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The Immunological Basis for Immunization Series

Module 1:
General Immunology

GLOBAL PROGRAMME FOR VACCINES AND IMMUNIZATION
EXPANDED PROGRAMME ON IMMUNIZATION

World Health Organization
Geneva

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Module 1:
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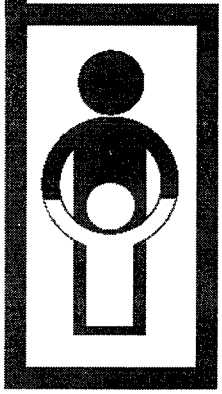

GLOBAL PROGRAMME FOR VACCINES AND IMMUNIZATION
EXPANDED PROGRAMME ON IMMUNIZATION

World Health Organization
Geneva

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The Immunological Basis for Immunization **series is available in English and French (from the address below). It has also been translated by national health authorities into a number of other languages for local use: Chinese, Italian, Persian, Russian, Turkish, Ukranian and Vietnamese. The series comprises eight independent modules:**

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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Contents

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 (EPI). 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. 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.

General Immunology

1. Antigens Inducing Immunity

1.1 Antigens and antigenic determinants

Immunity against infectious diseases develops in response to antigens. Antigens are defined as molecules which are recognized by the immune system and induce an immune response. An antigen stimulates the production of antibodies and/or cellular immune responses that will react specifically with that antigen. The reaction between antigen and antibody is similar to that between a lock and key. It is specific and antibodies produced against one antigen do not react, or react poorly, with other antigens.

The antigen may be a soluble substance produced by a microorganism (for example, toxin or its detoxified form, toxoid (Figure l), or a substance present on a bacterium, virus, other cell surface, or in the cell wall. Most antigens are proteins, but some are polysaccharides from bacterial capsules, or glycolipids.

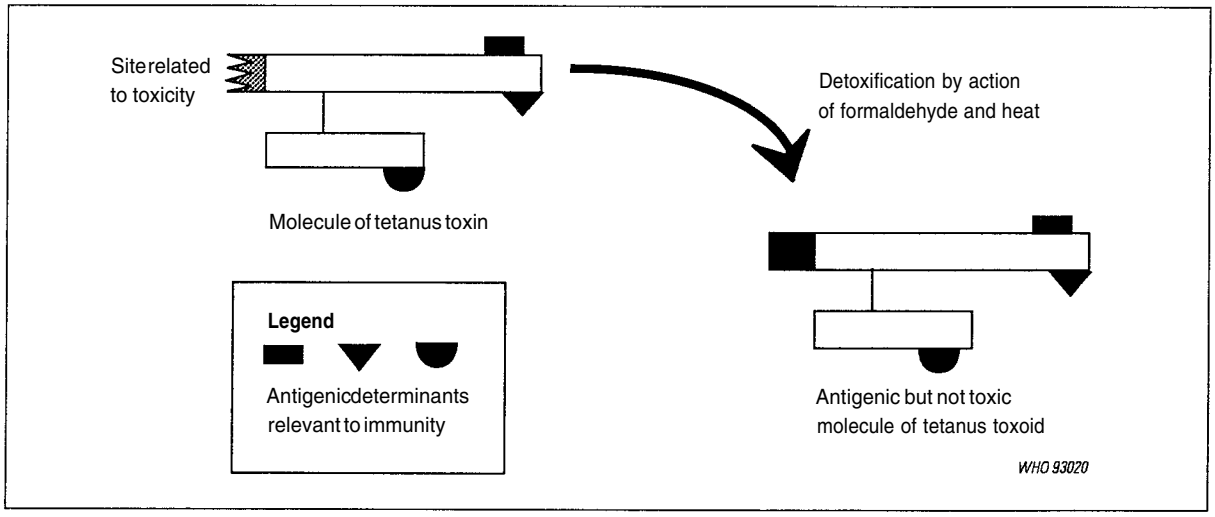
The part of the antigen to which an antibody binds is called an antigenic determinant, antigenic site, or epitope. Antigens usually contain many determinants which may be different from each other or may be repeated molecular structures.

A given microorganism contains many different antigens. Protozoa, fungi, and bacteria contain hundreds to thousands of antigens. Viruses contain from as few as three antigens (polyoma virus) to more than 100 antigens (herpesviruses and poxviruses). Immune responses develop to many of these antigens during infection. Resistance to infection, however, depends principally on the immune response to a smaller number of antigens on the surface of the microorganism. Relevant surface antigens have been isolated and characterized for some viruses. Much less is known about the antigens that induce resistance to bacteria, fungi, and protozoa. It is clear, however, that available vaccines consisting of killed bacteria induce a large number of irrelevant immune responses *(Mims 1982).* Whole cell pertussis vaccine, for example, contains several components, such as lipopolysaccharide, heat labile toxin, and tracheal cytotoxin, that although antigenically active, are not important in inducing immunity to pertussis (see Module 4).

1.2 T-dependent and T-independent antigens

There are two groups of antigens: T-dependent and T-independent. Antigens that require the intervention of T lymphocytes (see section 3.2) to trigger antibody production by B lymphocytes are called

Figure 1. Detoxification of tetanus toxin into harmless tetanus toxoid without loss of antigenic properties.



T-dependent antigens. Most protein antigens fall into this category. T-independent antigens are capable of stimulating B lymphocytes to produce antibody without the help of T lymphocytes. T-independent antigens are usually large compounds with multiple repeating subunits such as those from the bacterial capsular polysaccharides of *Neisseria meningitidis; Haemophilus influenzae type b,* or *group B streptococci.*

T-independent antigens are weak immunogens in persons below two years of age. The immunogenicity of T-independent antigens is increased when they are transformed into T-dependent antigens by coupling the antigen to a carrier protein. This phenomenon is utilized in preparing conjugated vaccines, such as a vaccine against *H. influenzae type b* in which the relevant polysaccharide (T-independent) is coupled to diphtheria toxoid, tetanus toxoid, or another carrier protein (T-dependent).

2. Vaccines Used in the EPI

2.1 Nature of EPI vaccines

The EPI vaccines contain preparations of many different kinds (Table 1).

Diphtheria and tetanus toxoids are protein toxins that have lost their toxicity through a detoxification process with formaldehyde (Figure 1). Fluid toxoids are relatively weak immunogens and in practice they are used in the adsorbed form with the addition of adjuvants (substances which increase considerably the immunogenic power of antigens). For diphtheria toxoid and tetanus toxoid the commonly used adjuvant is an aluminum salt.

The available pertussis vaccines commonly contain the whole killed bacteria of *Bordetella pertussis* and usually are used as components of diphtheria-pertussis-tetanus (DPT) vaccine. The pertussis component of DPT vaccine also has an adjuvant effect for the diphtheria and tetanus toxoids. Prospects for an improved acellular vaccine containing isolated protective antigens are discussed in Module 4.

Inactivated hepatitis B (HB) vaccine contains HB surface antigen (HBsAg) derived from the plasma of HBsAg carriers or made by recombinant DNA technology.

