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The Immunological Basis for Immunization Series

Module 4:

Pertussis

GLOBAL PROGRAMME FOR VACCINES AND IMMUNIZATION
EXPANDED PROGRAMME ON IMMUNIZATION

World Health Organization
Geneva

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Module 4:

Pertussis

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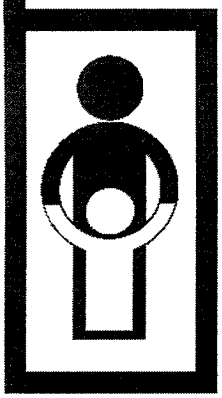
Expanded Programme on Immunization



GLOBAL PROGRAMME FOR VACCINES AND IMMUNIZATION
EXPANDED PROGRAMME ON IMMUNIZATION

World Health Organization
Geneva

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Module 1: General Immunology
Module 2: Diphtheria
Module 3: Tetanus
Module 4: Pertussis
Module 5: Tuberculosis
Module 6: Poliomyelitis
Module 7: Measles
Module 8: Yellow fever

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Preface

This series of modules on the immunological basis for immunization has grown out of the experience of persons working with the WHO Expanded Programme on Immunization (EN). The EPI was established in 1974 with the objective of expanding immunization services beyond smallpox, with emphasis on providing these services for children in developing countries.

Six vaccine-preventable diseases have been included within the EPI since its beginning: diphtheria, measles, pertussis, polio, tetanus, and tuberculosis. To protect newborns against neonatal tetanus, tetanus toxoid is administered to the mother either during her pregnancy or prior to pregnancy during the childbearing years.

Two more vaccine preventable-diseases will be addressed by the EPI during the 1990s. The World Health Assembly has set the target of including yellow fever vaccine in the EPI by 1993 in countries where this disease poses a risk. Hepatitis B vaccine is being added gradually, with the target date of 1997 for incorporation of this vaccine in the immunization programme in all countries.

Titles of the nine modules in this series are listed inside the front cover of this module. They are intended to provide information on the immunological basis for WHO-recommended immunization schedules and policies. They have been prepared for the following main audiences:

- immunization programme managers, whose questions and concerns caused this series to be written,
- consultants and advisers on immunization activities,
- teachers of courses on immunization at the university level and facilitators of workshops,
- medical and nursing students as part of the basic curriculum,
- laboratory scientists providing diagnostic or research services for vaccine-preventable diseases, and
- scientists involved in basic research aimed at improving the delivery of vaccines or providing improved vaccines.

Other modules in this series and additional materials on the EPI are available from the Expanded Programme on Immunization, World Health Organization, 1211 Geneva 27, Switzerland.

Pertussis

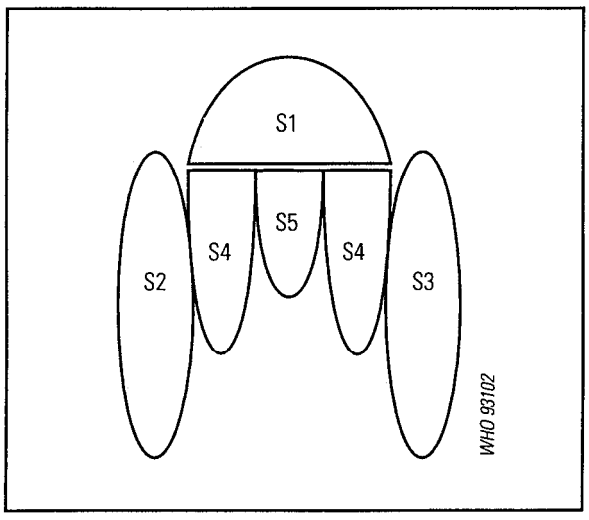
1. Antigens of Pertussis Organisms

Bordetella pertussis is a pathogenic organism with multiple biological activities. The first phase of pertussis infection is characterized by attachment of *B. pertussis* to the ciliated epithelium of the respiratory tract. The second phase of infection is thought to be the result of toxin(s) secreted by the organism. Recent studies on the immunochemistry of *B. pertussish*ave resulted in the isolation and characterization of several biologically active substances which are important in understanding of the pathogenesis of pertussis and the determinants of immunity after disease and vaccination. This knowledge has contributed to the development of acellular pertussis vaccines and to the improvement of the serological diagnosis of pertussis.

1.1 Pertussis toxin

The main toxin of the pertussis organism, pertussis toxin (PT), is responsible for different biological activities and, accordingly, is called lymphocyte promoting factor (LPF), histamine sensitizing factor (HSF), and islet activating protein (IAP). The name PT is used throughout this review. PT is a typical A-B toxin which is composed of two main subunits: an enzymatically active A (or S1) subunit and a B oligomer; which binds to receptors on target cells (Figure 1). The B oligomer is made of subunits S2 to S5. While the B oligomer is not toxic, it is necessary for efficient binding of the toxin to cells and it allows the Sl enzymatic subunit to reach its site of action within the target cell.

Figure 1. Schematic structure of pertussis toxin. The S1 subunit protein is responsible for the enzymatic activity of the toxin. The S2-S5 subunits form the B oligomer, which binds to receptors on target cells.



PT is a potent toxin which causes a variety of effects *in vitro* and *in vivo.* PT has been postulated to be the toxin responsible for most of the systemic symptoms of disease in patients with pertussis *(Pittman 1979).* PT induces production of antibodies, or antitoxins, which may be responsible for prolonged immunity to the disease. PT can be converted to nontoxic but immunogenic pertussis toxoid.

1.2 Adenylate cyclase

The second toxin of *B. pertussis* is adenylate cyclase (AC), an enzyme that is secreted in high concentration into the extracytoplasmatic space. AC toxin enters a variety of mammalian cells and can inhibit, probably together with PT, the microbicidal cytotoxic function of neutrophils, monocytes, and natural killer cells *(Thomas et al. 1989).* Its contribution to clinical pertussis may be through impairment of host defenses or through a direct effect on the respiratory mucosa *(Hewlett 1990).* AC is produced during pertussis infection in humans and it induces production of high titers of anti-AC antibodies that may persist into adulthood. These antibodies are also produced after vaccination, but in lower titers (*Farfel et al. 1990).*

1.3 Lipopolysaccharide endotoxin and other toxins

Other toxins of the pertussis organism are a lipopolysaccharide endotoxin (LPS) common to other gram-negative bacteria, a dermonecrotic heat-labile toxin (HLT), and tracheal cytotoxin (TCT). These toxins have been shown to cause a variety of toxic

effects in tissue cultures and laboratory animals, but so far they are not considered to be protective antigens. LPS is responsible for some of the adverse reactions in children following pertussis immunization and it has antigenic (although not protective) properties. The amount of LPS in whole cell pertussis vaccine has been shown to have a statistically significant association with the frequency of fever after vaccination *(Baraff et al. 1986).*

1.4 Filamentous hemagglutinin

Filamentous hemagglutinin (FHA) is a large-molecular-weight surface protein. It was originally thought, probably erroneously, to be associated with the bacterial fimbriae, but now FHA is considered to be a component of the cell envelope. FHA is a non-toxic antigen which probably plays a part in the initial colonization phase of infection by mediating the adhesion of *B. pertussis* to the ciliated epithelium of the upper respiratory tract. Antibodies to FHA confer short-term immunity.

1.5 Agglutinogens

Many authors believe that serotype-specific agglutinogens (AGG) are among the surface components of *B. pertussis* which are important factors in conferring immunity to the disease by mediating adhesion to the respiratory mucosa. It is now generally believed that there are three major agglutinogens, AGGl, 2 and 3 (these occur in nature in combinations 1-2, l-2-3, and l-3), while AGG4, 5, and 6, if they exist at all, are probably minor antigens *(Robinson et al. 1985).* Since the presence of AGG1, 2, and 3 in whole cell pertussis vaccine is believed to contribute to its protective efficacy, the WHO requirements for pertussis vaccine use a test to verify the presence of such agglutinogens in the vaccine *(WHO 1990).* Most manufacturers use several strains of *B. pertussis* to ensure the presence of all three types of agglutinogen *(Kudelski et al. 1978),* although some manufacturers base their production on only one strain *(Huovila et al. 1982).* Agglutinogens induce antibodies, called agglutinins, which cause bacterial cell agglutination. The presence of these antibodies in high titers in human serum has been correlated with clinical protection, although a relation between immunity and agglutinin presence has not always been found.

1.6 Other antigens

There is some evidence that response to antigens other than PT, FHA, AC, and agglutinogens may contribute to immunity after infection or vaccination. Some envelope-associated proteins of *B. pertussis* occupy an exposed location on the bacterium and therefore would be readily accessible to the host immune system. Some of these proteins may perform critical functions, either in mediating adhesion or in exchange of metabolites with the environment. Antibodies are elicited to several envelope proteins from *B. pertussis* after disease and after immunization with whole cell pertussis vaccine *(Arciniega et al. 1992).* An additional antigen of current interest is the 69-kd outer membrane protein *(Shahin et al. 1990).* The role of this component in disease pathogenesis is unknown. The human antibody response to individual antigens found in the complex mixture of envelope-associated proteins warrants further studies to determine what role, if any, these antigens play in immunity to pertussis.

Further understanding of the role of particular components of *B. pertussis* in the pathogenesis and immunity to the disease is impaired by the lack of an animal model which is equivalent to clinical pertussis in humans.

