The Immunological Basis for Immunization Series

Module 2: Diphtheria
Update 2009

Immunization, Vaccines and Biologicals

World Health Organization

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Contents

Abbreviations and acronyms

ACV	acyclovir
D	diphtheria
DPT	diphtheria-pertussis-tetanus vaccine
DT	diphtheria-tetanus vaccine, child- type
ELISA	enzyme-linked immunosorbent assay
EPI	Expanded Programme on Immunization
HA	passive haemagglutination test
Ig	immunoglobulin
IPV	inactivated poliomyelitis vaccine
IU	international units
Lf	limit of flocculation
mL	millilitre
PCR	polymerase chain reaction
PHLS	Public Health Laboratory Services
Td	adult preparation of diphtheria and tetanus toxoids with a low amount of diphtheria toxoid
Tdap	adult formulation of Td with acellular pertussis vaccine, adult formulation
ToBI	toxin binding inhibition test
WHO	World Health Organization

Preface

This module is part of the series *The Immunological Basis for Immunization*, which was initially developed in 1993 as a set of eight modules focusing on the vaccines included in the Expanded Programme on Immunization (EPI)[1]. In addition to a general immunology module, each of the seven other modules covered one of the vaccines recommended as part of the EPI programme - diphtheria, measles, pertussis, polio, tetanus, tuberculosis and yellow fever. The modules have become some of the most widely used documents in the field of immunization.

With the development of the Global Immunization Vision and Strategy (GIVS) (2005-2015) (http://www.who.int/vaccines-documents/DocsPDF05/GIVS_Final_EN.pdf) and the expansion of immunization programmes in general, as well as the large accumulation of new knowledge since 1993, the decision was taken to update and extend this series.

The main purpose of the modules - which are published as separate disease/vaccine-specific modules - is to give immunization managers and vaccination professionals a brief and easily-understood overview of the scientific basis of vaccination, and also of the immunological basis for the World Health Organization (WHO) recommendations on vaccine use that, since 1998, have been published in the Vaccine Position Papers (http://www.who.int/immunization/documents/positionpapers_intro/en/index.html).

WHO would like to thank all the people who were involved in the development of the initial Immunological Basis for Immunization series, as well as those involved in its updating, and the development of new modules.

[1] This programme was established in 1974 with the main aim of providing immunization for children in developing countries.

1. Diphtheria disease and toxin

Diphtheria is a bacterial disease in which most of the clinical manifestations result from the action of an extracellular protein, exotoxin, produced by *Corynebacterium diphtheriae*, a gram-positive bacterium whose genomic sequence was recently characterized (Cerdeno-Tarraga et al., 2003). Human cases or carriers are the reservoir for this infection. Diphtheria is acquired through direct contact or by sneezing or coughing; the incubation period is generally between two and five days. Upper respiratory tract infection is most common, involving the tonsils, pharynx, larynx, or nasal mucosa. Inflammation can be severe because of local cell damage caused by the exotoxin, manifesting as thick, adherent "membranes." Laryngeal diphtheria is life-threatening, while nasal diphtheria may be mild, often chronic. Skin diphtheria is also common in developing countries, with lesions indistinguishable from, or a component of, impetigo. Inapparent infections outnumber clinical cases. Late effects of diphtheria include cranial and peripheral motor and sensory palsies and myocarditis, which result from systemic distribution of the toxin. The case fatality rate is 5% to 10%.

Diphtheria (D) exotoxin is one of the most extensively studied and well understood bacterial toxins. It is an A-B type toxin consisting of two polypeptides. Fragment B is necessary for binding to surface receptors and penetration into cells. Fragment A is responsible for its toxicity, and it acts by interfering enzymatically with protein synthesis, ultimately causing cell death. Both native and recombinant forms of D toxin are available in highly purified form. D toxin amino acid sequence is determined and crystal structure established. Most aspects of the mode of D toxin action are well characterized on a molecular level and interpreted in terms of known structure (Holmes, 2000; Collier, 2001). Diphtheria toxin exerts its effects on distant tissues and organs, especially the heart (causing myocarditis), and the peripheral and cranial nerves (causing weakness progressing to paralysis), if absorbed from the site of infection.

All toxigenic strains of *C. diphtheriae* produce an identical toxin. For a diphtheria strain to become toxigenic, it must be infected by a particular bacterial virus, or bacteriophage, containing the toxin gene. This process is called lysogenic conversion. The introduction of a toxigenic strain of *C. diphtheriae* into a community may initiate an outbreak of diphtheria by clonal spread or by transfer of the bacteriophage to non-toxigenic strains carried in the respiratory tracts of individuals. Both toxigenic and non-toxigenic strains of *C. diphtheriae* may be isolated during such an outbreak (Mortimer, 1988). Identification of the diphtheria toxin gene allowed development of rapid, accurate polymerase chain reaction (PCR)- based methods for identification of toxigenic strains

(Nakao & Popovic, 1997; Mothershed et al., 2002). In highly immunized populations, toxigenic strains virtually disappear, although non-toxigenic strains may continue to circulate. Emergence of invasive non-toxigenic clones of *Corynebacterium diphtheriae* (Reacher et al., 2000; Romney et al., 2006) has been described, but such infections remain infrequent.

When treated with formaldehyde and heat, diphtheria toxin loses its ability to bind to cells and its harmful enzymatic activity, but retains its immunogenicity. This treatment converts diphtheria toxin to a toxoid, which is commonly used to immunize against diphtheria. Modern manufacturing procedures ensure that the toxoiding process is irreversible. Genetically altered, non-toxic, fully immunogenic mutants of diphtheria toxin are available e.g. CRM197, and can be used for immunization as potentially less reactogenic alternatives to the toxoid (Robbins et al., 2005). CRM197 is used as the protein carrier in several current polysaccharide-protein conjugate vaccines.

