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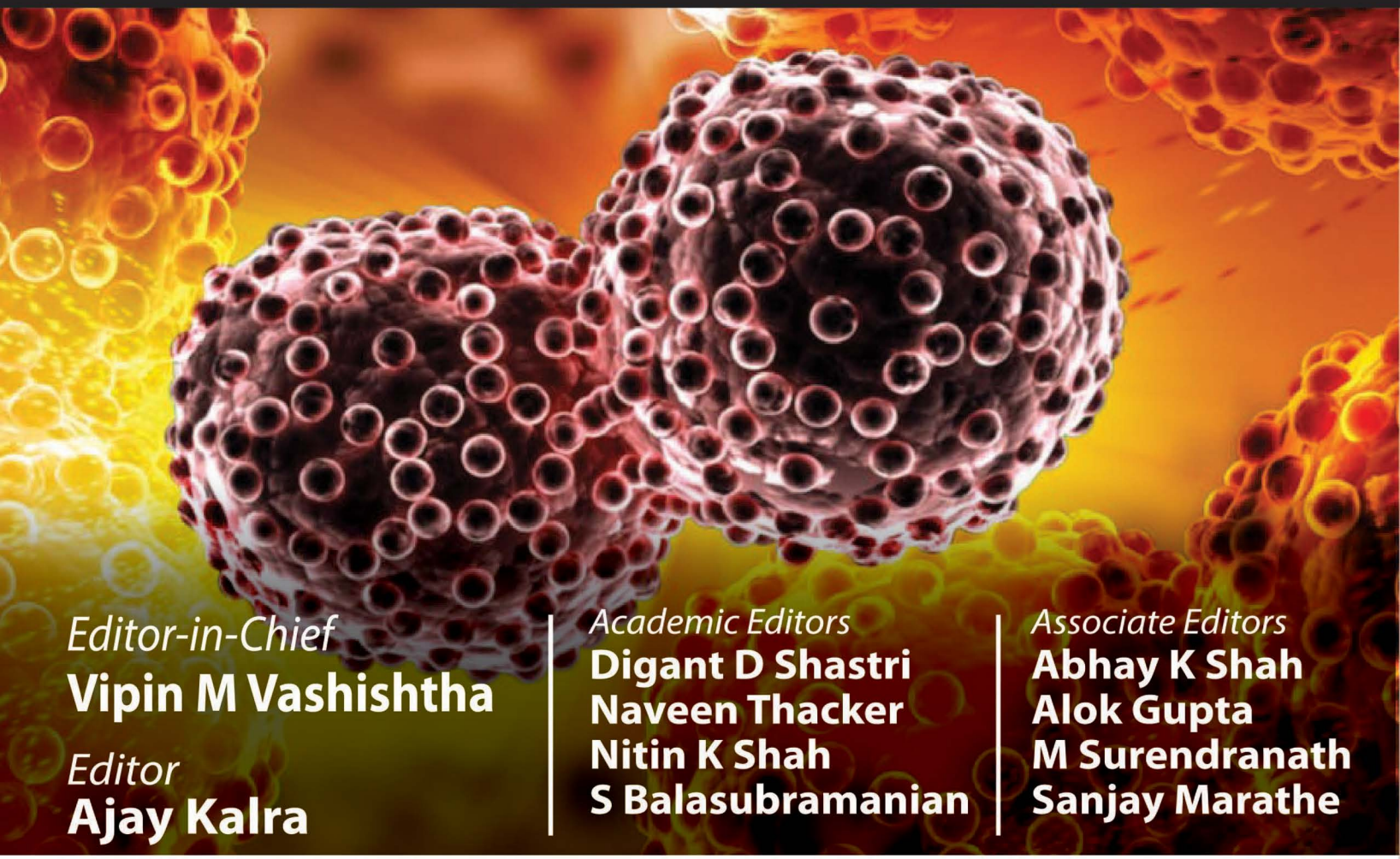
# IAP Textbook of VACCINES



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# Vaccine Immunology

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## ABSTRACT

Since the 1796 introduction of a vaccine against the smallpox virus, a large repertoire of vaccines has been developed against a myriad of infectious organisms resulting in stamping out some of the deadliest diseases in the world. However, the spectacular failure to produce vaccines against infectious agents such as malaria and human immunodeficiency virus (HIV) demonstrates the critical importance of understanding the mechanisms of immune interactions that will permit development of effective vaccines.

Immunizations protect individuals by generating immune responses against either T-cell-independent antigens (in which B-cells produce antibodies to the antigen without T-cell help) or T-cell-dependent processes (in which antigens are cleared by antibodies or by direct killing and clearance by mechanisms that involve interactions between T-cells, and their soluble factors, with B-cells and cytotoxic cells). While the innate immune system confers short-term shallow protection against foreign antigens, it is the adaptive immune system that plays a key role in generating strong, long-term "memory" response to those antigens. The cells of the immune system primarily responsible for the memory and long-term response are T-cells (both helper and cytotoxic T-cells) and B-cells which produce the protective antibodies upon interaction with the different types of T-cells and T-cell-derived cytokines, in conjunction with antigen-presenting cells.

Qualities of the ideal vaccine include the ability to confer protection against all strains of the infectious organism including the nonvaccine strains. Importantly, the ideal vaccine should not cause immune responses against self-antigens. Polysaccharide vaccines are poorly immunogenic in children under 2 years of age and, therefore, conjugated vaccines have been developed and used in this population.

Vaccines remain an important method to control and eliminate infectious diseases. More research is needed to understand the immune mechanisms by which the current vaccines protect against infections so that vaccines that are most effective in combating other infectious diseases can be developed.

*Keywords*: Immunization, vaccines, T-cell independent antigens, T-cell dependent process, innate immune system, adaptive immune system.

## INTRODUCTION

The first successful vaccine was developed by Edward Jenner in 1796 against smallpox and was based on Jenner's observations that milkmaids in general did not develop smallpox infection because they were either exposed to or had previously had cowpox infection.[1] Since that time, a large number of vaccines have been developed against various pathogens, but we have not been successful in developing vaccines against many others. In general, vaccines have been developed against only those organisms that have small genomes. This could be because organisms with small genomes have fewer genes involved in developing mechanisms to escape the host immune response as compared to organisms with larger genomes where many genes may be involved in that process.[2] While it was understood that vaccines elicit immune responses by activating complex processes involving myriad interactions between the cellular and secretory components of the

immune system, the precise mechanisms underlying this protection were not examined in detail until attempts to develop vaccines against organisms such as the human immunodeficiency virus (HIV), malaria, and *Mycobacterium leprae* failed. This failure to generate protective and sustained immune responses against certain organisms has made it imperative to understand the immune mechanisms involved in providing protection against infectious diseases. Understanding the immune response to vaccines will also help in the process of developing vaccines tailored specifically to the objectives of the vaccine: to prevent infection, to prevent spread of the infection and to prevent clinical manifestations of the infection.

