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NEW DIMENSIONS IN VACCINOLOGY: A NEW INSIGHT

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ABSTRACT

The development of vaccines to prevent infectious diseases has been one of the most important contributions of biomedical sciences. Increasing understanding in biochemistry, molecular biology, molecular genetics and related fields have provided an opportunity for the development of new generation vaccines that are based on rational design approaches. This is possible because of proper understanding of the microbial-genetics, biochemistry, host-pathogen interaction and recent developments in molecular immunology. Another important improvement made in the quality of vaccine production is the incorporation of immunomodulators or adjuvants with modified delivery vehicles viz liposomes, Iscoms and microspheres apart from alum being used as a gold standard. This article reviews the art of vaccination from Jenner period to present day context highlighting all the developments made at each stage of the vaccine development. Various criteria have been discussed regarding the selection of epitopes that expand B & T cells, its linkage with other accessory cells of the immune system, means to overcome MHC linked immune unresponsiveness, enhanced antigen processing and presentations that specially induce either helper or cytotoxic or mucosal immune responses were critically discussed.

KEY WORDS

Peptide, Vaccine, Epitope, Antigen, Antibody, MHC, Adjuvant, Liposomes, Iscoms, and Microsphere

INTRODUCTION

Ever since the time of Jenner and Pasteur, vaccination has been accepted as a part of life and constitutes one of the most successful achievements in the field of immunology. In spite of its imperfections, vaccination remains the best answer to infectious diseases. The art of deliberate immunization against infections has of course been practiced for centuries, but the mechanisms of protective immunity were not fully appreciated until the advent of modern immunology. With the discoveries of newer technologies and greater understanding of the molecular biology of pathogens, the conventional empirical approaches to vaccine development have given way to more rational vaccine design. Immunoprophylaxis through vaccination now offers the prospect of substantially reducing the mortality caused by microbial pathogens to human race. A prophylactic vaccine aims to elicit immune effector elements such as circulating antibodies and various antigen specific memory lymphocytes. These host elements are readily available for immediate neutralization of the pathogen upon entry or for production of cytotoxic molecules for destroying the infected cells. Rational vaccine design requires sensible formulated adjuvants. Adjuvant modulated antigen structures and associated immunological pathways therefore contribute to a better general understanding of immune effector mechanism(s) but eventually leads to more predictable efficacy. Depending on the types of the disease, vaccine has to fulfill a number of criterions. These may include an early onset of immunity, long duration of effector response through memory cells, the need to avoid booster immunizations in order to reduce the cost of vaccine and easy availability to mankind. Most of these features are strongly dependent on the choice of adjuvant.

This article briefly reviews the work on vaccine development from the past to the present with special emphasis on the work done in our laboratory and by others in the field of synthetic peptide vaccines starting from the identification of protective immunogen (s) and delineating the B& T cell determinants using algorithmic predictions followed by in vivo conformation and their effectiveness in eliciting humoral as well as cellular responses using immunoadjuvants or immunostimulators or immunomodulators with modified delivery vehicles with respect to various infectious diseases. The discussion includes the new approaches being explored in this field to increase the immunogenicity of the peptides by better vaccine design opted by scientists across the globe.

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History, from Past to the Present

"Never in the history of human progress has a better and cheaper method of preventing illness been developed than immunization at its best". This statement by Edsall in 1963 reflects the success of vaccines against diseases such as smallpox, poliomyelitis, measles, rabies, yellow fever, diphtheria and tetanus. The story of vaccinology began with Jenner in 1796 in his demonstration of the scientific principles and realities for preventing small pox by prior infection with the related but less virulent cowpox virus (1). The century that followed this primary event was marked with trial and error together with a series of new technologies and discoveries paving the path for present knowledge in vaccine design. Table 1 shows the vaccines licensed from the time of Jenner till date and table 2 shows the strategies for development of non-living vaccines with tables 3 and 4 showing the principal vaccine targets by and beyond 2005.The earliest vaccines were derived from killed pathogenic organism or subunit of the pathogenic organism. This is followed by live or attenuated virus or bacteria that do not cause disease but have been derived from the pathogenic parent organism. The killed or inactivated vaccines are made from virulent pathogens by destroying their infectivity to ensure the retention of full immunogenicity. These vaccines are relatively safe but however require periodic boosting to cope up flagging immunity. The era for live virus vaccines was made possible by the breakthrough in cell culture technology by Enders, Weller and Robbins which opened the way to the first and highly successful killed Salk (2) and live Sabin (3) vaccines against poliomyelitis that were introduced in 1960's. During this time, the infected organs of animals, embryonated chick eggs and primitive cell cultures of animal tissues were used to grow viruses for live attenuated and killed virus vaccines and whole cell bacteria and their secreted toxins were used in preparing vaccines against disease caused by them. Purified viral proteins or their subunits, and bacterial toxoids have certainly diminished the incidence of morbidity and mortality of a large number of infectious diseases, but their use is still associated with several major problems like excessive reactogenicity followed by serious delayed type hypersensitivity reactions as observed in earlier measles vaccine. Attenuation is generally achieved by growing the pathogens in an 'unnatural' host (passage); less commonly, known viruses have been adapted to grow at a temperature lower than normal (cold-adaptation) or have been rendered temperature-sensitive.

For inactivation, the pathogens are either subjected to autoclaving or fixed by agents such as formalin, b-propiolactone or more recently an imine. However, such vaccines may elicit side effects, which are frequently unacceptably harmful to the hosts. Even with the highly successful products such as the attenuated Sabin poliovirus or killed Salk poliovirus vaccine, or smallpox vaccines, there are a small but significant number of post-vaccination incidents. Killed vaccines may also present problems in that there is always the chance of some infectious pathogens surviving the inactivation process. Another problem is the constant evolution of some viruses into new strains with different serological specificity and sometimes viruses may produce oncogenic initiation. Thus, there is a need for new generation of molecularly defined vaccines, which would induce the desirable immune responses capable of controlling particular infectious agents. This phenomenon is even more severe in parasites / viruses that rapidly alter their antigenic structures as seen by the failure of protection of the influenza vaccine (4) or recently with HIV virus. In spite of their remarkable efficacy, vaccines based on the pathogen itself may sometimes lead to safety hazards. This indicates that certain parts of the pathogen actually induce some undesirable responses and should be eliminated from the vaccine formulations in order to obtain maximum efficacy. Many pathogens are virtually impossible to culture outside their natural host for eg. Hepatitis B virus and bacterium that causes leprosy have never been grown in vitro although they can be propagated in animal models. Consequently, it is not possible to generate live attenuated or inactivated vaccines culturing the agents. Attempts to produce sufficient quantities of either the organism or antigens for malaria research from in vitro cultures have not been very successful. Even if adequate quantities of malarial parasite can be obtained from in vitro cultures, the use of such blood derived malarial products for vaccination carries the risk of transmitting infectious agents such as HIV and hepatitis virus (5). Recombinant DNA technology allows the transfer of genetic information from these fastidious organisms to more amenable host such as E.coli, yeast or mammalian cells. It is not so easy to identify all protective antigens, clone and express in a suitable vector, though this technique helped to clone a gene of interest. It has its own limitations such as in a complex protein like lipoprotein or glycoprotein, the post-translational machinery may not be full proof and this may hamper the antigenicity or immunogenicity of the molecule as the translated protein may not acquire the actual conformation of the native protein. The current strategies of vaccine design include primarily at preventing the pathogen invasion and / or neutralization of the disease inducing toxins by antibodies and fine tuning the cellular immune responses to preferentially induce CD4+ Th1 or Th2 cytokine profile. The outcome of the progression of disease or the elimination of pathogen is decided by the outcome of cytokine involving Th1 and Th2 dichotomy. Also, the involvement of specialized antigen presenting cells for the induction of helper and cytotoxic T lymphocyte or both response by the use of

