Review Article

Immunological basis of vaccination

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The basic mechanisms of body defences against infectious diseases are both non-specific and specific immune systems. Non-specific immunity refers to mechanism of protection that do not require specific recognition of antigen, but that increase the protection afforded by specific immune mechanisms. Non-specific immune mechanisms include phagocytes, acute inflammatory responses, type-1 interferon, and tumour necrosing factor. Specific immunity consists of mechanisms of protection that require specific recognition of antigen. This immunity is highly specific, inducible, discriminatory and unforgotten T lymphocyte-dependent response.[1] Normal specific immunity operates under Major Histocompatibility Complex restriction.[2] It is the ability of this immune system to refine its antigen recognition domains and establish immunological memory that underlies the success of active vaccination.

The vaccine antigen

The ideal vaccine antigen will be taken up successfully by antigen presenting cells, lead to the activation, replication and differentiation of T and B lymphocyte, and result in the generation of a large pool of memory cells. The vaccine must therefore contain appropriate B cells epitopes that are able to stimulate neutralizing antibody responses. This is similar with relevant T cell determinants that will be incorporated into every recipient's MHC molecules so that the complex is recognized by the T receptor. Furthermore, ideal antigen must persist, conformationally intact, in lymphoid tissue to allow the continuing production of cells that secrete antibody of high affinity and the generation of memory cells. Recently, a variety of vaccine antigens are available, including live attenuated microorganisms, killed microorganisms, polysaccharides, surface antigen and bacterial toxoids.

The advances in vaccine antigens include the production of recombinant proteins by means of genetic engineering; the production of protein-polysaccharide conjugation that enhance the immunogenicity of polysaccharide antigens and fundamentally change their recognition by the immune system. Recent advances also include DNA vaccines, which was discovered by chance in 1989 during a gene therapy experiment, when it was shown that a gene inserted directly into mammalian cell could induce the cell to express the protein coded by the gene. Various current efforts are directed toward improving the immunogenicity of DNA targeting methods. Plasmid DNA encoding antigenic proteins is injected directly into the muscle of the recipient. The muscle cells take the DNA and the encoded protein antigen is expressed, leading to both a humoral antibody response and a T cell-mediated response.[3] The immunogenicity of vaccines can be enhanced by sev-

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eral ways. Adjuvant, such as alum, retain antigen at the site of application and by permitting slow release over time, increase the uptake of vaccine antigen by the Antigen Presenting Cells (APCs). They induce the synthesis of cytokines that recruit appropriate T helper cell. Other adjuvant, MF59, has been combined with sub-unit influenza antigen which demonstrated a consistently higher immune response.[4] The search for new and more potent adjuvants include the evaluation of Mono Phorphoryl Lipid (MPL) derived from lipopolysaccharide, and Saponin-derived QS21. In addition, particle adjuvant such as lipid-particle Immuno Stimulating Complexes (ISCOMs) is attracting much interest. DNA Vaccination has revealed CpG oligonucleotides as potential stimulators of selective immune responses.[5]

## Cellular responses to vaccines

Macrophages, dendritic cells, B cells and virtually any other cell type capable of expressing MHC class II molecules may function as Antigen Presenting Cells (APCs). CD4+T helper cells recognize and respond to antigen in the context of MHC class II.