In the past, it was believed that most vaccines provide protection through production of specific antibodies by B lymphocytes or B-cells (humoral immunity).[3] However, we now know that T lymphocytes or T-cells play a significant role in generating an effective and sustained humoral response.[4] T-cells are crucial in providing immune protection (cell-mediated immunity) against certain organisms.[5] It has also recently been demonstrated that innate immunity (described here) also plays a significant role in generating immune protection by vaccines.

## IMMUNE SYSTEM RELEVANT TO VACCINE IMMUNE RESPONSE

The immune system exerts its effects through the "innate"or the "adaptive" immune responses. The innate immune system destroys invading pathogens nonspecifically, i.e. without requiring specific responses to a particular antigen and without eliciting or requiring immunogenic memory to specific antigens. Adaptive immunity, on the other hand, elicits immune response to a specific antigen and has memory to that antigen. The innate immune system can act independent of the adaptive immune system, but may also work synergistically with the adaptive immune system to generate an effective immune response against the invading pathogen.

The innate immune response is brisk and short-lasting. It is nonspecific to a single pathogen but is specific to the conserved molecular patterns of all pathogens and thus lacks memory to one single specific antigen (organism). Skin, mucous membranes, gastric acid, lysozymes, and the complement system are the major components of the innate immune system.

Recently, pattern recognition receptors (PRRs) have been recognized as important components of the innate immune system.[6,7] PRRs are membrane proteins and are nonspecific to a pathogen. Mannose-binding lectin, C-reactive protein, heat shock protein, and Toll-like receptors (TLRs) are some examples of PRR. The PRRs recognize danger signals or pathogen-associated molecular patterns (PAMPs), proteins which are produced by microorganisms and not by human cells and thus they do not recognize and react to self-antigens.[7] Peptidoglycans which are found in the cell walls of bacteria and lipoproteins on bacterial capsules, bacterial DNA, and viral ribonucleic acid (RNA) are some examples of PAMPs. Engagement of PRRs by PAMPs leads to cytokine release, activation of complement, opsonization and phagocytosis of the pathogen.[7,8]

Monocytes, macrophages, and granulocytes are the cellular components of the innate immune system. Mature monocytes differentiate into tissue-specific macrophages or into dendritic cells (DCs), which present antigens to T-cells. DCs, which are present in lymph nodes, are known as follicular dendritic cells (FDCs). The main function of granulocytes is phagocytosis.

Adaptive immune system provides specific immune response to an antigen and has memory. Humoral immunity provided by B lymphocytes and cellular immunity provided by T lymphocytes are the two components of the adaptive immune system. Humoral immunity confers protection against extracellular organisms and toxins. B-cells respond to both T-cell-independent and T-cell-dependent antigens. T-independent antigens are naïve antigens and B-cells respond to them without the antigens being processed by antigen-presenting cells (APCs). Examples of such antigens are lipopolysaccharide and bacterial flagellin. The T-dependent antigens are protein antigens. The T-independent response is weaker and provides poor memory as compared to the T-dependent response. Upon recognition of an antigen, B-cells undergo clonal proliferation. These B-cells may mature to plasma cells producing specific antibodies, initially of immunoglobulin M (IgM) isotype and then switching to IgG and/or other isotypes. This is known as the primary immune response. Some of these B-cells may enter into the memory pool. These memory B-cells respond rapidly to subsequent exposure to the same antigen. The antigen-binding (Fab) portion of the antibody undergoes somatic mutation and the antibody specificity and affinity to antigen improves over a period of time, especially upon repeated exposure to the same antigen. This occurs due to selection of B-cells possessing receptors (antibodies) with higher affinity over B-cells possessing receptors with low affinity with repeated encounter with the same antigen.

Cellular immunity is conferred by T-cells, including T helper cells (CD4 cells) and T cytotoxic cells (CD8 cells). There are two types of T helper cells: (1) Th2 cells, which promote the humoral response and (2) Th1 cells, which promote the cell-mediated response. CD4 cells recognize antigens presented to them with major histocompatibility complex (MHC) antigen II, while CD8 cells recognize antigens presented to them in the context of the MHC antigen I. CD8 cells help control intracellular organisms. Like B-cells, T-cells proliferate upon exposure to specific antigen and then may either develop into effector cells or enter into the memory pool.

Thus, when the body is challenged with a pathogen, monocytes or macrophages of the innate immune system process this antigen for presentation to the effector cells. If the pathogen is a virus, the antigen is bound to MHC I protein and presented to CD8 cells, whereas if the pathogen is a bacteria or a parasite, the antigen is bound to the MHC II protein and presented to CD4 cells. This interaction elicits an immune response, i.e. an effector mechanism which rids the body of the offending agent via production of antibodies by B-cells, or generation of cytotoxic T-cells. Regulatory T-cells, which are responsible for maintaining immune tolerance, control these effector cells.[9]

## IMMUNE RESPONSE TO VACCINES

Immunization can be passive or active. Both immunization types can occur naturally or artificially. Natural passive immunization occurs when maternal antibodies are transferred transplacentally to the fetus, while artificial passive immunization is achieved by transfusing antibodies (plasma, antibody concentrate, or monoclonal antibodies) to protect the individual against organisms or toxins. Active immunization is a process whereby the body's immune system mounts either humoral, cellular or a combined humoral/cellular immune response. This can happen after a natural exposure such as following infection or can be induced clinically by vaccines. Passive immunization is short-lasting, while active immunization with the majority of currently available vaccines protects individuals for prolonged periods of time and also results in memory response.