2. The nature of immunity to diphtheria

Immunity against diphtheria is antibody-mediated. Because the lethality of diphtheria is almost entirely due to diphtheria toxin, immunity to diphtheria depends primarily on antibody against the toxin. This antibody, called antitoxin, is primarily of the immunoglobulin G (IgG) type and is measured in International Units per millilitre (IU/mL) of serum. Antitoxin is distributed throughout the body and can pass easily through the placenta, providing passive immunity to the newborn during the first few months of life. Diphtheria antitoxin may be induced by toxin produced by *C. diphtheriae* during the disease or the carrier state, or by diphtheria toxoid following immunization. These antibodies are identical and cannot be distinguished by any existing techniques. Cell-mediated immune responses to toxoid also occur and may be related to sustaining immunologic memory (Kniker et al., 1985; Yamamoto et al., 2002; Upham et al., 2006).

3. Techniques to measure antibody response

Two important properties of diphtheria toxin can be utilized to determine the activity of diphtheria antibodies (antitoxin). The first is the distinct dermonecrotic capacity of toxin, i.e. the ability to produce an inflammatory reaction when injected intradermally into the skin of humans or animals. This property was used for the Schick test in humans and to determine neutralization antibody in animals. The second property is the capacity of diphtheria toxin to block protein synthesis in cultured mammalian cells causing cell death. This capacity is used to determine diphtheria antibody levels in an in vitro neutralization test using cells sensitive to diphtheria toxin. Additional in vitro tests to measure diphtheria antibodies include the passive haemagglutination test and the enzyme-linked immunosorbent assay (ELISA).

3.1 Schick test

The standard procedure to detect immunity to diphtheria toxin in early studies was the Schick test in which a small amount of toxin was injected intradermally. A positive reaction was characterized by inflammation appearing after 24 to 36 hours, and this signified lack of antitoxin, while a negative result (lack of inflammation) indicated presence of antitoxin. A control test with inactivated toxin was performed to exclude allergic reactions to toxin. Although Schick test results usually correlated well with serum antitoxin levels, this technique is no longer used due to technical difficulties in performing intradermal injections, the requirement for two visits, discomfort when positive, and unreliability in cases of skin anergy (often found in newborns and young infants) where negative results can be erroneously interpreted as evidence for immunity (Wright & Clark, 1944; Vogelsang & Krivy, 1945; Papadatos et al., 1967).

3.2 Neutralization test on animals

The in vivo neutralization test, like the Schick test, is mainly of historical interest. It was usually performed on the depilated skin of rabbits (Jensen, 1933) or guinea-pigs (Glenny & Llewellyn-Jones, 1931). Different dilutions of serum mixed with fixed amounts of diphtheria toxin were injected into the depilated skin of the animal, and the antitoxin concentration was estimated based on the presence or absence of an inflammatory reaction. Results of the in vivo neutralization test may differ depending on the avidity of the antibody tested, the concentration of toxin used in titration, and the species of laboratory animal. The test is laborious, time-consuming and expensive, and requires suitable animals. However, the in vivo neutralization test showed the functional capacity of antibody to neutralize toxin.

3.3 Neutralization test on microcell culture

The neutralization test on microcell culture is based on the observation that the survival of mammalian cells in culture is inhibited by diphtheria toxin. This effect is neutralized when diphtheria antitoxin is present in serum samples (Miyamura et al., 1974a; Miyamura et al., 1974b). The titration of the antitoxin in the serum samples is done in plastic microtissue culture plates, in which dilutions of test sera are mixed with challenge toxin. After a short incubation, Vero (green monkey renal epithelium) or HeLa (cell suspension in a special culture medium) is added. After incubation for three or four days, results are read as a change in the colour of the reagents in the microtitre plate wells. The colour change is due to the normal metabolic formation of acid, which changes the pH. Vero cells are more sensitive to diphtheria toxin since they have large numbers of binding sites (receptors) and take up the toxin in a highly specific, time- and temperature- dependent manner (Middlebrook et al., 1978). When a serum dilution contains antitoxin in excess, the cells continue to grow, and the colour of the medium changes from red to yellow. Recent improvements in the microcell neutralization test include spectrophotometric determination of the equivalence point between toxin and antitoxin, and computer analysis of adsorption values (Aggerbeck & Heron, 1991).

The in vitro neutralization test in microcell culture is highly sensitive (minimum detectable level 0.005 IU/mL), is reproducible, and requires a minimum amount of serum. Up to 100 serum specimens may be titrated in one test run. The test has been used to determine the diphtheria antibody response of humans (Palmer et al., 1983) and animals (Kreeftenberg et al., 1985). For both human and guinea-pig sera, there is good correlation between the results of the in vitro neutralization test and the in vivo neutralization test on rabbit skin (Kriz et al., 1974; Miyamura et al., 1974a; Miyamura et al., 1974b; Kjeldsen et al., 1988). A modified in vitro neutralization test has been developed (Padovan et al., 1991). All cell-culture tests, however, require staff with special skills in tissue culture techniques, and a laboratory with special equipment.

3.4 Passive haemagglutination

The passive haemagglutination (HA) test was frequently used to test for diphtheria antibody (Fulthorpe, 1962; Galazka & Abgarowicz, 1967; Millian et al., 1967; Thorley et al., 1975; Ruben et al., 1978; Crossley et al., 1979; Allerdist & Ehrengut-Lange, 1982; Galazka & Kardymowicz, 1989; Koblin & Townsend, 1989; Cellesi et al., 1989a). In the HA test, sheep, turkey, horse, or human red cells (chemically stabilized and coated with diphtheria toxoid) are agglutinated by diphtheria antibody. The HA test is inexpensive and can be performed in a modestly equipped laboratory. The HA test is rapid (results available in one hour), reproducible, and sensitive. Results of the HA test for diphtheria correlate to some degree with results of the neutralization test, although the HA test tends to underestimate low concentrations of diphtheria antibody (Scheibel et al., 1962; Galazka & Abgarowicz, 1967; Simonsen, 1989). This is in contrast to the HA for tetanus, which tends to overestimate antibody titres (see Module 3). The results of the HA test for diphtheria can be distorted by non-specific agglutinins in the sera directed against antigens on the surface of the red cell requiring the use of control red cells without toxin. These effects can be minimized by several methods. Overall there is relatively poor correlation of HA with contemporary toxin neutralization tests considered as standard reference methods.