Soluble antigens

Soluble antigens are T cell-independent antigens that activate B cells in

a polyclonal manner in the absence of CD4+T-helper cells. Immunoglobulin receptors on the surface of B-cells are able to recognize soluble antigens.[6] The recognition of antigen via the surface immunoglobulin initiates the process of B-cells activation and differentiation. From this point of time, the subsequent differentiation of native B-cells is initiated and the sequence of antibody development secreting plasma cells progresses. Endocytosis of antigen bound to surface immunoglobulin occurs and processed antigen in the form of small peptides is re-expressed on the B cell surface in the context of MHC Class II molecules. This enables B cells to act as anti-
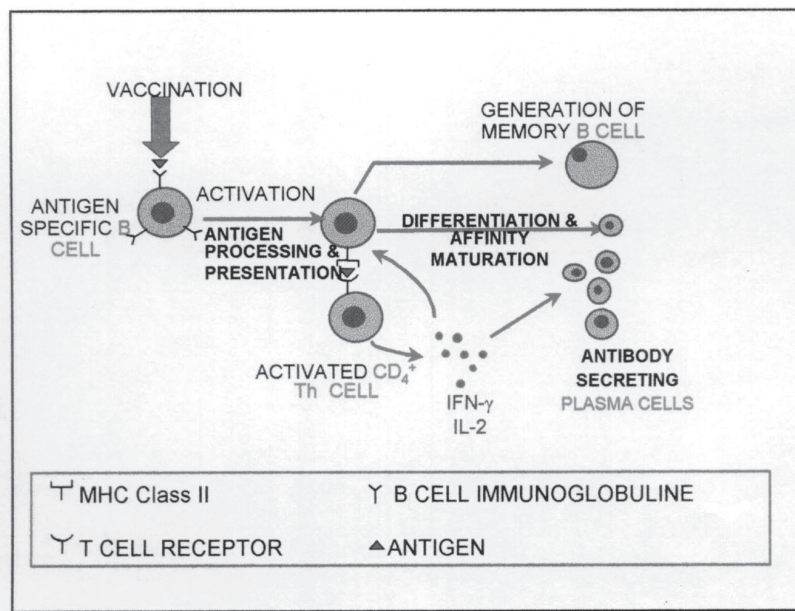
Figure 1. Visualizing concepts of the immunological mechanism of soluble antigen vaccination. Soluble antigens are T cell-independent antigens that activate B cells in a polyclonal manner in the absence of CD4+T-helper cells. Endocytosis of antigen bound to surface immunoglobulin occurs and processed antigen in the form of small peptides is re-expressed on the B cell surface in the context of MHC Class II molecules. This enables B cells to act as antigen presenting cells thereby recruiting T helper cells. The signals and soluble factors that result from such T cell help drive B cell process of affinity maturation, which result in the increasing affinity of secreted antibody and the generation memory B cells.

gen presenting cells thereby recruiting **T-helper cells**. The signals and soluble factors that result from such T cell help drive B cell process of affinity maturation, which result in the increasing affinity of secreted antibody and the generation of memory B cells (**Figure 1**).
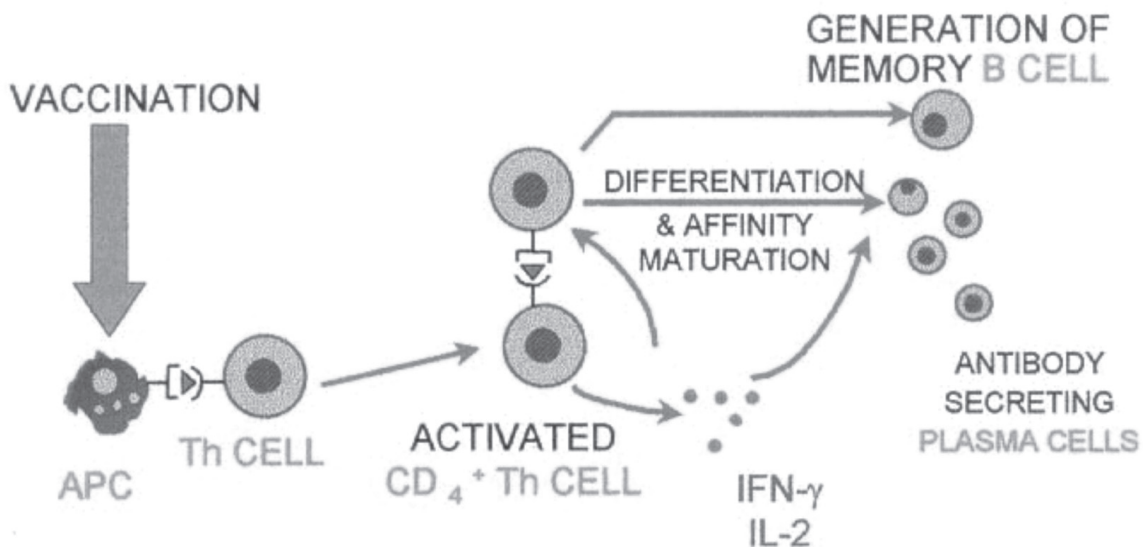
Peptides antigens

