IMMUNIZATION AGAINST BORDETELLA PERTUSSIS

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I. Introduction

Disease caused by Bordetella pertussis is serious and potentially fatal. Historically, this organism has been the cause of severe epidemics and was responsible for thousands of childhood deaths. Early in this century, pertussis was second only to measles as the cause of childhood fatalities (30). Due to the development of an effective vaccine in the 1940s, and its routine use since the 1950s, the incidence of pertussis has dropped dramatically. However, pertussis is not yet eradicated. Approximately 2,000 cases are reported in the U.S. each year with 5-20 deaths (19). Occassional localized epidemics occur, especially in populations with low vaccination rates. The current whole-cell vaccine is associated with a high frequency of adverse side effects. The majority of these reactions are transient and non-damaging. Rarely, however, severe permanent neurological damage or death results (25). In recent years, controversy over the use of the vaccine has led to a decrease in vaccine acceptance in several countries. England and Japan have experienced widespread epidemics with many deaths due to the discontinuation or curtailment of their pertussis vaccination programs (32). The production of pertussis vaccine has become a low profit, high liability venture. In the U.S., manufacturers are reluctant to supply pertussis vaccine, and currently only a single manufacturer produces the vaccine (59). The present vaccine is virtually the same as the original one which was developed half a

century ago, with only minor changes. Extensive research geared toward the development of a safer, more effective vaccine has identified several potential immunogens which could be included in an acellular vaccine preparation. Researchers in Japan have had some success with an alternative acellular vaccine that is highly immunogenic and less toxic (48). Advances in molecular biology in recent years have shown that synthetic vaccines may be a possibility for the future (1). Efforts to produce a safer more effective vaccine and the elimination of the disease pertussis will clearly benefit the world's children. In this paper, I will present a comprehensive study of the current core of knowledge regarding Bordetella pertussis, the disease it causes, and mankind's effective but controversial attempts to prevent it. An in-depth appraisal of the risks versus benefits of vaccination will ultimately lead to a discussion of pertussis research and new trends in vaccine development.

II. Discovery of Bordetella pertussis and Scientific Development of a Vaccine

Whooping cough was first recorded in Europe in the 16th Century and was responsible for severe epidemics and thousands of deaths (35). In 1906, the organism responsible for the disease was discovered in the sputum of children suffering from whooping cough by two Belgian scientists, Jules Jean-Baptiste Bordet and Octave Gengou (52). They developed a special blood agar for its cultivation which is still being used today. The organism was first called Hemophilus pertussis, but the name was later changed because of major nutritional differences between it and the Hemophilus genus (18). Pertussis comes from Latin and means severe cough. In 1952 Morena and Lopez proposed a new genus in honor of Bordet which includes three related species, Bordetella pertussis, Bordetella parapertussis and Bordetella bronchiseptica (37). Attempts to develop a vaccine began shortly after the discovery of B. pertussis and clinical trials began in 1912. Although many unsuccessful attempts were reported, Madsen demonstrated that a vaccine afforded some protection when it was given shortly before an epidemic in the Faroe Islands in 1925 (35). Sauer, Kendrick and Elderling are responsible for the effective whole-cell vaccines that are still being used today. Their success can be partially attributed to Leslie and Gardner who found that B. pertussis grows in both smooth and rough phases. Only the smooth virulent phase is

suitable for vaccine production (37). By the 1950s the vaccine was being used routinely. The current vaccine is virtually the same as the first vaccine with only minor change, such as the addition of aluminum salts as an adjuvant (32). Today an aluminum hydroxide triple vaccine is used which includes diphtheria toxoid, tetanus toxoid and killed whole cell pertussis. In 1964 an International Standard for pertussis vaccine was established, by the World Health Organization, which recommends 4.1 IU per dose (35). At least 4 doses of vaccine are recommended for children before they reach the age of 5. Although we have made great strides in the control of pertussis in this century, the precise mechanism of immunity to pertussis is not yet fully understood. Development of a safer, more effective vaccine will result from a deeper understanding of the characteristics and immunologic properties of B. pertussis.

III. Morphology and Culture Characteristics of Bordetella pertussis

The morphology and culture characteristics of B. pertussis make it readily distinguishable from other organisms. Bordetella pertussis is a very small, gram negative, short rod or coccobacillus which ranges in diameter from 0.3 - 0.6 um, and 3-8 um in length. It is non-motile and non-spore forming. The formation of capsules have been noted, but this is not a uniform feature. It is strictly aerobic, produces catalase, does not ferment carbohydrates, and utilizes amino acids as a source of energy (37). X and V factors (heme and NAD) are not required, indole is not produced, citrate is not utilized and urea is not hydrolyzed (6). This extremely fragile organism grows slowly and with difficulty outside the host and is easily killed by common disinfectants, pasteurization, boiling, drying and sunlight (18). Although the nutritional requirement of B. pertussis are relatively simple, good growth may be inhibited by various media ingredients, such as casamino acids, yeast extract and agar. These inhibitors have been neutralized by the use of blood, charcoal and ion-exchange resins (37). B. pertussis is best grown at 37°F with 10% CO_2 on media containing blood, serum or flesh extract. The addition of penicillin to the media is recommended to inhibit the growth of gram positive organisms. Cultivation of B. pertussis on Bordet-Gengou agar, which contains potato extract, glycerin

and blood, yields tiny colonies which appear as mercury droplets with partial, diffused hemolysis after 5-10 days incubation at 37°C (18). After 10 days incubation, more opaque colonies develop (37). Isolation of organisms from patients with whooping cough gives rise to virulent smooth, encapsulated, phase I colonies. Bordetella Pertussis exhibits transition to a phase IV, non-virulent, rough form with intermediate phases II and III. Morphologic and antigenic changes accompany these mutations. Strains of B. pertussis have been identified and classified according to their agglutination patterns with monospecific typing sera. Six agglutinogens (1-6) are species specific and two are genus specific. These surface antigens are important functionally in serotyping strains. However, little is known about their nature (37). These agglutinogens are found in various combinations in strains of B. pertussis. Strains 1, 2 and 1, 2, 3 are predominant and responsible for the majority of active pertussis cases (35).

Several components of B. pertussis have been identified including an endotoxin, heat labile toxin, and hemagglutinating substances (37). (See Appendix A) B. pertussis endotoxin is a heat stabile lipopolysaccharide similar to the endotoxin of other gram negative organisms. Its role in immunity to pertussis has not been studied extensively, but it is probably responsible for some of the adverse effects of the vaccine. Heat labile toxin is found in the protoplasm of the cell and is very unstable when

isolated. It produces inflammation and necrosis of respiratory tract tissue, but does not contribute to the ability of the organism to create infection. It is not believed to be involved in establishing active immunity. Hemagglutinins are known for their ability to agglutinate animal red blood cells and are found in the cell-free supernatant of broth cultures. Extensive research is being done to determine the protective activity of filamentous hemagglutinin (FHA) and lymphocytosis promoting factor hemagglutinin (LPF). LPF has several biological activities such as adjuvant activity, lymphocytosis promoting activity, and histamine sensitizing activity. LPF has also been called pertussigen and pertussis toxin.

Bordetella parapertussis and Bordetella bronchiseptica are organisms which are related to B. pertussis. B. parapertussis causes a less severe, pertussis-like disease in humans, and B. parapertussis can be distinguished from B. pertussis by its large colonies which produce a brown pigment on Bordet-Gengou agar. B. bronchiseptica differs in that it is motile, hydrolyzes urea and grows well at 25^{o}C on plain peptone media (55). These organisms share common antigens and may cross agglutinate with B. pertussis ,but specific antisera is available for their identification (6). In 1970 it was reported that a pertussis-like syndrome clinically indistinguishable from pertussis was caused by an adenovirus (18).