3.5 ELISA

The enzyme-linked immunosorbent assay (ELISA) involves the binding of antigen to polystyrene tubes. Exotoxins, such as diphtheria toxin (or toxoid), that have a highly lipophilic moiety in their molecule, coat the tubes efficiently (Svenson & Larsen, 1977). Results of the direct ELISA test are highly reproducible (Camargo et al., 1984; Melville-Smith & Balfour, 1988). When the antibody level is above 0.1 IU/mL, the results of the ELISA test correlate well with results of the in vivo neutralization test in guinea-pigs (Knight et al., 1986) and the results of the neutralization test in tissue culture (Melville-Smith & Balfour, 1988). When the antibody titre is low, the results of the ELISA test correlate poorly with results of the neutralization test. Better correlation has been reported with modified versions of the ELISA test (Knight et al., 1986; Hendriksen et al., 1989). For example, the toxin binding inhibition test (ToBI), shows good correlation (r = 0.91–0.93) with the in vitro neutralization test in Vero cells (Hendriksen et al., 1989) (Walory et al., 2000).

The main advantage of the ELISA test is its ability to measure class-specific diphtheria antibodies such as IgG (Dengrove et al., 1986).

4. Protective level of antibodies

It is assumed that a circulating diphtheria antitoxin level of 0.01 IU/mL, as determined by the neutralization test, and as explained below, provides basic clinical immunity against disease. This diphtheria antitoxin level corresponds to a negative Schick test. There is good correlation between clinical protection and the presence of serum antitoxin, whether this results from disease or immunization. In the 1984 diphtheria epidemic in the Kingdom of Sweden, all seven patients who died or showed neurological complications had antitoxin titres < 0.01 IU/mL, whereas 92% of symptom-free diphtheria carriers showed high antitoxin titres, above 0.16 IU/mL (Bjorkholm et al., 1986). However, it has also been shown that there is no sharply defined level of antitoxin that gives complete protection from diphtheria (Ipsen, 1946). A certain range of variation must be accepted; the same concentration of antitoxin may give unequal protection in different persons. Other factors may influence vulnerability to diphtheria including the infecting dose and virulence of the diphtheria bacilli, and the general immune status of the person infected (Christenson & Bottiger, 1986). Thus, an antibody concentration between 0.01 and 0.09 IU/mL may be regarded as giving basic immunity, whereas a higher titre may be needed for full protection. In some studies that used in vitro techniques such as passive haemagglutination, a level of 0.1 IU/mL was considered protective (Galazka & Kardymowicz, 1989; Cellesi et al., 1989a).

5. Development of antibodies due to natural stimulation

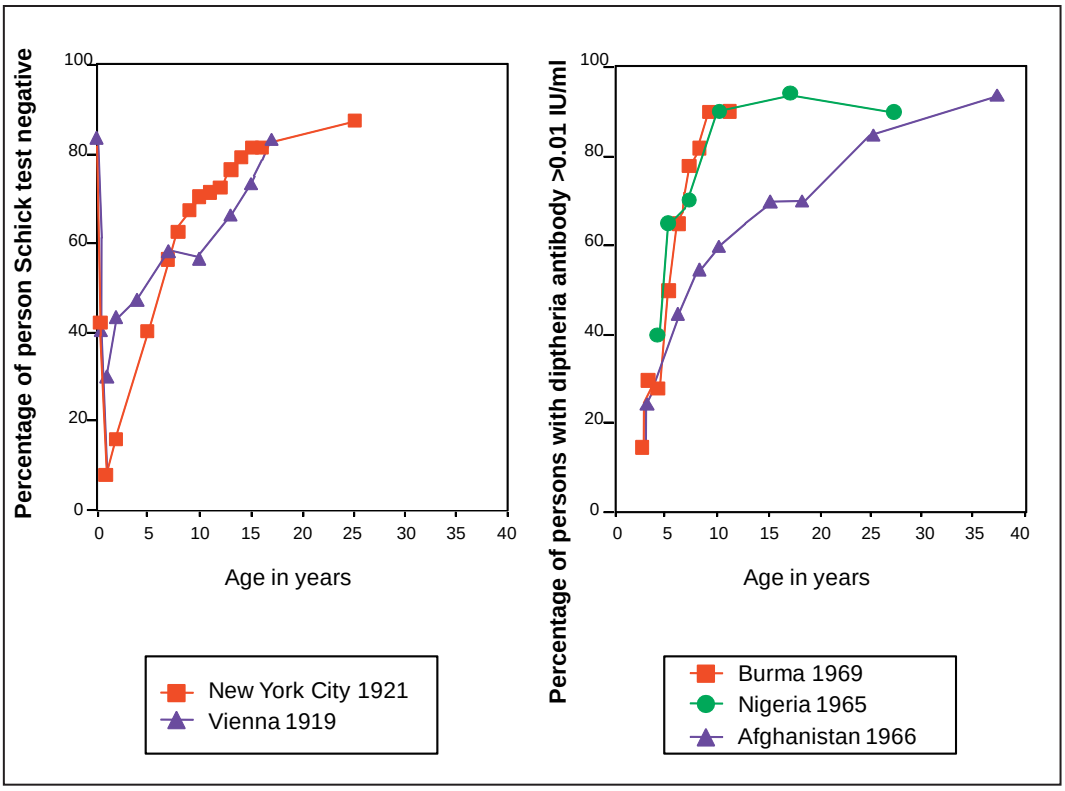
5.1 The pre-vaccine era in industrialized countries

In the pre-vaccine era, when circulation of *C. diphtheriae* organisms was frequent and the prevalence of diphtheria cases and carriers was high, natural immunity acquired by apparent or inapparent infection was the only mechanism of acquiring immunity. Diphtheria was primarily a disease of children. Early studies in Vienna in the Republic of Austria, in 1919, and New York City, the United States of America, in 1921, showed a typical immunity pattern. Most newborn infants had antibody acquired passively from their mothers; this antibody waned between 6 and 12 months of age. Then immunity rose rapidly in early childhood, reflecting increasing exposure to diphtheria organisms (Figure 1). By the age of 15 to 20 years, nearly all persons had acquired natural immunity to diphtheria. This pattern was observed in the United States in 1935 (Chason, 1936), the Republic of Poland from 1954 to 1955 (Daniel et al., 1957), and Japan in 1955 (Miyamura et al., 1983).