immunomodulatory molecules and new delivery systems like liposomes, ISCOMs and microsphere technologies has certainly improved the quality of vaccine efficacy. Since all the above qualities lie in peptide based vaccines this seems to be the most logical and likely answer in the present era to circumvent the above problems.

The current emphasis is on developing a formulation that can activate both systemic as well as mucosal immune responses especially where the transmission is occurring through oral/nasal/rectal routes.

Many infectious pathogens contain certain moieties, which are recognized by the phagocytes. This leads to release of proinflammatory cytokines, which eventually attract the lymphocytes at the site of infection as a part of innate immunity. This eventually leads to decrease in load of infection. When acquired immunity starts it is easy to handle the pathogen subsequently.

Recently, emphasis is made on Toll-like receptors that mediate innate immunity. The new class of adjuvants as well as delivery vehicles has shown to negate their action through these receptors only.

Protein Antigenicity vs Immunogenicity

Vaccination, in general, involves the use of an immunizing agent, which is expected to elicit protection by the formation of neutralizing antibodies against a biologically active molecule such as bacterium or virus

Table 1. Vaccine Development And Licensure 1945-1996

Viral vaccines	Bacterial vaccines and globulins	
Vaccine	**Vaccine**	**Date**
Killed	*Bacterial Subunit*	
Japanese B	Meningococcus A	1974
Pandemic A2 influenza	Meningococcus C	1975
Adenovirus	Combined	
Purified Poliovirus	A-C	1975
Purified Influenza	A-C-Y-W 135	1982
Adjuvanted Influenza	*Pneumococcus*	
Hepatitis B	14 types	1977
Plasma-derived	23 types	1983
Recombinant	*H. Influenzae*	
Hepatitis A	Conjugate	1989
Live		
Measles	*Immune Globulins*	
Edmonston B+	Hepatitis B	1978
Gamma Globulin	Hepatitis A	1979
More Attenuated		
Mumps		
Rubella		
Combined live vaccines		
Measles-smallpox		
Mumps-rubella		
Measles-mumps		
Measles, mumps, rubella (MMR)		
Varicella		
Marek's Disease		
(Cancer of Chickens)		

Table 2. Strategies for the development of Non-living Vaccines

* Whole chemically inactivated organisms
* Toxins-chemically inactivated
* Toxins-genetically inactivated
* Excreted antigens
* Capsular polysaccharides
* Protein-conjugated polysaccharides
* Subunits of organisms including OMPs and envelope antigens
* Subunit antigens produced in recombinant organisms
* Synthetic peptides with B and T epitopes
* Pseudoparticles produced in vitro lacking replicative capacity
* Anti-idiotypic antibodies
* Naked DNA

Table 3. Vaccines to be licensed by 2005

Hexavalent combination for infants (Phase-III clinical trials) H.Influenza (attenuated)
Lyme disease
MMR-varicella
Meningococcal A/C conjugates
Pneumococcal multiple serotype conjugates
Rotavirus (oral)
Leprosy

Table 4. Principal vaccine targets beyond 2005

Chlamydia pneumonia	Human papilloma
CMV	Malaria
Dengue	Meningococcal B
EBV	Otitis
Group B Streptococcal	Respiratory syncytial
Helicobacter	Tuberculosis
Herpes Simplex	Zoster
HIV	Anthrax
Cancer	Plague

or toxin. The exact mechanism of this protection is not clearly known, it is not certain whether all the antibodies elicited by the vaccine participate in the neutralization process. Moreover, these questions are not amicable to experimental elucidation due to huge size and complexity of the immunizing molecule. Infact, only a small percentage of the antibodies participate in the neutralization, whereas other determinants may frequently induce the opposite effect resulting in immunosuppression, which could be detrimental to the host defense mechanism. If we are able to find means to elicit only neutralizing antibodies, then immunization would be more effective and protective. The portion of the protein bound by specific antibody molecule is antigenic and recognized by that particular antibody. One viewpoint is that certain parts of protein are inherently antigenic and that this property would be intrinsic to the nature of the molecule and independent of the host to be immunized. On the basis of this concept attempts have been made to define the properties of certain protein sub-structures, which might make them inherently antigenic. This is possible, provided the actual epitopes recognized by B and T lymphocytes are mapped or predicted. Several tumor associated antigens (TAA) in melanoma and other cancers have been shown to be important in understanding tumor immunology (6). Most of the melanoma antigens have been identified by screening cDNA libraries (7) or identification of TAA involves the testing of known proteins for recognition of CTL property (8) or directly isolating and sequencing peptides eluted from the tumor cells (9). Most recently computer programs have been used to identify the peptide sequences of known proteins based on their binding affinity for selected HLA molecules (10). Three types of antigenic sites have been determined: sequential, continuous and discontinuous. In sequential determinants the antibodies recognize the linear sequence of aminoacids. A continuous antigenic site is a conformationally distinct portion of the protein that is comprised of aminoacids in continuous peptide bond linkage. A discontinuous site is one, which is conformationally distinct and is made of aminoacids not by peptide linkage but due to secondary or tertiary folding of the protein (11). The strength (affinity/avidity) of an antigen antibody complex is also governed by the molecule stereochemistry of its aminoacids. An effective immunogen will have two spatially distinct sites for B cell as well as T cell interactions. There are many approaches that have been developed from time to time with high fidelity of success to map or identify the antigenic sites on the protein molecules. During normal course of the antibody production, there are certain regions on a given protein molecule, to, which antibodies are predominantly generated called as immunodominant regions (12). With the identification of these immunodominant epitopes in large number of proteins interesting applications have been seen in diagnosis of the disease (13) or as T cell help to the hapten. Sometimes a single amino acid substitution in an immunodominant region refocuses the immune response and makes it a weak immunogen, thus proving the role of these immunodominant regions in vaccine design (14). A dominant region of a pathogen protein which has homology with host proteins may