Knowledge of the morphology and growth characteristics of B. pertussis and related organisms is essential for proper identification of the organism and diagnosis of the disease it causes.

IV. Laboratory Identification and Diagnosis

Laboratory identification and diagnosis of pertussis is accomplished by collecting material from the trachea, bronchi or nasopharynx. A traditional "cough plate" requires the patient to cough directly onto a plate containing the appropriate culture media. Specimens may be collected from infants by the use of nasopharyngeal swabs (52). During the first few days of infection the organisms exist in enormous numbers in the sputum. After 6 weeks, however, it is rarely possible to demonstrate bacteria in culture. Isolation of this organism is difficult but can be accomplished provided proper technique is employed. Studies have shown there is an advantage in using both charcoal agar and Bordet-Gengou agar for specimens suspected of containing B. pertussis organisms. While most strains are isolated from both media, a few are isolated from only one or the other of these. Therefore, using both increases the yield of positive cultures (2). A tentative diagnosis may be made by isolating a catalase positive, oxidase positive organism with colonial morphology typical of B. pertussis. The diagnosis should be confirmed by antigenic analysis or by fluorescent antibody techniques which are more rapid and productive than culture methods (16). The fluorescent antibody technique employs an antibody-fluorescent dye conjugate which reacts with the antigens of organisms in the patient's specimen. The antigen-antibody complexes can be visualized in an ultraviolet light microscope. Recently an

enzyme linked immunosorbent assay (ELISA), using fimbrial hemagglutinin of B. pertussis as an antigen, has been used for serological diagnosis of whooping cough (23). This assay measures serum IgG, IgM and IgA antibodies to the fimbrial hemagglutinin of B. pertussis. In a recent study, the use of ELISA testing detected twice as many positive cases of whooping cough than with culture alone. The advantage of this diagnostic method is that it is rapid and highly sensitive. Accurate diagnosis of pertussis is essential so that recommended treatments may be followed and also for epidemiologic purposes.

V. Pathogenicity

Pertussis, which is better known as whooping cough, is a respiratory infection caused by the bacteria Bordetella pertussis. Man is the only natural host of pertussis and the disease is extremely dangerous, especially during infancy. The pathogenicity of B. pertussis is achieved by attachment of the bacteria to the respiratory epithelium. There is no bacterial invasion of the blood or tissue and only a local infection results. The components of B. pertussis, which are responsible for the disease symptoms at the site of infection are not fully understood (34). Endotoxin which is released from disrupted cells may be the cause of inflammation of the trachea and bronchi. The organism multiplies rapidly on the epithelium of the trachea and bronchi and interferes with ciliary action. Therefore, the organisms are found in the saliva, sputum and nasal discharge. The disease is highly infectious during the first few days of infection when the organisms are found in the largest numbers. After an incubation period of about 10 days, symptoms similar to a cold develop eliciting a runny nose, slight fever and mild cough. This catarrhal stage lasts about 7-14 days and the disease cannot be clinically differentiated from other respiratory infections during this period. The second stage is characterized by the subsidence of fever and the onset of a paroxysmal cough which is provoked by the slightest stimulus (18). Attacks are commonly precipitated by excitement, running, inhaling moist

or dusty air, or eating, and are especially severe at night (37). The characteristic whoop results from a serial paroxysm of coughing followed by deep inspiration. The paroxysm is often prolonged and choking during these episodes can lead to insufficient oxygen delivery and convulsions. Frequent vomiting during these attacks can lead to malnutrition. Clinical findings at the height of the disease process include, acute lymphocytosis, congestion of the larynx and trachea, reduced blood sugar levels and acidosis due to the accumulation of CO_2 in the blood (16). Protein components of B. pertussis have been found to increase susceptability to various biological agents including histamine and serotonin (9). The second and most severe stage lasts 7-30 days and is followed by a convalescent period with a gradual decline of symptoms. The child usually returns to normal after 1-6 weeks convalescence.

Pertussis is a prolonged and frightening illness and is potentially fatal, particularly because of the secondary infections that follow. 97% of the fatal cases are in children under the age of 5, and 70% are in children under the age of 1 (16). Many fatalities are due to secondary infections. Pneumonia is the most common complication in children due to the secondary invasion of organisms other than B. pertussis. Bronchitis and rarely T.B. can also follow pertussis as complications (15). Of the 4,358 cases of whooping cough in the U.S. in 1982 and 1983, 3,159

individual case report forms were filed. Pneumonia was X-ray confirmed in 23% of the cases under 6 months of age, 17% of the cases 6-11 months and 12% of the cases 1-4 years. Nine deaths were attributed to secondary pneumonia (36). Recent research has found that B. pertussis produces a substance which inactivates macrophages and allows opportunistic organisms to proliferate. The substance is a soluble, heat stabile, highly active adenylate cyclase. The enzyme is internalized by phagocytic cells and catalyzes the unregulated formation of cyclic AMP, which disrupts normal cell function (15). The inability of macrophages to protect the body against invading organisms increases the host's vulnerability to secondary infections. Fatal cases of whooping cough are usually attributable to pneumonia or brain damage which is secondary to infection (31). Neurological involvement is less common but can result in death or permanent neurological sequelae. Mortality rates among patients with neurological symptoms can be as high as 60-90%. More than one-third of the survivors recover with no neurological sequelae (37). Of the 15 pertussis related deaths reported in 1982 and 1983, only 1 was attributed to encephalopathy in conjunction with pneumonia (36). In recent studies, evidence has suggested that an attack of whooping cough may be connected with abnormal pulmonary function later in life especially if it has been complicated by pneumonia (54). Pulmonary sequelae may be a result of the long course of the illness and pathological features

which occur in the acute phase. A British study, consisting of 360 school children with a history of whooping cough who were compared to over 700 controls, has disputed this finding (29). Although the children who had previously had whooping cough were more likely to suffer from various chest illnesses than the controls, there was no significant difference in the indices of lung function. This study suggests that subclinical abnormalities may exist which predispose certain children to excessive respiratory illness both prior to whooping cough and later in life. More extensive studies are needed to determine the relationship of pertussis to subsequent pulmonary sequelae.

Today a lower percentage of children infected with pertussis die. Although it is tempting to attribute the lower fatality rate to a reduction in the organism's virulence, it is doubtful that B. pertussis has mutated to a less virulent strain. On the contrary, lower fatality rates can be attributed to better supportive medical care. In developing countries, pertussis is consistently associated with high levels of morbidity and mortality. The severity, potential lethality, and possible long-term consequences of pertussis, in addition to its continued presence in the world's population has served as the motivational force behind continued research to develop more effective methods of treatment and vaccination.

VI. Treatment

Literature on the current treatment of whooping cough is sparse and the recommended methods of treatment utilize mainly symptomatic therapy. The general consensus is that it is doubtful that antibiotic therapy changes the course of the disease. However, it has been suggested that administration of erythromycin, ampicillin or tetracycline may shorten the period of communicability (18). A recent study by Broomhall and Herxheimer has determined that there is no evidence that prophylactic administration of erythromycin to unvaccinated contacts affords protection against infection with B. pertussis (34). The greatest advantage of the use of chemotherapeutic agents may be in preventing infections secondary to B. pertussis. In light of its poor response to traditional antibiotics, prevention is clearly the best approach to controlling the disease. Anti-spasmodic agents are of little use in the therapy of pertussis cases because smooth muscle is not involved in the cough. Corticosteroids and salbutamol have been used with some claim of lessening the severity of the symptoms and shortening the duration of the illness. The advantages of the use of sedatives to suppress coughing is yet controversial. It has been recommended that mothers of severely ill infants be taught to give a special type of physiotherapy which will help clear secretions. Hospitalization and isolation are essential if cyanosis and convulsions occur during coughing (57). Hyperimmune gamma

globulin has been used to hasten recovery, prevent complications and reduce mortality in unvaccinated children who are debilitated by other diseases. However, there is no evidence of its effectiveness in well-controlled trials. Unless it can be shown to be effective, it may lose its license in the U.S. (34). Treatment of pertussis should aim to reduce the severity of disease and mortality, and prevent the spread of the disease. A better understanding of the pathophysiology of B. pertussis is essential to guide additional clinical research which will lead to the development of more effective methods of treatment of pertussis. Ultimately, however, prevention via a safer, more effective vaccine is the most logical approach.