In general, all vaccines incite both humoral and cellular immune responses. However, whether the immune response is predominantly cellular or humoral in nature depends on the nature of the vaccine **(Table 1)**. Vaccines containing unconjugated capsular polysaccharide elicit T-cell-independent antibody responses.[10] These immune responses are weak and ill-sustained, especially in children

**TABLE 1:** Predominant immune responses to routine childhood vaccines.

| *Vaccines* | *Nature of vaccine* | *Humoral immune response* | *Cellular immune response* |
|---|---|---|---|
| Diphtheria | Toxoid | Yes | No |
| Hepatitis A | Killed | Yes | No |
| Hepatitis B | Protein | Yes | No |
| *Haemophilus influenzae* type B | Polysaccharide | Yes | No |
| *Haemophilus influenzae* type B (conjugated) | Protein conjugate | Yes | No |
| Human papillomavirus | Virus-like particles | Yes | No |
| Influenza (injectable) | Killed | Yes | No |
| Meningococcal | Polysaccharide | Yes | No |
| Meningococcal (conjugated) | Protein conjugate | Yes | No |
| Pertussis (whole cell) | Killed | Yes | No |
| Pertussis (acellular) | Protein | Yes | No |
| Pneumococcal | Polysaccharide | Yes | No |
| Pneumococcal (conjugated) | Protein conjugate | Yes | No |
| Polio (Salk) | Killed | Yes | No |
| Tetanus | Toxoid | Yes | No |

*Contd...*

*Contd...*

| *Vaccines* | *Nature of vaccine* | *Humoral immune response* | *Cellular immune response* |
|---|---|---|---|
| Influenza intranasal | Live attenuated | Yes | Yes |
| Measles | Live attenuated | Yes | Yes |
| Mumps | Live attenuated | Yes | Yes |
| Polio (Sabin) | Live attenuated | Yes | Yes |
| Rotavirus | Live attenuated | Yes | ?Yes |
| Rubella | Live attenuated | Yes | Yes |
| Varicella | Live attenuated | Yes | Yes |

under the age of 2 years because the marginal zone of the spleen (where the majority of this antibody production occurs) is not fully matured under the age of 2 years, and also because of the immaturity of B-cells (limited expression of molecule CD21 on their surfaces) as well as immaturity of the complement system.[11,12] When this capsular polysaccharide is conjugated to a protein carrier molecule, it now elicits a T-cell-dependent antibody response.[13] Examples of such vaccines are conjugated meningococcal and pneumococcal vaccines. Live attenuated vaccines such as varicella and measles, mumps, and rubella vaccines predominantly elicit cytotoxic T-cell immune responses.[14]

## EFFECTOR MECHANISMS GENERATED BY VACCINES

Most vaccines used in clinical practice today provide protection by generating antibodies (humoral immune response).[3] Antibodies are effective in preventing as well as reducing the severity of infections (clinical consequences of infections) by both extracellular and intracellular pathogens. Antibodies promote the opsonization and phagocytosis of extracellular bacteria and prevent colonization of mucosal surfaces with pathogenic bacteria. Antibodies also prevent viral replication by preventing binding of viruses to their cellular receptors and thus blocking viral entry into the cells. Antibodies neutralize toxins produced by the bacteria. This action can take place at the site of infection such as in wounds infected with tetanus or at the periphery/away from the site of infection. Even though antibody titers are usually checked to determine the response to vaccines and the protection offered by the vaccine, the affinity of the antibody to the vaccine antigen is more important and relevant for protection than the peak antibody titer.

Some vaccines such as bacillus Calmette-Guérin (BCG) generate antigen-specific cytotoxic CD8 T-cells.[5] These cells control, reduce, or clear infections produced by intracellular organisms. This is achieved by direct killing of the infected cells by releasing enzymes, such as perforin, and by killing of the infected cells by release of cytokines. Thus, CD8 cells do not prevent infection but decrease the intensity of infection and thus the severity of clinical disease caused by the infection.

The helper CD4 T-cells generated by vaccines do not by themselves prevent infection. However, the Th1 type of CD4 cells supports the activation and differentiation of cytotoxic T-cells and helps deal with infection caused by intracellular organisms. The Th2 variety of CD4 cells supports activation and differentiation of B-cells and thus helps prevent or clear infections caused by extracellular organisms, prevent viral infections, or neutralize the toxins produced by bacteria.

The immunological characteristics of the ideal vaccine are **(Box 1)**:

- Should provide immune protection in 100% of recipients of vaccine
- Should be immunogenic in recipients of all ages
- Should provide rapid and persistent primary immune response and thus prevent infection or if the person is already infected then reduce the clinical severity of illness

**BOX 1:** Immunological properties of ideal vaccine.

- Immunogenic in all recipients
- Immunogenic at all ages
- Should provide rapid and persistent primary immune response (prevent clinical illness)
- Should provide rapid and persistent secondary immune response (prevent clinical illness)
- Should provide lifelong protection (need few doses)
- Should provide protection against nonvaccine strains of organisms
- Should not cause immunological adverse reactions (allergic/anaphylactic reactions, autoimmune disorders)

- Should provide rapid and persistence secondary immune response and thus prevent infection or clinical illness
- Should provide lifelong protection. Thus, an individual would need only the primary series of vaccination and would not need repeated booster doses
- Should elicit sufficiently broad specificity to protect against nonvaccine strains of the organisms. For example, influenza vaccine should protect against all strains of the influenza virus, including those not included in the vaccine
- Vaccines should not cause allergic reactions due to cross-reactivity with common allergens or immune reactions against self-antigens leading to autoimmunity. Nonreactivity to self-antigens is achieved by immune regulatory mechanisms, especially by T regulatory cells as described earlier.[9] In fact, it is very rare to find clinical manifestations of autoimmune disorders exacerbated after vaccination.[15]

## INNATE IMMUNE RESPONSE TO VACCINES

Dendritic cells, which are APCs, travel throughout the body searching for foreign antigens. When APCs encounter vaccine antigens, the PRRs on APCs recognize the PAMPs, which lead to rapid maturation and activation of APCs resulting in expression of homing receptors on these cells. Whole cell vaccines provide strong danger signals or PAMPs as compared to noncellular vaccines and, therefore, the noncellular vaccines are poorly immunogenic and require adjuvants to incite strong immune response. Activation of DCs causes their migration to draining lymph nodes. Once the APCs reach the lymph nodes, they provide antigen-specific and costimulatory signals to T-cells and B-cells, leading to the activation of these cells **(Fig. 1)**.

After injection of live attenuated vaccine, viral particles disseminate throughout the body in a manner similar to natural infection with viruses. These vaccines are efficient in activating innate immune system through PRR by pathogen-associated signals such as viral RNA. Thus, DCs at multiple sites throughout the body become activated and migrate to a large number of draining lymph nodes. This leads to activation of B-cells and T-cells in lymph nodes throughout the body. Similar responses are elicited with live bacterial vaccines such as BCG, where lymphoid cells in both local and distant lymph nodes become activated. This mechanism makes live vaccines more immunogenic and is the reason that the site and route of injection for *live vaccines* is not particularly critical.[16]

Nonlive vaccines produce immune responses only in one group of local draining lymph nodes since there is

