hib to test the protein D - conjugate PRP vaccine's potential of protection. When tested with rats, it was shown how ActHIB was successful in invoking immunization and protecting the individual. It was shown how sera, which produces passive immunity, was in the largest amount when it was successful against a Hib strain and an NTHi strain.[44,46] Therefore, the usage of lipoprotein would help improve ActHIB. As the concern is the universal usage of the vaccine and the efficacy expecting less than 3 doses, it is important to develop the vaccine by the inclusion of LPD.

2. Vaxelis (DTaP-IPV-Hib-HepB)

Pentacel, is another vaccine that was targeted to prevent Hib. Like the ActHIB, Pentacel is also produced by Sanofi Pasteur is a subsidiary of Sanofi, a multinational pharmaceutical company headquartered in France. It consists of Diphtheria, Tetanus Toxoids, and Acellular Pertussis Adsorbed and Inactivated Poliovirus (DTaP-IPV) components. As vaxelis is not a monovalent vaccine, an ActHIB vaccine component is combined through reconstitution for intramuscular injection as well. To add, as it is a combined vaccine, it was aimed to protect people from diphtheria, tetanus, pertussis, poliomyelitis, and Hib. The conjugation with the ActHIB component significantly helps stimulate the immune response to protect from Hib. In fact, the presence of conjugate protein carriers are crucial as the polysaccharide capsule makes an effective vaccine. The FDA approved Pentacel for use as a four-dose series in infants and children at ages 2, 4, 6, and 15 -- 18 months in June of 2008. The child will need 3 shots: one shot at 2 months old, one shot at 4 months old, and one shot at 6 months old. The efficacy of the vaccine increases with the number of doses, as shown in figure 3. Vaccines use different carriers, and the polysaccharide helps immunize Hib as it creates the production of antibodies against Hib.[47,48]

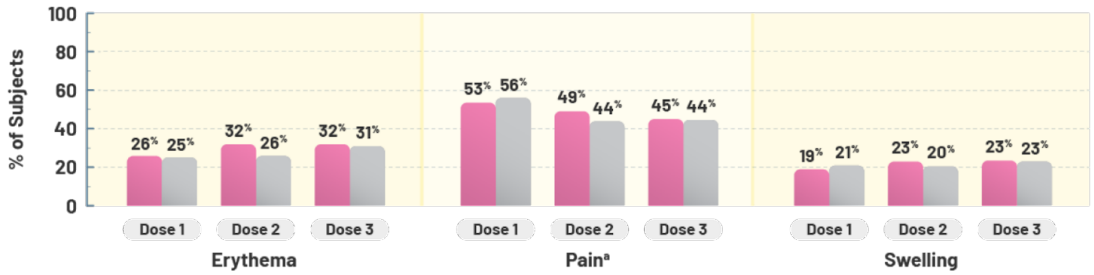

Figure 3. It is shown how the number of patients who showed side effects such as Erythema, Pain, and swelling decreased with the additional number of doses of Vaxelis. (Sanofi, 2023)

In a clinical study, consisting of more than 6800 children to test if it is suitable for premature individuals, the following results were shown: **"**Overall, 160 infants were considered premature (DTaP-IPV-Hib-HepB = 111 Control = 49). The incidence of adverse events (AEs) for DTaP-IPV-Hib-HepB was comparable between overall and premature populations for all AEs days 1-15 post vaccination (Overall = 96.3%; Premature = 97.3%;), solicited injection-site AEs days 1-5 post vaccination (Overall = 84.1%; Premature = 75.5%), and solicited systemic AEs days 1-5 post vaccination (Overall = 93.7%; Premature = 94.5%). A high percentage of premature infants mounted protective immune responses to antigens contained in DTaP-IPV-Hib-HepB vaccine. Response rates in preterm infants for all antigens (80-99%) were in a similar range to all infants (80-99%) for both DTaP-IPV-Hib-HepB and control vaccines." (Wilck, 2020). This result shows how the rate of the incidence of adverse events is pretty low. The immune responses were clearly shown, however to a satisfactory amount. The following do support that Vaxelis is safe for infants. [42,49]