lead to autoimmune diseases through breaking of the tolerance to the self determinants (13). In contrary, an immune recessive site is a site, which is less exposed, extremely buried. These regions may provoke induction of suppressor T cells that act to suppress the production of mature B cells. Immunosilent regions are the regions, which are immunologically silent, and they do not provoke any antibody response. In an exceptional study, it is amazing to understand that cryptic sequences (normally they do not provoke immune response in the native antigen) have shown to be protective epitopes (unpublished data). However, other regions are also accessible to the immune response but are called minor determinants of the protein. For generating an effective immune response for a peptide based vaccine, the need to understand the knowledge regarding immunodominance-a property where strong humoral response is mounted with the co- operation of the T cell, is needed (15,16). From the above studies, it is very clear that the knowledge of immunodominance, immunorecessive, immunosilent regions and cryptic sequences of a protein play a key role in generating overall immune response during the design of peptide vaccines.

Synthetic Peptide Vaccines

The basis for synthetic peptide vaccines was laid by the pioneering work of Anderer who showed that short fragments of a protein from tobacco mosaic virus could inhibit the precipitation of the virus by antiserum. Also, when a hexapeptide from the fragment is coupled with a carrier it induced specific virus precipitating and neutralizing antibodies (17). Further work by Arnon et al. extended this concept to show that chemically synthesized peptides could also induce antibodies that specifically recognize the intact virus particle from whose coat-protein, the amino acid sequence was derived and generated antibodies. With the advent of gene cloning and nucleic acid sequencing techniques, a large number of aminoacid sequences of biologically important proteins are now available. This is undoubtedly responsible for the greatly increased activity in the search for synthetic peptide vaccine by many laboratories. The first step in developing a synthetic peptide vaccine is to identify the relevant antigen(s) and determine its amino acid sequence. This is now mostly achieved by deducing from the nucleic acid sequence of the gene coding the protein. The next step is to identify the relevant antigenic determinant. This is perhaps the most difficult part and may be achieved by using the following parameters:

1. Chemical and enzymatic cleavage of purified proteins and subsequent analysis of their immunological properties.
2. The use of monoclonal antibodies to identify and select the smallest component of the antigen still capable of specific binding activity.
3. Predictions based on regions of hypervariability when amino acid sequences of a number of variants are available.
4. Predictions of secondary and tertiary structures by computer chemistry, which indicate regions of hydrophilicity, accessibility and mobility based on the state of lowest free energy.
5. Random synthesis of overlapping peptides.
6. Regions around disulphide bridges that locks the native conformation.
7. The use of hydrophilicity parameters of individual amino acids derived from the HPLC (high performance liquid chromatography) retention times to predict surface residues on the protein antigens. (18).
8. Isolation of the peptide sequence that is released during proteolysis of the antigen monoclonal antibody complex (19).
9. Developing a peptide microarray for the antigen of interest to delineate the helper, cytotoxic and humoral sites.

Concept of Synthetic Vaccines

The concept of synthetic vaccine must not only include the synthesis of immunogenic epitopes eliciting a protective antibody response, but also have the epitopes for sensitizing the specific T-helper / T-cytotoxic cells for long lasting immunity. This way, it can eliminate the intracellular pathogens through various effector mechanisms (20).

Thus for a peptide vaccine to be effective in a broader population of diverse HLA alleles, the following points should be met:

1. The immune response should be directed against the determinant(s) that are invariant within the population.
2. The immune response should be elicited in almost all the individuals of an outbred population i.e. there should be no genetic restriction at the host level against the synthetic vaccine.
3. Both the T and B cells should cooperate with each other in the immune response in order to produce long lasting immunity and memory.
4. Cross-reactive antigens should be eliminated in order to obviate any undesirable immune response.

Advantages of Synthetic Peptide Vaccines over Other Vaccines

The main advantages of using synthetic vaccines may be summarized as follows:

1. They can be produced in large quantity.
2. The peptide vaccines are stable at room temperature
3. The stability of the peptide vaccine makes it suitable for applications in delayed release vehicles and thus the slow release profile mimics the booster response of vaccine.
4. It is possible to attach several peptides, representing the relevant portions of different pathogens to the same carrier molecule effectively giving a multivalent vaccine.
5. Their use will eliminate immunization against many irrelevant antigenic determinants of the virus or irrelevant proteins that contaminate the viral preparation.
6. Many vaccines need an adjuvant to enhance the immune response. In synthetic vaccine, it is always highly desirable to introduce certain groups that augment antigenicity. Thus, the synthetic vaccine should contain built-in adjuvanticity and may prove to be less hazardous and of better quality for humans.
7. The peptide vaccine is defined in chemical terms and is free from infectious material/any contamination.
8. Unlike the conventional vaccine, synthetic vaccine need not to be propagated in the unnatural host hence, no fear of autoimmunity / cancer.

Antibodies against the synthetic peptide immunogens may provide reagents for passive immunity, antitoxin therapy, targeted immunotherapy of neoplasia, radioimaging of tumors and finally for use in immunodiagnostics (21).

Synthetic Vaccines can be Divided into Two Categories

1. Chemically synthesized or peptide vaccines
2. Recombinant vaccines

Peptide Design

The synthesis of peptides corresponding in sequence to the primary structure of antigenic regions of a pathogen represents another way of developing non-infectious surrogate vaccines. The potential effectiveness of a synthetic peptide vaccine is usually reflected in its ability to elicit the formation of neutralizing antibodies and/or immunological memory. Synthetic antigens might be of considerable importance in the future development of vaccines (22). They may be converted into potent immunogens by being coupled with proteins that activate the immune system and invite the participation of T-helper antigenic sites. The development of synthetic molecules along with the B-cell epitopes will allow the design and development of inexpensive, efficient vaccines. They should avoid possibly deleterious sites, such as suppressor T-cell epitopes or sites involved in tolerance (23).

The following guidelines may help in making vaccines better.