2. Antigens in Whole Cell Pertussis Vaccines

Whole cell pertussis vaccines contain PT, AC, LPS, FHA, and AGG antigens. The amount of LPS in whole cell pertussis vaccines ranges from 0.9 to 2.8 µg per ml and most of the LPS has been found to exist as free (not cell-bound) toxin. The release of LPS from cells during storage of vaccine is quite rapid; in the first few weeks 35% to 50% of the LPS is released and after 5 to 6 months 60% to 80% of LPS is released *(Ibsen et al. 1988).*

Considerable variation has been found in the amount of FHA and PT in different whole cell vaccines. Measured as antigen, FHA ranges between 0 and 1.6 µg per dose and total PT has been reported to be in the range of 0.02 to 0.68 µg per dose *(Ashwort et al. 1983; Bernier 1982).* The amount of AGG2 in Wellcome whole cell pertussis vaccine was estimated to be 4.7 µg per single dose *(Ashwort et al. 1983).*

The amount of pertussis antigens is low compared with the levels of protein in the tetanus and diphtheria toxoids in a dose of DPT vaccine. The purity of the toxoid components is about 1500 Lf per mg of protein nitrogen. A dose of DPT vaccine contains 20 Lf of diphtheria toxoid and 10 Lf of tetanus toxoid. These amounts of toxoid provide 80 µg of diphtheria antigen and 40 µg of tetanus antigen per vaccine dose.

3. Techniques for Measuring Antibody Response

Even though whole cell pertussis vaccine has been used successfully for several decades, there still

is no reliable measure of immunity to pertussis. Although many of serological techniques have proven useful as diagnostic procedures, it is unclear whether any of them is sensitive and specific as a measure of immunity to pertussis. The bacterial agglutination test that has been used for many years does not necessarily correlate with immune status. New assay techniques have been developed for the measurement of antibody to well-defined *B. pertussis* antigens that develop after immunization or natural disease. Their usefulness as a measurement of immunity is not yet proven.

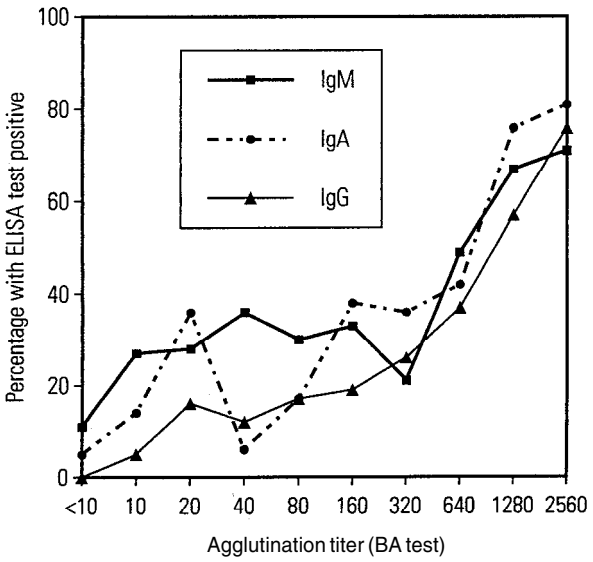
3.1 Bacterial agglutination test

The bacterial agglutination (BA) test was the first method developed to measure pertussis antibody and it is still the most frequently used method. It uses a simple technique for measuring antibodies induced by the agglutinogens of the antigenic form of *B. pertussis,* designated phase I. Freshly recovered, encapsulated pertussis bacteria generally belong to phase I. Passage of pertussis bacteria in culture may result in variant forms, which are deprived of immunogenic antigens and designated as phase II, III, or IV organisms. Phase I strains are required for transmission of disease and production of an effective vaccine. Early studies of Miller et al. *(1943)* and Sako *(1947)* suggested some correlation of agglutinins with immunity; vaccinated children with agglutinin titers 1:320 and higher (by the macro-technique) were protected from household exposure to pertussis. Recent studies have neither confirmed nor refuted this observation, which remains the basis for the recommendation by some authors to use the agglutinin response in evaluating vaccine efficacy *(Fillaster & Guerin 1987, Wilkins et al. 1987).*

However, vaccination or recovery from pertussis does not always induce agglutinins and some individuals lacking antibody are protected. One of the vaccines used in the 1950s in the Medical Research Council trial (based on sonically disintegrated pertussis bacteria called Pillemer antigen — see section 7) was shown to provide strong protection in children, but it had a weak capacity to stimulate production of agglutinins in mice and children *(MRC 1959).* The measured agglutinin titers seem to be markers of protection, rather than protective antibodies.

The BA test suffers from low sensitivity and it has not been standardized. The agglutinin titers strongly depend on the bacterial strain used in the agglutinogens *(Blumberg et al. 1992).* BA antibodies correlate best with IgG and IgA antibodies determined by the ELISA test. There is a better correlation between the results of these tests when the BA titer is above 1:320 than at lower BA titers (Figure 2).

Figure 2. Comparison of BA test and ELISA test for pertussis antibody in human sera *(Mertsola et al. 1983)*



3.2 Enzyme-linked immunosorbent assay

The enzyme-linked immunosorbent assay (ELISA) uses purified protein antigens of *B. pertussis* (such as FHA, PT, or AGG) to measure serum IgG, IgM, and IgA responses following disease or vaccination *(Ashwort et al. 1983, Baraff et al. 1984, Burstyn et al. 1983, Granstrom et al. 1982, Granstrom et al. 1988, Mertsola et al. 1983, Stroffolini et al. 1989, Thomas et al. 1989b, Zackrisson et al. 1990).* The ELISA test is sensitive, specific, relatively cheap, and requires only a small amount of serum. However, the accuracy of the test depends on the purity of the antigens involved. With mixed preparations (whole bacteria, sonicate or extract of bacteria), it is not possible to identify the particular antigens to which the antibody response is directed *(Thomas et al. 1989a).*

3.3 *In vitro* neutralization test

The *in vitro* neutralization test (NT) is conducted in a microplate culture of Chinese hamster ovary (CHO) cells. PT induces a distinct cytopathogenic effect with clustering of CHO cells in the microplate culture. Only a small amount of PT (about 1 ng) is needed to produce the clustering of CHO cells. This property of PT allows the *in vitro* neutralization test to measure antibodies neutralizing pertussis toxin *(Gillenius et al. 1985, Granstrom et al. 1985).* The NT is laborious, requires tissue culture facilities, and involves subjective readings. The NT is significantly less sensitive for the diagnosis of pertussis than the determination of the IgG response to PT by ELISA. Furthermore, not all patients develop measurable neutralizing antibodies after clinical and culture-confirmed whooping cough *(Granstrom et al. 1988).*

3.4 Techniques not widely used

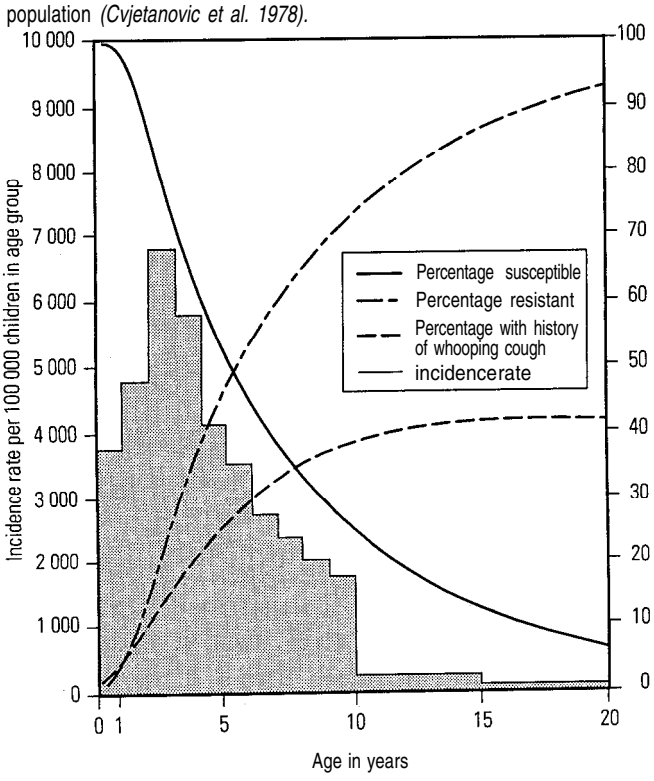
There are other techniques to measure pertussis antibody response, but these are not widely used. They include:

- Passive protection of mice against *B. pertussis* infection by serum antibody. This method is expensive, requires mice, and is poorly reproducible.
- An immunoblot technique in which antigens, separated electrophoretically, are allowed to react with antibodies that are then reacted with I^{125}-labelled antibody to human immunoglobulin and autoradiographed *(Thomas et al. 1989a).*
- Indirect hemagglutination, bactericidal reaction, immunodiffusion, and complement fixation (for a review see *Onorato and Wassilak 1987).*

4. Development of Antibodies Due to Natural Stimulation

The natural course of the disease is influenced by the age-specific proportion of susceptible and resistant persons in the community (Figure 3). Although no specific antibody against antigens of *B. pertussis* has been convincingly shown to be responsible for the immunity against the disease, the prevalence of these antibodies at different ages may be a valuable index of the exposure to pertussis antigens.

Figure 3. Simulated picture of pertussis epidemiology in a non vaccinated population *(Cvjetanovic et al. 1978).*



4.1 Passage of antibodies through the placenta

Newborns acquire antibodies passively from their mothers. IgG antibodies against FHA, PT, AGG2, and AGG3 have been detected in cord serum or in serum from healthy children before their first DPT immunization. These are thought to represent transplacentally acquired maternal IgG *(Baraff et al. 1984, Granstrom et al. 1982, Thomas et al. 1989b, Van Savage et al. 1990).* The infant's pertussis IgG antibody level against PT and FHA is comparable to the corresponding maternal level *(Van Savage et al. 1990).* In an early study, attempts were made to protect newborns against pertussis through maternal immunization. The mothers were immunized with 6 doses of unadsorbed pertussis vaccine, with a total dose of 150 million pertussis organisms. Most of the newborns showed agglutinin and mouse protective antibody titers equal to or higher than their mothers *(Cohen & Scadron 1943).* In most of the early studies with unabsorbed vaccine, the total dosage in terms of volume and numbers of organisms was large by today's standard.