All killed vaccines contain a preservative, which is usually merthiolate, a mercury containing compound, in a concentration of less than 0.1 mg per ml.

Other vaccines are live, attenuated vaccines. Bacille Calmette-Guérin (BCG) vaccine contains live BCG bacteria, an attenuated form of *Mycobacterium bovis* organisms (see Module 5). BCG vaccine contains no preservatives and after reconstitution may be easily contaminated. It should be therefore used quickly, during one immunization session.

Measles vaccine contains live measles virus of one of a number of attenuated strains (Schwarz, Edmonston-Zagreb, Moraten, L-16, CAM-70, AIK-C). These strains were attenuated in different ways but all of them induce similar anti-measles antibodies. Measles vaccine usually contains a small amount of antibiotic (neomycin, polymixin, or kanamycin, but never penicillin) as a conservative.

Trivalent oral poliovirus vaccine (OPV) represents a mixture of three distinct types of attenuated polioviruses (types 1, 2, and 3). A proper balance between different types of poliovirus is essential for ensuring induction of antibodies against all three types (see Module 6). OPV is stabilized with magnesium chloride or sucrose.

Yellow fever vaccine is a live attenuated vaccine produced in chick embryos from the 17D yellow fever virus strain (see Module 8).

There are important differences between killed and live vaccines. The amount of antigen in a killed vaccine is an important parameter for its efficacy. Killed vaccines must be given in repeated doses to induce an adequate immune response. The microorganisms in live vaccines, on the other hand, multiply in the host after immunization. The antigenic mass in the live vaccine is small but it is increased many thousand times following growth of the organism in the body, if there are favorable conditions for such growth.

Other vaccines (not shown in Table 1) are used in some countries. They include meningococcal polysaccharide vaccine and Japanese encephalitis vaccine.

2.2 Stability of EPI vaccines

In many situations, vaccines are not being properly stored and transported, and queries often arise about what to do with stocks of vaccines that have been exposed to elevated temperatures for various periods. Unfortunately, there is no straightforward and cheap method that can be used in the field to assess whether a vaccine that has been exposed to ambient temperatures has retained at least the minimum required potency. This can be determined only in the laboratory using expensive assays, the results of which are often delayed by several months. Such tests are only justified when a large number of doses (10 000 or, more) have been exposed to elevated temperatures. Specific instructions for when to order potency tests for heat-exposed vaccines and how to ship vaccines for these tests are provided in an EPI training module entitled "Manage the Cold Chain" (Document WHO/EPI/MLM/91.5).

Individual-vial thermal indicators have been developed. It is anticipated that they will be introduced by manufacturers in 1993. Such indicators, placed on individual vaccine containers, will change color when

Table 1. Basic data on EPI vaccines, with vaccines listed in order of heat stability.

Vaccine against	Nature of vaccine	Potency per dose	Amount per dose	Form	Adjuvant	Conservant	Mode of administration	Heat stability
Diphtheria	Toxoid (detoxified toxin)	At least 30 IU*	10 to 20 Lf**	Fluid	$Al(OH)_3$ or $AlPO_4$	Usually merthiolate	Intramuscular injection***	High
Tetanus	Toxoid (detoxified toxin)	At least 40 IU in TT and 60 IU in DPT	5 to 10 Lf	Fluid	$Al(OH)_3$ or $AlPO_4$	Usually merthiolate	Intramuscular injection***	High
Hepatitis B	Hepatitis B surface antigen (HBsAg)	2.5 to 20 mcg of HBsAg		Fluid	$Al(OH)_3$	Usually merthiolate	Intramuscular injection	High
Pertussis	Whole killed pertussis bacteria	At least 4 IU	10-20 x 10^9 of bacteria	Fluid	$Al(OH)_3$ or $AlPO_4$	Usually merthiolate	Intramuscular injection***	Medium
Measles	Attenuated live virus	At least 1000 $TCID_{50}$ or PFU****		Freeze-dried	None	Small amounts of antibiotics	Subcutaneous injection	High in dried form, low in reconstituted form
Yellow fever	Attenuated live virus	At least 1000 mouse LD_{50} or the equivalent in PFU		Freeze-dried	None	Stabilizing substances	Subcutaneous injection	High in dried form, low in reconstituted form
Tuberculosis	Attenuated live BCG bacteria	50 000 to one million live particles		Freeze-dried	None	None	Intradermal injection	Medium in dried form, low in reconstituted form
Poliomyelitis	Attenuated live viruses of three types	Type 1: at least 1 million; type 2: at least 100 000; type 3: at least 600 000 $TCID_{50}$		Fluid	None	Stabilizer: magnesium chloride or sucrose	Oral	Low

*IU = International units of potency as determined in animal test.
** Lf = flocculation value, the amount of toxoid which when mixed with one International Unit of antitoxin produces an optimally flocculating mixture.
*** In some countries deep subcutaneous injections are used.
**** $TCID_{50}$ = Tissue culture infective 50%; the quantity of a virus suspension that will infect 50% of cell cultures.
PFU = plaque forming unit; the smallest quantity of a virus suspension that will produce a plaque in monolayer cell cultures.

exposed to a given temperature for a given period of time. The change of color will show health workers that a given vial or ampoule has been exposed to potentially harmful elevated temperature.

Information about the stability of a vaccine, and especially about the rate at which it loses potency at a given temperature, can be useful in deciding whether it should be used, sent for testing, or destroyed.

Data on the stability of vaccines have been reviewed recently *(Galazka 1989).* Table 2 shows, in summarized form, stability data for the EPI vaccines at various storage temperatures. The stability of the EPI vaccines varies considerably. Based on their response to storage at 37°C they can be ranked from vaccines with relatively high stability (diphtheria toxoid, tetanus toxoid, and hepatitis B vaccine) to those with relatively low stability (OPV, reconstituted BCG vaccine, reconstituted measles vaccine, and reconstituted yellow fever vaccine). Work is in progress to improve the stability of OPV. Vaccines presented in the freeze-dried form have moderate or high stability, but after reconstitution these vaccines are unstable. Some vaccines, such as tetanus toxoid or hepatitis B vaccine, can withstand long periods of exposure without significant loss of potency. This characteristic could be important in the future for the use of these vaccines in outreach systems to immunize children or women in areas where the cold chain cannot be maintained. Studies are planned to examine the possible use of hepatitis B vaccine and tetanus toxoid without refrigeration in special situations.

Each exposure of a vaccine to elevated temperature results in some degradation, even if the residual potency still exceeds that considered to be the minimum immunizing potency. Moreover, each exposure to ambient temperature has a cumulative effect on reducing vaccine potency. Present recommendations are that all EPI vaccines should be stored routinely at the temperatures recommended by the manufacturers and the national EPI.

Table 2. Stability of EPI vaccines at various temperatures (Galazka 1989).