5.2 Developing countries in the 1960s

Data from developing countries suggest that the pattern of acquiring diphtheria immunity in the 1960s resembled the pattern seen in Europe and the United States in the pre-vaccine era (1920s). Such data are available from the Islamic State of Afghanistan, the Union of Myanmar and the Federal Republic of Nigeria (Kriz et al., 1980), the Republic of India (Robinson et al., 1964; Suri et al., 1967; Chakraborty & Choudhuri, 1969), the Democratic Socialist Republic of Sri Lanka (Gunatillake & Taylor, 1981), and the Democratic Republic of the Congo (Muyembe et al., 1972). The process of acquiring natural immunity was rapid; in some countries more than 80% of children were immune by 10 years of age (Figure 1).

Figure 1: Natural diphtheria immunity in the pre-vaccine era in industrialized countries, 1919 to 1921, and in developing countries, 1965 to 1969. (Zingher, 1923) for New York City; (Stransky & Felix, 1949) for Vienna; (Kriz et al., 1980) for Myanmar, Nigeria and Afghanistan.



5.3 Developing countries today

The World Health Organization introduced its Expanded Programme on Immunization (EPI) in the early 1970s, with the aim of improving global rates of infant immunization. Coverage of infants in developing countries with three doses of diphtheria-pertussis-tetanus (DPT3) vaccine increased from 20% in 1980 to 78% in 2005, with substantial rate variation among districts within many countries. Global coverage for DPT3 remained between 70% and 78% between 1990 and 2005, with the lowest rates in sub-Saharan Africa (67% in 2005) and South-East Asia (66% in 2005)(WHO, 2006). In populations achieving high rates of infant vaccination, the rates of diphtheria during childhood declined substantially, as did the population-wide circulation of toxigenic strains. However, these populations remain vulnerable to outbreaks of diphtheria among adolescents and adults, whose vaccine- or naturally-induced protection wanes without periodic contact with toxigenic strains or booster immunization.

There is a need for current studies of population immunity in developing countries with high uptake of infant vaccinations, to facilitate mathematical modelling of the most cost-effective booster vaccination strategies. Another variable needing study is the extent of residual cutaneous diphtheria as an ongoing source of natural immunity. This was once a major factor in the development of natural immunity against serious disease (Bray et al., 1972; Baum et al., 1985), but few data are available regarding the current prevalence of skin infections. Socioeconomic changes, especially migration from rural to urban areas, and sociocultural changes, including improved sanitation and hygiene, may change the epidemiologic patterns of diphtheria. What was once an endemic disease mainly of children, may evolve into one with periodic epidemics affecting adults, with severe respiratory forms of infection, until population-wide immunity is restored with broader vaccination programmes.

6. Immunity due to immunization

6.1 Vaccines

Discovery of toxoid and its immunogenic capacities in 1923, provided safe and effective means for mass vaccination. Diphtheria toxoid remains the basis of current diphtheria vaccines which have remained unchanged except for higher purity of toxoid and increased immunogenicity with addition of aluminium adjuvant. Recently, genetically- inactivated mutants of diphtheria toxin were proposed to be used instead of traditional toxoid (Robbins et al., 2005) in order to decrease the amount of protein needed for vaccination, and decrease reactogenicity, which becomes more of a problem with repeated immunizations. Mucosal delivery of adjuvanted mutated toxin shows promise (Mills et al., 2003; Rydell & Sjoholm, 2004; Rydell & Sjoholm, 2005), but licensure of a mucosal vaccine in the near future seems unlikely.

Current diphtheria vaccines perform satisfactorily when used in combination with other antigens. Diphtheria toxoid is used most commonly in combination with tetanus toxoid and whole cell or acellular pertussis vaccine. Newer combinations may also include inactivated poliomyelitis (IPV), hepatitis B and/or *Haemophilus influenzae* type b conjugate vaccines, as tetra-, penta- or hexa-valent products. Specific adult and adolescent formulations contain a reduced antigen dose to minimize injection-site reactions (Halperin et al., 2000). The dosage unit for diphtheria toxoid is the limit of flocculation (Lf). Vaccines for children typically contain 7.5–25 Lf per dose (all with a potency of no less than 30 International Units per dose) whereas those for adolescents and adults contain 2–3 Lf per dose. Conventionally, paediatric formulations are referred to as D vaccines, and adult formulations as d vaccines. Paediatric formulations are intended for use in children <7 years of age. Diphtheria vaccine for adolescents and adults is usually formulated with tetanus toxoid (Td). Other combinations with Td may include IPV and/or acellular pertussis vaccine, adult formulation (Tdap).

Although vaccine is very effective in preventing diphtheria-related death, its overall effectiveness against symptomatic disease is only estimated at 70%–90%. Diphtheria outbreaks were recently reported among highly-vaccinated populations in closed communities (Krumina et al., 2005; Ohuabunwo et al., 2005).

Diphtheria toxoid vaccines are generally well tolerated, reflecting their relatively simple composition. Injection-site reactions (erythema, swelling), occur infrequently in infants but increase in frequency and severity with booster doses in early childhood (Scheifele et al., 2001; Scheifele et al., 2005). Local reactions usually resolve within a few days and require no treatment. Transient febrile reactions may also occur in children and adults. Reactogenicity of alum-adsorbed or fluid formulations (without adjuvant) is comparable, but adsorbed vaccines are preferred for their superior immunogenicity.

Formulations with reduced doses of diphtheria toxoid are preferred for older children and adults as they cause fewer local and systemic adverse effects (Scheifele et al., 2005).

6.2 Development and duration of vaccine-induced immunity

The WHO recommendation for primary immunization of infants is to administer three doses of D-containing vaccine, starting as early as six weeks of age and given at least four weeks apart. The typical EPI schedule is for doses at 6, 10 and 14 weeks of age (Anonymous, 2006).

There is an age-related host response to immunization with diphtheria toxoid. The most important factor is the modifying effect of passively-acquired maternal antibodies in young infants (Halsey & Galazka, 1985). Early studies demonstrated that infants without maternal antibodies respond to diphtheria toxoid almost as well as older children (Vahlquist, 1949; Barr et al., 1950). A study in Japan found that the diphtheria antibody response to a diphtheria-acellular pertussis-tetanus (DPT) vaccine was similar in children 3–8 months and 24–30 months of age (Table 1).