Peptides antigens are T cell-dependent antigens that activate B cell only in the presence of CD4+T-helper cells. These antigens are non-specifically engulfed by macrophages by a process known as phagocytes, will associate with MHC class II molecules after processing.[2,6] Peptides are insoluble antigens capable to respond to T-cells in association with self-Major Histocompatibility Complex (MHC) molecules. Cellular responses to this type of vaccines are induced when antigen-presenting cells present antigen to T cells. MHC Class II is expressed on cells that participate in the immune response, and when occupied by foreign peptide is recognized via a subset of T cells

expressing the CD4 differentiation antigen. A major role of such T cells is to support the production of antibody by B cells, hence they are known as T helper cells (Th-cells).[7] Activated CD4+T-helper cells secrete IFN-γ, a cytokine which is very important in the maturation, differentiation of B cells into antibody secreting plasma cells, and generation of memory B cells.[8] It also has a central and pivotal role in all phases of immune responses. It is produced only by CD+Th-1 lymphocyte and NK cells.[2] Immunoglobulin of G class is the main product of plasma cells in this pathway and play an important role in the humoral immunity (**Figure 2**).

Attenuated pathogen vaccines

These vaccines have some advantages and some disadvantages. Because of their capacity for transient growth, such vaccines provide prolonged immune-system exposure to the individual epitopes on the attenuated organism, resulting in increased immunogenicity and production of

Figure 2. Visualizing concepts of the immunologocal mechanism of peptide antigen vaccination. Peptides antigens are T Cell dependent antigens that activate B cell only in the presence of CD4+T-helper cells. These antigens are non-specifically engulfed by macrophages by a process known as phagocytes, will associate with MHC class II molecules after processing. MHC Class II is expressed on cells that participate in the immune response, and when occupied by foreign peptide is recognized via a subset of T cells expressing the CD4 differentiation antigen. A major role of such T cells is to support the production of antibody by B cells. Activated CD4+T-helper cells secrete IFN-γ cytokine, which is very important in the maturation, differentiation of B cells into antibody secreting plasma cells, and generation of memory B cells
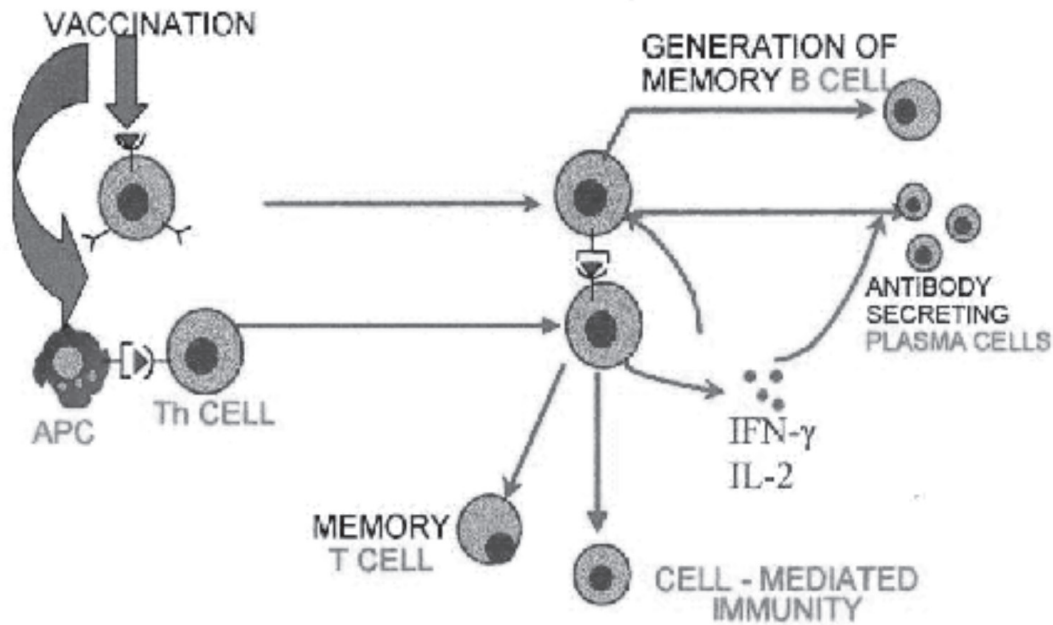
Figure 3. Visualizing concepts of the immunological mechanism of attenuated pathogens vaccination. Because of their capacity for transient growth, such vaccines provide prolonged immune-system exposure to the individual epitopes on the attenuated organism, resulting in increased immunogenicity enhancing the production of memory cells and the development of antibody secreting plasma cells. The ability of many attenuated vaccines to replicate within the host cells make them particularly suitable for inducing a cell-mediated response