VII. Epidemiology

Epidemics occur when viable organisms are present and are transferred among a large population of susceptible individuals. Limiting the number of susceptibles by active immunization at an early age reduces the possibility of outbreaks of the disease. Transmission of B. pertussis occurs by droplet infection (30). The organisms are transferred from person to person during coughing and by articles soiled with nasal and mouth secretions. During violent attacks of coughing a spray of sputum may be thrown 4-5 feet. New cases probably arise from early undiagnosed cases or from mild cases in adults (18). Although a cough may persist in individuals with whooping cough, carriers are generally not a problem, because it is rarely possible to demonstrate viable organisms after six weeks. The peak incidence occurs in late spring and summer and is most common in older infants and children. The disease is most severe in children under the age of one year (31). The risk of exposure to pertussis is relatively low in the U.S. because of the high proportion of vaccinated individuals and the effects of protective herd immunity. The risk is somewhat higher for children who attend day care centers or are in close contact with other young children, or for residents of institutions for the neurologically impaired where a high percentage of the residents are not adequately protected by vaccination. In underdeveloped countries and in certain developed countries where immunization programs

have been curtailed, there is a significant risk of exposure to pertussis (10).

At its peak in the 1930s pertussis affected more than 265,000 people per year with an annual fatality rate of about 8,000. Over 50% of the cases in the U.S. occured in children under one year of age (25). Today 1,000-3,000 cases, and 5-20 deaths are reported in the U.S. annually. In 1982 and 1983, 4,358 cases were reported in the U.S. 43% of the cases were in children under six months of age. New York and Pennsylvania reported localized epidemics among members of certain religious groups and Oklahoma experienced a statewide outbreak (36). Maryland experienced a marked increase in cases in 1982 and 61% of the patients six months and older had not been properly immunized (14). Morbidity and Mortality Weekly Report states that, "the crude incidence rate of reported pertussis in the U.S. in 1982 was 0.83/100,000 total population, and in 1983 1.05/100,000. The incidence rate for children under one year old in 1982 was 27.2/100,000 and in 1983 36.1/100,000." Fifteen deaths occured in 1982 and 1983 with a case fatality ratio of 0.5%. Thirteen deaths were in children under six months. The case fatality rate for this age group was 1%. Based on the 3,159 individual case forms received by the MMWR in 1982 and 1983, pertussis was laboratory confirmed in 68% of the cases, 9% by culture, 46% by direct fluorescent antibody and 13% by culture and direct fluorescent antibody. Nearly half the patients required hospitalization. The characteristic whoop

was described in more than 50% of the patients. Pneumonia occured in 16%, and encephalopathy in 0.3%. 68% were not properly immunized according to the current recommendations of the Immunization Practices Committee (36). Despite an active immunization program in the U.S., the continued existence of pertussis demonstrates the importance of an extensive vaccination program, and the need for more effective vaccines which may eventually eradicate whooping cough.

VIII. Immunity and Vaccination

Prevention of pertussis is essential due to the limited value of antibiotic therapy and the unestablished merit of passive immunization. The current whole-cell vaccine yields a certain level of toxicity which has been tolerated in order to achieve the level of potency sufficient to confer immunity. Originally, it was believed that immune factors to pertussis could not be transferred from mother to child through the placenta or milk (34) but recently, maternal antibodies have been demonstrated in cord blood. However, the duration of maternal antibody in the child and its role in passive immunity is not known (11). The newborn is especially vulnerable to infection with B. pertussis. Protective herd immunity due to the high percentage of vaccinated individuals in the U.S. has somewhat minimized the risk of exposure, but further reduction of risk during infancy can be accomplished by early immunization, usually as early as six weeks of age. Commonly, whole cell pertussis vaccine is mixed with diphtheria and tetanus toxoids, forming a triple vaccine, DPT. The Immunization Practices Advisory Committee recommends 3 doses of DPT by six months of age, a fourth dose approximately one year later and a booster dose between four and six years of age (14). The vaccine is not effective as a curative measure and must be given four to six weeks before exposure to allow immunity to develop. The precise mechanism of immunity to pertussis is not yet fully understood, but recovery from the

illness confers long-lasting immunity. Occasionally disease occurs in vaccinated individuals, but the illness is much less severe (25).

The vaccine currently being used in the U.S. is prepared from killed B. pertussis bacilli. Generally, phase I organisms which contain the major agglutinogen factors 1, 2 and 3 are cultured in antigenic-free medium. Medias used tend to vary by manufacturer. Large volumes of liquid media are usually inoculated and the cells are allowed to incubate at 37°C for a short period. Less than 3 days incubation is recommended to prevent phase variation. The cells are collected by centrifugation or precipitation and the cells are resuspended in saline. Culture purity checks should be performed at intervals during cultivation and harvesting to eliminate the possibility of contamination (34). Destruction of the heat labile substance is accomplished by heating the cells to 56°C for 30 minutes. Control tests for potency, toxicity, and sterility are performed before diluting the organism to the concentration appropriate for human use. Thimerosal and heat are used to kill the organisms. The mouse protection test is used to reflect the potency of the vaccine (37). An examination of the vaccine's toxicity can be achieved by utilizing the mouse weight-gain or mouse toxicity test. A group of 10 mice are injected intraperitoneally with half the human dose of vaccine. The test mice have an original weight of 14-16

grams. Normally mice lose weight for about 14 hours after injection and they begin to regain it. The World Health Organization recommendation requires that the test mice regain their starting weight within 3 days after injection, and achieve 60% of the weight gain of a control group. The U.S. requires that the mice gain an average of 3 grams per mouse, 7 days after injection (12). The International Standard for pertussis vaccine recommends 4.1 IU/dose (14). Alum, aluminum hydroxide or aluminum phosphate, which acts as an ajuvant, is added to pertussis vaccine and it is mixed with diphtheria and tetanus toxoids. The importance of immunization to pertussis should not be underestimated and is essential in reducing the possibility of whooping cough epidemics.

IX. Vaccine Controversy

Although the killed whole-cell pertussis vaccine has been estimated to be 80-90% effective in preventing disease, it has been associated with a high rate of transient, non-damaging side effects and a much lower frequency of potentially dangerous neurological reactions which occasionally result in brain damage or death. The following data on the nature and rate of DPT associated reactions is derived from records of nearly 16,000 DPT immunizations. Approximately 50% of children receiving pertussis vaccine experience pain at the injection site, fever, and fretfulness within 48 hours. Between 20-30% experience anorexia, and drowsiness, and between 1-10% experience persistent crying, vomiting, and redness and swelling at the site of injection. Reactions occurring in less than 1% of the children include a high pitched, unusual cry, convulsions and collapse with a shock-like syndrome (59). The risk of first seizures subsequent to vaccination with DPT is approximately 1 in 1,750 (10). The vaccine may be responsible for toxic effects on the central nervous system in a small number of children. There is speculation that the pertussis in DPT may be associated with sudden infant death syndrome (41), but the existence of a causal relationship has not been proven (20). A comparative study of infants receiving either DPT or diphtheria and tetanus (DT) vaccine demonstrated that reactions in children receiving DPT were substantially higher than in those

receiving DT. The implication of this study is that the pertussis component of DPT is responsible for the post injection symptoms (31). Administration of the entire bacterium requires the host to sort out the effective immunological response, but also to deal with components that may be toxic.