A serum concentration of passive antibody greater than 0.1 IU/mL temporarily interferes with active immunization of infants, whereas a concentration below 0.02 IU/mL does not (Vahlquist, 1949; Barr et al., 1950). In areas where *C. diphtheriae* circulates in the population, and especially where cutaneous infection is common, mothers and their infants may have high diphtheria antitoxin titres, exceeding 0.1 IU/mL (Allerdist et al., 1981). However, the half-life of antitoxin is about 30 days (Anderson et al., 1988), so there is rapid loss of passively-acquired antitoxin in babies, averaging 14% per week (Barr et al., 1949). Thus passive antibody may suppress responses to the first or second vaccination, but not a third.

Table 1: Diphtheria antibody response to DPT vaccine containing acellular pertussis component in children of various ages (Kimura et al., 1991).

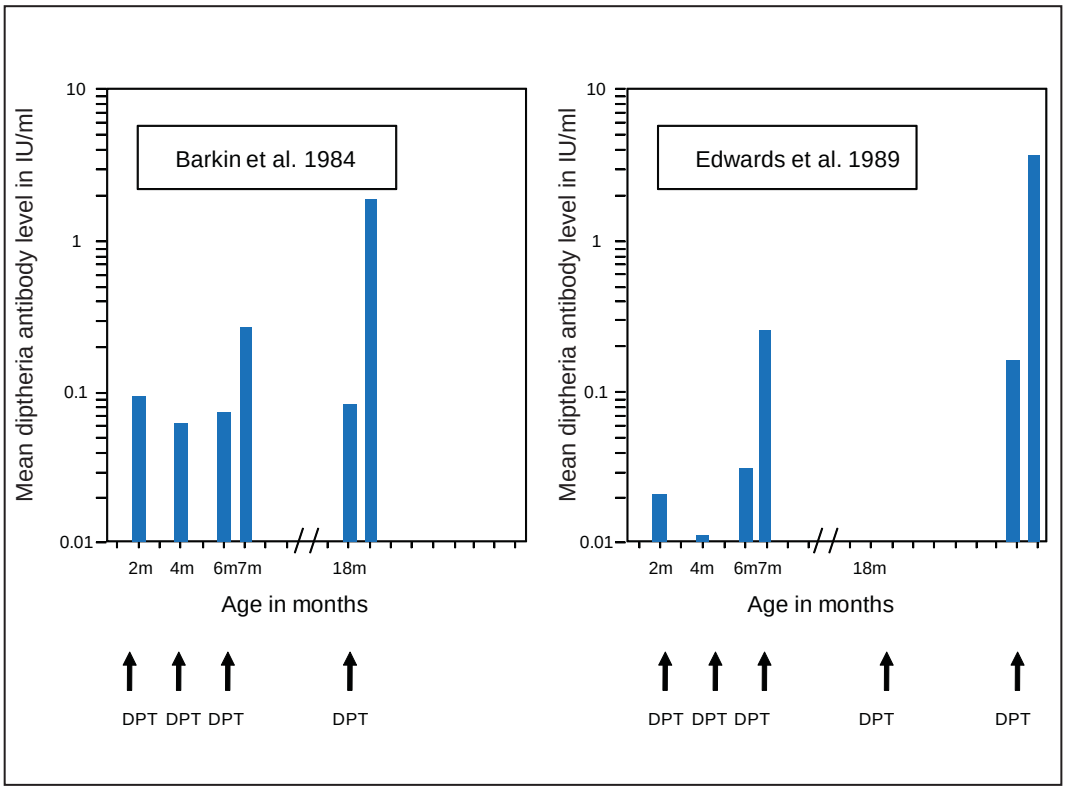
Age (months)	Geometric mean diptheria antibody titer in IU/ml				
	Before 1st dose*	Before 3rd dose	After 3rd dose	Before booster**	After booster
3 to 8	<0.01	0.8	1.6	0.3	6.7
9 to 23	<0.01	0.5	1.5	0.3	10.2
24 to 30	<0.01	0.7	1.7	0.3	8.3

* First three doses given at intervals of 6 to 10 weeks.
** Booster (4th) dose given 12 to 18 months after the 3rd dose.

Primary immunization with three doses of DPT vaccine stimulates antibody levels that considerably exceed the minimum protective level (Figure 2). In the two studies presented in Figure 2, the primary series of DPT vaccine was given at 2, 4, and 6 months of age, and a booster dose was administered at 18 months of age. The antibody level starts to increase after the second dose of DPT vaccine, and the level is considerably higher after the third dose. After the primary series, 94%–100% of children have antibody levels higher than 0.01 IU/mL (Chen et al., 1956; Bhandari et al., 1981; Barkin et al., 1984; Pichichero et al., 1986; Schou et al., 1987; Guerin et al., 1988), with the mean level ranging between 0.1 and 1.0 IU/mL (Barkin et al., 1984; Barkin et al., 1985; Anderson et al., 1988; Edwards et al., 1989; Kimura et al., 1991), or more (Bhandari et al., 1981). In a compressed and more immunologically challenging EPI schedule, diphtheria antibody responses one month after the third primary vaccination dose are comparable (Hussey et al., 2002; Gatchalian et al., 2005).

The nature of the pertussis component of DPT vaccine does not seem to affect the immune response to the diphtheria component of the vaccine. Several studies show that the diphtheria antibody response following DPT-containing whole cell- or acellular- pertussis components is similar (Pichichero et al., 1987; Anderson et al., 1988; Edwards et al., 1989). However, in one study, the antibody response to diphtheria toxoid given in combination with pertussis toxoid was lower than to diphtheria toxoid given alone (Trollfors et al., 2005) .

Figure 2: Diphtheria antibody levels in children immunized with a primary series of DPT vaccine at 2, 4, and 6 months of age, and following one or two booster doses.



6.3 Booster immunization

WHO has recommended that industrialized countries add childhood boosters of diphtheria toxoid to the primary series in infancy, to compensate for the loss of natural boosting that accompanies effective disease-control. Boosting at 12–24 months of age, at school entry or at school leaving, are all possible options, the choice of which should be based on disease surveillance and programmatic considerations. People living in low endemic and non-endemic areas may require additional boosters at about 10-year intervals to maintain life-long protection.

