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Principles of Vaccine Development

Constantin A. Bona

INTRODUCTION

Two hundred years ago, Jenner devised the first vaccine able to prevent variola. This vaccine was based on the observation that subsequent to injection of a boy with cowpox, he was protected against two successive inoculations with smallpox virus. After 200 years, global administration of vaccinia has led to almost total eradication of the smallpox virus from the earth. There is no other example in medicine of a new drug or biologic substance leading to eradication of the causative agent and extinction of disease. Vaccinations against other bacteria or viruses prevents the death of millions of people yearly. However, currently vaccines do not cover the entire spectrum of diseases. There is a long list of microbes, among which are HIV and malaria, affecting millions of individuals, for which we do not yet have vaccines. This is why the development of new vaccines is a permanent aim of medical research. This interest grew because scientists have understood that vaccines can be used not only for prevention of infectious diseases but also for therapy, leading to the concept of therapeutic vaccines.

Classical vaccines pioneered by the discoveries of Jenner, Pasteur, and Ramon was based on the principle of inactivation of pathogenicity of a microbe without altering its capacity to induce a protective immune response.

Developments during the past decades in biochemistry, molecular biology, and immunology have provided new tools for the development of a new generation of vaccines. Biochemistry and Immunochemistry contributed to the identification of epitopes endowed with protective capacities. The identification of such antigenic determinants, also called epitopes, on antigens of protein origin allowed for preparation of synthetic peptides or subunit vaccines in the case of antigens of nonprotein origin. Recombinant DNA technology, which revolutionized biomedical research, contributed to the development of genetically engineered antigens used as vaccines, as recombinant protein molecules, microbial vectors, or fusion proteins. Immunology provided the framework for understanding the mechanisms responsible for the activation of lymphocytes following vaccination as well as functional analysis of various epitopes that induce a protective immune response. This is particularly important as antibodies mediate the protection against some bacteria; cellular immune responses are prevalent against obligatory intracellular microbes.

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The immune system is composed of two major populations: B-cells, producing antibodies, and T-cells, mediating cellular immunity. T-cells are divided into CD4 and CD8 subsets and the CD4 T-cells are divided, based on the pattern of cytokine secretion, into Th1, Th2 and Th3 cells. The differences between B- and T-cells are not only functional but are also seen in the mechanism of recognition of antigens.

The B-cells, via the Ig receptor, recognize both conformational and linear epitopes directly on the surface of native macromolecules. In certain cases the recognition of epitopes leads to activation and differentiation of B-cells directly, i.e. T-independent antigens. In other cases they need the help of CD4 T-cells, i.e., T-dependent antigens. The isotype of antibodies is dependent on collaboration with T-cells. Whereas Th1 cells polarize the response to IgG2, the collaboration with Th2 leads to IgG1 and IgE *(1)*.

The polarization of isotypes is caused by cytokines secreted by these subsets that represent second signals: interleukin-2 (IL-2), and interferon-γ (INF-γ), in the case of Th1 and IL-4, IL-5, and IL-10 in the case of Th2 cells *(1)*. Antibodies exert their protective capacity by blocking the microbial receptor through which they bind to the cellular receptor of permissive cells, promoting phagocytosis via opsonins and complement-dependent lysis.

In contrast to B-cells, T-cells are unable to recognize the antigens on the surface of native macromolecules. They recognize only fragments of degraded antigens in association with MHC molecules. CD4 T-cells recognize peptides or glycopeptides in association with class II MHC molecules. The peptides are produced from the processing of exogenous proteins in the endosomes of professional antigen-presenting cells (APCs; B-cells, macrophages, and dendritic cells), where they bind to nascent and empty class II molecules. The peptide-class II complex is translocated to the membrane, where interaction with the T-cell receptor (TCR) of T cells occurs.

Figure 1 illustrates cellular events leading to generation of a class II-peptide complex within professional APCs. CD8 T-cells recognize the peptides in association with class I MHC molecules. The peptides are derived from endogenous proteins, including proteins of intracellular microbes. The proteins are fragmented by proteasomes, and the peptides are bound to transporter proteins (TAPs), and taken to the endoplasmic reticulum (ER), where they are released and bind to nascent class I MHC molecules. The peptide-class I complex is transferred via the Golgi apparatus to membranes and is recognized by CD8 T-cells *(2)*.