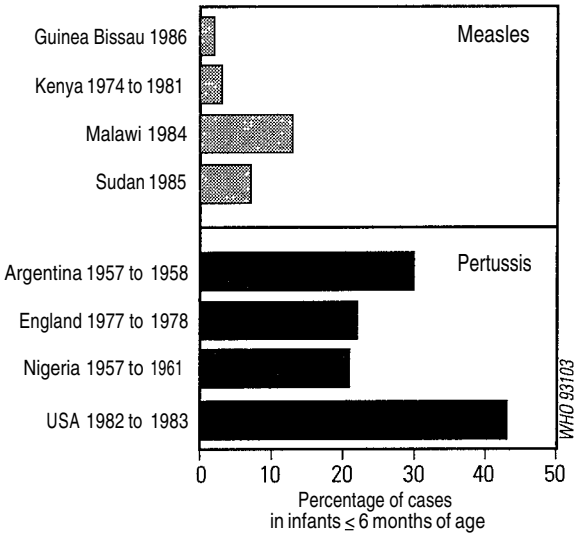
Only 5% of infants have IgA pertussis antibody *(Thomas et al. 1989b)* and there are no IgM antibodies detected in cord blood *(Baraff et al. 1984).*

Pertussis agglutinins are found in the cord serum in varying concentrations. In Los Angeles, USA, the titers of agglutinins in cord serum ranged from 1:30 to 1:50 *(Baraff et al. 1984).* In Poland, agglutinins were seen in 33% of newborns, but the titers were low with the average titer around 1:4 *(Adonajlo et al. 1971).*

Although there is placental passage of some pertussis antibodies, infants do not seem to be protected against clinical disease during the first months of life. The susceptibility of small infants to life-threatening pertussis has been well documented. There is a high incidence of pertussis in the first six months of life (Figure 4). This contrasts with the low incidence of measles in the first six months, which is due to passively acquired maternal antibodies against measles.

Anti-pertussis antibodies were found in samples of human milk in Nigeria and the United States. IgG antibody levels were higher in maternal sera than in breast milk. On the other hand, the mean IgA antibody levels to pertussis (as well as to *Hemophilus influenzae type b, Streptococcus pneumoniae, and Neisseria meningitidis)* were higher in breast milk than in either maternal serum or infant serum *(Kassim et al. 1989).* In Indonesian women 87% of colostrum samples had at least one of the pertussis antibodies — agglutinins, anti-PT, or anti-FHA. Colostrum containing anti-PT antibodies or agglutinins was able to protect suckling mice from aerosol challenge with *B. pertussis,* whereas colostrum lacking these antibodies

Figure 4. Percentage of measles and pertussis cases in infants aged 6 months and below *(Aaby et al. 1986, CDC 1984, EPI 1982, 1986, Morley et al. 1966, Robinson et al. 1981, Wahab et al. 1988, Zamora et al. 1962)*



but containing anti-FHA gave Iittle protection *(Oda et al. 1985).* The significance of pertussis antibodies in milk in enhancing an infant's immunity to pertussis is not known.

4.2 Development of natural antibodies

Passively acquired antibodies fall to a nadir after several months *(Baraff et al. 1984, Grumtrom et al. 1982, Van Savage et al. 1990).* At the age of six months only 4% of children have neutralizing pertussis antibody *(Blennow et al. 1988).* The half-life of anti-PT, anti-FHA, and agglutinin antibodies is estimated to be 36, 40, and 55 days, respectively *(Van Savage et al. 1990).*

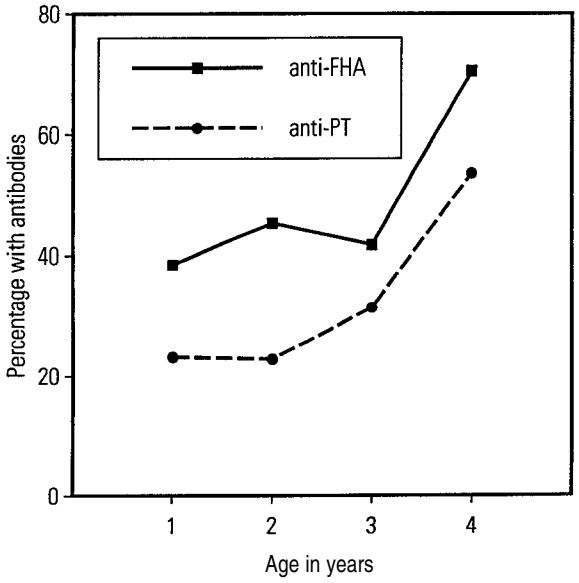
The prevalence of pertussis antibody in various age groups in the general population depends on the status of immunization against pertussis in childhood and the extent of exposure to circulating pertussis organisms.

Serological studies show that the proportion of persons with measurable IgG antibodies against pertussis toxin increases with age, reflecting contact with *B. pertussis* organisms. The rate of this increase is different in various countries. The proportion of seropositives exceeds 80% by the age of 10 years in Cameroon *(Stroffolini et al. 1991),* while only 40% have measurable antibody by the age of 15 years in New Zealand *(Lau 1989).* In New Zealand, the percentage of persons with ELISA IgG antibody against pertussis toxin increased with age, from 16% in 5 year olds to 63% in the 40 to 49 year age group. The percentage of individuals with antibody dropped to 45% in the 50 to 65 year age group. A low percentage of young children with antibody to pertussis is considered cause for concern, since this may reflect a potential reservoir for disease *(Lau 1989).*

In Sweden, where vaccination against pertussis was stopped in 1979 and the pertussis incidence rate has been very high for more than decade, the prevalence of antibodies in children below five years of age increases with age. There is a good correlation between the presence of antibodies and a history of whooping cough. Anti-FH4 antibodies are more commonly found than anti-PT antibodies (Figure 5). The data may indicate that exposure to other Bordetella species might be important for development of anti-FHA antibodies or that many infections with *B. pertussis* are subclinical or atypical *(Zackrisson et al. 1990).* In another study, only 25% of children had measurable titers of ELISA IgG anti-FHA antibody at the age of two years, even though at least 80% of them had been vaccinated with DPT vaccine in infancy. Antibody titers reached a high level in older teenagers, and 90% of young adults had measurable antibody titers *(Granstrom et al. 1982).* This is in agreement with the results of studies in the pre-vaccination era,, which showed that a high proportion of children had experienced pertussis infection by age 10 *(Fine & Clarkson 1984).*

In Palermo, Italy, where the coverage rate with DPT vaccine is very low, the results of a seroepidemiological study suggest a high exposure of children to *B. pertussis,* resulting in increasing rates of seroprevalence of IgG anti-PT antibodies with age. The overall prevalence of these antibodies determined by the ELISA test was 56%; it increased from 24% in 1 to 3 year old children to 67% in 11 to 12 year old children *(Stroffolini et al. 1989).*

Figure 5. Percentage of unvaccinated children with antibodies to FHA and PT, Sweden 1984 to 1986 *(Zackrisson et al. 1990)*



A study of serum samples collected at the end of the 1960s and the beginning of the 1970s from several countries shows two different patterns of agglutinin titers *(Maixnerova et al. 1979).* In one group of countries (Algeria and Yugoslavia) the frequency of positive titers was highest in preschool and early school children, with a downward trend afterwards. In the second group of countries (Afghanistan, Kenya, Mongolia, and Niger) there was no clear elevation of titers in early childhood, but titers increased gradually with age, reaching a high level of positivity in older ages.

In some European countries, a decreasing seroprevalence of pertussis agglutinins has been observed with age. In Czechoslovakia, the percentage of seropositivity decreased from 81% in persons 15 to 19 years to 16% in persons 30 to 34 years. These data show that at least some individuals have long-term persistence of agglutinins following a 6-dose immunization schedule, with the last dose given at 6 years of age *(Celko et al. 1984).*

In Poland, the highest BA levels were observed in children aged 3 months to one year; the proportion with a titer 1:40 or higher was 60%, while the proportion with a titer of 1:160 or higher was 29%. In older Polish children BA titers declined considerably, regardless of vaccination history. Only 7% of persons aged 15 to 19 years had a BA titer of 1:160 or higher. On the other hand, the percentage of sera with high titers of antibodies against *B. parapertussis* increased with age, from 8% in the youngest children to 51% in older age groups *(Adonajlo et al. 1971).*

These observations may reflect differences in the development of immunization programmes and changes in pertussis epidemiology. It is possible that success in controlling pertussis by immunization has altered its epidemiology by reducing the likelihood of repetitive casual exposure to pertussis and consequent reinforcement of the immune response *(Mortimer 1991).* In countries where the incidence of pertussis has been reduced by mass immunization, the highest seroprevalence is observed in age groups covered by vaccination. In older groups, the rate of seropositivity declines depending on the time of administration and the number of doses of vaccine given. In countries where the coverage rate with pertussis vaccine is low and pertussis organisms circulate freely, seroprevalence increases with age, reflecting the high proportion of infection with pertussis organisms.