The duration of immunity after the primary series of diphtheria toxoid in infancy has been studied in the Kingdom of Denmark, where primary vaccination used DT vaccine (1950–1961), DPT vaccine (1961–1970), or DT-polio vaccine (after 1970). Except for military recruits, who receive a dose of Td vaccine, revaccinations are not routinely given. Serum antitoxin concentration showed a steep decline immediately after vaccination, followed by gradual fall-off. Studies show that diphtheria antitoxin levels in schoolchildren were steadily declining from the 1940s to 1985, although the number of doses of diphtheria vaccine administered has remained the same. Tetanus antitoxin concentration does not show such a decline. The lower diphtheria immunity current among schoolchildren in Denmark may be due to less exposure to diphtheria organisms and so a reduced opportunity to become naturally immune (Schou et al., 1987; Simonsen et al., 1987; Simonsen, 1989).

The duration of post-vaccination immunity also differs between early and more recent studies performed in the United States. In the 1960s, only 10% of children had lost diphtheria immunity 7 to 13 years following primary immunization with diphtheria toxoid (Volk et al., 1962). In more recent studies, diphtheria immunity declined more rapidly; 10% of children lost immunity by one year following the primary series (Pichichero et al., 1987), 67% of children lacked adequate immunity after 3 to 13 years, and 83% after 14 to 23 years (Crossley et al., 1979). During the first year after the primary series of three DPT vaccine doses, the mean level of diphtheria antibody declined four to fivefold (Pichichero et al., 1986; Kimura et al., 1991). By contrast, studies in the United Kingdom and the Republic of Italy showed that 96% to 100% of children immunized with three doses of DPT or DT still had protective diphtheria antibodies four to eight years later (Jones et al., 1989; Cellesi et al., 1989a). Differences in these results may be caused by different vaccines, different vaccination schedules, or different levels of exposure to *C. diphtheriae* with natural reinforcement of diphtheria immunity.

Many national immunization programmes offer one to two booster doses, for example one during the second year of life and a second at between four and six years of age. A booster dose administered at either of these times stimulates abundant production of diphtheria antibody with mean levels above 1.0 IU/mL (Barkin et al., 1984; Lewis et al., 1986; Anderson et al., 1987; Pichichero et al., 1987; Edwards et al., 1989; Kimura et al., 1991).

The outcome of revaccination of adults depends on several factors including the schedule and potency of toxoids used for primary immunization, the time since the last dose of diphtheria toxoid, and the age of the vaccinees. In the Kingdom of Denmark, toxoids with a large dose of antigen are used for primary immunization. Revaccination of Danish adolescents, military recruits, or adults with Td vaccine containing a reduced amount of diphtheria toxoid, stimulated rapid and vigorous production of diphtheria antitoxin, with the mean level exceeding 1.0 IU/mL (Volk et al., 1962; Simonsen et al., 1986a; Simonsen et al., 1986b). Revaccination response decreased with increasing time from the primary vaccination, but even if more than 20 years had elapsed, adequate individual protection could be obtained by a single booster dose (Simonsen, 1989). This was confirmed by a German study in which individuals primed in childhood and not boosted for at least 10 years achieved full protection in 95.4% of cases after one dose, and in 97.5% after two doses (Nicolay et al., 1999). A similarly high rate of protection (89.7%) after a single booster dose was reported among more than 500 patients in a Viennese hospital (Marlovits et al., 2000). A similar investigation in Belgium however, documented that one booster dose given to adults primed in childhood with four doses, secured full protection in only 76% (Vellinga et al., 2000), while a second booster dose raised the protection rate to 92% (Vellinga et al., 2001). The need for two booster doses to confer full protection with near certainty, was confirmed by other studies (Bayas et al., 2001; Hasselhorn et al., 2004).Overly frequent booster immunizations should be avoided, as they pose an increased risk of local reactions and do little to improve protection (Edsall et al., 1967; Danilova et al., 2005; Scheifele et al., 2005).

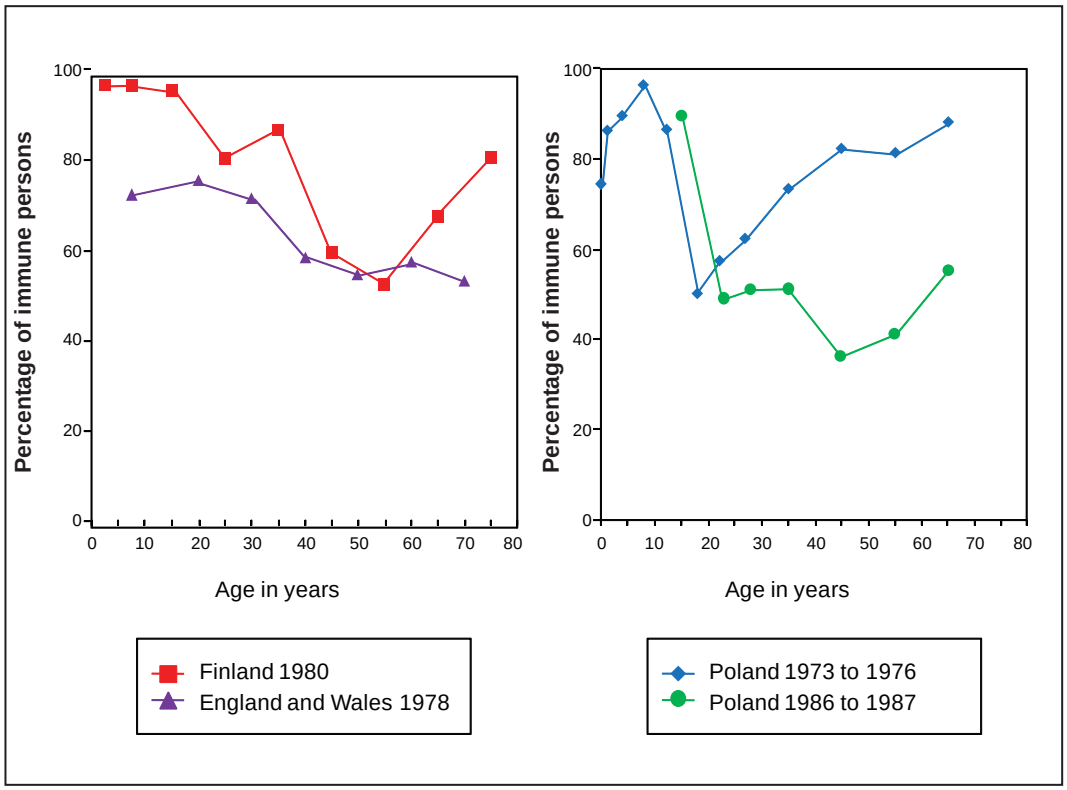
Although a small amount of diphtheria toxoid is effective in inducing a secondary response in previously primed schoolchildren or adults, it is insufficient to stimulate an effective immune response in those who have never been actively immunized, or who have not acquired basic immunity by natural means (Galazka & Olakowski, 1962; Trinca, 1975; Feery et al., 1981). An effective course of primary adult immunization should include three doses of adult formulation toxoid (reduced potency) with an interval of four to six weeks between the first and second dose and 6 to 12 months between the second and third dose (Feery et al., 1981).