Figure 2 illustrates the generation of a class I-peptide complex. γ/δ T-cells or natural killer (NK) cells can recognize lipopeptides or glycopeptides in association with CD1 molecules, which are less polymorphic than MHC molecules *(3)*.

Table 1 depicts the major functions of cells involved in host response to vaccines.

Interdisciplinary contributions of accumulated knowledge and new methodologies have led to the development of new vaccines "à la carte," stimulating production of antibodies, cytokines, and cytotoxic responses, contributing to the recovery process from infectious diseases. This also contributed to the development of immunotherapeutic vaccines. Ideally, a vaccine should display the following properties:

1. The antigen should be pure and chemically well defined.
2. It should induce a protective immune response.
3. It should exhibit a constant antigen specificity without being the subject of genetic variation
4. The protection should be lifelong or induced promptly after a booster dose.

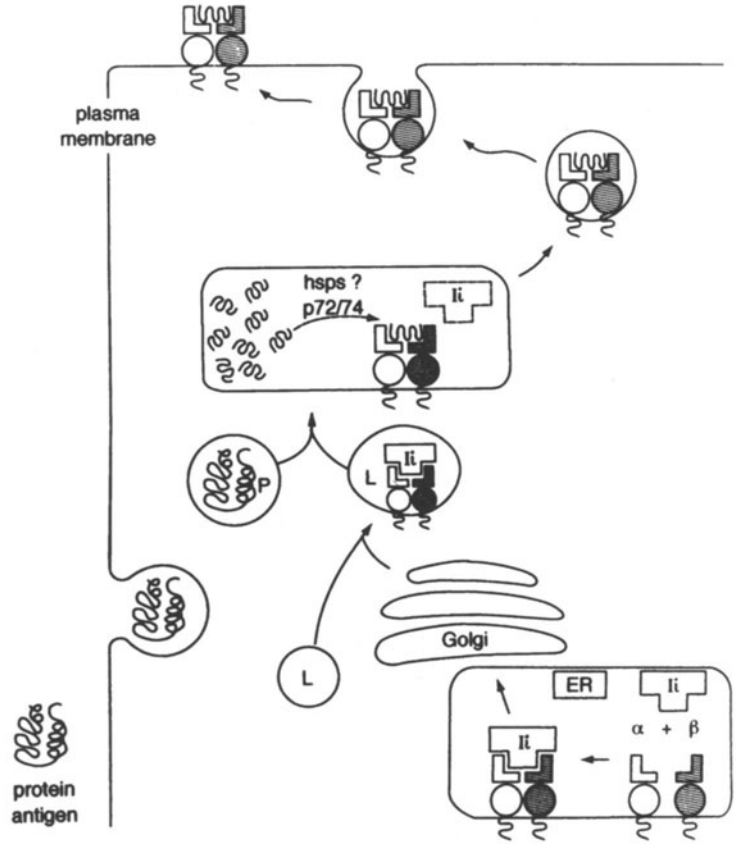

Fig. 1. Intracellular events leading to generation of class II-peptide complex expressed on the surface of professional antigen-presenting cells (APCs). After internalization, a vaccine is digested within endosomes of APCs. Class II molecules are synthesized in the endoplasmic reticulum (ER) as a trimeric complex made up of α, β, and invariant chains. It migrates to endosomes where the invariant chain is partially degraded and a short peptide called CLIP remains attached to class II molecules. After CLIP is released, DM functions as a chaperone that stabilizes the empty class II molecule, allowing the binding of peptides derived from degradation of foreign protein to class II empty molecules. Another molecule called DO stabilizes the class II-peptide complex, which is then pulled to the membrane, where it may be recognized by CD4 T-cells. hsps, heat shock proteins.

5. It should be devoid of side effects.
6. The manufacturing should be inexpensive.

CLASSICAL AND NEWLY DEVELOPED VACCINES

Inactivated Vaccines

The development of inactivated vaccines resulted from the development of methods to grow microbes and to purify the toxins. The preparation of inactivated vaccines is based on a golden rule emerging from Pasteur and Ramon's studies leading to preparation of anti-rabies and toxoid vaccines, respectively: a vaccine should be devoid of pathogenicity but should preserve intact its immunogenicity.