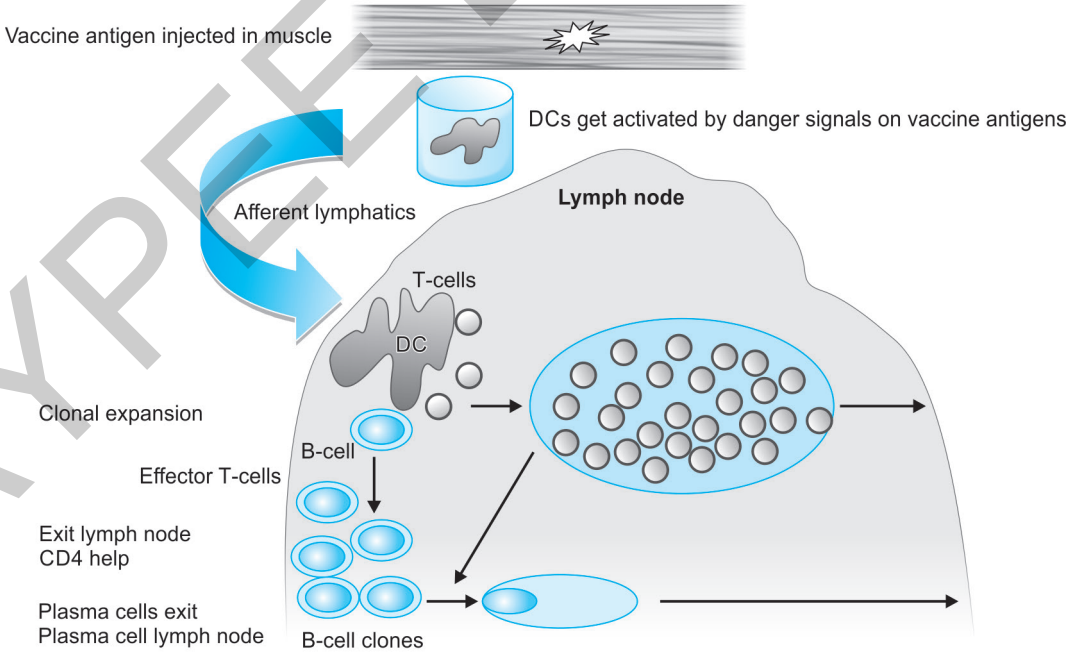


**Fig. 1:** Initial steps in immune response to vaccine. The figure shows that (1) activation of dendritic cells by vaccine antigen; (2) movement of DCs via lymphatics to lymph node; (3) presentation of antigen to B and T-cells by DCs; (4) activation and clonal expansion of B and T-cells; (5) CD4 cells assist the maturation of B-cells into plasma cells, and (6) effector and memory cells enter circulation. (DCs: dendritic cells)

no systemic dissemination of the antigen. This makes the immune response to nonlive vaccines weaker than that produced by live vaccines and, therefore, the site and route of injection of *nonlive vaccines* is very important. Since the skin and muscles have large numbers of DCs as compared to subcutaneous tissue, immune response is generally better when the vaccines are administered intradermally or intramuscularly as compared to those administered subcutaneously especially with vaccine antigens which are not strongly immunogenic.[17] At present, mucosal surfaces are not suitable for administering nonlive vaccines because of significant physical, chemical, and immunological barriers posed by mucous membranes.

Since nonlive vaccines produce immune responses in the local draining lymph nodes, multiple vaccines can be administered simultaneously without interfering with the immune response. In addition, there is no evidence to suggest that simultaneous administration of multiple vaccines either overwhelms or weakens the immune system.[18] Since the immune response is weaker with nonlive vaccines, these vaccines are usually manufactured with adjuvants.[19] Adjuvants serve two functions: first, they lead to the slow release of antigen from the site of the nonlive vaccine deposition which leads to large number of APCs being activated over a period of time, and second, they act as immune modulators providing additional activation signals to APCs. The adjuvant alum has been shown to generate Th2 type of immune response.[20]

## HUMORAL IMMUNE RESPONSE TO VACCINES

Currently, the majority of the vaccines in use provide protection by producing specific antibodies.[21] Both T-cell-independent (polysaccharide) and T-cell-dependent (protein) antigens in the vaccines elicit humoral immune responses. These vaccine antigens reach the lymph nodes by either diffusion or in association with APCs. T-dependent vaccine antigens activate both T and B-cells **(Fig. 2)**, while T-independent antigens activate only the B-cells in lymph nodes. However, there is recent evidence that T-cells are involved in the immune response to polysaccharide antigens as well.[4,22]

### Immune Response to T-dependent Vaccines

#### *Primary Immune Response*

The primary immune response has two components: (1) extrafollicular reaction and (2) germinal center reaction **(Fig. 2)**. Naïve B-cells recognize and bind the vaccine

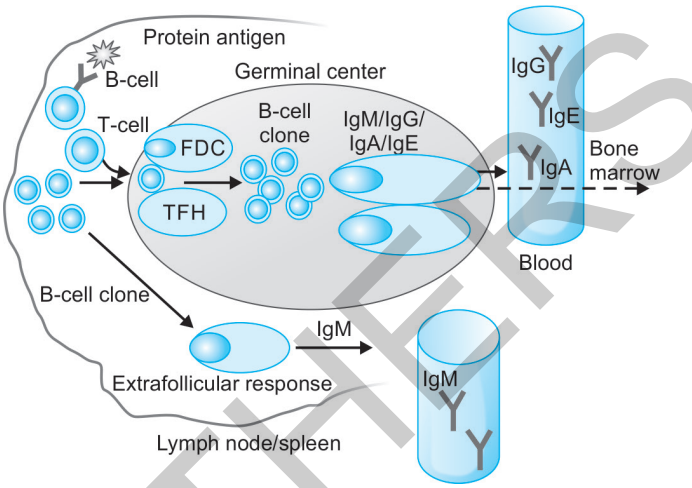


**Fig. 2:** Extrafollicular and germinal center reaction to protein antigen. The figure shows that (1) protein antigen activates B-cells with Ig receptors specific to that antigen; (2) some of the activated B-cells from the B-cell clone rapidly differentiate into plasma cells secreting low affinity, low titer IgM antibodies (extrafollicular response); (3) in the germinal center response, antigen-specific T-cells drive these B-cells toward FDC; (4) with support from the TFH B-cells undergo clonal proliferation and mature into plasma cells initially secreting IgM but then switching to IgG/IgA and in special circumstances IgE antibodies; and (5) some plasma cells migrate toward survival niches in bone marrow. (Ig: immunoglobulin; FDC: follicular dendritic cell; TFH: follicular T helper cell)

antigen through the specific IgM antibody (receptor) on their cell surfaces. This leads to activation and proliferation of B-cells as well as upregulation of chemokine receptor 7 (CCR7). Some of the B-cells from this antigen-specific B-cell clone rapidly mature into plasma cells, which produce low affinity IgM antibodies in a process known as extrafollicular reaction.[23] This reaction is quite rapid leading to the appearance of specific IgM antibodies in just a few days after vaccination. Subsequently, there is class switching of antibodies from IgM to IgG, IgA, and under certain circumstances to IgE isotypes.