Vaccine	Stability at different storage temperatures			
	0°C to 8°C	**22°C to 25°C**	**35°C to 37°C**	**Above 37°C**
Diphtheria and tetanus toxoids	3 to 7 years	Several months	About 6 weeks	2 weeks at 45°C; loss of potency after a few hours at 60°C to 65°C
Pertussis vaccine	18 to 24 months, although with continuous slow decrease of potency	Variable; some vaccines stable for 2 weeks	Variable; some vaccines with a 50% loss of potency after one week	About 10% loss of potency per day at 45°C; rapid loss of potency at 50°C
Freeze-dried BCG vaccine	1 year	Variable; 20% to 30% loss of potency after 3 months	Variable; 20% loss of potency after 3 to 14 days	Unstable; 50% loss of potency after 30 minutes at 70°C
Reconstituted BCG vaccine	Reconstituted BCG vaccine should not be used during more than one working session. This recommendation is based on concern about the risk of contamination (since BCG vaccine contains no bacteriostatic agents) and concern about the loss of potency.			
Freeze-dried measles vaccine	2 years	Retains satisfactory potency for 1 month	Retains satisfactory potency for at least 1 week	50% loss of potency after 2 to 3 days at 41°C; 80% loss of potency after 1 day at 54°C
Reconstituted measles vaccine	Unstable; should be used in one working session	Unstable; 50% loss of potency after 1 hour, 70% loss after 3 hours	Very unstable; titer may be below acceptable level after 2 to 7 hours	Inactivation within 1 hour at temperatures above 37°C
Oral polio vaccine	6 to 12 months	Unstable; 50% loss of potency after 20 days; some vaccines may retain satisfactory titers for 1 to 2 weeks	Very unstable; loss of satisfactory titer after 1 to 3 days	Very unstable at 41°C, 50% loss of potency after one day, complete loss of potency after 1 to 3 hours at 50°C

2.3 Use of EPI vaccines

The basic principle guiding the use of the EPI vaccines is that protection against the EPI diseases should be achieved prior to the time that infants are at risk from these diseases. For countries where pertussis, poliomyelitis, and measles pose serious health problems in very young infants, the EPI recommends the immunization schedule shown in Table 3.

In countries where neonatal tetanus is an important cause of infant mortality, immunization of women of childbearing age, and especially pregnant women, is recommended.

Reasons for a particular age for starting immunization, the number of doses, and intervals between doses in the recommended schedule are presented in the disease-specific modules of this series. The modules also discuss alternative schedules of immunization.

Results of available studies indicate benefits from the simultaneous use of some vaccines (i.e. given simultaneously at different places) or the use of combination vaccines (i.e. a mixture prepared in advance such as trivalent OPV or DPT vaccine). The administration of several vaccines simultaneously simplifies routine childhood immunization and reduces the number of contacts. All the EPI vaccines can be administered simultaneously *(Galazka 1991)* and it is common practice to give DPT vaccine and OPV at the same visit. BCG vaccine is compatible with DPT vaccine, measles vaccine, and OPV.

Hepatitis B (HB) vaccine is compatible with the infant vaccines and it is used in several integrated immunization programmes simultaneously with other EPI vaccines. Immunization schedules should be designed to provide the first dose of HB vaccine as early as possible, consistent with the epidemiology of the disease and within the capacity of the vaccine delivery system (see Module 9). Three doses are considered a primary series. Where perinatal transmission of HB virus is common, the first dose should be given as soon after birth as possible, the second within two months, and the third within the first year (Table 3, Scheme A). If early transmission is not a problem, the first dose of HB vaccine may be given at six weeks (or later) with the first dose of DPT vaccine and further HB vaccine doses may be administered simultaneously with each dose of DPT vaccine or measles vaccine (Table 3, Scheme B). In any case, the second and third doses of HB vaccine should be timed to coincide with visits required for other childhood immunizations.

EPI recommends that countries at risk for yellow fever should incorporate yellow fever vaccine into the routine activities of the national immunization programme. Yellow fever vaccine can be given at 6 months of age or with measles vaccine at 9 months of age. Most African countries that have incorporated yellow fever vaccine into their EPI give it at 9 months of age at the same visit as measles vaccine.

Mixing vaccines in one syringe before injection (for example using DPT vaccine as the diluent for measles vaccine) is not recommended since the presence of preservatives or stabilizers in one vaccine can interfere with the action of the other vaccines *(Galazka 1991).*

The first priority for routine immunization programmes is to ensure that infants are completely immunized against the target diseases, with appropriate primary immunization at the youngest age possible. Immunization programmes considering immunization schedules that include additional vaccine doses should evaluate the epidemiological pattern of the target diseases in their country. The additional resources required and any potential negative impact on sustaining high coverage in infants should be considered prior to implementing such schedules.

Table 3. The immunization schedule recommended by the EPI.

Age	Vaccines	Hepatitis B (HB) vaccine	
		Scheme A	Scheme B
Birth	BCG, OPV0 *	HB1	
6 weeks	DPT1, OPV1	HB2	HB1
10 weeks	DPT2, OPV2		HB2
14 weeks	DPT3, OPV3	HB3	
9 months	Measles, yellow fever **		HB3
Women of childbearing age, and especially pregnant women	TT1 -as soon as possible in pregnancy or as early as possible in the childbearing years		
	TT2 - at least 4 weeks after TT1		
	TT3 - at least 6 months after TT2		
	TT4 and TT5 - at least one year after the previous TT dose		

* OPV at birth (OPV0) is recommended in countries where poliomyelitis has not been controlled.
** Yellow fever vaccine is recommended in countries at risk for yellow fever.

3. Types of Immunity

The defense mechanisms of the body are complex. Despite constant microbial challenge from the environment, the body prevents infections by a number of non-specific and specific mechanisms working on their own or together (Figure 2).

3.1 Nonspecific defense mechanisms

Nonspecific defense mechanisms are present in all normal individuals. They are effective at birth and function without requiring prior exposure to a microorganism or its antigens. They include physical barriers (e.g. intact skin and mucous membranes), chemical barriers (e.g. gastric acid, digestive enzymes, bacteriostatic fatty acids of the skin), phagocytic cells, and the complement system. The complement system contains several enzymes and consists of at least 19 separate serum proteins. Complement plays a major role in initiating the inflammatory response, clearing immune complexes, modulating immunoglobulin production, opsonizing microbial pathogens (see section 4.1.2), and killing certain gram-negative bacteria.