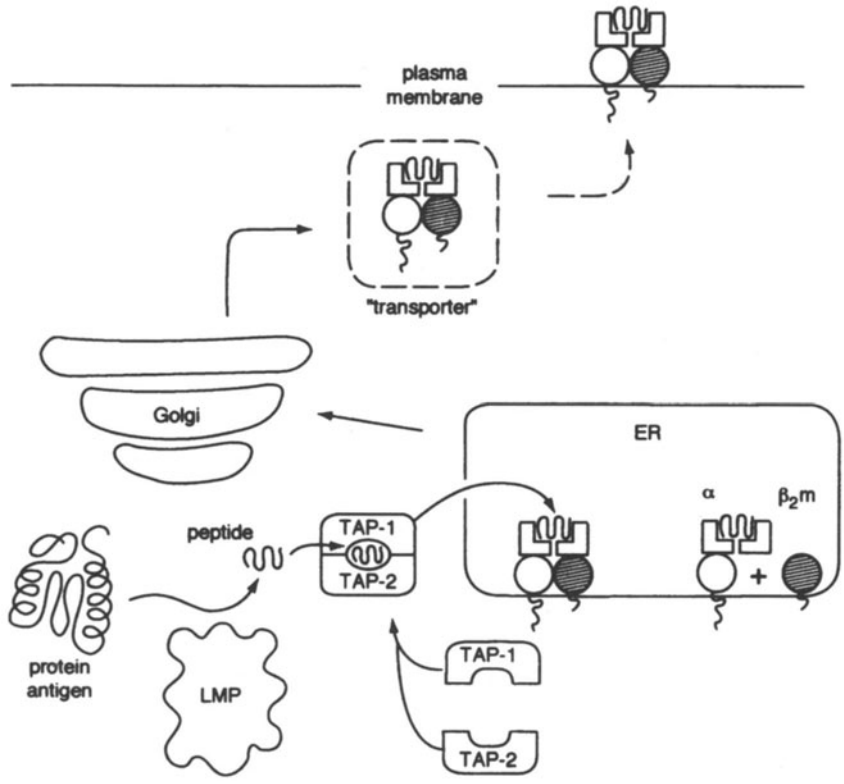

Fig. 2. Intracellular mechanisms leading to generation of class I-peptide complex. Endogenous proteins are degraded by lysosomes and the resulting peptides are translocated to the endoplasmic reticulum (ER) by transporter protein (TAP) molecules. In the ER, the peptides are released from TAP by chaperons such tapasin, calreticulin, and calexin and then bind to the heavy chain of class I molecules, which in turn bind β_2-microglobulin (β_2m). The class I-peptide complex is then transferred via a Golgi secretory pathway to the membrane, where it is recognized by CD 8 T-cells.

The killing of bacteria can be achieved by physical means (heat) or by chemical agents. For example, currently used influenza and Salk polio vaccines are produced by inactivation with formalin. Similarly, the conversion of toxins to toxoids was obtained by treatment with formalin. Table 2 lists currently used inactivated vaccines.

Immunity

Inactivated vaccines can induce only a humoral response. Functional antibodies are produced subsequent to recognition by the Ig receptor of B-cells of a protective epitope on the bacterial membrane or secreted toxins. Activation and differentiation of resting B-cells into antibody-forming cells requires a second signal by cytokines produced by activated CD4 T-cells. The activation of CD4 T-cells is achieved by APCs, which take up the microbes, process them in endosomes, and present the peptide-class II complex to CD4 T-cells.

Advantages

Inactivated vaccines display several advantages:

1. Simple manufacturing methodology.
2. Inability of reverse mutations that might lead to pathogen microbes.

Table 1
Function of Immunocytes in Immune Responses Elicited by Vaccines

Cell type	Function
Dendritic and B-cells	Presentation of epitopes
Macrophages	Phagocytosis of opsonized microbes
B-cells	Synthesis of antibodies against T-independent antigens
B+CD4 T-cells	Synthesis of antibodies against T-dependent antigens
CD4 T-cells	Secretion of antiinflammatory cytokines
CD8 T-cells	Lysis of infected cells