The development of pertussis antibodies following pertussis disease has been studied by various authors *(Aleksandrowicz & Pstragowska 1980, Granstrom et al. 1988, Nagel & Poot-Scholtens 1983).* There is a significant rise of ELISA IgG titers to PT and FHA. A combination of these two determinations and the ELISA IgA for FHA seems to be the most sensitive method for diagnosing pertussis in unimmunized children *(Granstrom et al. 1988, Nagel & Poot-Scholtens 1983).* The presence of serum IgA antibodies indicates natural contact with *B. pertussis* or related bacteria. In infants, 6 to 7 weeks is needed for the serum IgA antibody to reach a high level *(Nagel & Poot-Scholtens 1983).*

5. Development of Antibodies Following Vaccination with Whole Cell Vaccine

5.1 Response to whole cell vaccine

Vaccination results in an increase in the ELISA antibody titers to all known antigens of *B. pertussis* organisms. Children vaccinated with whole cell pertussis vaccine show increasing levels of antibodies against FHA, PT, AGG, LPS and outer membrane protein *(Ashworth et al. 1983, Baraff et al. 1984, Barkin et al. 1984, Blumberg et al. 1991).* The extent of the response is proportionate to the number of doses administered (Figure 6). Note the elevated level of antibodies to outer membrane protein (OMP) and lipopolysacharide (LPS) in sera of unvaccinated children, presumably against cross-reacting non-pertussis antigens *(Ashworth et al. 1983).*

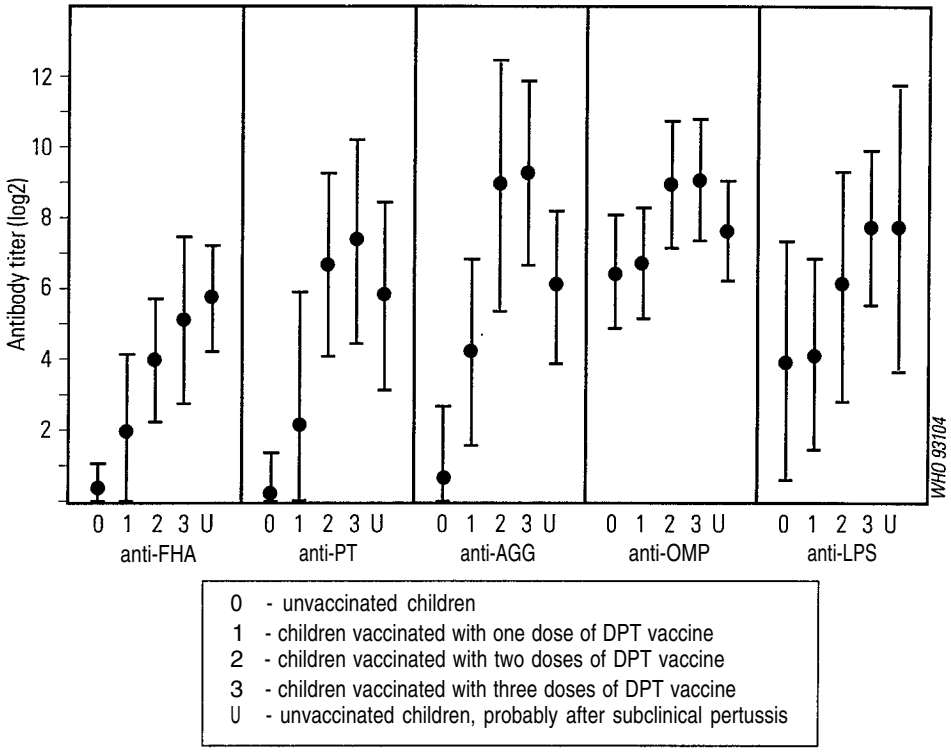
Neutralizing antibody also increases following immunization. Three doses of the whole cell vaccine caused a moderate response in neutralizing antibody titers, with 59% seropositive *(Blennow et al. 1988).* The mean titer of neutralizing antibodies against LPF (determined by the CHO assay) increased from 45 units/ml before immunization at 2 months of age to 407 units/ml one month after the third dose of DPT vaccine *(Blumberg et al. 1991).*

More than 70% of children respond to three doses of DPT vaccine containing the whole cell component with an agglutinin titer of 1:80 or more (by microtechnique, equivalent to 1:160 by macrotechnique) (Table 1).

5.2 Differences in antibody response to various whole cell vaccines

Whole cell pertussis vaccines from various manufacturers differ considerably in stimulating production of antibodies. A recent study performed at two centers in the USA showed that two commercially available whole cell vaccines consistently differed in their ability to induce antibody to PT. Infants receiving the Lederle vaccine produced a 46-fold increase in antibody to pertussis toxin, compared with a 2.4-fold increase for infants receiving the Connaught vaccine. The FHA and AGG responses to the two whole cell vaccines were comparable *(Edwards et al. 1991).*

Figure 6. Mean serum antibody response (±1 standard deviation) to filamentous hemagglutin (FHA), pertussis toxin (PT), agglutinogens (AGG), outer membrane protein (OMP) and lipopolysaccharide (LPS) following immunization with DPT vaccine containing whole cell pertussis component *(Ashworth et al. 1983).*



The mean agglutinin titer after three doses of DPT vaccine ranged from a high value of 1:1826 *(Barkin et al. 1984) to 1:87 (Blumberg et al. 1991).* In a study in France, three doses of DPT-polio vaccine (adsorbed on calcium phosphate) failed to stimulate an agglutinin level of 1:l0 in 25% of children and the mean titer was very low, 1:23 *(Relyveld et al. 1991).* These differences may be partly due to different techniques for determining agglutinin titers (including use of different bacterial strains as the test antigen) or to different techniques used in vaccine production. It is also possible that whole cell vaccine produced in fermentors could contain lower amounts of surface antigens than vaccine produced under stationary conditions *(Relyveld et al. 1991).*

Table 1. Pertussis agglutinin response to DPT vaccine in infants who received the first dose of DPT vaccine at 4 to 20 weeks of age *(Wilkins et al. 1987).*

	Percentage of children responding one month after		
Agglutinin titer	**1 dose**	**2 doses**	**3 doses**
< 1:10	78	12	2
1:10 to 1:40	21	35	25
≥ 1:80	1	53	73

5.3 The age for beginning immunization

Pertussis vaccine should be administered at a time prior to exposure, yet at an age when the infant is capable of responding *(Cherry et al. 1988).* A review on the early immunization of infants with DPT vaccine concluded that although pertussis antibodies passively acquired from the mother may modify or block the immune response during the first few weeks of life, immunization with a primary series of three doses of whole cell pertussis vaccine beginning after four weeks of age should result in a satisfactory antibody response and protection against disease *(Halsey & Galazka 1985).*

Age is an important factor influencing the serological response to whole cell pertussis vaccine. Antibody titers are generally higher with increasing age of immunization. Very early administration of pertussis vaccine does not produce advantageous results. A dose of DPT vaccine given at the time of nursery discharge (mean age 3.5 days), in addition to a routine series of three doses given at 2, 4 and 6 months of age, did not produce better agglutinin, anti-PT, and anti-FHA responses than three standard doses of the vaccine *(Baraf et al. 1984).* One group of investigators has suggested that immunization within 24 hours of birth may result in "immunologic paralysis" *(Provenzano et al. 1965).*

Several studies have shown that the ability of an infant to produce serum IgG anti-PT after immunization with pertussis vaccine is inversely related to the cord blood serum IgG anti-PT titer. A good antibody response is observed in infants with low cord blood titers, and a poor antibody response is seen in infants with high cord blood titers *(Baraff et al. 1984, Burstyn et al. 1983).* The same relationship between between preimmunization titer and response to the vaccine has not been universally observed; in a recent study, infants with high preimmunization of ELISA antibody against PT had a higher response to three doses of DPT vaccine than those with low preimmunization values *(Blumberg et al. 1991).*

The neutralizing antibody response to whole cell vaccine is influenced by the prevaccination titers. Vaccinees with lower preimmunization anti-PT neutralizing antibody titers have higher anti-PT neutralizing antibody values after immunization than subjects with high preimmunization antibody values *(Blumberg et al. 1991, Granstrom 1985).* In contrast, the preimmunization level of antibodies does not influence the immune response to the acellular pertussis vaccine (see section 7).

The optimal agglutinin response to DPT vaccination is best achieved by beginning vaccination late, at five months of age or later, with the second dose given at an interval of 8 weeks or more (Wilkinson *et al.* 1987). However, delaying the schedule for DPT vaccine results in the occurrence of additional cases of the disease among young infants not protected by early immunization *(Funkhouser et al. 1987).* The optimal immunization schedule is therefore a reasonable compromise between immunological principles and epidemiological needs.

5.4 Number of doses and interval between doses

As mentioned above, the pertussis response is proportionate to the number of doses administered. Epidemiological observations on the efficacy of pertussis vaccine suggest that three doses are needed to provide effective immunity at an early age *(Fine & Clarkson 1987).* Since longer intervals between doses result in a better immune response, several studies have compared two- and three-dose regimens to assess the interval between doses that will produce an adequate antibody response.

A two-dose regimen of DPT vaccine given with an interval of more than 60 days between doses has been shown to induce an agglutinin response comparable to that of 3 doses given one month apart *(Wilkins et al. 1971).*

In another study, the agglutinin response of Kenyan children to two DPT doses 6 months apart was compared with that of children receiving three DPT doses 3 months apart. There was no difference in titers between the two- and three-dose groups at one month after the last immunization, but waning of antibodies was more rapid in the two-dose group than in the three-dose group. The differences were statistically significant after 2 years *(Muller et al. 1984).* In a similar study in India, pertussis agglutinin responses were similar after two-dose (two month interval) and three-dose (monthly intervals) schedules, but the diphtheria and tetanus titers were significantly higher after the three-dose regimen *(Bhandari et al. 1981).* Two doses of DPT polio vaccine administered in 3 to 24 month old Senegalese children at a six month interval, with or without simultaneously administration of hepatitis B vaccine, induced agglutinin titers of 1:80 in only 40% of children *(Coursaget et al. 1986).*

Two doses of DPT cannot be recommended for a routine immunization programme where the first dose is administered early, e.g. at 6 weeks to 2 months of age. It is possible that passively transmitted antibody could suppress the response if the first DPT dose is delivered early. This might lead to an unsatisfactory level of immunity against all three components of DPT vaccine following a two-dose schedule.