Most of these plasma cells die within a few days by apoptosis and therefore, this response is very short-lived. Because antibodies produced by extrafollicular response are of low affinity and since this immune response is short-lasting, the extrafollicular immune response to vaccination plays a minor role in protection provided by vaccines.

In contrast the germinal center interaction gives rise to antibodies of higher titer and affinity.[24] The upregulation of CCR7 on B-cells leads to migration of the activated B-cells to the outer T-cell zone of lymph nodes.[25] Here, these B-cells, activated by specific vaccine antigen, encounter T-cells recently activated by the same antigen. The DCs carrying

specific antigens play an important role in this reaction by attracting the antigen-specific B and T-cells. This encounter between B and T-cells leads to higher expression of CXCR5, a receptor belonging to CXC chemokine family, on B-cells.[26] DCs express CXCR13 (also known as B lymphocyte attractant), which binds CXCR5 and this leads to migration of B-cells and initiation of formation of a germinal center. These B-cells undergo clonal proliferation in the germinal center; thus, a single germinal center contains B-cells specific to a single antigen. This clonal proliferation is associated with class switching from IgM to other isotypes and selection of B-cells producing antibodies of higher specificity and affinity. It takes about 2 weeks for the germinal centers to develop.[27]

A large number of somatic hypermutations in the variable region of immunoglobulin genes lead to the improvement in the affinity of surface antibodies produced by these B-cells in the germinal center. The somatic hypermutation process produces a large number of B-cells which express surface antibodies of low affinity and a very small number of B-cells expressing surface antibodies of high affinity. B-cells expressing antibodies of high affinity effectively compete with B-cells expressing surface antibodies of less affinity for follicular T helper (TFH) cells. These TFH cells, in turn, provide signals and help for survival, maturation and proliferation of B-cells with surface antibodies of the highest affinity to the vaccine antigen. The TFH cells also provide signals to these B-cells to differentiate either into antibody-secreting plasma cells or into memory cells **(Fig. 2)**. Thus, during the primary immune response, i.e. after the 1st dose of vaccine, the antibodies with specificity to vaccine antigens first appear in the blood between 1 week and 2 weeks after vaccination and the peak response (highest antibody titer) takes place at about 3–4 weeks after vaccination. The antibody titers drop fairly rapidly due to the short half-life of these antibody-producing plasma cells **(Fig. 3)**.[27,28]

A strong secondary antibody response is seen in individuals with strong primary antibody responses implying that it is important to generate large numbers of B-cell clones of high specificity during primary immune response.

*Intensity of primary humoral response*: The intensity of the peak antibody generation to vaccine antigen depends on a number of factors. The most important factor is the nature and the immunogenicity of the vaccine antigen.[24] Live vaccines elicit better immune response than nonlive vaccines and vaccines containing polysaccharide antigens

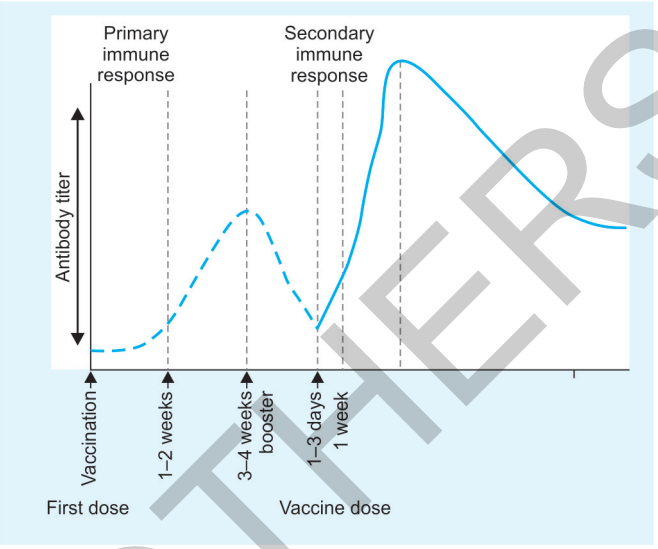


**Fig. 3:** After 1st dose of vaccine it takes about 1–2 weeks for the antibodies to appear in the blood with the peak response at about 3–4 weeks. The antibody titers wane rapidly due to the short half-life of plasma cells. This is the primary immune response. After the booster dose, the antibodies appear in serum in 1–3 days with the peak antibody response in 1 week. The antibodies remain in the circulation for a long time due to the presence of memory B plasma cells. This is the secondary immune response.

elicit poorer antibody response than those containing protein antigens. Among protein antigens, tetanus toxoid is a stronger immunogen than diphtheria toxoid. Another important determinant of antibody response is the dose of vaccine antigens. The optimal dose of vaccine antigen for the highest antibody responses can be determined only by clinical trials. A higher dose of nonlive antigens, up to a threshold, will generate higher antibody titers in the primary response. As stated earlier, a strong secondary response needs a strong primary response. However, it is important to note that even though a small antigen dose gives rise to a weak primary antibody response, it may result in a stronger secondary response with antibodies of higher affinity. The weak primary immune response due to a small antigen dose is due to generation of small number B-cells. However, these B-cells have high specificity and they respond vigorously to booster dose of antigen resulting in a strong secondary immune response. The dose of antigen used during primary immunization also determines whether the immune response will result in plasma cell induction and hence antibody production or memory B-cell generation. A smaller antigen dose drives the immune reaction toward the generation of memory B-cells, while the larger antigen dose leads to the

production of plasma cells. Therefore, if rapid protection against infection is not required, then smaller vaccine antigen doses may be administered, but if rapid protective humoral immune response is desired, large doses at shorter intervals may be administered.[24] This form of rapid vaccine series is particularly useful in instances where passive immunization is either not available or not acceptable such as in the instance of travel to a country endemic to a particular infection.

The genetic makeup of an individual also influences the primary antibody response.[29-31] The antibody responses are poor at the extremes of ages and in individuals with congenital or acquired immunodeficiency conditions.[12,32-35]

With the majority of nonlive vaccines, the primary antibody response wanes rapidly after 4 weeks and, therefore, even in healthy immune-competent individuals a single dose of vaccine will not provide protection against an infection for longer than 4–6 weeks. Therefore, for sustained primary immune response and longer protection, two or more vaccine doses at intervals of 3–4 weeks should be administered to produce additional clones of antigen-specific B-cells. A memory response is required to confer protection from the infection or from clinical manifestations of the infection.