Table 2
Inactivated Vaccines

Vaccine	Licensed	In trials
Rabies	Yes	
Influenzavirus	Yes	
Salk polio	Yes	
Hepatitis B	Yes	
Japanese encephalitis	Yes	Yes
Bordetella pertussis	Yes	
Mycobacterium leprae		Yes
Vibrio cholerae	Yes	
Salmonella typhi	Yes	
Tetanus toxin	Yes	
Diphtheria toxin	Yes	

3. Stability.
4. Good induction of antibody synthesis.
5. Can be administered as combined vaccines such as trivalent or quatrivalent vaccines, e.g., influenza vaccine composed of H1N1, H3N3, and a subtype B-strain, or diphtheria, tetanus, and pertussis trivalent or quatrivalent when polio vaccine is added to trivalent vaccine. Combined vaccines induce similar responses, as do monovalent vaccines, indicating that is no antigen competition.

Disadvantages

The inactivated vaccines exhibit some drawbacks:

1. Poor antibody response is seen owing to weak generation of memory B-cells; several boosts are often required.
2. The antibody-mediated response against the protective epitope can be diluted by production of antibodies against the multitude of bacterial macromolecules bearing nonprotective epitopes.
3. There is an inability to stimulate the cell-mediated immune responses that contribute to recovery from disease or alter the course of disease in the case of therapeutic vaccines.
4. Some inactivated vaccines exhibit side effects (e.g., pertussis vaccine *[4]*).

Subunit Vaccines

Subunit vaccines represent a variant of inactivated vaccines. These vaccines can easily be developed when the disease is caused by a single or a few serotypes of infectious agents (e.g., *Neisseria meningitidis, Streptococcus pneumoniae,* and *Hemophilus influenzae* b serotype). They cannot be generated when multiple serotypes are involved in pathogenicity, as in the case of the nosocomial infection caused by *Klebsiella pneumoniae.*

Subunit vaccines are produced by purification from bacteria of antigens bearing protective epitopes or by molecular methods of expression and purification of recombinant proteins. With the exception of the hepatitis B subunit vaccine (which is of a protein nature), these are bacterial polysaccharides

Immunity

Polysaccharide vaccines are generally poor immunogens and induce T-independent responses dominated by IgM. The immune response results from direct activation of a subset of B-cells subsequent to the crosslinking of B-cell receptor (BCR) by antigens exibiting repetitive epitopes. This subset is under the control of an X-linked gene. Mutation of this gene, as in Wiscott-Aldrich syndrome, makes such patients unresponsive to subunit polysaccharide vaccines.

Advantages

Subunit vaccines are very stable and safe. Antibody response is more restricted than that induced by inactivated vaccines.

Disadvantages

Antibody response is generally weak, requires several boosts, and is dominated by low-affinity IgM antibodies. Generally, the vaccines are inefficient in newborns and infants because of the ontogenic delay of expression of a B-cell subset responding to polysaccharide antigens. Induction of high-affinity IgG antibodies can be obtained by coupling the polysaccharide to a protein bearing strong T-cell epitopes. This procedure was successfully used in the case of *H. influenzae* b serotype vaccine, in which the polysaccharide was coupled to tetanus toxoid. This vaccine is efficient not only in adults but also in infants. Finally, subunit vaccines cannot induce cytotoxic T-lymphocyte (CTL) responses. Table 3 lists licensed subunit vaccines.

Live Attenuated Vaccines

The possibility of preparation of live attenuated vaccines is based on Enders *(5)* discovery of a method of culturing viruses in vitro in permissive cells. Live attenuated vaccines are produced by culturing the microbe in special conditions, leading to loss of pathogenicity without altering immunogenicity. To achieve this goal, several methods were and are currently used:

1. Passage of virus many times in tissue culture or chicken embryonated eggs.
2. Selection of temperature-sensitive mutants that do not grow above 37°C.
3. Selection of naturally occurring mutants (e.g., Sabin vaccine).
4. Deletion of pathogenic genes.

Immunity

Live attenuated vaccines induce humoral and cellular responses. The humoral response results from the interaction of CD4 T-cells recognizing a peptide generated by APCs,

Table 3
Licensed Subunit Vaccines

Hemophilus influenzae
Streptococcus pneumoniae
Neisseria meningitidis
Bordetella pertussis (acellular)
Hepatitis B (recombinant protein)

which have taken up the microbe with B-cells able to recognize an epitope on the surface of virus. The CD4 T-cells secrete cytokines, which represent the second signal required for the activation of B-cells responding to T-dependent antigens. The infected cells can produce peptides subsequent to fragmentation of endogenous viral or microbial proteins. The peptide associated with class I molecules is expressed on the membrane. The CD8 T-cells are activated subsequent to recognition of the class I-peptide complex.