Table 2. Immunization schedules for DPT vaccine used in countries of the six WHO Regions *(Galazka 1992).*

WHO Region	Schedule for primary series of 3 DPT doses				Schedule for booster doses	
	6, 10 and 14 weeks or 2, 3, and 4 months	3, 4, and 5 months or 4, 5, and 6 months	2, 4, and 6 months	3, 5-6, and 7-15 months	12-24 months	3-6 years
	Percentage of countries using a given schedule					
Africa	64	36	–	–	31	–
Americas	19	10	57	14	57	33
Eastern Mediterranean	30	25	30	15	45	5
Europe*	11	48	15	18	63	11
South East Asia	64	36	–	–	9	9
Western Pacific**	23	35	23	16	48	35

** No pertussis vaccine is given in Sweden. In Denmark, monovalent pertussis vaccine is given at 5 weeks, 9 weeks, and 10 months of age.*
*** In 5 countries in the Western Pacific Region, the DPT booster dose is given at school entry as a fourth dose.*

There have been efforts to give reduced doses of DPT vaccine in full term and preterm infants in order to lessen local and febrile responses *(Bernbaum et al. 1989, Rogers 1990, Warfield 1989).* However, there is no evidence that serious reactions to pertussis vaccine (encephalopathy, convulsions) are dose-related or that they would be less frequent with reduced doses. The agglutinin response was lower in children receiving a half-dose of DPT vaccine compared with those receiving a standard dose *(Bernbaum et al. 1989).* This difference disappeared after a booster dose *(Barkin et al. 1984).* Giving smaller doses does not appear to be prudent and is not recommended *(Cherry et al 1988, Wassilak et al. 1985).*

Giving the second or third dose in a fractionated manner (0.2 ml and 0.3 ml at an interval of 2 to 4 days) does not improve the immune response to any of the vaccine components *(Vodickova & Svandova,* 1986). Delivery of split doses also adds the inconvenience of additional injections and is not recommended.

5.5 Duration of immunity after different immunization schedules

Many studies have provided strong evidence that the whole cell pertussis vaccines in recent use are effective in protecting the individual against typical pertussis, either by preventing its occurrence altogether or by markedly reducing its severity *(Griffith 1988).* However, the duration of immunity following pertussis vaccination is still an open issue. This is reflected in differences in the immunization schedules used. Usually, the primary series consists of three doses of DPT vaccine given during the first year of life. In the African and South East Asia Regions, most countries use the immunization schedule recommended by EPI, which calls for three doses of DPT vaccine at 6, 10 and 14 weeks; some countries use a 3, 4 and 5 months schedule (Table 2). In the American Region, the schedule used in the United States, with two month intervals, is mostly used.

It should be mentioned that for 14% to 18% of countries in the American, Eastern Mediterranean, European, and Western Pacific Regions, the third dose of DPT vaccine is recommended at a late age, some time after six months of age. Various immunization schedules used by countries in the European Region are shown in Figure 7.

Epidemiological observations suggest that the efficacy of pertussis vaccine is high only for a limited period of time and falls gradually with the time after immunization. In the United Kingdom, the vaccine

Figure 7. Immunization schedules for diphtheria, pertussis, and tetanus vaccines in various countries of the WHO European Region.

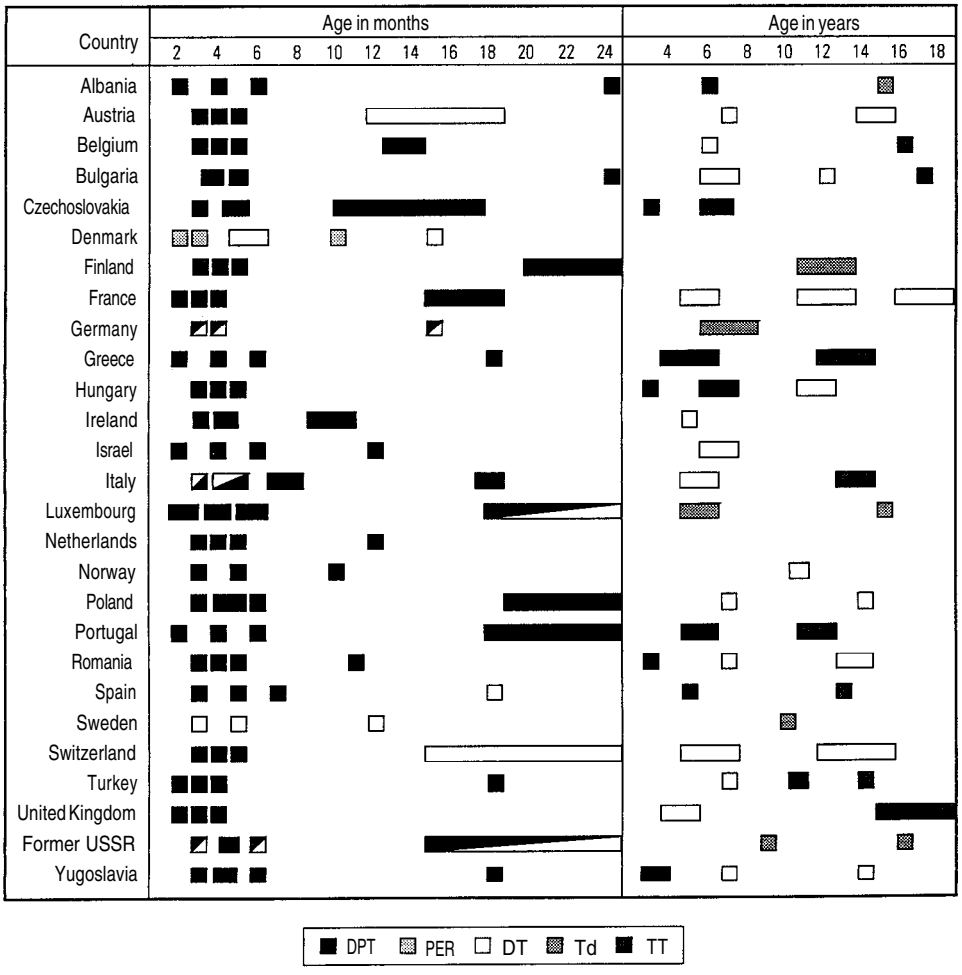
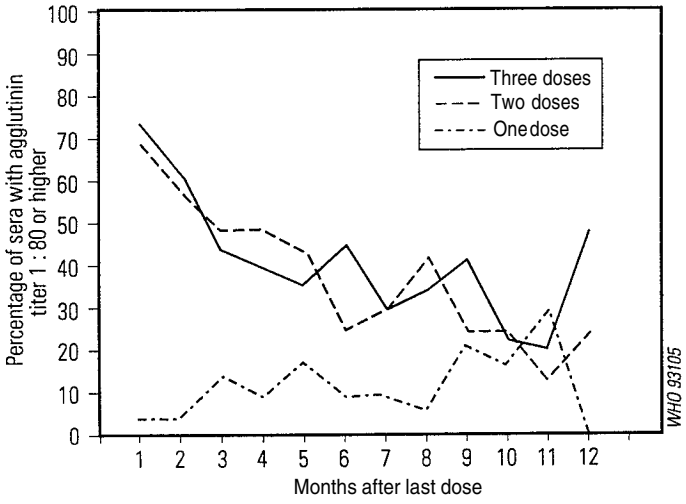


Figure 8. Agglutinin response after one, two, and three doses of whole cell DPT vaccine *(Wilkins et al. 1987).*



efficacy fell from 100% in the first year following three doses of DPT vaccine to 46% in the seventh year *(Jenkinson 1988).* In Sweden, the efficacy of three doses of unadsorbed whole cell pertussis vaccine declined from 89% in 6 to 11 month old children to 76% in children at the end of the second year of life *(Blennow et al. 1988).* Other cohort and case-control studies suggest a slight but continuous decrease of vaccine efficacy with time *(Fine & Clarkson 1987).*

Serological studies show a much steeper decline of postvaccination antibody levels against various *B. pertussis* antigens. Agglutinin titers decay to low levels by 12 months after the last dose of DPT vaccine (Figure 8). Levels of antibody against PT, FHA, and outer membrane proteins, as well as agglutinins and neutralizing antitoxins, decline considerably during the first year after the completion of a primary series *(Barkin et al. 1984, Blennow & Grandstrom 1989a, Blumberg et al. 1991, Edwards et al. 1991, Relyveld et al. 1991).*

The policy of using booster doses of DPT vaccine varies considerably. The United Kingdom recommends only a primary series of three doses of DPT vaccine with no booster, while in the United States two additional booster doses are administered at 1.5 months and 4 to 6 years of age. A fourth dose of DPT vaccine is recommended at 12 to 24 months of age by 9% to 63% of countries (Table 2). The situation in Europe is shown in Figure 7. The importance of a booster dose of DPT vaccine has been shown in Finland. In spite of high immunization coverage for the primary series of DPT vaccine (exceeding 90%) an outbreak of pertussis was reported in Finland from 1976 to 1977. An analysis performed in 1982 suggested that the most probable reason for this pertussis outbreak, mostly among children of school age, was very low coverage with the booster dose of DPT vaccine; only 25% of children had received the fourth dose before the age of four years. Results of the analysis suggested that primary immunization in infancy protects children against pertussis for about one year and that the booster immunization provides additional protection for two to three years *(Huovila 1982).*

Serological studies provide strong evidence for the booster effect of the fourth dose of DPT vaccine administered at the end of the second year of life. The antibodies against PT, FHA, and agglutinins increase significantly following the booster dose *(Barkin et al. 1984, Chen et al. 1957, Edwards et al. 1989, Lewis et al. 1986, Pichichero et al. 1987, Relyveld et al. 1991).* An example of the response of four different antibodies to the booster doses of DPT vaccine is shown in Figure 9. The extent of the antibody increase differs considerably; for example, the agglutinin titer rose from 1:100 to 1:7000 in one study and from 1:18 to 1:213 in another *(Barkin et al. 1984, Relyveld et al. 1991).* These differences may be related to the potency of vaccines used, the different schedules of immunization, and various methods of determining the level of antibodies.