The British National Encephalopathy Study estimates that rare serious consequences occur in approximately one in 100,000 children receiving a complete primary series of 3 doses, or once in 300,000 injections of DPT (59). Concern that the vaccine may constitute a greater risk than the disease itself has been spurred by the decline in disease incidence. However, the disease is not yet eradicated. Bordetella pertussis is still widely present in the population and can be the source of an epidemic if vaccination rates begin to decline. Dramatic illustration of this occured in England and Japan where declining immunization rates were followed by widespread epidemics. Due to negative publicity in England, immunization acceptance rates fell from 79% in 1973 to 30% in 1978. Between 1977 and 1980 over 100,000 cases of pertussis were reported with 36 deaths (32). A similar outbreak extrapolated to U.S. population would be equivalent to nearly half a million cases and 160 deaths. Japan had a similar experience. In 1975 the Japanese Ministry of Health and Welfare discontinued the use of vaccine when two children reportedly died as a result of vaccine

complications. The reduction of the immunization rate was followed by an epidemic resulting in 35,000 cases and 118 deaths. After resumption of the vaccination program, acceptance rates were poor. Brain damage resulting from an active case of whooping cough occurs approximately once in 8,000 cases, in comparison to once in 100,000 children vaccinated with B. pertussis (31). A comparison of the costs attributed to disease and costs associated with a vaccine program demonstrate that approximately 11.1 dollars in benefits are accrued for every dollar spent for an effective pertussis vaccination program (36). Clearly, these statistics favor vaccination as the appropriate method for the control of pertussis, but the controversy persists.

In the U.S. nearly 95% of all children entering school in 1983-1984 had received at least 3 doses of DPT. Legal vaccination requirements exist in many states. Twenty-seven U.S. states require 4 doses of DPT for school entry, 13 states and the District of Columbia require 3 doses, and one state requires an unspecified number of doses (36). Despite the arguments favoring pertussis vaccine, careful attention should be paid to the contraindications for its use. The vaccine should not be administered if the child has an underlying neurological disorder or allergy to the vaccine components. Additional contraindications include any of the following reactions which have occured within 48 hours after a previous dose of pertussis vaccine: 1) persistent, inconsolable screaming for 3 hours or longer duration; 2)

collapse or shock; 3) temperature greater than 105°F; or 4) convulsions or alterations of consciousness (31). A recent study evaluated the post DPT reactions and serologic response with a standard dose, and with a reduced dose of pertussis vaccine (7). The results indicate that the modified dose schedule was associated with significantly less adverse reactions and elicited a serologic response equivalent to the standard dose. Although these results seem to demonstrate an effective method of reducing the side effects without sacrificing protection, they should be viewed with cautious optimism. Variation in the potency of individual lots of vaccine creates difficulty in predicting whether other lots will provide similar results with the modified dose schedule. Additionally, a clear correlation between agglutinin titers and protection from disease has not been established. An examination of the immunization records of nearly 600 children in England provided the data necessary to predict that a immunization target level of 80% is realistic and achievable. The incidence of contraindications and parental refusal will unfortunately exclude appoximately 20% of the children from proper protection against pertussis (28). Vaccination of 80% of the population with a vaccine efficacy of 90% implies a protection level of only 72% (17).

As previously mentioned, involvement of the media in the pertussis vaccine controversy has led to serious public health consequences in Great Britain. An inflammatory 1974

television documentary on the risks of vaccination had tremendous impact on young parents and physicians. Ultimately, acceptance rates were dramatically reduced and the incidence of active pertussis began to rise (24). Alarm that similar media involvement in the U.S. will lead to devastating consequences is not entirely unfounded. Excerpts from a 1-hour documentary entitled, "DPT: Vaccine Roulette," aired around the nation in April 1982. The report focused on neurological damage allegedly resulting from pertussis vaccine and was accompanied by heartrending footage of damaged children. Little attention was given to the devastating effects of the actual disease, and the risk of infection in unimmunized children. Such distortion of the issues lead to inaccurate public perception of the vaccine benefits (22). Following the report, physicians noted that parents began to question immunization practices, and DPT sales fell while DT sales increased (14). Recently, several manufacturers have discontinued production of the vaccine because it is viewed as a low profit, high liability venture (59). Ensuing parental concern led to a hearing of the U.S. Senate Subcommittee on Investigations and General Oversights which examined the topic of DPT and continued government funding for pertussis immunization. Subsequently, the House Commerce Committee called for a 2-year study of the safety of DPT. The Center for Disease Control and the American Association of Pediatrics have reaffirmed the necessity of a continued immunization program

and have indicated that the benefits of vaccination far outweigh the risks (22). The Immunizaton Practices Advisory Committee continues to recommend 3 doses of DPT by six months of age followed by two boosters (14). The American Association of Pediatrics is making every effort to ensure that accurate informed consent is obtained. They provide an informaton sheet which is used by public health clinics as part of their immunization program procedure (22). A recent (February 5, 1985) special report on ABC's "20/20," presented a fairly accurate description of the vaccine dilemma. Fundamentally, their research agreed with mine, but they overemphasized the risks and the vaccine's unproven relationship with SIDS. They implied that the risks of vaccination are much higher than currently reported by the American Association of Pediatrics, and led the public to believe that pharmaceutical companies have developed a new vaccine in the U.S. which has been conclusively proven to be safer and more effective, yet it is not being marketed for reasons associated with cost. Although Japan has reported success with a new vaccine in clinical trials, I have found no evidence in the literature of such advanced clinical trials in this country. However, in the interest of preserving their commercial advantage, drug companies may not make their research available to the general public. Numerous reports indicate that researchers are rigorously studying components of the <u>B. pertussis</u> bacillus. These studies may prove useful in the development of a new

vaccine.

Although the public has a right to be informed, over dramatization of the issues surrounding vaccination may result in the erosion of public confidence and a mass rejection of the vaccine. Research endeavors should concentrate on the development of a safer, more effective vaccine. However, until a new vaccine is available, achieving the highest possible immunization rate is the only rational approach to disease control.

X. Current Trends in Vaccine Development

Since chemotherapeutic agents are of little value in alleviating the symptoms of the devastating disease, whooping cough, prevention is essential. The current vaccine is only 80-90% effective and yields a level of toxicity which is responsible for the previously discussed, undesirable side effects. These disadvantages, coupled with diminishing public confidence demonstrate the need for research aimed at the development of a new pertussis vaccine. The goal of current research efforts is to define the immunochemical structure of B. pertussis and understand the host-parasite interactions involved in its pathogenicity. Ultimately, assembly of the necessary purified antigens and modification of the toxins may make a new vaccine a reality. Extensive research is focused on the protective activities of components of the B. pertussis bacillus. Clinical trials with consideration of ethical and legal issues will be essential and will be most accurate if they are done on a large scale with frequent observations for side effects. The majority of pertussis research focuses on the isolation, purification and characterization of biologically active components of the cell, such as, filamentous hemagglutinins, lymphocytosis promoting factor, adenylate cylcase, hemolysin and the outer membrane proteins of the organisms, which I will discuss in great detail (34). Additionally, modern developments in the field of molecular biology and contributions from immunology create new

possibilities for vaccines through genetic engineering techniques. Synthetic vaccines may ultimately provide effective protection from many infectious agents, including pertussis (1).