*Persistence of primary humoral response*: As discussed earlier, antigen-specific antibodies after primary immune response wane after a few weeks because the plasma cells generated following vaccination have a short half-life. A small number of these plasma cells, however, migrate and settle in "long-term survival niches" in bone marrow **(*see* Fig. 2)**. The stromal cells in bone marrow are responsible for attracting these plasma cells to the bone marrow and supporting them for years once they arrive there.[36] These plasma cells produce antibodies for long periods of time. The persistence of antibodies may be predicted by measuring the antibody titers between 6 months and 12 months after vaccination.[37,38] By this time, the short-term plasma cells have disappeared and in the absence of repeat vaccination the antibodies detected in the serum are produced only by the long-term surviving plasma cells.

Some of the determinants of long-term antibody responses are: the nature of the vaccine, the interval between vaccine doses, as well as the age and health status of the recipient.[39] Attenuated live viral vaccines generate antibody responses that persist for years. This is due to the continuous activation of B-cells by viral particles. In contrast, the polysaccharide vaccines generate short-lasting antibody response because of lack of germinal center development and, therefore, absence of generation of long-term surviving plasma cells.[11] If vaccines are administered at short intervals, the generation of long-term surviving plasma cells is poor and, therefore, the antibody response is short-lasting. The duration of antibody response is shorter in very young and very old individuals. Also in individuals suffering from conditions in which antibodies are lost through gastrointestinal or urinary tracts, the antibody titers last for shorter duration after immunization.

### *Secondary Immune Response*

To achieve protection, the individual must produce antibodies specific to vaccine antigen that persist in sufficient titers for prolonged periods of time, or develop good memory response, which will respond to the invading pathogen rapidly before the pathogen has the opportunity to multiply and cause clinical disease. As discussed earlier, memory B-cells are generated in parallel to antibody-secreting plasma cells during the development of germinal centers during the primary immune response to T-dependent antigens **(*see* Fig. 2)**. These memory B-cells migrate to the extrafollicular regions of spleen and lymph nodes. They do not secrete antibodies but differentiate into antibody-secreting plasma cells after they are re-exposed to the antigen, either from the vaccine or from natural infection.[40] The longevity of memory B-cells is important for secondary humoral immune responses and thus vaccine efficacy. The production of memory B-cells with prolonged survival depends on the persistence of antigen and therefore, in general, attenuated live-virus vaccine produce memory B-cells which survive for decades even in the absence of booster doses.[24]

After the primary immune response, the variable segments of immunoglobulin genes in memory B-cells undergo somatic hypermutation over a period of 4–6 months, thus producing a few B-cell clones with hypermutation for variable portions of antibodies with very high affinity.[40] These memory B-cell clones are the ones that are activated on re-exposure to antigen because of a better fit between the antigen and the surface immunoglobulin on B-cells. Thus, the booster dose is administered at least 4–6 months following the primary series is completed so that antibodies with high affinity are produced.[41,42]

After re-exposure to vaccine antigens, B-cell activation, proliferation and differentiation to antibody-secreting plasma cells occur at a rapid pace. Since the B-cells bearing immunoglobulin with very high affinity are activated in this

process, the dose of antigens required to obtain immune response is much smaller than that required for the primary immune response. Thus, antibodies of higher affinity and higher titers are produced at a rapid rate with a significantly short lag time after booster dose during the secondary humoral immune response as compared to the primary humoral immune response **(*see* Fig. 3)**.[43]

As discussed earlier, the intensity of the secondary humoral immune response depends on the intensity of the primary immune response: if the primary humoral response is robust, one should expect a robust secondary response and vice versa.[44] This could be due to the high number of memory B-cells produced during primary immunization. However, for a strong secondary response, the interval between primary series and booster dose should be at least 4–6 months and a large dose of vaccine antigen should be administered. This leads to recruitment of large numbers of memory B-cells with the ability to produce high-affinity antibodies resulting in the production of higher titers of antibodies with high affinity.[41,42,45] An interval between primary series and booster doses shorter than 4 months leads to poor antibody response.

Antibodies produced during the primary humoral immune response persisting at the time of the booster vaccination may influence antibody production during the secondary humoral immune response. For live attenuated vaccines, these antibodies neutralize the virus particles, thus preventing their multiplication and dampening the secondary humoral immune response. This can be circumvented by using different vaccines for priming and boosting.[46] For nonlive vaccines, the antibodies generated during primary immune response bind the vaccine antigens administered during the booster dose, thus preventing them from binding to B-cells and consequently weakening the secondary immune response.[24]

Once again, the secondary humoral immune response, which is brisk and produces antibodies of high affinity, is due to activation of memory B-cells. Thus, the persistence of memory B-cells has many practical implications for vaccination:

- Vaccine series should be continued and never restarted even if decades have gone by between the doses
- Attenuated live virus vaccines may not need multiple boosters because of the production of a pool of memory B-cells with prolonged survival due to continued activation of memory B-cells by vaccine viral particles
- Travelers would need booster doses of vaccine for infections endemic to the host country just prior to departure and would not need any boosters in the home country since the risk of infection is low
- If natural boosting occurs frequently (population is exposed to infections frequently), then booster doses of vaccines may not be required
- If it is recognized that the secondary immune response to a pathogen occurs rapidly after exposure and prevents the microbial invasion, then a booster dose of vaccine may not be needed for certain infectious diseases. If, however, the memory response to infection is slower and poorer, then the infection and clinical disease will be established even after a successful primary vaccine series against that infection. This is especially true with infections with short incubation periods such as diphtheria. In this setting, even though an individual is not protected from infection, the severity of the illness has been observed to be lower as compared to those who did not receive the primary series of vaccine.

## Immune Response to T-independent Vaccines

Polysaccharide antigens from bacteria such as *Haemophilus influenzae, Streptococcus pnuemoniae* and *Neisseria meningitidis* elicit T-independent immune responses. After administration of polysaccharide vaccine, the polysaccharide antigen reaches the lymphoid tissues (lymph node and spleen) through blood. At the marginal zone of these lymphoid tissues, these antigens bind to the B-cells. This leads to the activation of these B-cells, which mature into plasma cells and during this maturation process isotype switching from IgM to IgG, IgA, or IgE occurs. These plasma cells then move to the red pulp of spleen and continue to produce antibodies and then die by the process of apoptosis within a few weeks. Since there is limited somatic hypermutation of the variable region of immunoglobulin genes in this response, the antibodies produced are of low affinity.[47]

Antibodies of low affinity and low titer appear in circulation within 1 week and remain in circulation for a few months after administration of polysaccharide vaccine. Since memory B-cells are not generated by T-independent immune responses, subsequent immunization with polysaccharide vaccines results in the same immune response as that generated upon the first immunization. In fact, after repeat immunization with group C *N. meningitidis* polysaccharide vaccine, lower antibody response is elicited as compared to the first response.[48,49] This is known as hyporesponsiveness; this phenomenon has not yet been well explained.