1. Identification of precise epitopes within the predicted sequence by synthesizing overlapping peptides with single amino-acid deletions from the N-or C-terminus of the predicted sequence which is finally recognized by T-cells in association with many MHC class II / class I molecules.
2. Construct multiple antigen system.
3. Inclusion of a universal T-helper epitope into the vaccine since the immune response to a given epitope is normally under genetic restriction.
4. Mimicking of the conformation of the native epitope by disulphide bonding to form a circle that enhances the antigenicity of the synthetic oligopeptides.
5. Linking of hydrophilic epitopes between hydrophobic segments to provide correct folding to stimulate immune responses against different epitopes in a single synthetic chain.
6. Introduce some motif that allow polymerization/ cross-linking of each epitope.
7. Palmitoylation of peptides leads to CTL response.

Structural Factors in Peptide Vaccine Design

In order to be effective, a synthetic peptide vaccine must possess a high level of immunogenicity and induce antibodies that cross-react extensively with the pathogen. Developing peptides suitable for vaccination is a far more difficult task than selecting peptides able to induce antibodies that simply cross-react with the cognate protein. At times, certain structural modifications have shown a marked increase in the antigenicity/immunogenicity of synthetic vaccines. Little evidence support the role of carbohydrates as immunogens in protection or prevention of a disease but majority of the studies show that they have a very little role to play in elimination of the pathogen. The majority of the studies involving bacterial pathogens show that anti-carbohydrate antibodies have a negative effect either in enhancing the multiplication of the

pathogen or are involved in pathogenesis of a disease. The role of carbohydrates observed in majority of diseases is in receptor recognition and also the site of protein modification or protection of the antigen from degradation by burying some potential tryptic and chymotryptic sites (24). Addition of carbohydrate residues also helps in the modulation of antigenicity by stabilizing the three-dimensional structure of the molecule. Masking and unmasking of the protein is another important function of the carbohydrate. This may lead to exposure of the antibody binding sites in the areas previously buried in the native molecule. Thus, efficacy may be greatly enhanced by altering the position and number of carbohydrate residues in a synthetic protein vaccine. Local secondary structures can also be dramatically altered by changes in the sequence. Substitution of a polar residue for a hydrophobic residue may lead to the exposure of buried residues e.g. foot and mouth disease peptide VP1 (25). Further in some cases disulphide bridges have been shown to be critical for the maintenance of the native antigenic and/ or immunogenic activity. In the hepatitis B surface antigen peptide, the presence of an intra-chain disulphide bond (between 124 and 137) makes this cyclic peptide exceptionally immunogenic (26). Acylation of the N-terminal of synthetic peptides by long chain fatty acids has also been shown to increase the immunogenicity. Myristylated, as opposed to unmyristylated pre-S peptide of the hepatitis B envelope protein has been shown to be immunogenic in sub human primates (27). Unless the protective determinants are identified and isolated, it is easy to make tailor made sequences retaining their immunogenic properties, though it would not be possible to produce a highly specific vaccine free from infectious material.

Mapping of T-and B-Cell Antigenic Determinants

Successful induction of immunity to most antigens requires the recognition by T and B cell of every different epitope. T cells are important regulators of immune responses and it has become increasingly clear that the class of T cell preferentially activated in an immune response is of pivotal importance for its strength and for the generation of effector mechanism. Hence, the ability to predict regions of protein sequence most likely to elicit T cell immunity would be of potential use in vaccine development.

A computer algorithm designed to predict antigenic regions in a given protein does so by: -

1. The use of hydrophilicity parameters of individual amino acids derived from the HPLC retention times to predict surface residues on the protein antigens (18).
2. The use of hydrophilicity-recognition profiles of proteins.
3. Predicting local secondary structure, i.e. α-helix, β-pleated or random structure.
4. Looking for an amphipathic structure, i.e. a structure in which the hydrophobic residues tend to occur on opposite faces. These two sides may serve to interact with the MHC molecule on the antigen-presenting cell and with the T cell receptor (28).
5. The isolation of the peptide sequence that is released during the proteolysis of the antigen-MoAb (monoclonal antibody) complex (19).
6. Constructing peptide sequences that are comers of the folded polypeptide chain.
7. Sequence accessibility, antigenic index and hydrophilicity parameters.

Role of MHC in Peptide Vaccine Design

The ability of an individual to respond to a given antigen is controlled by Immune response (Ir) genes. In the epitope or peptide based vaccines, the immune response is restricted since the peptide binds to a restricted MHC molecule. This allele specific nature of peptide binding to MHC molecules indicates that single peptide vaccine will be ineffective except in the most limited homogeneous population. In general, multiple peptides or proteins yielding peptide fragments will be necessary to ensure that all members of a heterogeneous population possessing diverse MHC alleles can capture and present to their T cells at least one effective antigen. Normally the size of the peptide recognized by class II molecule are usually between 10-20 amino acids in length while the size for class I molecule is 9-10 amino acids in length. Peptide class II interaction has been analyzed in detail both at the structural and functional level and peptide-binding motifs have been proposed for various mouse/human class II specificity (29). In some cases, the peptide produced from the proteins of an infective agent will not contain optimal motifs for binding to prevalent MHC molecules in the populations. Predictions based on these motifs appear to be less accurate for class I molecules. This is because the peptide binding groove for class II molecule are open on both the sides thereby allowing different motifs to bind to single MHC molecule (30). This methodology helped to engineer non-natural T helper epitope by modulating either MHC binding affinity or alteration of TCR (T cell Receptor) contact residue or both. One such epitope identified for class II binding is PADRE peptide (31). This methodology helped to engineer non-natural T helper epitope by modulating either MHC binding affinity or alteration of TCR (T cell receptor) contact residue or both. In such cases, the introduction of suitable motif residues or the elimination of dominant negative residues (32) can make some improvement in the

vaccine material. Under proper circumstance, such peptides will stimulate T cells and still be able to recognize the native peptide bound to the same MHC molecule. This is because primed T cells require lower levels of TCR ligand for stimulation, for the lower efficacy of the natural peptide in forming the complexes with MHC molecule will still permit activation of the T cells previously primed by the modified peptide. It is also possible to identify the key residues controlling the T cell specificity (epitope residues) rather than MHC molecule binding and produce the vaccine material with multiple substitution at such position to preclude escape from immune destruction due to pathogen sequence variation at these sites (33). With reference to class I pathway, the antigen/peptide should have the access to the cytoplasmic delivery. This can be accomplished either by using live vehicles or liposomal delivery. Another important point is, it is crucial to ensure that load of the distinct antigens in a combined vaccine does not exceed the capacity of the presentation system because help for B cell antibody production involves recognition of peptide-MHC class II complex on the B cell. Sequestering the circulating antibodies can minimize another level of peptide competition.