6.4 Changes in the immune profile of various age groups following mass immunization

Mass immunization programmes result in considerable reduction of diphtheria incidence. They also result in profound and important changes in the immune status of different age groups by markedly reducing circulation of toxigenic strains and providing opportunities for natural boosting. Although direct comparison of the immunity levels in different countries is complicated by the different methods used to determine diphtheria immunity, some common characteristics may be noted.

Children acquire a high level of diphtheria immunity if fully vaccinated with three or more doses. Depending on the schedule of immunization with diphtheria toxoid and the incidence of diphtheria, the level of immunity declines in late childhood and adolescence (Figure 3). High levels of immunity in children result in reduced incidence of disease.

Figure 3: Diphtheria immunity (antitoxin level ≥ 0.1 IU/mL) in the post-vaccine era in the United States (McQuillan et al., 2002), Australia (Gidding et al., 2005), England and Wales (Maple et al., 2001), and the French Republic (Ballereau et al., 1998), contrasts with the Republic of Poland (Galazka & Sporzynska, 1979; Galazka & Kardymowicz, 1989; Walory et al., 2000), where routine immunization began several decades later.



Adults might become susceptible to diphtheria again due to reduced opportunities to boost immunity through subclinical infections. The likelihood of having protective antibody levels decreases with age, and in some industrialized countries less than 50% of adults may be immune to diphtheria. The age groups respectively with the lowest level of diphtheria antibodies was 20 to 40 year olds in the Federal Republic of Germany (Naumann et al., 1983), various areas of the former Soviet Union (Maksimova et al., 1984; Dalmatov et al., 1986; Schwartz et al., 1987), and Japan (Miyamura et al., 1974a); 40 to 50 year olds in the Republic of Poland (Galazka & Kardymowicz, 1989), Australia (Forsell, 1972), and the United Kingdom (Public Health Laboratory Services — PHLS, 1978); and persons older than 50 years in the Kingdom of Denmark (Kjeldsen et al., 1988), the Republic of Finland (Kerttula et al., 1980), the Kingdom of Sweden (Christenson & Bottiger, 1986), and the United States (Sargent et al., 1984). In some countries elderly persons are still immune to diphtheria, and this is probably due to natural immunity. In one province of the People's Republic of China where diphtheria incidence has been considerably reduced following immunization, the lowest levels of immunity were noted in persons aged 10 to 20 years (Expanded Programme on Immunization., 1988a).

In some countries significantly fewer adult females than males are protected against diphtheria. This trend was evident in four out of seven western European countries participating in a sero-epidemiological investigation (Edmunds et al., 2000), and in the United States (McQuillan et al., 2002) and Australia (Gidding et al., 2000), but not in Canada (Yuan et al., 1997) or the Republic of Poland (Galazka & Kardymowicz, 1989). A number of factors may contribute to this gender-related difference, such as immunization of males during military service (de Melker et al., 1999) and higher rates of injury, and consequent immunization of males with combined diphtheria/tetanus vaccine (Golaz et al., 2000). However, a recent German study (Volzke et al., 2006) noted that the odds of being unprotected given a booster dose in the past 10 years, were higher among women than men, suggesting gender-related differences in immune responses. This accords with the observation that fewer unprotected women seroconvert after one booster dose (Marlovits et al., 2000).

A large pool of susceptible persons creates an epidemic potential. An increased incidence of diphtheria has been noted in several European countries. During the early and mid-1980s, small outbreaks of diphtheria were reported from the Kingdom of Sweden, the Federal Republic of Germany, and the Portuguese Republic (Bjorkholm et al., 1986; Expanded Programme on Immunization, 1988b; Galazka & Keja, 1988; Rappuoli et al., 1988). In the former Soviet Union, diphtheria incidence started to increase in the early 1980s, reached its first peak in 1983 to 1985 and its second peak in 1994 to 1995. In one report, 1876 and 3897 diphtheria cases were reported in 1991 and 1992 respectively in Russia (Galazka, 1992; Expanded Programme on Immunization, 1993). The epidemic spread to Ukraine, where 1552 cases were reported in 1992, and to other states of the former Soviet Union. In many of these outbreaks, adolescents and adults were mainly affected. Multiple contributing factors were identified, among which were the limited uptake of primary and booster immunizations among children, allowing renewed circulation of toxigenic strains. Such strains may have been re-introduced by soldiers returning from the Islamic State of Afghanistan, where disease was endemic. Population movements among and within the new republics aided the spread of disease and taxed medical resources. Vaccine quality was determined to be satisfactory. A full analysis of these epidemics has been published (Galazka, 2000).

6.5 Strategies for immunization against diphtheria

There is no simple and universal schedule for immunization against diphtheria. The choice of an appropriate schedule in each country depends on the epidemiological pattern of diphtheria and on the level of development of immunization services.

In developing countries where the reservoir of *C. diphtheriae* is still large, and natural immunity plays a significant role in protection against the dangerous pharyngeal forms of the disease experienced mostly by children, the first priority is to ensure high coverage of infants with the primary series of three doses of DPT vaccine. Priority should be given to achieving at least 90% coverage.