XI. Pertussis Research

Phase I strains of Bordetella pertussis synthesize factors such as lymphocytosis promoting factor hemagglutinin (LPF), and filamentous hemagglutinin (FHA) which are believed to play a role in pathogenicity and immunity. LPF is believed to be the same as mouse protective antigen (PA), histamine sensitizing factor (HSF), and islet activating protein (IAP) which have been described by some workers. Therefore, the name pertussigen or pertussis toxin has been suggested and is used interchangeably with LPF. In order to study their protective capabilities, it is necessary to separate and purify LPF and FHA. LPF has a relatively low hemagglutinating activity and induces a variety of physiological responses including lymphocytosis, histamine sensitization, adjuvant effects and potentiation of insulin secretion. It appears as a spherical structure 6 nm. in diameter by electron microscopy and is demonstated on polyacrylamide gel electrophoresis as a single band with a molecular weight of 105,000. FHA has a hemagglutinating activity 5-7 times greater than LPF. It appears as fine filaments 2 nm. in diameter and 40-100 nm. in length by electron microscopy. It has been suggested that FHA is derived from the fimbriae of B. pertussis and may be involved in attachment of the organism to the respiratory surface. Immunodiffusion has shown that LPF and FHA are antigenically distinct. A method for the separation and purification of LPF and FHA has been described by Sato et.

al. which yields preparations of LPF and FHA which contain less than 0.01% of each other based on the ELISA test (46). Polyacrylamide gel electrophoresis demonstrates that these proteins are highly purified by this technique. The method of separation utilizes a co-precipitation technique from supernatants of stationary, Tohama strain, phase I cultures. FHA binds to a column of spheroidal hydroxylapatite at a high pH when culture supernatant is passed through it. LPF does not bind to the column and is eluted. It is recovered and is passed through a column of haptaglobin-coupled Sepharose 4B. LPF binds to this column but FHA does not. The Sepharose 4B is used to further purify LPF and remove small amounts of LPF which are still present in the FHA eluted from the hydroxylapatite column. These chromatographic techniques allow for sufficient quantities of purified substances to be obtained for further study and may prove useful in the development of a new pertussis vaccine.

Arai and Munoz have developed a method for crystallizing pertussigen (3). The supernatant fluid from Tohama strain, phase I cultures of B. pertussis are pooled and concentrated by ultracentrifugation through a dialysis membrane and passed through and Sepharose 4B column. The bound pertussigen is eluted and further concentrated by ultrafiltration under vacuum in a dialysis membrane. Passage through a Sepharose CL6B column removes contaminating high and low molecular weight substances, and

the addition of ethanol breaks hydrophobic interactions between pertussigen and other substances. The solution is further concentrated by ultrafiltration and is centrifuged to remove solid materials. After 2-3 weeks at 2-4^{o}C, this purified pertussigen solution precipitates to form fine crystals. Recovery of pertussigen by this method is low. However, attempts are being made to improve the yield. The advantage of this method is that crystallographic techniques may be used to study the structure and chemical composition of pertussigen. Pertussigen induces physiologic changes in mice leading to an increased susceptability to histamine, serotonin and other shock inducing agents. Furthermore, it acts as an immunological adjuvant, induces lymphocytosis and hypoglycemia. It also has the ability to agglutinate erythrocytes at relatively high concentrations.

The ability to crystallize pertussigen has led to studies of its biological activities in mice (38). Biological activities which have been examined include, increased production of insulin, induced leukocytosis, hypersensitivity to histamine, adjuvant effect with increased production of IgE and IgG1 antibodies to hen egg albumin, increased susceptability to anaphylactic shock and increased vascular permeability of striated muscle. In one study of these activities, toxicity tests were performed on mice which were given intraperitoneal injections of specified doses of pertussigen. Individual weights and the number of deaths were recorded daily. Crystalline

pertussigen killed 50% of the mice tested at a dose of 546 ng/mouse. With a dose of 5ng/mouse the mice did not gain weight and died within 5 days. This verifies a previously noted characteristic of pertussigen called delayed toxic death in mice. The mice designated for insulin studies were given an intraperitoneal dose of 25% glucose in physiological saline 3 days after an intravenous dose of pertussigen. After 15 minutes, blood was extracted and insulin levels were determined by radioimmunoassay. 2 ng/mouse of pertussigen was found to be sufficient to induce increased secretion of insulin. A dose of 8-40 ng/mouse induced leukocytosis which was defined as a doubling of the leukocyte count in the peripheral blood. The level of histamine sensitivity was determined by giving groups of 70 mice varying doses of intravenous pertussigen. The intraperitoneal, 50% lethal dose of histamine was determined 7,21 and 84 days after injection. A dose of 0.5 ng of pertussigen produced hypersensitivity to histamine which could still be detected 84 days later. The adjuvant activity of pertussigen and its ability to stimulate the production of IgE and IgG1 antibodies to hen egg albumin was tested by giving mice varying amounts of pertussigen and 100 ng of hen egg albumin at day zero. Control mice received only hen egg albumin. After 21 days, the test mice received a booster dose of 5 ng of hen egg albumin and were bled 7 days later. The presence of anti-hen egg albumin in their serum was analyzed using the passive cutaneous anaphylaxis

test. Control mice had undetectable levels of anti-hen egg albumin IgE and IgG1. The test mice showed an increase in IgE and IgG1 with a dose of 0.1 ng/mouse of pertussigen. The antibody titers increased as the dose of pertussigen increased and the IgE class showed a better response than IgG1 overall. A significant increase in the permeability of striated thigh muscle of mice to ^{125}I labeled human serum albumin was noted at a pertussigen dose of 0.5 ng/mouse. The induction of experimental allergic encephalomyelitis (EAE) due to the adjuvant effect of pertussigen was tested by injecting a mixture of guinea pig spinal cord emulsion and crystalline pertussigen intraperitoneally into Lewis rats. A total EAE score was derived from the degree of paralysis and rapidity of onset and demonstrated that 20 ng/mouse of pertussigen was capable of increasing the EAE score significantly. Even when spinal cord emulsions are given without an adjuvant, this strain of rat is mildly susceptable to EAE. The addition of pertussigen as an adjuvant results in an accelerated and more severe form of EAE when the right dose is used (39) (37).

In another study, pertussigen was found to have a chemotactic activity on human peripheral blood monocytes (40). The chemotactic assay was performed by using a cell fraction containing 20% monocytes and 80% lymphocytes prepared from human donor blood, a purified preparation of pertussis toxin at various dilutions, and a multiwell

chemotaxis assembly. Each well of the bottom plate was filled with 25 ng of diluted test or reference solution and covered with a polycarbonate filter sheet of 5 um pore size. The top plate contained 50 ul of the cell suspension in each well. The assembly was incubated and the filter was removed, fixed and stained. A microscopic determination of the number of monocytes which passed through the filter indicated a significant dose-dependent chemotactic enhancing effect of pertussigen on human monocytes. This activity of pertussigen was completely destroyed, however, when it was heated at 100^{o}C for 30 minutes. Monocytes play an important role in natural and acquired immunity. Therefore, studies on the effects of bacterial cell components on monocytic activity may shed some light on the host defense mechanisms responsible for immunity to pertussis.

Treatment of crystallized pertussigen with glutaraldehyde reduces its toxic effects and some of its biological activities. Immunization of mice with glutaraldehyde detoxified pertussigen protects them from intracerebral challenge with B. pertussis (38).