Immunogenicity of Peptides

Despite considerable research over many years the only adjuvant currently approved as gold standard for use with vaccines is alum but comparative studies show that it is a relatively weak adjuvant for antibody induction and a poor adjuvant for the induction of cell-mediated immunity. There is an urgent need to supplement this adjuvant with improved delivery systems, which are potent and safe and can be used with new generation vaccines. In order to increase the immunogenicity of peptide vaccine two important criteria should be satisfied. Firstly, efficient presentation of the processed antigen to the T-cell receptor. Secondly, immune response to be uniformly generated in outbred population. In an attempt to achieve the above goal, we have used two approaches. Simultaneously, present the antigen in a particulate form so that antigen can be released slowly into circulation in depot formulation more so with liposomes or ISCOMs (Immunostimulating Complex's) or microspheres (34) or in continuous and pulsatile form (35). The influence of immunoadjuvants on the qualitative aspect of immune response should not be ignored and hence, we have used panel of immunoadjuvants that are non-toxic, permissible and water-soluble. Adjuvants include a polymer of tuftsin (36), bio-active fragment of IL-1 α (37), MDP analog such as murabutide (38) and casein immunomodulatory sequence (39). A combination of adjuvants with modified delivery vehicles undoubtedly increased the immunogenicity of otherwise non-immunogenic peptide fragments of CS protein of P. vivax, RESA antigen of P. falciparum (40,41) and envelope & core peptide sequences of HIV-I. Such an approach had generated high titer and high affinity antibodies. The quality of the generated isotype is polarized towards $IgG_{2a/2b}$, which are known to be cytophilic in nature, activates complement, enhance phagocytosis and clear the pathogen through ADCC mechanism. Most importantly the generated antibodies inhibited the growth of the relevant pathogen in-vitro. The influence of such an approach had also contributed to cell-mediated immunity through activation/ expansion of splenic lymphocytes and generated cytokines, which are predominately of IL-2 and IFN-γ ($CD4^+$ TH1)(42,43). As most of the studies were done in inbred mice with different genetic background as well as in outbred strains, the outcome of the study undoubtedly proves that there is no MHC linked immune response with any of the above strains.

One of the rationales for designing engineered vaccines is based on putting together individual defined epitopes for eg. as in HIV one can select epitopes that induce neutralizing antibodies, cytotoxic and helper T cells that might be protective thereby avoiding epitopes that induce enhancing antibodies, autoimmune responses or suppressor cells. One can combine the epitopes in various ways to make them potentially active. This can be achieved by producing multivalent constructs (44) or combining some neutralizing sequences arising from different clades of pathogens (45,46) or instead improvise on these epitopes by tinkering with the internal structure of the antigenic determinant or antigenic site. It is always mandatory that for generating neutralizing antibodies that cross-react with native protein, the selected peptide should assume the necessary requisite conformation adopted by the native protein. In such circumstances, the importance of physical parameters like free energy of conformation plays a major role in critical binding of antigen antibody complex. The hypothesis is that if MHC molecules can combine to host of peptides and only a few specific side chains are necessary for positive interaction, then negative or adverse interactions at nonessential positions should playa role in determining the specificity of peptide MHC binding. Therefore by identifying the different residues in a peptide may suggest ways to improve the antigenic activity and the nonessential residues might be replaced to enhance the function (47). In another study as in the case of VP1 protein of foot and mouth disease virus it is observed that the most variable regions of VP1 would be those subject to immunological pressure for mutation, which would be the site of greatest antigenicity. The obvious property associated with genetic polymorphism of class I and class II molecules determine the specificity and affinity of peptide binding is in T cell recognition. In other words, individuals with different haplotypes will vary in CMI response to the same antigen. It is therefore important

to identify the peptides recognized by T cells for efficient protection against the disease. In theory all these studies suggest that it is possible to map the specificity of CTL clones by using a panel of recombinants expressing the overlapping peptides to create targets but in practice it is however more common to use synthetic peptides in conjunction to computer predictions.

In one of the studies in cancer it was observed that identification of peptide sequences recognizing CTL has led to direct induction of CTL responses in-vivo (48). To stimulate the $CD4^+$ T cells that respond to peptides presented by class II molecule, proteins must be delivered efficiently to the endosomal-processing compartment. Thus the delivery vehicle is important in maximizing such delivery. Also particulate antigen and delivery in a concentrated form may help class II pathway. Another way is to use ligand conjugation so that cellular receptors are used to enhance endocytic uptake of the antigen. Another way is to regulate the stage in the endosomal pathway when the antigen is available during processing. In one of the study it was observed that a fusion peptide with an endoplasmic reticulum-signal sequence at the amino terminus was more effective in generation of CTL response than the peptide itself (49). Numerous reports are available in the literature regarding peptide vaccination for cancer. It was demonstrated that there is tumor regression by immunisation with MAGE-3 derived peptide even in the absence of any adjuvant (50). Furthermore there is generation of CTL specific responses for gp100 derived (51) peptides immunised with Incomplete Freunds adjuvant. Many peptide vaccines are being studied currently e.g. peptides derived from MART-I, tyrosinase, gpl00, MAGE-3 (52,53), prostate specific antigen (54). Several strategies for the modification of these peptides such as lipidification or changing anchor residues that binds to HLA motif are also being attempted (Rosenberg et al. 1998) to produce an efficacious vaccine against all types of cancers. Many studies have been coming up for a better design of peptide vaccine by exploring the immunological specificity using synthetic peptide combinatorial libraries (55). The use of this approach has four major effects: first, the definition of high affinity ligands for both T cells and antibodies; second, the application of alternative means for identifying immunologically relevant peptides for use as potential preventive and therapeutic vaccines; third, a new appreciation of the requirement for TCR interactions with peptide -MHC complexes in immunogenicity; fourth, the establishment of new principles regarding the level of cross reactivity in immunological recognition. Though peptide based vaccines have enormous advantages, it has few disadvantages:

1. Synthetic peptides are poor immunogens.
2. They are mono specific in the induction of immune response.
3. Generated immune response is not uniform in outbred populations.
4. As the length of the peptide fragment is short it may contain insufficient information to fold into the correct shape necessary to mimic conformationally dependent epitope.

Plausible Ways of Overcoming the Disadvantages

The above drawbacks can be circumvented by use of adjuvant or controlled delivery vehicles i.e. ISCOMs, Liposomes or Microspheres. Chemical conjugates with antigen derived viral or bacterial proteins controlled polymerization of some of the epitopes and lipopeptide conjugation or using MAP (Multiple peptide antigens) comprising of T and B cell epitopes coming from same antigen or from different antigens also provide an effective method of synthesis of many epitopes in a well defined orientation using a branched oligolysine matrix. All these approaches produce a long lasting B cell, T helper and CTL response provided there is no epitopic competition in between multiple antigens and thus leading to suppression of antibody production to otherwise dominant epitopes. Strategies to enhance immunogenicity of these candidate vaccines are therefore critical.