3.2 Specific immunity

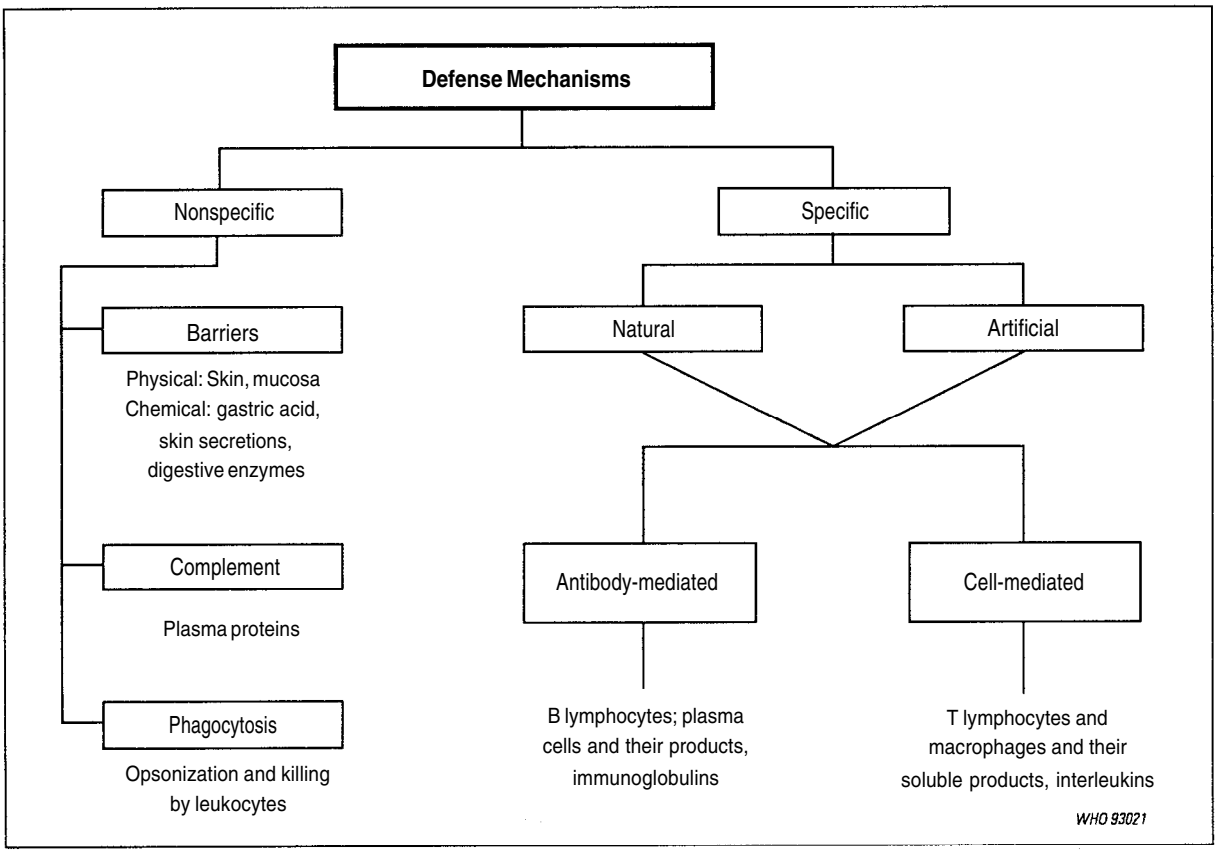
In contrast to nonspecific defense mechanisms, specific immune defense systems are not effective fully at birth and require time to develop after exposure to the infecting agent or its antigens. Specific immunity may be acquired naturally by infection or artificially by immunization.

Specific immunity is divided into antibody-mediated and cell-mediated components. Reactions carried out by antibodies are called humoral immune reactions. The most convenient indicator of immunity is antibody, as antibodies are the best known of the many products of the immune system. Antibody-mediated immunity is related to B lymphocytes (or B cells), and to their direct descendents, known as plasma cells. The plasma cells produce immunoglobulins (antibodies). When a B cell encounters an antigen, recognized by the antibody expressed on the antigen's surface, the B cell is stimulated to proliferate. This leads to expansion of the number of lymphocytes capable of secreting antibody to this antigen. Replication and differentiation of B cells into plasma cells is regulated by contact with antigen and by interactions with T cells, macrophages, and complement.

B lymphocytes develop in fetal liver and subsequently in bone marrow. The name "B" cell comes from the bursa of Fabricius, a specialized organ in birds that acts as a site of B cell development. Mammals do not possess this organ. Approximately 10% of the blood lymphocytes are B cells; most B cells and almost all plasma cells reside in peripheral lymphoid organs, e.g. the spleen, lymph nodes, tonsils and appendix.

Cell-mediated immunity is conferred by T lymphocytes and effected by lymphocytes and macrophages. It involves the function of T lymphocytes (T cells) of

Figure 2. Defense mechanisms of the body



various types and their soluble products, lymphokines (interleukins), that act as signals for communication between different types of cells involved in an immune response.

These two components of specific immunity are closely related to each other. T cells interact with B cells in the production of antibody against most antigens. Specific antibodies and CMI are induced in all infections, but the magnitude and quality of these two components varies in different infections.

4. Antibody-mediated Immunity

4.1 Immunoglobulins

4.1.1 Classes of immunoglobulins

Antibodies are comprised of a family of globular proteins termed immunoglobulins (Ig). Five different classes of immunoglobulins have been identified (IgG, IgM, IgA, IgD, and IgE), based on structural differences in the composition of their heavy chains.

Some of the immunoglobulin classes contain subclasses. For example, IgG has four subclasses: IgG1, IgG2, IgG3, and IgG4 that display differences in their heavy chains. Each IgG subclass has distinct physiochemical and biologic properties. For example, IgG3 has a much shorter serum half-life than IgG1, IgG2, or IgG4. IgG1 and IgG3 activate complement, whereas IgG4 does not. The antibody responses to most protein antigens are found primarily in the IgG1 subclass, although significant amounts of antiviral antibodies occur in IgG3 as well. Small amounts of antiprotein antibodies also occur in IgG4.

There are two subclasses of IgA: IgA1 is the predominant form in serum (90% of total); IgA2 is the predominant form in secretions (60% of total).

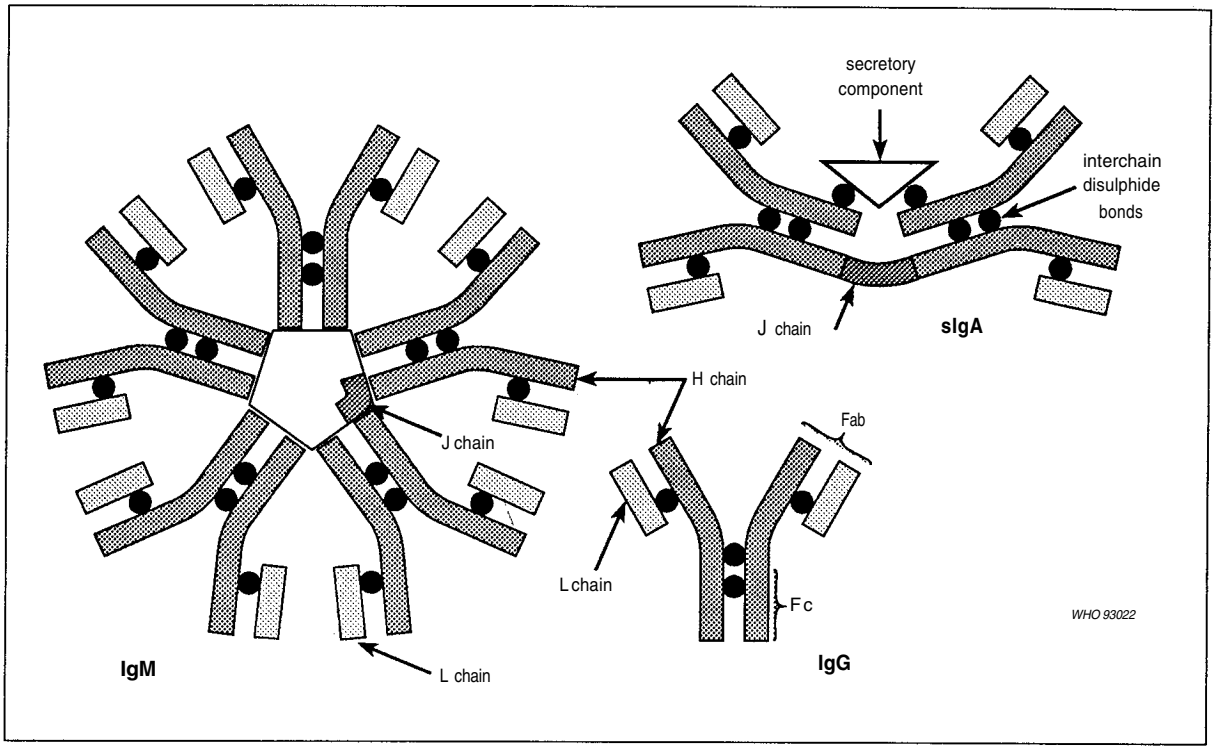
The most abundant immunoglobulins are IgG, IgM, and IgA. IgE antibodies play a major role in allergic reactions and the role of IgD antibodies is not yet fully understood.