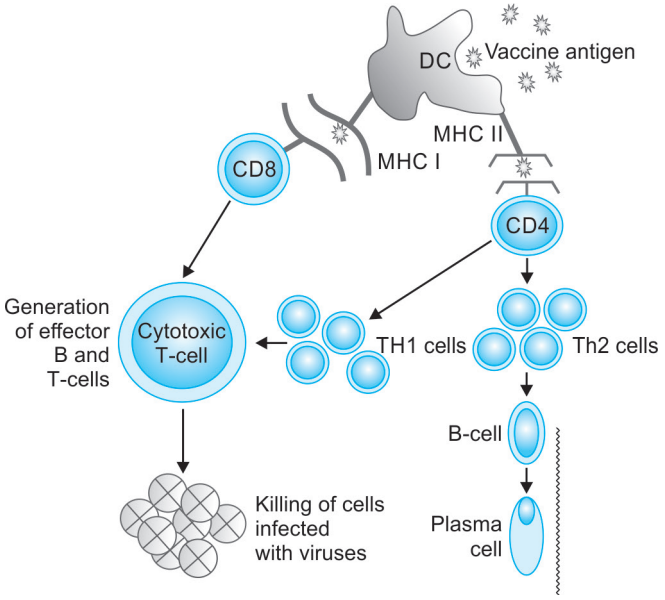


**Fig. 4:** The figure that (1) dendritic cells present antigen to CD4 T-cells with MHC II molecule; (2) dendritic cells present antigen to CD8 T-cells with MHC I molecule; (3) CD4 cells differentiate into two subtypes: (i) Th2 cells which help humoral immune response, i.e. production of specific antibodies, and (ii) Th1 cells which help cellular immune response, i.e. killing of infected cells. (DC: dendritic cell; MCH: major histocompatibility complex)

## CELLULAR (T-CELL) IMMUNE RESPONSE TO VACCINES

Live as well as nonlive vaccines elicit T-cell responses, with both CD4 as well as CD8 T-cells involved in this immune response. Th1 type of CD4 cells supports the development of CD8 cytotoxic T-cells, while the Th2 type of CD4 cells supports differentiation of B-cells **(Fig. 4)**.

Following vaccine administration, an inflammatory response occurs at the site of administration, and the vaccine antigen is taken up by immature DCs. This leads to the activation and migration of these DCs to the draining lymph nodes. During migration, as these DCs mature, the antigen is broken down into small pieces and expressed on the cell surface along with MHC molecules. As discussed earlier, CD4 cells recognize antigens, most often from nonlive vaccines and expressed along with MHC II antigen, while CD8 cells recognize antigens, most often from live attenuated virus or bacterial vaccines and expressed along with MHC I antigen. The cellular immune response tends to be quite variable among different individuals because the antigen-specific receptor expressed on the surface of T-cells binds to a specific-human leukocyte antigen (HLA) molecule and every individual expresses different HLA molecules. Just the presentation of antigen along with MHC molecule is not sufficient to activate T-cells. The DCs express costimulatory molecules after engagement of their PRR and T-cell activation occurs only after they receive the necessary signals through these costimulatory molecules.

After activation by DC, CD4 cells differentiate into two mutually exclusive cell types: (1) Th1 and (2) Th2. In vitro, studies have shown that interleukin (IL)-12 is the key cytokine produced by DCs responsible for generating Th1 cells, while Th2 cells are generated by IL-4 secreted by DCs.[50] Th1 cells secrete interferon-gamma and tumor necrosis factor-alpha and kill the cells infected with pathogen directly by secreting cytokines or indirectly by activating CD8 cytotoxic cells. The Th2 cells secrete IL-4, IL-5 and IL-13 and support the activation and differentiation of B-cells into antibody-producing plasma cells and thus eliminate extracelluar organisms and toxins. The activation of DCs by innate immune system determines the differentiation of CD4 cells to either the Th1 or the Th2 pathway.[51] This implies that selection of an adjuvant for a vaccine, can drive the CD4 differentiation to either the Th1 or Th2 direction and thus can effectively control generation of the desired type of immune response (humoral or cellular).

Immune memory is important for T-cell-dependent vaccines because the effector T-cells are very short-lived. However, memory T-cells live lifelong. To mount a good memory response, it is essential that the vaccine produces a strong T-cell response after the primary vaccination; this, in turn, depends on the amount of vaccine antigen injected. Thus, live-virus vaccines produce strong primary T-cell responses and nonlive vaccines fail to do so. Adjuvants help nonlive virus vaccines in delivering the antigen slowly over longer periods of time and thus produce a strong T-cell response. This can also achieved by administering booster doses of vaccines. Even though antibodies are primarily responsible for protection against extracellular bacteria and toxins, CD4 cells have been shown to provide protection against clinical pertussis even after the specific-antibody titer had waned to nonprotective levels.[52]

Two types of memory T-cells have been identified: (1) effector memory cells and (2) central memory cells.[53] The effector memory cells can function in a cytotoxicity capacity and patrol nonlymphoid tissues; if these cells encounter foreign antigens associated with cells, they destroy those cells. The central memory cells have weak cytotoxic capability but possess strong proliferative potential.[54] They settle in lymph nodes and bone marrow and upon encounter with antigens presented by DCs they proliferate and help clear the foreign antigens.

The T regulatory cells play an important role in preventing the development of allergic and autoimmune disorders by vaccines by inhibiting CD4 positive Th2 and Th1 cell types, respectively.[9] These T regulatory cells also suppress CD8 cells and are antigen-specific. T regulatory cells inhibit the proliferation of CD4 and CD8 cells; this suppressive action is achieved by direct cell to cell contact as well as by production of cytokines, specifically IL-10 and transforming growth factor-beta.

## IMMUNE RESPONSE TO VACCINES IN INFANTS

Immune responses in newborn infants are weaker because of the immaturity of the immune system.[11,32] Antibody responses to vaccines (live attenuated, inactive and subunit) administered before 12 months of age have a small peak and short duration.[55,56] Thus, antibody titers drop to prevaccination levels rapidly, making these infants vulnerable to infections. However, it has been shown that the morbidity and mortality associated with measles are reduced even when immunized in the presence of maternal antibodies.[57,58] As discussed earlier, the immune response to polysaccharide vaccines is poor under the age of 2 years and is the reason why the polysaccharide vaccines currently used are conjugated vaccines, i.e. the polysaccharide antigen is conjugated to carrier proteins. Even after conjugation to carrier protein, the immune response elicited against the polysaccharide antigens is much weaker than that observed in older children.[59]

The antibody responses to vaccines in infants depend on the gestational as well as the postnatal age of an infant.[12,60] Premature babies have a weaker antibody response as compared to term babies.[18] The intensity of antibody responses depends on when the last dose of vaccine for primary series was administered; the older the infant, the better the immune response.[25] Germinal center development in lymphoid tissues is slow and delayed during early infancy and that results in the weaker and limited antibody response during the neonatal period.