memory cells. As a consequence, these vaccines often require only a single immunization, eliminating the need of repeating boosters (**Figure 3**). According to the most conventional model, memory cells are derived from effector cells that revert to a quiescent state. Typical immune responses lead to the prominent clonal expansion of antigen-specific T cells followed by their differentiation into effector cells. Most effector cells die at the end of the immune response but some of the responding cells survive and form long-lived memory cells. Recent evidence suggests that T memory cells arise from a subset of effector cells. The longevity of T memory cells may require continuous contact with cytokines, notably IL-5 for CD8^{+} cells.[9,10] But if we believe to the new model of progressive T cell differentiation, the duration of antigenic stimulation and the type and amount of cytokines present during priming lead either to fully differentiated effector cells that home to peripheral tissues or to intermediate cells that are devoid of effector function and home to lymph nodes. These two cell types can be identified according to the differential expression of the T-GFP marker transgene and the lymph node-homing receptor CCR7. Both cell types are maintained in the memory pool and upon secondary challenge, mediate immediate protection in non-lymphoid tissues or secondary responses in lymph nodes.[9] The ability of many attenuated vaccines to replicate within the host cells make them particularly suitable for inducing a cell-mediated response.[11] Varicella vaccine as one example among these group of vaccines, is highly effective in inducing persistent immunity and long-term protection against breakthrough varicella infection. This fact is accompanied by consistently high seropositive rates, suggesting that antibodies detected by gpELISA are indicative of long-term protection after vaccination. In support with this contention, varicella antibody titers measured by gpELISA 6 weeks after vaccination have been shown to correlate with VZV-specific cell-mediated immune responses and with VZV neutralizing antibody titers.[12]

DNA vaccines

The injected gene is expressed in the injected muscle and in nearby APCs.

The peptides encoded by the DNA are expressed on the surface of both cell types after processing as endogenous antigen by the MHC class I pathway. Cells that present the antigen in the context of class I MHC molecules stimulate development of cytotoxic T cells and generates T-cells memory. The protein encoded by the injected DNA is also expressed as a soluble, secreted protein, which is taken up, processed, and presented in the context of class II MHC molecules. This pathway stimulates B-cell immunity and generates antibodies and B-cells memory against the protein.[13]

Response to vaccination

Antigen exposure for the first time induces an antibody response that can be measured in the serum after a lag period of about 6 to 12 days.[1] The antibody isotype produced during a primary response is mostly IgM. The level decreases shortly after peak level has been obtained, and generation of memory B cells occurs. In contrast, exposure to the same antigen for the second time induces an antibody response that can be measured in serum within a few days of antigen exposure.[14] The antibody isotype produced is primarily IgG. The level of antibody in the serum persists from weeks to months, and the affinity of the antibody for antigen is greatly increased (**Table 1**).