4.1.2 Basic structure of immunoglobulins

Each Ig class has a similar basic unit structure consisting of two longer peptide chains, known as "heavy" or H chains, bound by disulfide bridges to two shorter peptide chains, known as "light" or L chains (Figure 3). Heavy chains are of five major types (γ, μ, α, δ, and ϵ) that determine the antibody class. Light chains are of two major types (κ and λ).

IgG is a monomer with four chains. IgM is a

Figure 3. Structural models of IgG, sIgA and IgM.
IgG is a monomer; sIgA is composed of two units, a J-chain and a secretory component; IgM is a pentamer composed of five basic units plus the J-chain. Each Fab fragment has one binding site. The Fc fragment is responsible for transport of IgG across the placenta. Black circles denote interchain disulfide bonds.



pentamer composed of five basic units plus an additional chain, the J or joining chain. Accordingly, the molecular weight of IgM is about 6 times greater than the weight of IgG. IgA exists in two forms, one in serum and one in the secretions. Serum IgA is a monomer, with a single basic unit. Secretory IgA (sIgA) is dimeric, composed of two units, plus J-chain and a secretory component (Figure 3).

Immunoglobulins can be split into active fragments by enzymatic digestion. The main fragment, $F(ab')_2$, is the "head" of a Y-shaped structure and is composed of two subfragments, Fab. The term Fab is used because it is this fragment that binds antigen. Each Fab fragment has one binding site, so there are two binding sites on the IgG molecule. The IgM has ten binding sites (2 x 5). The fragment Fc (the "leg" of the Y-shaped structure) has no antigen-reactive sites, but it gives the molecule certain biologic activities, including the ability to activate complement and combine with receptors on macrophages. These properties are important for opsonic activity. Invading microorganisms are coated by specific antibodies, opsonins, which make microorganisms more easily attacked by macrophages. Macrophages engulf antibody-coated microorganisms by the process of phagocytosis. The Fc fragment is also responsible for the transport of IgG across the placenta.

4.1.3 Functions of immunoglobulins

The primary function of immunoglobulins is to serve as antibodies. This is accomplished through the molecule's antigen binding (Fab) portion.

The size of the immunoglobulin molecule is one of the factors determining its tissue distribution. IgG is the major immunoglobulin in the bloodstream and accounts for about 80% of the total immunoglobulin pool. IgG is also present in the tissue spaces. It easily passes through the placenta (Table 4). IgG is responsible for neutralization of viruses and bacterial toxins, facilitating phagocytosis, and lysing (destroying) bacteria.

IgM, the largest immunoglobulin, is confined mainly to the bloodstream and is less able to pass through capillary walls. IgM does not pass through the placenta. With its 10-valent antigen-combining site, IgM has a high affinity, i.e. a strong ability to bind firmly with antigen. IgM is particularly effective in the complement-mediated lysis of microorganisms.

IgA is the second most abundant immunoglobulin in serum. IgA is the predominant immunoglobulin in secretions of the gastrointestinal and respiratory tracts as well as in human colostrum and milk. IgA provides local mucosal immunity against viruses and limits bacteria overgrowth on mucosal surfaces. IgA also

Table 4. Properties of immunoglobulins.

Property	IgG	IgM	IgA*
Molecular weight	150 000	900 000	385 000 (170 000)
Half life in days	25	5	(6)
Concentration in adult serum, mg/dl	1 000	100	(250)
% of total Igs	80	6	(130)
Proportion in serum (%)	50 to 60	90	0
Proportion in extracellular fluids (%)	40 to 50	<10	0
Proportion in secretions (%)	0	0	100
Complement fixation	++	++++	0
Opsonic activity	++++	+	0
Lytic activity	++	++++	0
Viral neutralization	+++	++	+++
Transfer to offspring	via placenta	no transfer	via colostrum/milk

* Data for secretory IgA; data for serum IgA in brackets.

functions in the gastrointestinal tract and shows a greater resistance to proteolytic enzymes that other classes of antibodies.

4.1.4 Placental transfer of immunoglobulins

Maternal IgG (but not IgM or IgA) is transported across the placenta from about 16 weeks onwards. This reflects passive transport, which increases progressively with gestation and is proportional to the maternal IgG concentration. It also reflects active transport, which tends to normalize neonatal IgG concentration, suggesting that low maternal values stimulate and high maternal values inhibit transport. At full term, umbilical cord levels of IgG can be equal to or even higher than maternal levels. Premature infants have lower IgG levels than those delivered at term. Passively-acquired IgG antibodies are responsible for the protection of newborns and young infants against viral and bacterial diseases.

The transfer of IgG antibodies from mother to fetus across the placenta provides the newborn with a portion of the mother's immunologic experience. This experience is different in areas where infectious agents circulate at high levels in the population and adults are naturally immune, compared to areas where the circulation of infectious agents is limited and adults have low levels of immunity. In developing countries, passive transfer occurs for diphtheria, measles, polio, and rubella antibodies. Also, tetanus antibodies induced in the mother following immunization with tetanus toxoid easily pass across the placenta providing protection against tetanus in the newborn (see Module 3). In developed countries, where women of child- bearing age may have low levels of polio and diphtheria antibodies, transfer of these antibodies is more limited. If protective maternal antibodies are not of the IgG type, as is usually the case for the gram-negative pathogens such as *Escherichia coli* and *Salmonella,* the fetus does not receive antibodies from the mother and the neonate is not passively protected against these infections.