Several types of immunoenhancers are under investigation. They work in a variety of ways by changing the conformation of the antigen thereby enhancing the antigen presentation, by preventing the proteolytic destruction of the antigen in the stomach thus allowing it to pass into the intestines intact for presentation to gut associated lymphoid tissues or by targeting the antigen directly to M cells of the gut to induce mucosal immune response or by the induction of various immunomodulatory cytokines such as GMCSF, IL-12, TNF-α that act directly on the thymus/derived helper T cells to stimulate specific arm of immune responses. The exact molecular or cellular mechanism required for the generation of an effective immune response in-vivo depends on the co-injection with adjuvant, which need proper understanding (56). Therefore, they are still surrounded by obscurity and called as "immunologists dirty little secret" (57). Therefore for the formulation of a highly effective subunit vaccine, the inclusion of strong immunoadjuvants and / or proper delivery vehicle has become essential to elicit optimal immune response in the host.

Adjuvants and Delivery Systems

The poor immunogenicity of peptide vaccines and thus, their ineffectiveness in soluble form to generate an effective immune response necessitate the following guide lines:

1. Increase the antigen absorption
2. Prevent its degradation and
3. Show the outcome of immunization to a desired goal (protective response against infectious diseases vs tolerance, B vs T cell response; mucosal vs systemic).

Adjuvants are defined as a group of structurally heterogeneous compounds, used to evoke or increase an immune response to an antigen (58). The concept of an adjuvant, an immunity stimulating substance, enhances the specific immune response, both humoral and cell mediated, to a protein antigen (59). Classically recognized examples include oil emulsions, saponins, aluminium or calcium salts, non-ionic block polymer surfactants, derivatives of lipopolysaccharides, MDP, mycobacteria and others (like Vitamin E and Fluoride). Theoretically each molecule or substance is able to favor or amplify a particular situation in the cascade of immunological events, ultimately leading to a better immunological response is defined as an adjuvant (60). Adjuvants or delivery systems modulate the immunogenicity of antigens either by simply prolonging the half-life in the recipient or through activation or combination of effector mechanisms. This is achieved by acting as a depot for slow release of antigens or preserve the conformational integrity of the antigen for better antigen presentation or secrete immunomodulatory cytokines or induce helper or cytotoxic response or by targeting the antigen to cell surface receptors for better opsonization. They are known to modulate antibody avidity, specificity, isotype or subclass distribution. They enhance the immune responses in immunologically immature or senescent individuals. At the start of this century, there was almost no adjuvant research or report other than aluminium salts. Although aluminium salts are the only adjuvant registered for human use, numerous immunomodulators have been developed during the past decade to allow for both an increase in immunogenicity of peptide antigens, but also to allow for delivery to new sites such as mucosal area. Adjuvants come in many different forms and are generally considered both for delivery of antigen(s) and as immunostimulant that has a direct effect in the immune system. Delivery systems for vaccines have been designed to this date either to improve parenteral delivery or to allow for a new approach in vaccinology such as in mucosal delivery (61). Although the immune response obtained after parenteral or mucosal administration differ, the various types of delivery systems that are being developed are to induce both mucosal & systemic immune response. The currently available adjuvants and delivery vehicles can be classified as particulate, nonparticulate and others. Particulate adjuvants include the aluminium salts, surface-active agents, slow releasing vehicles like FCA, liposomes, Novasomes, IRIV's, ISCOMs (62) and microspheres (63). Nonparticulate includes non-ionic block copolymers, glycopeptides and lipopeptides, and peptides of microbial origin. Other adjuvants being used are proteosomes, cytokines, Trat, polytuftsin, QS21, MF59, Montanide, mucosal adjuvants like cholera toxin-B, CpG DNA, lectins, LT (R192G), IgA and cochleates (64). The induction of immune response at a desired site can be accentuated with the use of bacterial or live vectors (65), which contain the genes from unrelated microbial species that encode important virulence factors and antigens. These vectors have been used especially in mucosal immunizations i.e. Salmonella, E.coli, mycobacterium, lactobacilli, polio, adeno, rhino, meningo, influenza, vaccinia and canary pox virus have been used in various animal models with only salmonella typhi and adeno virus used in human system (66). As the parenteral route(s) of immunization generates systemic immune response only hence, we in our laboratory are currently emphasizing the relevance of both systemic (IgG) as well as mucosal immunity (secretory IgA). We are targeting the antigens to the M cells of Peyer's patches (GALT / NALT) after entrapping the antigen in microspheres or by using a ligand having specificity for M cell in diseases like HIV (67) and Plague (unpublished data).

By use of delivery systems like ISCOMs in which Quil A is combined with cholesterol or other lipids like phosphatidylcholine to form honey comb matrix of micelle and thus the Ag attaching itself by hydrophobic interactions. By these interactions multiple sites of the Ag are presented to the antigen presenting cells and thus it enhances the immunogenicity of the antigen (68).

The use of liposomes, which are bilayered phospholipid vesicles, are non toxic and safe for human use. They have been viewed as potential replacement for aluminium based vaccines due to their flexibility biocompatibility and biodegradability. They present no toxicity in human and provide efficiency in many experimental protocols.

The use of microspheres provides a delivery vehicle both for mucosal as well as systemic immunization (69). They are designed to slowly release the antigen at various time points in the tissues, various polymers have been used for preparation of such microspheres with the most-widely used being Poly lactic co-glycolide. They also come under the category of nontoxic, biocompatible and biodegradable adjuvants (70).

Fluoride, the agent responsible for reduction of dental carries is shown to be a potent adjuvant when given intra-gastrically to rats. Ingestion of fluoride can modulate the immune response to orally and

parenterally administered antigens have shown to stimulate the proliferation of intestinal lymphoid tissue (71). Vitamin E and Vitamin A are antioxidant vitamins and are proved to be good immunopotentiators because they protect the sensitive rapidly proliferating cells of the immune system from oxidation damage and increase cell interaction by membrane alteration. The adjuvant emulsion amplifies local inflammatory reactions, attracting polymorphonucleocytes, dendritic cells, macrophages and lymphocytes to the site of injection, allowing optimal interaction between antigen, antigen processing cells and the vitamin. The vitamin acts as a physical constituent of the emulsion as well as a potent immunoenhancer (72).