Mechanisms of antibody production

The selective theory

This theory proposed that an antigen combined with one out of several molecules on the surface of immune cells stimulates the generation and release of that one particular molecule more than others. This theory became invalid when it was realized that each cell would be required to express a vast number of different molecules to recognize a vast numbers of structurally different natural and synthetic antigens.

The instructive theory

In contrast with the former theory, instructive theory proposed that antigen acted as a template for instructing a non-specific antibody on the cell surface to fold itself around the antigen to make a specific antibody against that antigen.

Clonal selection theory

This is the most accepted theory to date which proposes that antigen receptor diversity is generated before antigen enters the system. The role of antigen is to selectively stimulate specific receptors on a specific clone of cells to induce these cells to proliferate and produce specific antibody against the antigen. This theory implies that all secreted antibody produced by cells from the expanded clone will have antigen-combining sites, identical to the antibody receptors on the surface of the original clone. All of antibody produced against a single antigen is the result of the selection, activation and expansion of cells expressing a restricted antigen receptor specificity.[1]

Immunity resulted from vaccination.

Humoral immunity involves the production of antibody that can be transferred in the donor's serum to unimmunized recipient, to confer immediate specific immunity within the recipient.

TABLE 1. COMPARISON OF PRIMARY AND SECONDARY ANTIBODY RESPONSES

Primary Response	Secondary Response
Response to first antigen exposure	Response to second antigen exposure
Lag time in response	Immediate response
IgM	IgG
Low affinity antibody	High affinity antibody

Mechanisms by which antibodies provide immunity are: neutralization of antigen, Fc-receptor mediated phagocytosis and complement-mediated lysis.

Neutralization of antigen

Antibodies can bind to soluble or cell-associated antigen to prevent them from inducing any detrimental effects. The antibodies are able to bind to the cell surface antigens, presented on an intact microorganism. Since some of these antigens are important to gain entry into a host cell, the binding of a specific antibody to these antigens will neutralize the ability of the microorganism to enter a cell.

Fc receptor-mediated phagocytes

Antibodies can bind to soluble and cell-associated antigen, and subsequently bind to phagocyte cells via the Fc portion of the antibody to the Fc receptor expressed on phagocyte cells. In this manner, the antibody coating the antigen, known as opsonization, delivers antigen to the phagocyte and thereby enhances both the phagocytes and the ultimate destruction of the antigen by these cells.

Complement-mediated lysis

Antibodies can bind to cell-associated antigens and subsequently activate complement proteins that form pores in the cell to destroy the cell through lysis.[1] Use of DNA vaccines and attenuated pathogens vaccines raise both humoral and cellular immunity.[15,16] DNA vaccines offer advantages over many of the existing vaccines. The encoded protein is expressed in the host in its natural form, no denaturation and modification. The immune response will be directed to the antigen exactly as it is expressed by the pathogen. DNA vaccines induce both humoral and cell-mediated immunity. This stimulation of both arms of immune response normally requires immunization with a live attenuated vaccine. DNA vaccines cause prolonged expression of antigen, which generates significant immunological memory.[11]

In conclusion, soluble antigens are T cell-independent antigens that activate B cells in a polyclonal manner in the absence of CD4+T-helper cells. Peptides antigens are T cell dependent antigens that activate B cell only in the presence of CD4+T-helper cells. The immunity resulted from these antigens is humoral immunity. Use of DNA vaccines and attenuated pathogens vaccines raise both humoral and cellular immunity.

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