In developing countries which have already achieved high coverage with three doses of DPT vaccine in children under one year of age, the policy of using a booster dose of DPT vaccine during the second year of age and/or a dose of DT at school entry should depend on the pattern of diphtheria and the availability of the vaccines. If diphtheria poses a significant health problem in preschool or school-age children, booster doses of diphtheria toxoid may be warranted. Data from serological studies which show declining antibody levels with age, may serve as a valuable guide in deciding when booster doses are warranted. The main issue may be whether or not the target age group is conveniently accessible for preventive health activities, in which case administering a fourth DPT dose may be appropriate.

The use of DT vaccine at school entry or Td vaccine at school-leaving may be important for providing anti-tetanus immunity for these age groups and is discussed in the module on tetanus. Health authorities need to consider the resources required to deliver these additional vaccine doses and balance this against the resources needed for other services.

In developed countries, primary immunization usually consists of three doses of DPT vaccine given at intervals of four or more weeks, beginning at two or three months of age, and reinforced by a fourth dose given in the second year of life or later. In some countries, booster doses of DT-containing vaccine are given at primary-school entry and doses of Td-containing vaccine at school leaving. Many countries, however, give only monovalent tetanus toxoid to older schoolchildren, thus missing an opportunity to efficiently reinforce both types of protection. Use of Tdap vaccine affords an additional opportunity to reinforce protection against pertussis among adolescents and young adults.

As noted above, serologic surveys can be useful tools for refining immunization schedules. Detecting the existence of a cohort of susceptible adults identifies the potential for disease to occur, so the introduction of Td vaccine for adults at high risk should be considered. Some controversy surrounds this recommendation. Some authors propose immunizing adults with adult-type Td vaccine every 10 years and giving Td vaccine whenever tetanus toxoid is indicated, e.g. in treating wounds in emergency rooms (Karzon & Edwards, 1988). Where 10-yearly adult boosters are recommended, compliance has typically been limited for lack of workable delivery methods. Other authors recommend using Td vaccine for high-risk groups, especially those persons vulnerable to the acquisition of virulent *C. diphtheriae*, such as those travelling to developing countries, military personnel, medical staff, kindergarten and childcare centre personnel, teachers, and alcohol and drug abusers (Galazka & Kardymowicz, 1989; Edwards, 1990). This approach has also met with little success. New programmes aiming to boost pertussis protection among young adults (e.g. parents of young infants) using Tdap vaccines may be better accepted, improving diphtheria protection in the process. In Canada, the need for general revaccination of adults against diphtheria has been questioned (Mathias & Schechter, 1985) on the grounds that diphtheria is rare in Canada despite evidence of declining adult antibody levels. A paradox is evident; seroepidemiology data indicate that many adults in developed countries are susceptible to diphtheria yet cases are rare. Adults may be indirectly protected so long as childhood immunization programmes are well supported by the public, thus minimizing circulation of toxigenic strains.

7. Summary : Implications for immunization programmes

The global recommendation for diphtheria immunization is to apply an effective primary immunization in infancy and to maintain immunity throughout life. The immunization schedule should be tailored to specific conditions in a given country, taking into account the actual epidemiological pattern of diphtheria and the level of development of the immunization services.

In all countries, priority should be given to efforts to reach at least 90% coverage with three doses of DPT vaccine in children below one year of age.

In developing countries where a high immunization coverage rate has been achieved in children under one year of age, the policy of using further doses of vaccines containing diphtheria toxoid should depend on the epidemiology of diphtheria. If diphtheria poses a significant health problem in preschool or school-age children, booster doses of diphtheria toxoid should be considered. A fourth dose of DPT vaccine in the second year of life and/ or a dose of DT vaccine at school entry are the most frequently selected options.

In countries where diphtheria has been successfully controlled, the immunity level acquired through immunization in infancy and early childhood should be maintained through properly-timed booster doses of DT- or Td-containing vaccines. Td vaccine should be used for older children (≥7 years) or adolescents leaving primary or secondary schools. Periodic booster doses are required to sustain protection throughout adulthood.

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The World Health Organization has provided technical support to its Member States in the field of vaccine-preventable diseases since 1975. The office carrying out this function at WHO headquarters is the Department of Immunization, Vaccines and Biologicals (IVB).

IVB's mission is the achievement of a world in which all people at risk are protected against vaccine-preventable diseases. The Department covers a range of activities including research and development, standard-setting, vaccine regulation and quality, vaccine supply and immunization financing, and immunization system strengthening.

These activities are carried out by three technical units: the Initiative for Vaccine Research; the Quality, Safety and Standards team; and the Expanded Programme on Immunization.

The Initiative for Vaccine Research guides, facilitates and provides a vision for worldwide vaccine and immunization technology research and development efforts. It focuses on current and emerging diseases of global public health importance, including pandemic influenza. Its main activities cover: i) research and development of key candidate vaccines; ii) implementation research to promote evidence-based decision-making on the early introduction of new vaccines; and iii) promotion of the development, evaluation and future availability of HIV, tuberculosis and malaria vaccines.

The Quality, Safety and Standards team focuses on supporting the use of vaccines, other biological products and immunization-related equipment that meet current international norms and standards of quality and safety. Activities cover: i) setting norms and standards and establishing reference preparation materials; ii) ensuring the use of quality vaccines and immunization equipment through prequalification activities and strengthening national regulatory authorities; and iii) monitoring, assessing and responding to immunization safety issues of global concern.

The Expanded Programme on Immunization focuses on maximizing access to high quality immunization services, accelerating disease control and linking to other health interventions that can be delivered during immunization contacts. Activities cover: i) immunization systems strengthening, including expansion of immunization services beyond the infant age group; ii) accelerated control of measles and maternal and neonatal tetanus; iii) introduction of new and underutilized vaccines; iv) vaccine supply and immunization financing; and v) disease surveillance and immunization coverage monitoring for tracking global progress.

The Director's Office directs the work of these units through oversight of immunization programme policy, planning, coordination and management. It also mobilizes resources and carries out communication, advocacy and media-related work.

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