4.1.5 Normal development of serum immunoglobulins

Immunoglobulin synthesis starts before birth. IgM has been shown to be present at 10 weeks, IgG at 12 weeks, and IgA at 30 weeks of gestation. Most of the antibody synthesized by the fetus is IgM. Nevertheless, the fetus grows in a sterile environment and production of immunoglobulins by the healthy fetus is extremely limited until birth. In some fetuses immunoglobulin synthesis may be delayed or may not occur at all.

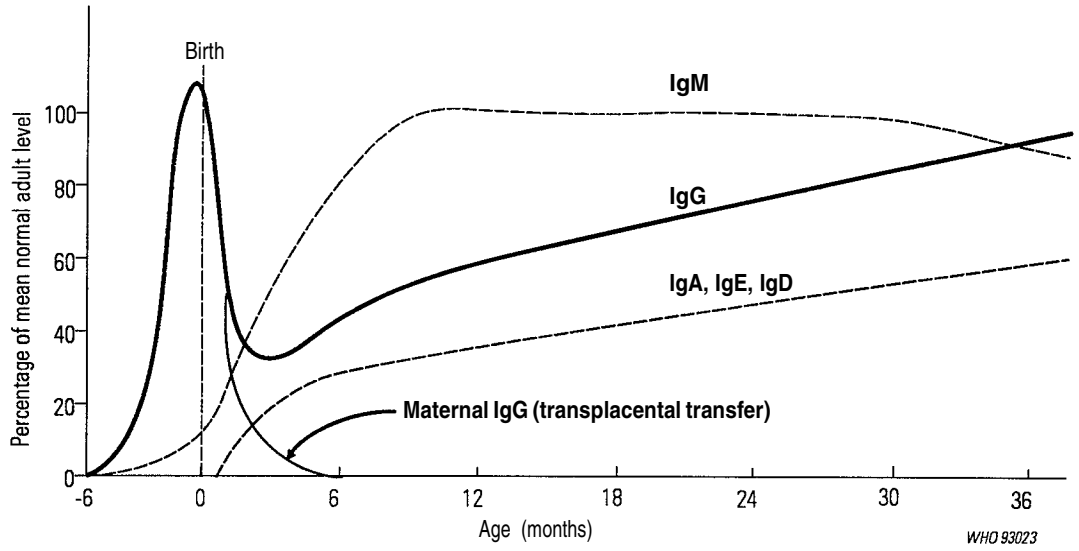
In the first year of life immunoglobulin levels increase quickly under the influence of antigenic challenge from the environment (infections) and through the contact with vaccine antigens (Figure 4). By one year of age, values for the IgG, IgM, and IgA concentrations are approximately 60%, 100%, and 30%, respectively, of those in adults.

The newborn infant is capable of responding to a number of diverse antigens, but to fewer and at a much reduced level compared with an adult. There is little or no response to polysaccharide antigens. The relative inefficiency of the fetal and newborn humoral immune response reflects both immaturity in the antibody-producing B cells and plasma cells and poor T cell — B cell cooperation.

Passively transferred antibody, especially at high levels, may transiently suppress the response of the infant to specific antigens. This phenomenon has influenced the schedule for some immunizations. Measles immunization, for example, is postponed until 9 months of age when placentally transferred antibodies have fallen to low concentrations (see Module 7). A high level of passively acquired diphtheria, tetanus, and pertussis antibodies may inhibit the response to all components of DPT vaccine during the first weeks of life. This is the reason for delaying the administration of the first dose of DPT vaccine until 6 weeks of age. This inhibitory effect is transient and it diminishes following subsequent doses of DPT vaccine (see Modules 2, 3, and 4).

Infants born prematurely and infants small for gestational age respond to immunization as well as term infants of a similar postnatal age.

Figure 4. The normal development of serum immunoglobulin levels.



4.2 Measurement of antibody activity – serological assays

4.2.1 When are serological studies useful?

Because it is rather easy to estimate immunity by measuring circulating antibody, there is a tendency to identify antibody with immunity. However, the antibody level does not reflect the total immunity of the body *(Ipsen 1961).* The presence of serum antibody does not always mean that immunity exists, but it does indicate that the individual has had a previous encounter with the microorganism. Furthermore, for most of the EPI diseases the level of antibody considered to be protective is defined somewhat arbitrarily or is based on artificial laboratory models (see Module 3). The level of protection depends not only on the amount of antibody assayed, but also on the affinity of antibodies (see section 4.3.3), their classes and subclasses, and their complement fixing capacity — properties not measured in routine tests. The concentration of antibodies that exists in an individual does not reflect the degree of priming for a booster response after subsequent exposure to the microorganism.

Finally, currently available serological techniques cannot distinguish between antibodies induced by contact with circulating microorganisms or their toxins (natural immunity) and antibodies induced by immunization. All these factors cause serological methods to have a limited value in routine monitoring of immunization programmes in developing countries. Other tools, such as immunization coverage surveys or different surveillance techniques for target diseases, may be more useful for that task.

On the other hand, serological techniques can be very useful for providing answers to clearly defined questions on the epidemiology of target diseases or the efficacy of immunization programmes. They have been used successfully for to assess seroconversion following administration of various measles vaccines in different age groups (see Module 7), to determine antibody titers following different vaccines and immunization schedules against poliomyelitis (Module 6) and tetanus (Module 3), to assess the diphtheria immune status in various age groups in areas where circulation of *Corynebacterium diphtheriae* is reduced (Module 2), to evaluate the rate of decline of passively-acquired antibodies, and to evaluate the duration of vaccine-induced immunity against different target diseases.

4.2.2 Methods to measure antiviral antibodies

Antiviral antibodies can be measured by the neutralization test on tissue culture, the hemagglutination inhibition (HI) test, and the enzyme-linked immunosorbent assay (ELISA).

The basis for the neutralization test is the property of viruses to propagate and to produce degenerative morphological changes (cytopathic effect) in a susceptible cell culture. If antibody is present in the sample, the virus will be neutralized and rendered inactive and will not produce a cytopathic effect. Although neutralizing antibody is the most important antibody for the resolution of infection and the establishment of immunity, neutralization tests are not performed routinely because they are expensive and time-consuming.

Some viruses have hemagglutinating properties, i.e. the virus has the ability to bind to erythrocytes and form a lattice of hemagglutinated erythrocytes in the bottom of a tube or well. Selective blocking of hemadsorbtion by antibodies is the basis for the

commonly used hemagglutination inhibition test (see Module 7).

In the indirect ELISA test, antibody in the test solution is allowed to react and form a complex with the antigen, a specific virus or viral antigen which has been passively adsorbed to a surface of polystyrene microtiter wells or plastic beds. An enzyme-labelled antibody against bound human antibody (usually anti-IgG) is then attached to the antigen-antibody, complex. The uptake of label to the solid phase shows the amount of antibody in the test sample and can be measured by the degree of degradation of the suitable enzyme substrate. Usually, the substrate is chosen so that the final result is a color change which can be assessed visually or photometrically.

4.2.3 Methods to measure antibacterial antibodies

Antibacterial antibodies are determined by two main groups of tests: *in vivo* neutralization tests and *in vitro* techniques.