3. **Pentacel (DTaP-IPV/Hib)**

Pentacel, consisting of DTaP and conjugated toxoid is a vaccine also targeted to children 6 weeks to 4 years old age for the prevention of Hib. It is approved for 3-dose series.[49,50] However, the safety and efficacy of Vaxelis in infants less than 6 weeks of age have not been established, and no data are available. A problem with this vaccine is that it has a low effectiveness to people with a weakened immune system. They are at higher risk of polio, Hib, and tetanus even after they receive the vaccine. Pentacel is a prevalent vaccine as it combines five components (Hib conjugate vaccine, toxoid, acellular pertussis vaccine, inactivated poliovirus vaccine) into a single vaccine. Specifically, it was shown that infants treated with the vaccine had a low possibility of getting fever, vomiting, and drowsiness compared to the ones who did not take the vaccine.[50,51] Finally, the manufacturing data sent proved that the manufacturing process of the vaccine is safe and reliable. Pentacel, like Vaxelis, is also designed so that it can prevent more than Hib: other invasive diseases. To add, Pentacel is a vaccine that is still talked about, regarding the future advancements to make. Skibinski Et al. mentioned about Addition of pneumococcal or meningococcal vaccines to DTaP-based vaccines and providing a New carrier protein strategies for conjugate vaccines is going to be innovative.

4. **Discussion of all three vaccines.**

ActHib differs from the previous two vaccines as this one is only composed of one antigen aimed to combat Hib. For example, a difference among the three vaccines is that Vaxelis includes protection against hepatitis B, one of the secondary infections of Hib, which Act-HIB and Pentacel do not cover.[34–36] It is also true that all of these vaccines can be used interchangeably. Further exploration on the types of proteins used are still in the process. However, knowing how the combination vaccines help preventing invasive diseases will be beneficial to

Conclusion

This paper introduced the severe problems of Hib and the effectiveness of the approved vaccines. To this day, Hib, which can cause meningitis, epiglottitis, and pneumonia, is still not completely prevented. After decades of global vaccine use, impressive control of Hib invasive

disease has been achieved through mass vaccination. However, the disease is still prevalent and has severe consequences. High vaccination coverage has been achieved in most parts of the world and the number of cases remains low, although there are still a few countries where Hib vaccination has not been implemented in the National Immunization Program (NIP) . The burden of disease and associated mortality in these countries is very high, and introduction of mass vaccination should be considered. It is clear that vaccines should be enhanced as the vaccines were not effective at identifying nontypeable strains, especially in young people. In order to make vaccines against NTHi, the conjugate vaccines mentioned previously should be enhanced to increase the effectiveness. In order to do this, there needs to be more studies of corresponding suitable carrier proteins and antigens specific to NTHi. Multivalent vaccines should be designed to protect multiple serotypes as well.

To conclude, by comparing the three mentioned vaccines, Act-HIB is specifically designed to protect against Hib infections as a monovalent vaccine. Therefore, it is considered the most effective way to stimulate the immune system.[23,31] Vaxelis and Pentacel, however, are effective in building an immune system in a broader range as they provide protection to several diseases in a single vaccine.

Another difference among the three vaccines is that Vaxelis includes protection against hepatitis B, one of the secondary infections of Hib, which Act-HIB and Pentacel do not cover.[23,31] A merit about Act-HIB is its proven effectiveness as it significantly reduced Hib-related diseases so far. However, its disadvantage is that children might require additional vaccines to guard secondary infections as this vaccine only protects against Hib. An advantage of Vaxelis is its comprehensive protection and the fact that it requires fewer shots. Children don't need to receive multiple doses of shots as it is comprehensive in its protection against multiple secondary diseases and Hib. A disadvantage of this vaccine is its risk of secondary diseases and worsened condition due to the numerous vaccine doses. Pentacel is really effective in its comprehensive and exhaustive protection against Hib as well.

Currently, Hib vaccine coverage has increased over 90%. Still, there is a growing number of adults and children with secondary infections due to Hib.[4,5] As the foundation and the development of Hib vaccine is necessary, further research should be done. Researchers should continue working on the identification of antigens, using the Hib Bacteria to identify the immune responses. The carrier proteins and adjuvants, which are cofactors that enhance the immune responses, should be focused as well. Most specifically, researchers should work on non-typeable vaccine creation; to do this, they should focus more on vaccines that contain protein D of nontypeable H. Influenzae, and use this as a carrier protein for vaccines.

After all, it can be concluded that the emergence of vaccines such as Vaxelis, Pentacel, and ActHIB significantly decreased the Hib infection rate.[34–36] There have been many countries with robust vaccination programs, actively promoting these vaccines and future studies. However, the persistence of non-typeable strains of Hib and the sporadic outbreaks still remain and vaccines should be advanced as there should be more innovative strategies to fight against Hib and its tremendous effects. As mentioned above, more studies should focus on protein D and carrier proteins.

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