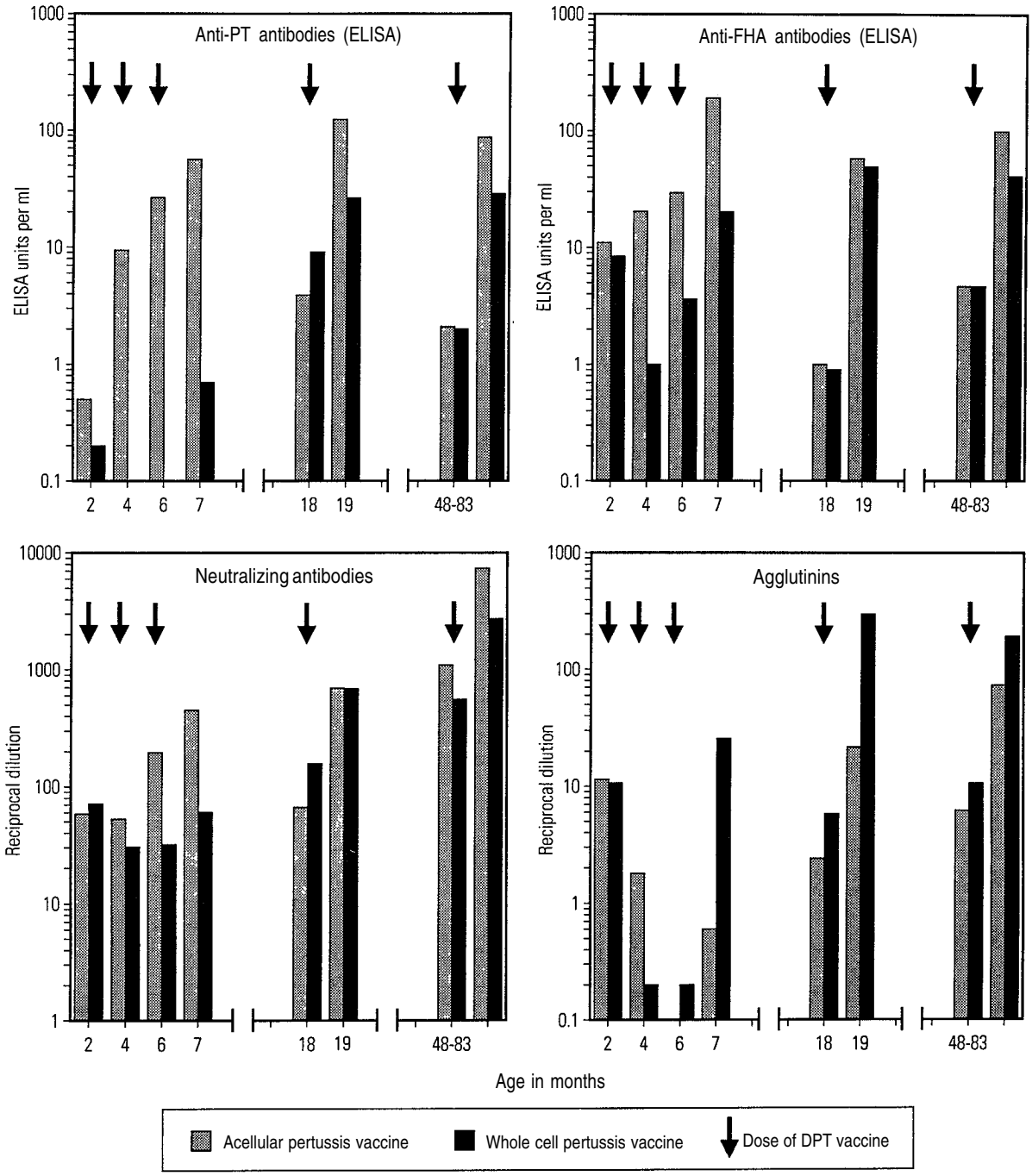
Several countries give a fifth dose of DPT vaccine ("second booster"). About one-third of countries in the American and Western Pacific Regions do so (Table 2). The need for a fifth dose of DPT vaccine and its importance in controlling pertussis in other regions remains to be proved. Serologically, this additional dose of DPT vaccine seems to exert a clear booster effect *(Edwards et al. 1989, Morgan et al. 1990).*

Recently, there have been several reports of pertussis in adults. This may reflect a shift in the age distribution of pertussis, with and an increasing proportion of adult cases as the disease is effectively controlled among children. On the other hand, sevela1 authors have expressed concern that pertussis immunity may be only partial among adults *(Fine & Clarkson 1987).* The decreased immunity among adults may be related to the reduced circulation of pertussis organisms in well vaccinated populations, with subsequent less frequent exposure to the pertussis bacteria and less natural boosting. The importance of late booster doses of pertussis vaccine may lie in maintaining immunity against pertussis in older children or adolescents.

6. The Need to Monitor Pertussis Vaccine Efficacy

During the past 15 years, successful programmes of immunization have been implemented in most countries of the world. In many countries, pertussis has gradually disappeared as a major health problem

Figure 9. The geometric mean antibody titer (GMT) to acellular and whole cell pertussis components of DPT vaccines given to infants as primary immunization and to 18 to 24 month and 4 to 6 year old children as booster doses *(Edwards et al. 1989)*



in infants and children. However, in some countries in the developed world (Germany, Italy, Russia, Sweden, and the United Kingdom) there has been a recrudescence of pertussis or its persistence *(Galazka 1992, Mortimer 1988).* The underlying causes have been apathy and complacency on the part of physicians and parents, negative attitudes toward immunization spread by anti-immunization pressure groups, and litigation over alleged vaccine-related injuries.

In the developing world, the main problem is high drop-out rates between the first dose and the third dose of DPT vaccine. Immunizations are often delayed, leaving infants unprotected during the most vulnerable period of the first six months of life.

Apart from problems with the delivery of pertussis vaccine, the use of potent pertussis vaccine is of prime importance in pertussis control efforts. According to the WHO requirements, pertussis vaccine should contain at least 4 international units of potency per dose, as determined in the intracerebral mouse protection test *(WHO 1990).* This standard is based on the correlation between protection in children and

the results of the mouse test, which was established by the Medical Research Council in the United Kingdom *(MRC 1959).* However, the intracerebral mouse test is technically difficult and its results vary widely. There is a continuing need to monitor vaccine efficacy. The differences in antigenicity of different whole cell vaccines discussed in section 5.2 may or may not have clinical significance. However, there are reports on the low clinical efficacy of locally produced pertussis vaccines in Canada *(Halperin 1989)* and South Africa *(Strebel 1991).* Such reports should be considered warning signals on the quality of pertussis vaccines.

Incorrect storage and administration as well as changes in the timing, frequency, and age at vaccination may also be associated with reduced vaccine efficacy. All these logistical issues should be strictly monitored.

7. Immunological Aspects of Acellular Pertussis Vaccine

7.1 The nature of acellular pertussis vaccine

The first acellular pertussis vaccine was probably the Pillemer vaccine. The Pillemer vaccine was prepared by sonic disintegration of *B. pertussis* and treatment of the cell extract with autoclaved human group O, Rh-negative red-cell stromata to give a stromata-antigen complex which contained only a small fraction of the whole bacterial cell. The Pillemer vaccine was tested in a British Medical Research Council field trial and it induced a high degree of immunity in children. Unfortunately, the Pillemer vaccine caused severe reactions in children and therefore it was never licensed for clinical use *(Griffith 1988).*

An extracted pertussis vaccine was marketed by Eli Lilly in the United States from 1962 to 1977. The extracted vaccine was never well characterized and, in terms of current knowledge, it is not clear what the vaccine contained. The extracted vaccine has not been available since the manufacturer withdrew from the vaccine market.

Recognition of the roles of PT, FHA, and AGG in the pathogenesis of and immunity against pertussis has led to interest in the use of these proteins as the basis for new acellular pertussis vaccines. The use of monovalent pertussis toxoid and bivalent toxoid-FHA vaccine has been reviewed previously *(Galazka 1988).*

One of the main advantages of the introduction of acellular pertussis vaccines has been the removal of most nonprotective toxic components, especially LPS, during the purification of soluble antigenic material.

There is general agreement that pertussis toxin (PT), suitably treated to destroy its toxicity but retaining its immunogenicity (i.e. transformed into pertussis toxoid, PTd), should be included in acellular pertussis vaccine. There is also agreement that FHA should be part of an acellular vaccine. Routinely produced or experimental acellular vaccines contain different amounts of PTd, FHA, AGG and 69kd protein (Table 3).

In Japan, where acellular pertussis vaccines are routinely used for children above 2 years of age, two different vaccines are available. The first is Takeda-type vaccine, produced by Takeda Chemical Industries in Osaka and four other manufacturers (Kitasato Institute, Tokyo; Chiba Serum Institute, Ichikawa; Denka Seiken, Tokyo; and Kaketsu Chemo-Sero-Therapeutic Institute, Kumamoto). Takeda-type vaccine contains a substantial predominance of FHA over PTd (30 to 40 µg of FHA and about 5 µg Ptd per dose) and a small amount of AGG (about 1 µg per dose) *(Aoyuma et al. 1989).* The proportion of individual antigens on a weight basis is usually 90:10:1 for FHA:PTd:AGG (Table 3), although the proportion of FHA:PTd in commercially available vaccines can be as low as 4:1 *(Tomoda et al. 1991).* Takeda-type acellular pertussis vaccine also contains a small amount of 69kd protein from the outer membrane. Takeda-type acellular vaccine was formulated as a trivalent DPT vaccine by Wyeth and Lederle Laboratories and this product underwent several clinical studies in infants and children in the United States *(Andersen et al. 1987, 1988, Blumberg et al. 1991, Lewis et al. 1986, Morgan et al. 1990, Pichichero et al. 1987).* Takeda-type vaccine has also been extensively studied in infants and children in Japan *(Aoyama et al. 1989, Kimura et al. 1991, Mortimer et al. 1990, Tomoda et al. 1991).*

The second type of acellular pertussis vaccine routinely used in Japan is produced by Biken (Research Foundation for Microbial Diseases of Osaka University). The Biken vaccine contains equal amounts of FHA and and PTd (usually 12.5 to 24 µg per dose). In a study of household contacts of pertussis cases, the secondary attack rate was 14.3% for Biken-type acellular pertussis vaccine, 7.5% for Takeda-type vaccine, and 13.5% for whole cell vaccine. The attack rate for unimmunized children was 61.3%. This information allows calculation of vaccine efficacy estimates of 88%, 77%, and 78% for Takeda-type vaccine, Biken-type vaccine, and whole cell pertussis vaccine, respectively *(Aoyama et al. 1988).* These vaccine efficacy estimates have wide confidence limits which overlap, so that it is only possible to conclude that the Takeda and Biken vaccines are of similar efficacy and these acellular vaccines do not differ from the efficacy of 78% estimated for whole cell vaccine.