Different properties of bacterial toxins are utilized for *in vivo* neutralization tests. The dermonecrotic property of diphtheria toxin is used to demonstrate the presence of neutralizing diphtheria antibody on the skin of guinea pig or rabbit. An alternative is the Schick test on human skin. Tetanus toxin has no dermonecrotic effect and the proportion of mice that survives after injection with a mixture of toxin and test sample is used to measure neutralizing antibodies (see Module 3).

Neutralizing tests *in vivo* are sensitive and show. the functional capacity of antibodies -the neutralization of toxin — and not only a reaction between relevant and nonrelevant antigen-antibody systems, as is be the case with *in vitro* tests. However, *in vivo* tests are laborious, expensive, require well trained personnel, use a large number of costly animals, and need a relatively large amount of serum for determination of low concentrations of antibodies.

Diphtheria neutralizing, antibodies may also be tested *in vitro* on microcell culture (see Module 2). Neutralizing antibody to pertussis toxin can be measured on microplate culture of Chinese hamster ovary cells (see Module 4).

Many other *in vitro* tests are used to measure bacterial antibodies. The most common are: passive hemagglutination (HA) and ELISA for diphtheria, tetanus, and pertussis antibodies; and bacterial agglutination for pertussis agglutinins. In general, these tests are simple, sensitive, rapid (for example, results of an HA test for tetanus may be known after one hour), and inexpensive. However, *in vitro* tests are less specific than *in vivo* neutralization tests, which are more sensitive in detecting IgM than IgG antibodies, particularly in the early periods of the primary response to immunization or infection. Therefore, the results of *in vitro* techniques should be interpreted carefully and verified against *in vivo* neutralization tests.

Details of particular techniques are discussed in relevant modules for specific target diseases.

4.3 Immune response

4.3.1 Class-specific response

Immunization and natural infection induce production of antibodies of the IgG, IgM, and IgA classes. During acute infection IgM antibody usually appears within the first few days after onset of symptoms and it reaches its peak concentration by 7 to 10 days. IgM gradually declines to undetectable levels over the next several months with resolution of the infection. Thus, the presence of IgM antibody in the serum indicates a current or recent infection, although there are exceptions to this rule.

In natural infection or after immunization, serum IgG antibody usually appears simultaneously with IgM, or within a day or two after. IgG rapidly increases in concentration thereafter (Figure 5). IgG antibody usually persists for years at low levels, which are detectable with appropriate tests of sufficient sensitivity. On reinfection or revaccination, a booster response occurs (section 4.3.2).

The route of immunization or infection determines whether the IgA antibody response will be mainly systemic or mucosal. A systemic IgA antibody response occurs with parenterally injected vaccines or infections with microorganisms that replicate in and disseminate to inner organs and to the systemic circulation. The serum IgA antibody response varies in onset, level, and duration, and is less predictable than IgM and IgG antibody responses.

4.3.2 Primary versus secondary immune response

On the first introduction of an antigen into the body, the antibody response takes up to 10 days to develop. This period is called the lag time, or lag phase. Lymphoid cells encounter the antigen, divide repeatedly to form a clone of cells with similar reactivity, differentiate, and start to synthesize antibody. The antibody levels rise steeply, reach a plateau, and then decline.

The antibody response following the first (primary) encounter with antigen differs from that following the second (secondary) contact. The primary response has a longer lag phase, reaches a lower plateau, and declines more quickly than the secondary response. A proportion of persons immunized with a killed vaccine (tetanus toxoid, for example) will be "primed" but will not show an antibody response. On reexposure to antigen, there is an accelerated response with a shorter lag period, a higher plateau and persisting levels of antibody.

A major component of the primary immune re-

sponse is IgM, whereas IgG is the main immunoglobulin class represented in the secondary immune response. The difference between the primary and the secondary response is most marked when the antigen stimulates both B lymphocytes and T lymphocytes (T- dependent antigens).

Subsequent doses of antigen result in a faster and more vigorous antibody response. The capacity to respond to a booster dose of a vaccine also is prolonged following several doses. For example, the booster response to tetanus toxoid has been observed to last for up to 20 years following the third dose of tetanus toxoid (see Module 3).

A so-called "negative phase", with a transient drop in antibody levels a short time after a secondary stimulus, has been observed. Further research is needed to determine the importance and magnitude of this phenomenon. Following a booster dose of tetanus toxoid, one study showed no change in the level of tetanus antitoxin level; however, resistance to tetanus toxin started immediately *(Ipsen 1961).* This may be related to an increase in the avidity (see section 4.3.3) of the antitoxin produced.

4.3.3 Maturation of the immune response — avidity of antibodies

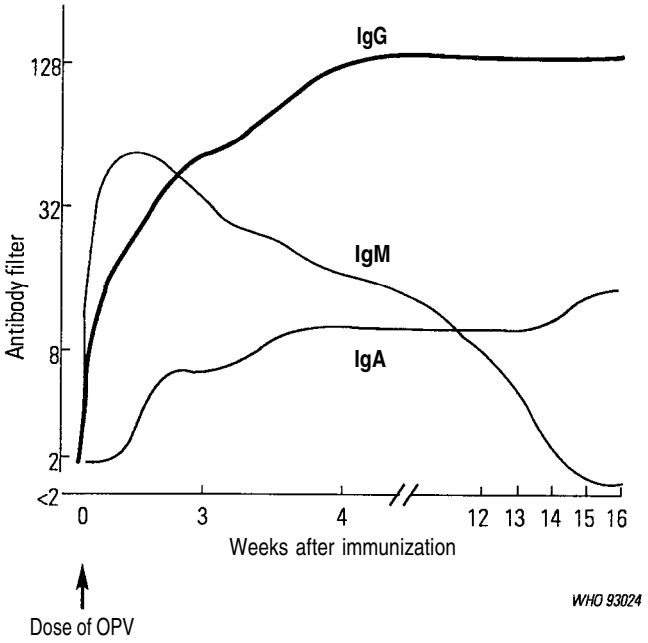
The immune response is characterized not only by the amount of antibody produced, but also by the quality of the antibody. One of the measures of quality is the strength of the bond between a single antigen-combining site of the antibody and an antigenic determinant of the antigen. This property is termed antibody affinity and the sum of all binding forces is called antibody avidity. The avidity of antibody matures during the immune response. B lymphocytes producing high-affinity antibodies are more likely to be triggered on rechallenge, so that the average binding affinity of the antibody increases following subsequent exposure to the antigen. High-affinity antibody with strong binding capacity is much more effective in neutralizing viruses or bacterial toxins than low-affinity antibody.

5. Cell-Mediated Immunity

5.1 The nature of cell-mediated immunity

In many infections the immunological responses of the host include not only the synthesis of antibodies against various antigenic determinants, but also the development of cell-mediated immunity to some of components of the microorganism. The term cell-mediated immunity is a generic designation for immune responses that can be transferred to a nonimmunized recipient by lymphoid cells, but not by antibody.