Other Types of Vaccines

Poly topic vaccines

In its present and simplest form, the new science of vaccinology might be considered to be the science of epitopes. These are immunological determinants of antigens whose presentation, either in native or processed form to the B or T cell receptor in live or non-living forms, induces humoral and/ or cellular immune responses and finally generates protection against infection and disease for a sufficient period of time. The administration in single vaccine of a highly complex combination of appropriately selected epitopes permits the prevention of many different diseases in a practical way with single preparation. These are referred to as poly topic vaccines (73). In seeking individual epitopes, the focus has been on the continuous or sequential epitopes that lie within the confine of a short amino acid sequence, which can be synthesized. Discontinuous epitopes consist of conformational contributions by several different segments of folded chains and are difficult to synthesize. However, they may be approached through the synthesis of covalent peptides that in their conformations mimic the three-dimensional surface of the antigenic site as well as through the synthesis of anti-idiotypic antibodies. Sequential epitopes normally form part of the corners of the folded polypeptide chains.

At least four assumptions underpin the current research endeavors into new and improved molecular vaccines:

1. A subset of epitopes of the pathogen from perhaps several different antigenic molecules is sufficient for induction of host-protective immunity in the genetically diverse host population.
2. Immune effector mechanisms will be identified through basic research that is necessary or sufficient for the expression of resistance.
3. New, potent, acceptable and selective immunostimulating agents (adjuvants) should be made available easily with low cost.
4. The safety of the vaccine can be assured by exclusion of the epitopes and contaminants, which lead to undesirable side effects whether they are immunogenic or inflammatory.

Idiotypic Vaccines

Antigenic determinants on antibodies are invariably immunogenic in other species since B- and T-cells are linked mutually through idiotypic complementarity (74). The antigen-binding site is called the paratope and the antigenic structure associated with the variable region of antibody is called the idiotype. Each idiotype is composed of a set of distinct antigenic structure called idiotopes. Antigen-binding and anti-idiotypic antibodies belong to the same family. This implies that each antibody molecule can bind both an epitope on an antigenic molecule and an idiotope. The latter appears as the internal image of the foreign epitope. This formed the basis for using internal image determinants as vaccines for infectious disease. Based on the ability of the anti-idiotypes to mimic foreign antigens, these have found use as alternatives to conventional vaccines in inducing anti-parasitic, anti-viral, anti-bacterial immunity (75). They have been particularly effective in the following case:

1. When the protective antigen of the infectious agent is a polysaccharide or the carbohydrate moiety of a glycoprotein.
2. When the microbes show antigenic variation such as Influenza virus, Human Immunodeficiency virus (HIV) or Trypanosoma.
3. By producing an idiotope, which is capable of preventing the binding of the HIV to its receptor of $CD4^+$(or T helper) cells.
4. To possibly provide a mechanism to combat cancer by inducing a tumoricidal immune response.

Dendritic cells (DC's) in vaccines

A number of studies show the successful use of DC's for inducing antitumor immune responses in both animals and patients (76). DC's are known to be potent antigen presenting cells and they initiate antigen specific immune responses (77). In addition to this they express high levels of MHC class I and II as well as co-stimulatory molecules essential in antigen presentation. They have the ability to cross present the phagocytized dead antigens to the T cells. It was found that peptide pulsed DC is superior to injection of peptide in adjuvant in inducing potent CTL responses (78). A possible disadvantage of this method is the short half life (2-10 hrs) of most MHC restricted epitopes, which

creates the requirement for several injections of peptide pulsed DC to achieve effective immune response (79), Thus, development of different methods of loading antigens allowing DC to utilize their own intracellular pathways is highly desirable.

Edible Vaccines

Plants can be made to synthesize immunogenic proteins and by eating these transgenic plants vaccine antigens can be expressed for eliciting mucosal and systemic immune response. Thus plants can be manipulated to produce bio-medically important substances. One of the limitations observed is, expressing the desired antigen(s) in leaves of plants may provide toxic effect, as it contains high levels of toxic alkaloids. Hence, the approach has been shifted either in tubers or fruits or seeds of plants. Though such an approach yielded encouraging results, but there is concern regarding such vaccine in humans being less immunogenic (80).

Combination Vaccines

Breakthrough in molecular biology, biochemistry, related fields have resulted in the development of many new vaccines as well as in the improvement of several existing ones. The successful development of combinational vaccines is a major way to reduce the number of vaccine administrations, thereby assuring improved compliance with immunization schedules. A combinational vaccine is a mixture of individual vaccines before administration with the result that multiple vaccines are administered together in a given host. There are two types of combinational vaccines multidisease (individual vaccines for different diseases), multivalent (directed to different serotypes or sero groups of the same viral or bacterial antigen). There are three types of which two of them are developed into combinations i.e. live and non-live (includes inactivated, killed, subunit vaccines) but there are not yet any examples of combination of live and non-live. Till today there are six combination vaccines available, they are DTPa, influenza, polio, MMR, pneumococcal and meningococcal. An increasing number of new combinations are being developed (81).

DNA Vaccines

Direct inoculation of expression plasmids, which results in the induction of long lasting immune of both humoral and cellular responses against the expressed antigens are DNA vaccines. Studies show that intramuscular injection of DNA generated best response where as inoculation of DNA coated to gold particles using gene gun significantly lowered the immunizing dose of DNA. Studies clearly show that uptake of the injected DNA is an active energy dependent process (82). The plasmid DNA can get into the nuclear membrane or muscle cells and persists as a non-replicating episomal molecule, which explains the long-lived foreign gene expression in case of DNA vaccines.

Heat Shock Proteins in Vaccines

An interesting approach in vaccine development is the use of heat shock proteins -peptide complexes for vaccination. Heat shock proteins derived from any given cell type associate with a wide variety of peptides generated during protein degradation (83). Vaccination of mice and rats with HSP-peptide complexes has showed powerful immune responses against peptides bound to them, but not to HSP itself. More recently it was reported that HSP-peptide complexes can be used as prophylactic and therapeutic agent even in diseases like prostate cancer (84) .The heat shock proteins like Hsp 60/70 also have a receptor on antigen presenting cells that helps in targeting to class I or class II MHC molecules. Four classes of heat shock proteins such as gp96/90/70/30 & calreticulin have been used successfully to immunize against cancer and other infectious diseases in prophylactic and therapeutic protocols. Two recent studies reported significant enhancement in DNA vaccine potency by the linkage of antigenic genes to HSP genes (85,86). The disadvantage of HSP in the clinical settings is the requirement of generation of customized, patient specific vaccines for cancer however, their ability to elicit specific CTL response in mice of any haplotype makes them very attractive vaccine therapeutics against cancer.

Microarray

Microarray is a technique that provides a global analysis of gene expression at the level of transcription. Genetic and epigenetic changes underlie neoplastic transformation, cardiovascular disease, some psychiatric illness, and a growing list of disease pathogenesis and therapeutic responses. The profile of genes expressed by different cells(gene up and down regulation under different conditions) determines their phenotype, and thus provides insights in to the molecular basis for health and disease. DNA microarrays, which are also called DNA arrays or gene chips are a tool that uses genome sequence information to analyse the structure and function of tens of thousands of genes at a time.