Table 3. Composition of acellular pertussis vaccines (revised from *Robinson and Ashworth 1988).*

Vaccine	Proportion of antigen				Stage of development
	FHA	PTd	AGG	69kd	
Takeda-type (produced in Japan; incorporated in DPT vaccine produced by Lederle, USA)	90	10	0.1 to 1	1	Used to immunize children over 2 years of age in Japan; clinical studies in infants and children in Japan and USA
Biken-type (a) (produced in Japan and at Merieux Institute, France)	1	1	-	-	Used in Japan for children; field trial and clinical studies in Sweden; clinical trials in USA
Biken-type (b)	-	1	-	-	Used in Swedish trial
Centre for Applied Microbiology and Research (CAMR), UK	1	1	1	-	Clinical studies in UK
Sclavo, Italy	1.4	1*	-	1	Clinical studies in adults

** Genetically detoxified pertussis toxin without the S1 component.*

The Biken-type acellular pertussis vaccine is also produced by the Merieux Institute and contains 12.5 µg of PTd and FHA. This vaccine, combined with diphtheria and tetanus toxoids, has been studied in infants and children in the United States *(Edwards et al. 1989, 1991, Van Savage et al. 1990).*

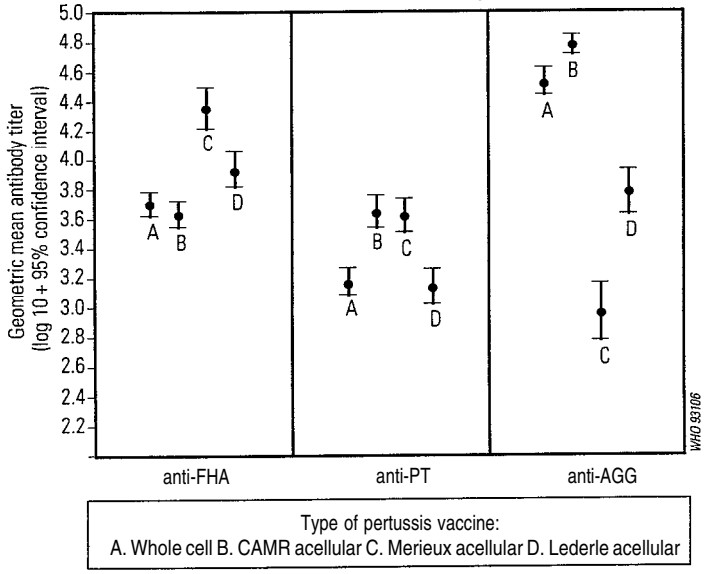
The Biken-type acellular pertussis vaccine and monovalent pertussis toxoid were tested in a field trial in Sweden from 1986 to 1987. Protection against culture-confirmed pertussis, including cough of any duration, was estimated to be 69% for Biken-type vaccine and 54% for monovalent pertussis toxoid *(Olin 1990).* The estimated vaccine efficacy was influenced considerably by the case definitions used. In culture-confirmed cases, the efficacy of monovalent pertussis toxoid increased when cases with longer duration of cough were considered: efficacy was 72% in protecting against cough lasting 14 days and 86% in protecting against cough lasting 28 days. When 21 days of coughing spasms and whoops was used as the case definition, the efficacy of the monovalent pertussis toxoid was 90% *(Blackwelder et al. 1991).*

Unfortunately, the Swedish trial failed to identify serologic correlates for clinical immunity; postimmunization levels of antibody to PT were the same in subjects who did and did not acquire pertussis. Furthermore, the acellular pertussis vaccines were not combined with diphtheria and tetanus toxoids and the study subjects were 6 to 11 month old children. Therefore, the results of the Swedish trial cannot be directly applied to infants younger than 5 months of age, who are the target group for routine EPI immunization with the combined DPT vaccine.

The Biken-type vaccine has been tested in several clinical studies in Sweden *(Blennow et al. 1988, Blennow & Granstrom 1989a, 1989b, 1990).* The data indicate that two doses of acellular pertussis vaccine provide protection for at least 3 years *(Olin 1990).* The results suggest that the monovalent toxoid (PTd only) greatly modifies the clinical course of the disease, but does not protect against pertussis infection. The addition of FHA provides more protection against infection. The results suggest that both acellular vaccines, given in a two-dose schedule to infants, provide solid protection against typical pertussis with prolonged paroxysmal coughing or whoops. The two-component vaccine containing FHA and PTd seems to protect better against mild or asymptomatic pertussis infection. The Swedish studies suggest that the efficacy of acellular pertussis vaccines against pertussis infection and disease might be increased by adding other purified protective components of pertussis organisms *(Storsaeter et al. 1990).*

At the Centre for Applied Microbiology and Research (CAMR), Porton, United Kingdom, an acellular pertussis vaccine has been developed which consists of equal amounts (10 µg/dose) of separately purified FHA, PTd, and AGG *(Robinson & Ashworth 1988)* (Table 3). To select an acellular vaccine for a phase-III trial comparing the efficacy of acellular and whole cell vaccines, a phase II study was carried out in the United Kingdom with three vaccines: CAMR vaccine, Biken-type vaccine prepared in the Merieux Institute, and Takeda-type vaccine made in Japan. A total of 432 children were allocated to one of the three acellular vaccine groups or to a whole cell vaccine group by a double-blind, random method. Each vaccine was delivered as a component of DPT vaccine. Children were immunized at 3, 5, and 8 to 10 months of age. The trial confirmed the low reactogenicity of the acellular vaccines. The results showed significant differences in antibody response to PT, FHA, and agglutinogens among the three acellular vaccines and between each acellular vaccine and the whole cell vaccine (Figure 10). In May 1990, the primary immunization schedule for DPT vaccine in the United Kingdom was changed to a 2-, 3-, and 4-month schedule. Therefore, a new phase II trial will

Figure 10. Geometric mean antibody titers against FHA, PT, and AGG after three doses of DPT vaccine at 3, 5, and 7 months of age *(Miller et al. 1990)*



be required to decide which acellular vaccine should be included in the phase III trial.

There is considerable interest in preparing acellular vaccine without the active S1 enzyme subunit and containing only subunits responsible for binding to the cell surface. Many sites crucial for toxin action are located on the S2-S4 and S3-S4 dimers and these subunits are able to elicit a neutralizing antibody response, suggesting that they may be possible candidates for inclusion in acellular pertussis vaccine *(Burns et al. 1988).* The absence of the active S1 subunit would ensure that the vaccine would be free from PT-induced toxicity. A vaccine which is devoid of the toxic properties of PT and maintains all the immunological properties of the "wild" toxin has been constructed by genetic manipulation and was evaluated in a phase I study on a small group of adult volunteers. The vaccine has been shown to be safe and highly immunogenic *(Podda et al. 1991).*

In spite of encouraging evidence for the feasibility of a PT subunit vaccine, some observations call for caution. Results of the study of Thomas et al. *(1989)* showed that after administration of whole cell vaccine or pertussis infection, the human humoral response to PT is directed almost exclusively to the S1 subunit. If serum antibody responses to *B. pertussis* antigens accurately reflect immunity to pertussis after infection or immunization, these results suggest that unless the binding subunits of PT can be modified to increase their immunogenicity in humans, they are unlikely to fulfill their theoretical promise as components of a safe acellular vaccine.

The ability to isolate the gene sequences coding for FHA and PT and to insert them in other bacteria suggests that pertussis vaccines might be manufactured using recombinant DNA technology. Such vaccines would be purer than those derived from *B. pertussis* cultures and could be designed to contain the parts of the FHA and PT molecules thought most likely to be protective.

7.2 The use of acellular pertussis vaccine

Japan is the only country which has succeeded in introducing acellular pertussis vaccine into the routine immunization programme. The acellular vaccine is given in combination with diphtheria and tetanus toxoids to children aged 24 to 48 months. Three doses are given with intervals of 3 to 8 weeks and the fourth dose is given 12 to 18 months after the third dose. Since the introduction of the acellular pertussis component to the DPT vaccine in Japan in 1981, the number of reported pertussis cases has decreased from some 5000 to 13 000 in 1979 to 1980 to about 500 to 1000 in 1987 to 1988. This is still higher than the pertussis incidence in 1970 to 1974, when the whole cell pertussis vaccine was used in infants. The decline of pertussis incidence included all age groups; however, the rate of decline is higher in older children than in children below 2 years of age. Since December 1988, DPT vaccine in Japan could be given to infants over 3 months *(Kimura & Kuno-Sakai 1990, Kimura et al. 1991),* but as of 1990 only about 10% of infants younger than 12 months of age were immunized with DPT vaccine *(Kimura & Kuno-Sakai 1990a, 1991).*

In general, the reactogenicity of acellular vaccine is much lower than the reactogenicity of whole cell vaccine. Acellular vaccine is associated with a significantly reduced frequency of systematic reactions (fever, vomiting, fretfulness, anorexia) and local reactions (swelling, redness, warmth, tenderness). However, the available experience on the reactogenicity of acellular vaccine in very young infants is limited and based mainly on clinical studies involving small groups of infants. In some studies, questions have been raised concerning the reactogenicity of acellular vaccine given in repeated doses beginning in infancy. Older children have significantly more local reactions (redness or swelling) and systemic reactions (fever or fretfulness) than younger children following DPT vaccine containing Takeda-type acellular vaccine *(Anderson et al. 1987, Kamiya et al. 1992).* Biken-type acellular vaccine produced lower rates of fever and local reactions following primary immunization than whole cell vaccine; however, following a booster dose in 2 year old children, rates of these reactions were higher in those whose primary immunization was with acellular vaccine than in children whose primary immunization was with whole cell vaccine *(Blennow 1988a, Blennow & Granstrom 1989).* More data are needed to assess the reactogenicity of repeated doses of acellular pertussis vaccine.