The first step in the infectious process is the attachment of B. pertussis to the epithelial cells in the respiratory tract. Fimbriae play an important role in the attachment of bacteria to mucosal surfaces. Research suggests that a component of the organism B. pertussis called filamentous hemagglutinin is derived from fimbriae and may be an adherance factor of B. pertussis. Studies

of FHA are aimed at identifying the immunogenic potential of this component and its possible contribution to an acellular vaccine. Tuomanen and Hendley studied the ability of B. pertussis to interact with human respiratory epithelial cells (56). The tests utilized normal human tracheal epithelial cell suspensions and freshly isolated virulent strains of B. pertussis. The cell and bacterial suspensions were mixed, incubated and rinsed. Organisms which adhered to the ciliated cells were stained with pertussis specific fluorescent antibody and Evans blue counterstain, and counted microscopically. B. pertussis organisms adhered exclusively to the ciliated cells of the natural host, close to the cell body. They did not adhere to nonciliated cells or the body of ciliated cells. Variation in the extent to which organisms adhered was dependent on the concentration of organisms and the incubation time. In contrast, pneumococcus did not attach to cilia, although it too is a respiratory pathogen. Virulent phase I B. pertussis organisms adhere more readily than non-virulent phase III and IV. The effect of trypsin, periodate and formaldehyde on adhesion was studied by treating either the organisms or cells with the test substance for limited periods. Treated cells were mixed with untreated organisms and visa-versa, and the adhesive activity was analyzed. Neither formaldehyde nor trypsin altered the adherance, suggesting that ciliary motility and active metabolism are not requirements for adhesion. The

periodate treated cells and organisms demonstrated a decrease in adherance. Additionally, portions of the human bronchus were incubated with the B. pertussis suspension then rinsed, embedded and sectioned. The sections were stained with lead citrate and uranyl acetate so that the ultrastructure of adherance could be examined by electron microscopy. B. pertussis organisms interacted with ciliary tufts either by connection by bacterial filamentous material or by apposition of bacterial and ciliary surfaces.

Strains of B. pertussis that possess agglutinogen 2 are fimbriated whereas strains that lack it are non-fimbriated. This suggests that agglutinogen 2 is a fimbrial antigen and that agglutinogen 3 is not. Attachment of bacteria to the respiratory cells by fimbriae may explain the predominance of strains possessing factor 2 (stains 1, 2, 3 and 1, 2) in non-vaccinated communities. Types 1, 3 and 1 are rarely isolated from active cases of pertussis (13). Definitive studies of the adhesive properties of B. pertussis may provide information applicable to more effective methods of vaccine production.

A report on the substructural units of B. pertussis fimbriae suggest that filamentous hemagglutinin (FHA) originates from the fimbriae of B. pertussis (8). The phase I organisms exhibit long peritrichously arranged fimbriae on the cell surface which are approximately 3 nm thick. Purified FHA has been found to contain similar, but shorter structures 3 nm thick and varying in length from 40-100 nm.

Hemagglutinating activity is greatest during the first day of culture when fimbriation reaches its peak, suggesting a correlation between the hemagglutining activity of FHA and fimbriation of the bacillus. Examination of the substructural components of the fimbriae reveals that the fimbriae are made up of substructures 12.5 nm long which are connected by fragile regions. The concept of such regions would account for the structural heterogeneity of size in purified FHA due to breakage of the long fimbrae at these fragile regions during the purification procedure. Although at least one study has disputed the relationship between FHA and B. pertussis fimbriae (4), the evidence in this study and others supports the hypothesis that FHA is derived from fimbriae and may be important in the preparation of an acellular vaccine. The purified preparation of FHA contains only protein which can be separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis. On SDS-PAGE, the purified protein exhibits multiple polypeptides ranging in molecular weights from 220,000 to 58,000. The chemical composition of FHA is not known. Monoclonal antibodies provide a means of analyzing the structure of complex proteins due to their inherent specificity (26). Monoclonal antibodies to several of the smaller FHA proteins also react with the highest molecular weight polypeptide indicating that the lower molecular weight proteins have antigenic determinants identical to the 220,000 m.w. protein. Many of the smaller polypeptides may be fragments derived from the

highest m.w. protein.

Studies of the protective capabilities of antibodies to LPF and FHA elucidate the role these components may play in preventing infection with pertussis in immunized individuals. Sato and his colleagues have done extensive research on the role of antibodies to these factors in immunity to pertussis (47). They purified LPF and FHA by the co-precipitation technique, previously described, using Tohama strain I bacillus. Antiglobulins to LPF and FHA were prepared by immunizing New Zealand white rabbits with the purified preparations of FHA and LPF. Normal gamma globulin was obtained before immunization. Passive immunization data showed that mice receiving anti-LPF or anti-FHA 30 minutes before aerosol challenge with B. pertussis had a rate of weight gain equivalent to mice not exposed, and no deaths occured in the passively immunized or uninfected groups. In contrast, 80% of the mice receiving normal gamma globulin 30 minutes before aerosol challenge with B. pertussis were dead within 14 days and showed a significantly reduced weight gain. Both anti-LPF and anti-FHA enhanced the clearance of B. pertussis from the lungs. Peripheral white blood cell counts were done periodically on the three groups of mice. A significantly lower WBC count in the group given anti-LPF demonstrates that only anti-LPF has the capability of neutralizing leukocytosis promoting factor. HeLa and Vero tissue cell cultures were used to test the attachment of B. pertussis to mammalian cells. Suspensions of B. pertussis

cells were incubated with either anti-FHA, anti-LPF, or normal gamma globulin prior to incubation with the tissue cell cultures. Results demonstrated that only anti-FHA neutralization of pertussis cells prevented their attachment. Neutralization of the biological activities of LPF was tested by mixing purified LPF with various dilutions of normal gamma globulin, anti-FHA, or anti-LPF. After 30 minutes incubation, the mixture was injected into the tail vein of mice. Histamine sensitivity was determined by injecting the mice intraperitoneally with histamine on day 4. Mortality was used to determine histamine sensitivity. Anti-LPF neutralized the histamine sensitizing activity of LPF up to a dilution of 1:108. Even at the lowest dilution, normal gamma globulin and anti-FHA did not neutralize LPF. Further evaluation of the specificity of these antibodies was accomplished by the immunodiffusion of anti-LPF and anti-FHA against a mixture of B. pertussis antigens. Anti-LPF and anti-FHA were found to be distinct from each other and specific for their respective antigens. A paper by Robinson and Irons (45) discusses the enhancing effect that LPF has on the protective activities of various Bordetella antigens. They demonstrated a synergistic effect which is not well understood and seems to be unrelated to the adjuvant effect of LPF. It is possible that this phenomenon is related to LPF's biological activities, including the ability to increase vascular permeability of the blood-brain barrier, because glutaraldehyde inactivated LPF does not exhibit this

synergistic effect. These studies suggest that LPF and FHA have immunologically protective capabilities, and may be essential components of an acellular vaccine. Vaccination would allow the host to elicit an antibody response to FHA and LPF prior to contact with the organism. Anti-FHA may protect the host from infection by preventing the attachment of B. pertussis to the respiratory epithelium. Anti-LPF protect the host by neutralizing the harmful effects of LPF which is released during infection.

The human antibody response to FHA and LPF after routine immunization or disease has been evaluated using the ELISA procedure (11). IgG and IgM antibodies directed against LPF and FHA rise after initial vaccination with DPT, and antibody titers increase with subsequent immunizations. Generally, no serum IgA is detected. Maternal IgG antibodies can be found in cord blood indicating placental transfer of antibodies, but it is not known how long these antibodies circulate in the infant's system. Children with high cord blood titers of anti-LPF give a poor serological response to an initial dose of vaccine given during the first week of life. Children with low cord blood titers give a good serological response. This depressed antibody response may be important in defining a vaccine schedule which will optimize the immune response in children. Elevation of antibody titers to LPF and FHA in patients with active cases of pertussis indicate that the response is of prolonged duration. In addition to IgG and IgM, IgA was

detected in patients with the disease. These observations show that LPF and FHA are immunogenic constituents of the current whole cell vaccine and their inclusion in a subcellular vaccine may be important in eliciting a protective immune response (45).