Figure 5. The temporal appearance of different classes of serum antibodies following primary immunization with live oral poliomyelitis vaccine



5.2 The T lymphocyte — a key cell in the immune response

Cell-mediated immunity is mediated by a subset of lymphocytes called T lymphocytes, or T cells. These cells circulate in the bloodstream and lymphatic vessels and also migrate through the intracellular space. Immunologically-reactive T lymphocytes control immune responses; each immune response is controlled by different lymphocytes. T cells mediate three principal functions: help, suppression, and cytotoxicity. T lymphocytes called helper cells stimulate the immune response of other cells (i.e. T cells stimulate B cells to produce antibodies). The helper functions are mediated primarily by a subset of T-cell helpers that express CD4 surface antigen.

Other T lymphocytes, called suppressor cells, play an inhibitory role and control the level and quality of the immune response. Another function of T cells is recognizing and destroying infected cells and activating phagocytes to destroy pathogens they have taken up. The suppressor and cytotoxicity functions are mediated primarily by T cells expressing CD8 surface antigen.

5.3 Signals between cells of the immune system — lymphokines

When a T lymphocyte meets a foreign antigen, it will attach itself to the antigen or antigen-containing cell. Antigen-activated T cells respond by secreting lymphokines, proteins that act as molecular signals for communication between cells of the immune

system (B cell — T cell interaction) and as systemic mediators of the host's response to infection. The group of lymphokines includes some interleukins, B cell growth and differentiation factors, and interferon gamma.

Cytokine is a more general term. The cytokines include lymphokines produced by T cells as well as similar substances produced by other cell types, particularly mononuclear phagocytes. Lymphokines help B cells produce antibody and phagocytes deal more effectively with pathogens.

6. Hypersensitivity

The term hypersensitivity is used when an immune response occurs in an exaggerated or inappropriate form causing tissue damage. Four types of hypersensitivity reaction are known; the first three are antibody-mediated, the fourth is mediated primarily by T cells and macrophages.

- **Type I,** or immediate hypersensitivity, is characterized by an allergic reaction immediately following contact with the antigen (which is referred to as the allergen). The immediate hypersensitivity reaction is dependent on the specific triggering of IgE-sensitized cells by antigen, resulting in the release of pharmacological mediators of inflammation (for example, histamine). An example of immediate hypersensitivity is the reaction to bee venom. Atopic diseases, such asthma, eczema, hay fever, and urticaria, also belong to this category.
- **Type II,** or antibody-dependent cytotoxic hypersensitivity, occurs when antibody binds to antigen on cells and this leads to phagocytosis, killer cell activity, or complement-mediated lysis. The most clear-cut example of a type II reaction is the response of an individual to red blood cells following an incompatible blood transfusion.
- **Type III,** or immune complex-mediated hypersensitivity, develops when antibody-antigen complexes are formed in large quantities, or cannot be cleared adequately by the reticuloendothelial system, leading to serum sickness-type reactions. Chronic immune complex formation, with the deposition of complexes in the tissues, occurs in streptococcal and staphylococcal endocarditis, malaria infection, and hepatitis B virus infection. Neurological reactions following hyper-immunization with tetanus toxoid belong to this category and are due to the immune complexes that are formed between preformed antibody and injected toxoid. These immune complexes attract complement and leukocytes, which produce localized vascular damage (see Module 3). Serum sickness following injection of heterologous serum is another example of type III hypersensitivity.
- **Type IV,** or delayed type hypersensitivity, develops when antigen trapped in a macrophage cannot be cleared. T lymphocytes are then stimulated to elaborate lymphokines, which mediate a range of inflammatory responses. Delayed hypersensitivity is seen with a variety of viral, bacterial, protozoal, fungal and helminth infections. The cutaneous delayed hypersensitivity reaction to tuberculin is a classic example. Tuberculin is the lipoprotein obtained from *Mycobacterium tuberculosis* organisms. The tiny fraction of T cells (less than 1 in 1000) innately reactive to tuberculin proliferates to form a clone of reactive cells after initial exposure (a clone is a group of cells derived from a single original cell). An individual who has been exposed to tubercle bacilli or immunized with BCG has T lymphocytes that are sensitized to tuberculin. When such an individual is injected intradermally with tuberculin, there is a positive inflammatory reaction at the injection site after 24 to 48 hours. Further discussion on the tuberculin reaction can be found in Module 5.

Abbreviations

BCG	Bacille Calmette-Guérin, vaccine against tuberculosis
DPT	diphtheria-tetanus-pertussis vaccine
ELISA	enzyme-linked immunosorbent assay
EPI	Expanded Programme on Immunization
HA	hemagglutination test
HB	hepatitis B
IU	international unit of potency
LD_{50}	dose which kills 50% of test animals
Lf	Flocculation value, the amount of toxoid which when mixed with one International Unit of antitoxin produces an optimally flocculating mixture
OPV	oral polio vaccine
PFU	plaque forming unit; the smallest quantity of a virus suspension that will produce a plaque in monolayer cell cultures
$TCID_{50}$	Tissue culture infective dose 50%; the quantity of a virus suspension that will infect 50% of cell cultures
TT	tetanus toxoid

References

Cremer NE. Antibodies in serodiagnosis of viral infections. In: Lennette EH, ed. Laboratory diagnosis of viral infections. New York: Marcel Dekker;l985: 73-85.

Galazka A. Stability of vaccines. Document WHO/EPI/GEN/89.8. Geneva: World Health Organization, 1989.

Galazka A. Simultaneous administration of vaccines. Document EPI/RD/9l/WP.7/APR/Rev.l. Geneva: World Health Organization, 1991.

Halsey NA, Klein D. Maternal immunization. Pediatr Infect Dis J 1990;9:574-581.

Heinzel Fp Root RK. Antibodies. In: Mandell GL, Douglas RG, Bennett JR, eds. Principles and practice of infectious diseases, 3rd ed. New York: Churchill Livingstone;l990:41-61.

Ipsen J. Changes in immunity and antitoxin level immediately after secondary stimulus with tetanus toxoid in rabbits. J Immunol 1961;86:50-54.

Mims CA. The pathogenesis of infectious disease, 2nd ed. London: Academic Press;1982.

Moxon ER, ed. A Lancet review: Modern vaccines, current practice and new approaches. London: E. Arnold;1990.

Roitt I, et al. Immunology. London: Gower Medical Publishing;1989.

Smith TF. IgG subclasses. Adv Pediatr 1992;39:101-126.

Wilson CB. The cell immune system and its role in host defense. In: Mandell GL, Douglas RG, Bennett JR, eds. Principles and practice of infectious diseases, 3rd ed. New York: Churchill Livingstone;1990:101-138.

The **Global Programme for Vaccines and Immunization,** established by the World Health Organization in 1994, defines its goal as "a world in which all people at risk are protected against vaccine-preventable diseases". The Programme comprises three units:

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.

The **Global Programme for Vaccines and Immunization** produces a range of documents, audiovisual materials and software packages to disseminate information on its activities, programme policies, guidelines and recommendations. It also provides materials for group and/or individual training on topics ranging from repair of health centre equipment to curricula guidelines for medical schools, nursing colleges and training of vaccine quality control personnel.

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