Maternal IgG antibodies are actively transferred to the fetus during the third trimester of pregnancy and these antibodies last up to 6–9 months of postnatal age. The titer of maternal IgG is higher in babies born at term as compared those born earlier and this titer decreases with increasing postnatal age. Maternal antibodies neutralize the vaccine antigen, which is then unavailable to bind to antigen-specific receptors on B lymphocytes, thereby preventing a strong immune response. This effect is seen against all vaccines but is more marked against live attenuated vaccines.[61] This maternal antibody-associated immune inhibition is dependent on the antibody titer, i.e. the higher the maternal antibody titer, the lower the antibody responses produced by the infant.[62] In other words, the higher the ratio between maternal antibody and the antigen, the lower the immune response. Thus, either waiting until the maternal antibody titer wanes or administering higher doses of vaccine antigen may circumvent this problem. In babies born before the third trimester, interference by maternal antibodies in antibody responses is not a significant factor because of low maternal antibody titers in these infants. It is believed that even though maternal antibodies interfere with antibody responses to vaccines, they may facilitate memory B-cell production by allowing only a small amount of vaccine antigen (escaped from the maternal antibodies) to prime the B-cells.

Antibody responses to vaccines also depend on the interval between the primary series doses as well as on the interval between the primary series and the booster dose. A shorter duration between doses of the primary series as well as a shorter interval between the primary series and the booster dose results in poor antibody responses.[25]

The process of affinity maturation, which involves isotype switching and somatic hypermutation, is functional in term[63] as well as preterm[64] babies and is similar to adults. However, affinity maturation is a prolonged process occurring over months; thus, the affinity maturation in infants may not be as strong in infants as compared to adults.

Even though the primary immune response to vaccines during early childhood is weaker, immune memory against these vaccine antigens, at least against hepatitis B vaccine and poliomyelitis vaccine, is induced.[24] Again, the memory induced by primary series during early infancy may not be as strong as seen in older individuals because of poor germinal center induction during primary vaccination and therefore low number of memory B-cell production. This has clinical significance, especially for vaccines such as hepatitis B, where the primary series may be completed during early childhood and the booster doses are not recommended. At least with hepatitis B vaccine, it has been shown that even though the immune response and therefore memory B-cell production is low during neonatal period, the secondary immune response to booster doses of hepatitis B vaccine administered up to a decade after the primary series is detectable. This implies that there is persistence of memory B-cells to hepatitis B vaccine even when the primary series is completed during early infancy.

It is possible that same is true with other vaccines. Some of the strategies to circumvent the poor primary response to vaccines in presence of maternal antibodies include using high-titer vaccines and administering the vaccine when the maternal antibody titer in infant is at lowest.[65] However, animal studies have shown that maternal antibodies have significant effect on the development of infant B-cells, antibody diversification, and in removing potentially autoreactive B-cells and therefore may play a significant positive role in immune response to vaccines.[66,67]

Similar to weak B-cell responses to vaccines, neonates and young infants, also exhibit poor T-cell responses to various vaccines.[12] This is due to immaturity of DCs in this population: they do not respond to PRR as efficiently as do DCs in adults and therefore do not produce sufficient amount of cytokines required to activate T-cells.[68] Maternal antibodies do not negatively influence T-cell responses to vaccines. In fact, it is believed that maternal antibodies may enhance T-cell responses to vaccines.[69] This happens due to the fact that once the vaccine antigen is bound to maternal antibody, the resultant immune complex is taken up by the APCs and the vaccine peptide is then presented to T-cells along with the appropriate MHC molecules. Thus, antibodies facilitate the process of presenting antigen to T-cells and enhancing the T-cell responses.

## IMMUNIZATION OF MOTHER DURING PREGNANCY

Infections are the major cause of morbidity and mortality in neonates and infants because of their immature immune system, and immature mucosal barrier. Many of these infections are vaccine preventable. However, as we discussed earlier, vaccines are not very effective in neonates and young infants because of their immature immune system and interference by maternal antibodies. However, because maternal antibodies are transferred during third trimester and breast milk contains sufficient quantities of antibodies specific to vaccine antigens; it is possible that immunizing mothers during pregnancy will help protect infants from the vaccine preventable infections. In addition, these vaccines will also protect mothers from the vaccine preventable infections and thus the neonates and infants will not be exposed to these infections. Influenza infection during pregnancy is associated with high morbidity and mortality. Similarly, infants under 6 months of age, who cannot receive influenza vaccine, are also at risk of high morbidity and mortality with influenza vaccine. Killed influenza virus vaccine has been successfully used in pregnant women without any adverse events.[70] Neonates and young infants are associated with high morbidity and mortality with pertussis and first dose of pertussis vaccine is not recommended to be given under 6 weeks of age. Thus, the neonates and infants are at risk of getting infected with pertussis because 6 weeks is the earliest age at which the pertussis vaccine can be administered and therefore, pertussis vaccine administered to mother during pregnancy will have protective effect on neonates and infants. In fact, Tdap vaccine has been given to pregnant woman without any adverse effects to mother or the infant. Initial studies have shown that infants whose mothers received Tdap during pregnancy responded well to DTaP immunization.[71] Live attenuated vaccines such as MMR and varicella are contraindicated during pregnancy because of a theoretical risk of the vaccine virus crossing the placenta and infecting the fetus. However, there is no clinical evidence of adverse effect to mother or fetus when the mother receives live virus vaccines. Therefore, this area of vaccinating pregnant women with live attenuated vaccines is important and needs to be studied further.[72]

## CORRELATES OF IMMUNE PROTECTION BY VACCINES

Correlates of protection are the immune mechanisms produced by vaccines which are responsible for protection from infections. It is often confused or interchanged with "surrogate" which is a measurement of immune response and not a measurement of protective mechanism. For some vaccines, the correlates of protection are not known or difficult to measure. In those situations, one measures "surrogates" as predictors of protection against infection. There may be more than one correlate for protection against infection or disease and those are known as co-correlates. Correlates depend on the end point selected and, therefore, vary both qualitatively and quantitatively. The end points usually looked for are: prevention of infection, prevention of disease, prevention of severe disease, or death. As discussed earlier, the correlates for most of the vaccines being used currently are specific antibody titer, as long as these antibodies are functional. However, certain vaccines (specifically live attenuated viral and bacterial vaccines) may have cell-mediated immunity as a correlate of protection, and humoral immunity may also function as the co-correlate in those cases.

VACCINES

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ISBN 978-93-5270-989-2
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