In addition to LPF and FHA, B. pertussis produces a heat labile toxin (HLT). The mechanism of action and the involvement in pathogenesis of heat labile toxin is unknown. Studies have shown that anti-HLT is absent in the convalescent sera of pertussis patients and detoxified HLT provides no protection against intracerebral challenge with B. pertussis in mice. Consequently, it is doubtful that HLT plays a significant role in immunological protection against pertussis (33).

Experiments with tracheal cytotoxin (TCT), which is produced by B. pertussis, have demonstrated that this toxin has the ability to mimic the pathogenesis of B. pertussis on the respiratory epithelium (21). The purification of TCT and its interaction with hamster trachea cells has shown that its biological activities include inhibition of DNA synthesis and epithelial cytopathology with a marked reduction in ciliated cells. The relationship between these activities is not well understood, but further studies of TCT may prove useful in developing a better understanding of the pathogenesis of B. pertussis.

B. pertussis also synthesizes adenylate cyclase and hemolysin. Adenylate cyclase and hemolysin-deficient

mutants have been used to study the role these factors play in the virulence of B. pertussis (60). Double mutants deficient in both of these factors are avirulent, whereas hemolysin deficient mutants show moderately reduced virulence. The loss of adenylate cyclase must be responsible for the difference in virulence in these two mutants. The enzyme adenylate cyclase is toxic to phagocytes and reduces the natural killer activity of lymphocytes by catalyzing the unregulated formation of cyclic AMP (15). Its role in the disease process may be related to the interference of normal host defenses which allows the infectious organism to become established.

Transposon Tn5 mutagenesis has been used to generate lines of B. pertussis organisms each with single site mutations (61). The site at which Tn5 is inserted in the gene is marked physically by the transposon DNA and genetically by the expression of antibiotic (Kanamycin) resistance by the mutated organisms. Selection for the mutants is accomplished by using Kanamycin containing media for cultivation. Researchers have isolated mutants deficient in LPF, FHA, hemolysin and adenylate cyclase. Experimental studies using lines deficient in factors which are believed to be involved in virulence may be useful in elucidating the roles these factors play in pathogenesis and immunity. A better understanding of the molecular organization of virulence and immunogenic determinants may

arise from the ability to clone gene fragments marked by Tn5.

In addition to the components of B. pertussis which can be isolated from the cell-free supernatants of phase I cultures, the outer membrane proteins of organisms have been studied and should be considered for inclusion in an acellular vaccine (44). Several phase I specific outer membrane proteins have been isolated by detergent extraction with molecular weights ranging from 30,000 to 90,000 on SDS-PAGE. The major protein found on all phases of B. pertussis cultures has a molecular weight of 41,500 and certain outer membrane proteins are found to be common to all members of the Bordetella genus. In a study of the phase I envelope, the purified proteins were found to be protective in mice against intracerebral infection and in rabbits against respiratory infections with B. pertussis. Furthermore, a strong correlation existed between the protective potency, lymphocytosis promoting activity and histamine sensitizing activities of different preparations of the isolated phase I outer membrane proteins. Removal of LPF from the protein preparation by haptaglobin-Sepharose 4B chromatography or inactivation of LPF by glutaraldehyde treatment resulted in a marked reduction in LPF activity and a lesser reduction in protective potency. Native LPF was found to be toxic and non-protective in mice. The evidence suggests that the protective potency of the envelope proteins is enhanced by the presence of LPF (3). The

serologic response to the outer membrane proteins of B. pertussis has been evaluated in vaccinated mice and in vaccinated human infants (43). The sera extracted from mice contained antibodies to a large number of pertussis outer membrane proteins ranging in molecular weight from 15,000 to 114,000. No antibody response to the major 41.5K outer membrane peptide was detected, nor were antibodies to two major phase I specific proteins, 30,000 and 33,000 m.w. The mice elicited strong antibody responses to high molecular weight proteins which appear to correspond to FHA components. Immunized infants also developed antibodies to the high molecular weight proteins but responded only weakly to the major 41.5K outer membrane protein. The infant sera also reacted with a low molecular weight protein (27,000) which corresponds to the major subunit of LPF. These findings indicate that although active LPF appears to play no role in mouse protection, it may be important in protecting children from pertussis. This finding questions the suitability of using the mouse protection test in determining vaccine potency. The major outer membrane protein is known to be accessible and exposed at the cell surface, but the reason for its failure to elicit a good immune response is not understood. Purified envelope proteins may be found to be a necessary component of an acellular vaccine. Recent success has been reported in cloning the two major outer membrane proteins of Phase I B. pertussis organisms (30,000 and 33,000 m.w.) (49). The

chromosomal fragment which codes for the synthesis of these proteins can be inserted and expressed in Escherichia coli bacilli. Additional research in the field of genetic engineering aimed at identifying and cloning the protective antigens of B. pertussis may demonstrate possibilites in pertussis vaccine production far superior to the whole cell and acellular types.

XII. Japan - Acellular Vaccine Trials

Y. Sato and his co-workers at the National Institute of Health in Japan are responsible for the development of a new acellular vaccine which has been used in Japan since the autumn of 1981 and is claimed to be as effective as the whole-cell vaccine, but less toxic (48). This alternative vaccine contains lymphocytosis promoting factor-hemagglutinin (LPF) and filamentous hemagglutinin (FHA) which are purified from the culture supernatant of B. pertussis (48). As previously described, antibodies to the LPF and FHA components of B. pertussis have been shown to protect laboratory animals from infection with B. pertussis. LPF and FHA are precipitated from the culture supernatant of Tohama strain, phase I B. pertussis which has been cultivated on Stainer-Scholte medium. Contaminating substances, such as endotoxin, are removed by ultracentrifugation. LPF is treated with formalin for detoxification. Formalin also inactivates the adjuvant activity of LPF, so aluminum hydroxide is added to enhance the immune response to these antigens. Diphtheria and tetanus toxoids are mixed with the pertussis component vaccine to form a triple vaccine, DPT. The World Health Organization test for potency in mice has been used to determine the protection capabilities of the new vaccine, and the mouse weight-gain test has been used to determine its toxicity. The component vaccine was found to be as effective as the whole-cell vaccine and about one-tenth as toxic. The component vaccine has been compared to the

conventional whole-cell vaccine in clinical trials of 5,000 children in Japan. The incidence of fever and local side effects with the component vaccine was much lower than with the whole-cell vaccine. The incidence of pertussis among children who were exposed to the disease by a member of the household was 82.8% for those who were unvaccinated, 14.3% for those receiving the whole-cell vaccine, and 11.1% for those receiving the acellular vaccine. Antibody titers to LPF and FHA rose considerably and were well maintained in children immunized with both the whole-cell and component vaccines. It is believed that antibodies to FHA in the host's tracheal epithelium and tracheal secretions prevent adherance of the organisms to the cells of the respiratory tract, thereby preventing colonization and replication. Circulating LPF antibodies neutralize the harmful biological activities of the LPF component of B. pertussis. Although the success of these preliminary trials seems impressive, it is important to note that this study consisted of only 5,000 children, half of which were immunized with the new component vaccine (42). Additionally, most of the children immunized with the new vaccine were over one year old and the incidence of home exposure to pertussis infection was relatively low. Extensive long-term studies are needed to determine the incidence of side-effects and protective capabilities of the vaccine, especially in children at the usual vaccination age of 3-6 months. The preliminary findings are, however, very optimistic.