Each Microarray is made up of many bits of single stranded DNA fragments arranged in a grid pattern on the glass or plastic surface. When DNA or RNA is applied to the array, any sequence in the sample that find a match binds to a specific spot on the array. A computer then determines the amount of sample bound to each spot on the Microarray.

In a typical Microarray experiment, cDNA from one

sample(sample A) is labeled with red dye and cDNA from another (sampleB) with green dye. The fluorescent red and green cDNA samples are then applied to a Microarray that contains DNA fragment corresponding to thousands of genes. If a DNA sequence is present both on the array and one or both samples, the sequences bind, and a fluorescent signal sticks to a specific spot on the array. The result of a gene expression experiment are referred to as a gene expression 'profile' or 'signature'.e.g. microarrays can be used to diagnose different cancers by comparing the profile of a cancer cel with that of a normal cell. Similarly, microarrays can determine which genes are turned on during cell division by comparing the expression profile of a cell that is in resting state to the profile of one that is dividing (87).

Protein Arrays

Protein arrays that are used to identify proteins typically consist of many antibodies arrayed on a glass or plastic slide. Each antibody can bind to a different target protein. Bound proteins can be detected either by adding a second antibody tagged with a fluorescent molecule or by chemical labeling the proteins. Each bound protein can therefore be detected as a signal on the array, and the intensity of the signal roughly represents the amount of the protein present.

Medical application of micro array technology

The most important application of microarrays is in the study of differential gene expression in disease and health, and in normal and abnormal physiologic and immunologic responses. Diversion in normal physiology is frequently accompanied by panoply of histological and biochemical changes including in gene expression patterns. The up-or down-regulation of gene activity can either be the cause of pathophysiology or the result of disease. To study the fine changes in the expression of these thousands of genes affected in a diseased state is almost impossible without the help of this powerful microarray technology. Microarrays promise to accelerate the understanding of the host as well as the pathogen side of the host-pathogen interaction. A large fraction of the genome can be simultaneously interrogated, and clustering of the data may identify groups of genes that influence activation or repression of key regulatory pathways. The opportunity to compare the expression of thousands of genes in varied pathophysiological conditions allows the identification of virulence factors that can aid better and inform vaccine design (87,88)

In conclusion, in the context of vaccine research, the power of microarrays combined with transcript profiling and cluster analysis is such that it allows the sub-classification of disease types and identification of molecular targets, which may have relevance for diagnosis, therapy and vaccine development (87,89)

Medical Applications of Synthetic Peptides

1. Use of synthetic peptides in serological assays: Synthetic peptide technology permits mass production of peptides for specific proteins, thereby adding new information regarding the host immune response during natural infection, age dependent immunity in relation to intensity or duration of exposure to a given pathogen. In fact, the synthetic peptides have largely replaced conventional antigens in the detection of antibodies in the sera. They are also preferred for raising antibodies to detect the parent antigen, thereby increasing the mono specificity.

   It is important to remember that there is an age dependent increase in antibody level, which was observed by us while studying malaria endemicity, using repeat sequence of CS and Ring infected erythrocytic stage antigen (RESA) peptides of P. falciparum and P. vivax as test antigens. This observation correlates well with parasitaemia on the one hand and with antibody levels on the other. However, interestingly no correlation was found between the levels of CS antibodies and blood stage antibodies in the same population. Thus the response to CS peptide appears to develop at an earlier stage than the response to blood stage peptides, though this approach discriminates current infection with past infection.

2. The synthetic peptide vaccines have been used towards the development of diagnostic assays as in bovine tuberculosis using ESAT-6, MPB6-4 and MPB83 mycobacterial antigens expressed at high levels in M. bovis and at low level in BCG, pasture. Hence, bovine T cell epitopes are identified and formulated into peptide cocktail. (90).

3. These peptide vaccines are used in vaccination with heat shock proteins for tumors thus generating anti-tumor responses. The immunogenicity of HSP is derived from the antigenic peptides, which they associate with these HSP-peptide complexes and can be used as human vaccine for cancer immunotherapy. (91).

4. They have a role in regulation of specific immune responses to chemical and structural modifications of allergens. This is achieved by modifying B cell epitope in order to prevent IgE binding and effector cell cross linking while preserving T-cell epitope to retain the ability of inducing tolerance. Thus developing novel vaccines for the treatment of allergy (92).

5. They can been used in the treatment of various microbial infections by the use of diverse array of natural, synthetic and recombinant immuno-modulators and thus stimulating host defense mechanism for prophylaxis (93).

6. Preparation of safe malarial vaccines which are well tolerated and gives high titer while expending CD4+ and CD8+ lymphocyte response by using synthetic peptides of CS and of various plasmodia (94).

7. Development of pre-erythrocytic malarial vaccine leading to complete resistance to malarial infection. This can be achieved by use of synthetic peptide vaccines, multiple antigen peptides and polyoximes from CS protein the first pre-erythrocytic antigen identified and present in all malarial species (95).

8. Their use as vaccines against auto-immuno diseases e.g. COP-I is a synthetic amino acid copolymer in suppression of experimental allergic encephalomyelitis (EAE) (96).

9. Their role in generating rabies vaccines by use of synthetic peptide technology besides employing molecular biology tools (97).

10. An important aspect of producing large amounts of protein or peptide is to make the product easily purifiable. This has been done by attaching peptides or protein to easily purified units such as virion particles or by exporting proteins to apoplast so that purification begins with a highly enriched product (98).

11. Use of synthetic peptide combinational libraries in exploring immunological specificity. (55).

12. For intervening the pregnancy at various stages attempts have been made to develop peptide based agents based on FSH, hCG (99), ZP3 (100) and Sperm surface protein (101).

Future Directions for New Generation

The immediate future of the synthetic peptide immunogen in medicine or in the veterinary field is clear, but the results expected in the laboratory must be translated into safe application in the field. In any case, the ideal vaccine will most likely consist of a cocktail of antigens or proteins. One must also consider the dose of antigen and speed of antigen release in the vaccine formulations. High doses of antigen released faster may induce B& T cell tolerance. Immune tolerance may be due to fast expansion and subsequent elimination of specific T cell clones or apoptosis induced by repeated stimulation of already existing B & T cells in cell cycle (102). Protective epitopes from various stages of parasite can be linked in one formulation to generate a response across all the stages of the parasite. Some of the vaccine requires the right animal model to study the protective efficacy. Therefore it is essential to choose the right immunogen with the right adjuvant and the best delivery vehicle to produce an efficacious vaccine against any pathogen.

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