7.3 Antibody response to acellular pertussis vaccine

There is no direct comparison of the clinical efficacy of whole cell pertussis vaccine and acellular pertussis vaccine. However, there is a large body of evidence on the antibody response following these two types of vaccine. Some results are presented in Figures 9, 10, and 11.

In general, the PT response to primary and booster immunization with acellular pertussis vaccine is equal to or better than the response to whole cell vaccine *(Anderson et al. 1988, Edwards et al. 1989, Lewis et al. 1986, Miller et al. 1990, Morgan et al. 1990, Pichichero et al. 1987, Van Savage et al. 1990).* Compared with whole cell vaccine, significantly higher FHA response has been seen in all studies with Biken- and Takeda-type acellular vaccines.

On the other hand, DPT vaccine with whole cell pertussis component stimulates a higher agglutinin response than DPT vaccine containing acellular pertussis component. Lower agglutinin titers following acellular pertussis vaccine may be explained by the small amounts of AGG present in the Takeda-type vaccine and lack of AGG in the Biken-type vaccine. The CAMR acellular vaccine, which contains a significant amount of AGG2 and AGG3, induces a better agglutinin response than whole cell pertussis vaccine (Figure 10).

There may be differences in the kinetics of the antibody responses to acellular and whole cell vaccines. The response to acellular vaccine seems to start after the first dose, without the delay period seen following whole cell vaccine (Figure 9). A difference has been found between responses to acellular and whole cell vaccines in infants with various preimmunization levels of IgG ELISA antibody to PT (Figure 11). The response to acellular vaccine was independent of the preimmunization antibody titer, while the response to whole cell vaccine was strongly dependent on the preimmunization titer. It is not known whether the better response to acellular vaccine among those with higher anti-PT titers was due to greater immunogenicity of PTd in the acellular product, the absence of some component of the whole cell vaccine, or other as yet unidentified factors *(Van Savage et al.* 1990). These observations enhance the attractiveness of acellular vaccine for early immunization against pertussis.

The diphtheria and tetanus responses in children receiving acellular- and whole cell-pertussis component DPT vaccines are similar *(Anderson et al. 1988, Edwards et al. 1989, Lewis et al. 1986, Pichichero et al. 1987).* This indicates that acellular pertussis vaccine has adjuvant properties similar to whole cell pertussis vaccine.

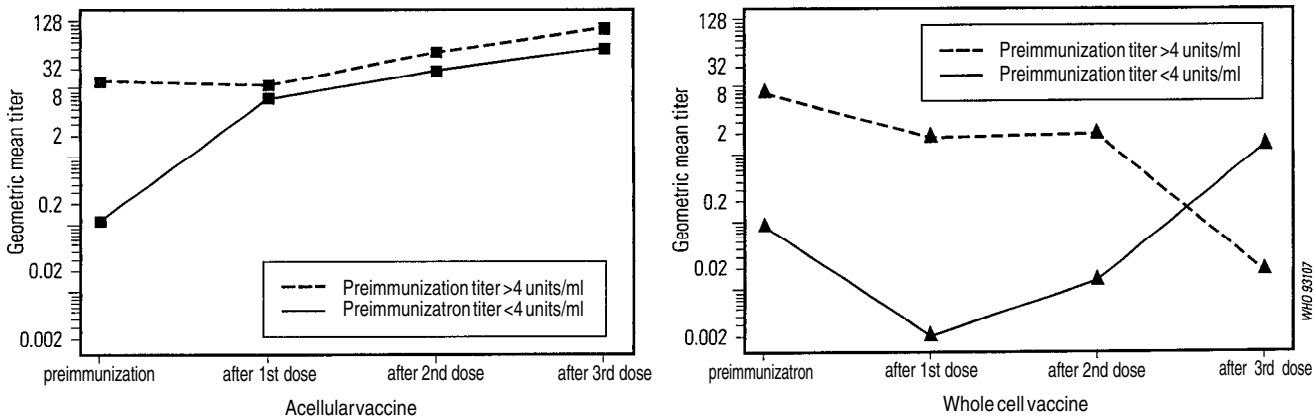
7.4 Future use of acellular pertussis vaccine

Should acellular vaccine replace conventional whole cell pertussis vaccine? Observations in Japan and trials in Sweden show that acellular vaccine confers some protection against pertussis.

In Sweden, the acellular vaccine study found the bivalent PTd-FHA vaccine to be 69% effective in preventing all culture-proven pertussis and 79% effective in preventing severe cases. A post trial, non-blinded follow-up study gave higher vaccine efficacy estimates, 83% and 97%, respectively. However, the postimmunization levels of antibodies to PT and FHA did not appear to correlate with clinical protection. This suggests that PT and FHA may not be the only antigens responsible for protection and that the response to other antigens may also contribute to immunity after infection or vaccination.

In the United States, the Food and Drug Administration has licensed acellular pertussis vaccine for use in the fourth and fifth doses of the childhood DPT immunization schedule *(ACIP 1992a, ACIP 1992b).* The whole cell component DPT vaccine is still recommended in the United States for the primary three doses in infancy. Despite a substantial body of existing data on acellular pertussis vaccines, several major

Figure 11. Antibody to PT (ELISA units/ ml) in infants receiving acellular and whole cell pertussis vaccine *(Van Savage et al. 1990)*



questions continue to delay the licensure and availability of these or similar vaccines outside of Japan and the United States. These questions refer mainly to the uncertainty about the overall "best estimate" of efficacy of acellular vaccine relative to existing whole cell vaccines and the efficacy of acellular vaccines in infants under 6 months of age. Also, questions about the risk, if any, of rare but serious adverse events associated with acellular vaccines have not been fully answered and continue to limit acceptance of these vaccines. Obtaining answers to these questions will be costly, logistically difficult, and take several years to accomplish.

Several groups, including government agencies and private manufacturers, are now planning or performing additional vaccine efficacy trials. Among issues that must be resolved are:

1. selection and development of an appropriate site for a trial from a limited number of suitable populations (Germany, Italy, Sweden, United Kingdom),
2. selection of the most promising vaccines or vaccines from a multiplicity of candidates,
3. choice of whole cell vaccine to use as a positive control,
4. the possibility of including a placebo control group,
5. selection of an immunization schedule for young infants,
6. agreement on a clear cut case definition,
7. the frequency of any rare adverse events that should be measured.

If successfully launched, a trial is expected to run for approximately 3 years, cost several million dollars, and provide data by the mid 1990s at the earliest *(Bernier 1990).*

8. Implications for Immunization Programmes

To effectively control pertussis in the world, all countries should use available pertussis vaccines in immunization programmes for children. In October 1991, the EPI Global Advisory Group recommended that since acellular pertussis vaccines are not yet widely available, the use of DPT vaccine containing the whole cell pertussis component should be continued *(Expanded Programme on Immunization 1992).*

In all countries, and particurlarly where pertussis is still an endemic disease and poses a serious health problem in infants and young children, the priority should be to ensure that infants are completely immunized with a primary series of three doses of DPT vaccine at the youngest age possible. All efforts should be made to complete the primary series at or before 6 months of age, and to reach at least 90% coverage in all districts.

Duration of immunity following pertussis immunization is still an open issue. An additional dose of DPT vaccine administered one year after completing a primary series prolongs the duration of immunity and maintains immunity against pertussis in older children. Countries considering the use of additional doses of DPT vaccine should evaluate the cost-benefit and potential impact of such doses on the epidemiology of pertussis, and especially on the age distribution of the disease. The additional resources required, the likelihood of continued availability of these resources, and any potential negative impact on sustaining high coverage in infants should be carefully analyzed prior to implementing such schedules.

Countries which have already included additional doses of DPT vaccine in their immunization programmes should monitor coverage with these doses, with the objective of reaching at least 90% coverage.

Surveillance of pertussis morbidity should be strengthened in all countries, and pertussis should be a reportable disease.

Abbreviations

AC	adenylate cyclase
AGG	agglutinogens
BA	bacterial agglutination
DPT	diphtheria-pertussis-tetanus vaccine
ELISA	enzyme-linked immunosorbent assay
FHA	filamentous hemagglutinin
HLT	heat-labile toxin
Lf	floculation units of toxoid
LPF	lymphocyte promoting factor
LPS	lipopolysaccharide endotoxin
PT	pertussis toxin
PTd	pertussis toxoid

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Expanded Programme on Immunization

Vaccine Research and Development

Vaccine Supply and Duality

The **Expanded Programme on Immunization** focuses on the prevention of selected childhood diseases and, through support to national immunization programmes, aims to achieve 90% immunization coverage of children born each year. Its goals are to eradicate poliomyelitis from the world by the year 2000, reduce measles deaths and incidence, eliminate neonatal tetanus as a public health problem and introduce hepatitis B vaccine in all countries.

Vaccine Research and Development supports and promotes research and development associated with the introduction of new vaccines into the Expanded Programme on Immunization. This includes research and development of new vaccines, improvement of immunization procedures and support to epidemiogical studies.

Vaccine Supply and Quality ensures adequate quantities of high quality, affordable vaccines for all the world's children, supports the efforts of governments to become self-reliant as regards their vaccine needs, and assists in the rapid introduction of new vaccines.

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