XIII. Synthetic Vaccine Research

The goal of vaccination should be to confer immunity without risk of dangerous complications. Contributions from the fields of molecular biology and immunology may make safer, effective vaccines a reality. These approaches to vaccine development are based on the assumption that proteins from the pathogenic organism can elicit an immune response which will protect the host from disease. The antigenic proteins can be synthesized by inserting the DNA coding for a particular peptide into a virus, bacteria or nucleated cell (1) (51) or by chemical or enzymatic methods (53) (50).

If the gene responsible for the production of a particular peptide can be identified, it can be inserted into a bacterium which will express the gene and produce the protein. Currently, synthetic vaccines are being developed against viruses by cloning the viral proteins in this way. Bacteria are more complex, and isolating the immunologic portions is more difficult. Monoclonal antibodies have been used to identify segments of proteins which are antigenic. If a monoclonal antibody can be shown to confer passive immunity, it can be used to identify a possible protective antigen (1). As previously mentioned, researchers have been successful in cloning the outer membrane proteins of B. pertussis, but it is not known whether or not these proteins promote an immune response which confers adequate protection against disease. Perhaps the isolation of the genes for

components of B. pertussis such as LPF or FHA would be more suitable for producing this type of synthetic vaccine. Tn5 pertussis mutants and cloned gene fragments marked by Tn5 might be helpful in elucidating the DNA coding for the protective antigens of B. pertussis.

The advantages of vaccines made from peptides cloned by bacteria or nucleated cells are that they will contain simple ingredients free of toxic factors, such as those found in whole-cell pertussis preparations. They will be easy to prepare in bulk, and may overcome antigenic variation which is often a problem in conventional vaccine production. These protein molecules will be small and may have to be coupled to a larger molecule to be immunogenic. The addition of adjuvants will probably be necessary to confer lasting immunity.

Researchers at the Health Department's Center for Laboratories and Research in Albany, N.Y. have spliced foreign viral genes into vaccinia virus, which can ultimately be used as a vector for vaccination (58). Vaccinia virus is used for smallpox vaccination and is large enough for genetic manipulation. The vaccinia virus expresses the characteristics of the foreign virus and by inserting the DNA of several viruses into vaccinia, vaccines could produced which confer immunity to several different diseases at once. Animals tested with these vaccines produce large amounts of antibodies, but actual protection from disease has not yet been proven. One disadvantage of

this method of vaccination is that vaccinia vaccine is rarely associated with severe complications. Thus far, this research has dealt exclusively with viral preparations, but may eventually be applied to bacterial preparations as well.

The third possibility for synthetic vaccine production is to synthesize peptides of an infectious agent by chemical or enzymatic methods. DNA sequencing methods are rapid and efficient for reading the primary chemical sequences of genes. Originally, it was thought that synthetic peptides must be constructed with the same complexity and tertiary conformation as the native protein in order to elicit antibodies that could react with the native protein. Therefore, chemical sequences seemed impractical because sequencing of large biologically important proteins would be a tedious process. Recently, however, it has been shown that it is not necessary to reproduce the exact tertiary conformation, and small fragments of a protein can elicit antibodies to the intact protein (53) (50). In order to develop a vaccine of this sort, immunologic studies are necessary to identify which proteins are the target of an immune response. The amino acid sequence of the protein is determined and peptides are synthesized for use as possible immunogens. This alternative vaccine has an advantage over biologically synthesized vaccines in that it contains no contaminants by any biological substances, including genetically engineered proteins from other bacteria. Currently, the synthetic vaccines being studied in animals

are directed against viral pathogens and are not yet suitable for human use. As the research progresses, these methods may be applied to the production of vaccines directed against bacterial pathogens. If the immunogenic peptides of B. pertussis can be identified, isolated and sequenced, a synthetic vaccine may ultimately be the answer to the pertussis vaccine dilemma.

XIV. Conclusion

Whooping cough is an acute, prolonged respiratory illness, which has plagued mankind for centuries. It is caused by B. pertussis, a tiny, non-invasive, human pathogen which is resistant to antibiotic therapy. The advent of an effective vaccine in the 1940s was followed by a dramatic reduction in the incidence and number of deaths attributed to pertussis. Approximately 2,000 cases and 5-20 fatalities are reported in the U.S. annually. Although the current vaccine is estimated to be 80-90% effective, its safety has been questioned in recent years. The vaccine is prepared from whole, killed B. pertussis organisms and has been associated with a high frequency of transient, non-damaging side effects and a lower frequency of brain damage and death. Use of the current vaccine should be approached cautiously with careful attention to the contraindications for its use. Research efforts are geared towards developing a safer, more effective vaccine which may eventually eradicate the disease. The most promising prospect for the immediate future is an acellular vaccine which will contain immunogenic components of B. pertussis and will delete the toxic factors which are responsible for the harmful effects of the whole cell vaccine. Several potential immunogens have been identified, such as lymphocytosis promoting factor and fimbrial hemagglutinin, which could be included in a component vaccine preparation. Japanese researchers have developed an acellular vaccine

which may be highly immunogenic and less toxic. Additional research and evaluation will be necessary before such a vaccine will become available in this country. Synthetic vaccines may be the ultimate solution to the vaccine dilemma in the more distant future. Despite the controversy surrounding the current pertussis vaccine, continued immunization is essential in controlling this potentially dangerous disease. Efforts to develop a safer, more effective, and universally accepted vaccine should proceed expeditiously. The eventual eradication of the disease is foreseeable, and when accomplished, will clearly benefit the world's children.

Appendix A - Individual components of *Bordetella pertussis*

Component	Biological Activities	Role in Immunity
Agglutinogens	used in typing strains of *B. pertussis* (different strains exhibit various combinations of agglutinogens)	?
Endotoxin	probably responsible for adverse effects of whole-cell vaccine	?
Heat labile toxin	inflammation and necrosis of respiratory tissue - not involved in infectious process	-
Pertussigen - also called: lymphocytosis promoting factor (LPF) mouse protective antigen (PA) islet activating protein (IAP) histamine sensitizing factor (HSF) pertussis toxin (PT)	lymphocytosis promotion, insulin potentiation, histamine sensitizing activity, monocyte chemotactic activity	+
Fimbrial hemagglutinin (FHA)	attachment to tracheal mucosa	+
Tracheal Cytotoxin	mimics pathogenesis of *B. pertussis* in respiratory tract	?
Adenylate Cyclase	inactivates macrophages	?
Outer Membrane Proteins	?	?

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IMMUNIZATION AGAINST

BORDETELLA PERTUSSIS

by

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AN ABSTRACT OF A MASTER'S REPORT

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Whooping cough is a severe, prolonged, and potentially fatal disease caused by the microorganism Bordetella pertussis. The disease affected 265,000 people and was responsible for about 8,000 deaths at its peak in the 1930s. Following the development of a vaccine in the 1940s, the incidence of whooping cough dropped dramatically, but it is not yet totally eradicated. Despite its success in effectively controlling disease and widespread epidemics, the current whole-cell vaccine has been associated with a high rate of side effects, a small percentage of which are severe or fatal. A worldwide controversy over the use of the vaccine has led to a decreased vaccine acceptance in several countries. This Master's Report presents a comprehensive study of the current core of knowledge regarding Bordetella pertussis, the disease it causes, and mankind's effective, but controversial attempts to prevent it. An in-depth appraisal of the risks versus benefits of vaccination leads to a discussion of pertussis research and new trends in vaccine development. An analysis of the pertussis research culminates in the conclusion that the most promising prospect for the immediate future is an acellular vaccine which will contain immunogenic components of B. pertussis and will delete the toxic factors. Additional commentary is included which hypothesizes that synthetic vaccines may be the ultimate solution to the vaccine dilemma in the distant future. Conclusions drawn from the report are that continued immunization with the current whole-cell vaccine, despite some concomitant adverse

effects, remains essential in controlling this potentially dangerous disease pending the foreseeable development of a component vaccine.