Advantages

1. Live attenuated vaccines elicit a long-lasting immunity comparable to that induced during natural infection. Therefore, immunity can be induced by a single or several injections.
2. These vaccines induce both humoral and cellular immunity.

Disadvantages

The preparation of live attenuated vaccines requires a tedious procedure to select the microbes that are devoid of pathogenicity, and manufacturing is costly. A possible drawback is the occurrence of reverse mutations. Table 4 lists currently used live attenuated vaccines.

Internal Image Idiotype Vaccines

Idiotypes are phenotypic markers of antigen receptors of lymphocytes. The diversity of antigen receptors is reflected in the diversity of idiotypes. Idiotype are immunogenic and able to induce antiidiotypic antibodies (Ab2s), which in turn express their own idiotypes. As a statistical necessity, Jerne *(6)* introduced the concept that the idiotypes of antiidiotype antibodies could mimic the antigen recognized by antibody-Ab1. This concept is not a simple consequence of the "lock and key" rule of complementary of antigen-antibody interaction but can be owing to molecular mimicry or sharing of similar sequences between antigen and Ab2.

This hypothesis is strongly supported by crystallographic studies. Fields et al. *(7)* had determined the crystal structure of an antibody specific for lysozyme and of its corresponding antiidiotype antibody. Of the 18 residues that contact Ab1 with Ab2, and the 17 that interact with lysozyme, 13 were in contact with both lysozyme and Ab2. This important information clearly demonstrated that some antiidiotypic antibodies are internal images of antigens and therefore they may function as antigen surrogates because they represent the positive imprint of antigen.

An Ab1 antibody specific for a protective epitope is prepared, and then Ab2 antiidiotype antibodies are generated. Antigen-inhibitable Ab2, which then can be used as internal image idiotype vaccines, is then selected *(8)*.

Table 4
Live Attenuated Vaccines

Vaccine	Licensed	In trials
Vaccinia	Yes	
Sabin polio	Yes	
Measles	Yes	
Mumps	Yes	
Rubella	Yes	
Adenovirus	Yes	
Varicella-zoster	Yes	
Cytomegalovirus		Yes
Dengue		Yes
Rotavirus		Yes
Parainfluenza		Yes
Japanese encephalitis		Yes
Hepatitis A	Yes	
Influenza (cold attenuated)		Yes
Salmonella typhi (aromutant)		Yes
Bacille Calmette-Guérin	Yes	

In various animal models it has been shown (Table 5) that antibodies produced subsequent to injection of internal image idiotype elicited a protective response. However, this type of vaccine was not introduced in human trials.

Immunity

The immune response elicited by idiotype vaccines results either from activation of B-cells subsequent to the binding of Ab2 to BCR of Ab1 or by interaction of B-cells with CD4 T-cells that recognize idiopeptides produced by digestion of antiidiotype by APC.

Advantages

The internal image idiotype vaccines are safe, induce humoral immunity, and are able to circumvent the ontogenic delay responsible for unresponsiveness of infants to some vaccines *(8)*.

Disadvantages

Internal image vaccines are poor immunogens and require coupling with carrier protein, which increases their immunogenicity. Generally they do not induce memory cells, an intrinsic property of a good vaccine. In addition, they are unable to induce mucosal immunity or CTL activity *(9)*.

Recombinant Protein Vaccines

The preparation of this type of vaccine is limited to microbial proteins bearing protective epitopes. The generation of recombinant proteins is based on cloning a gene encoding a protein, which is then aligned with a promoter and inserted into a suitable plasmid replicon. The plasmid is used to transform bacteria such as *E. coli* or to stably transfect mammalian, insect or yeast cells. Recombinant proteins can also be obtained from genetically engineered viruses. In this case, the flanking region of the

Table 5
Idiotype Vaccines

Microbe	Antigen mimicked by internal image	Property of antibodies
E. coli	Capsular polysaccharide	Protective
Streptococcus pneumoniae	Phosphocholine	Protective
Streptococcus pyogenis	Group A carbohydrate	Protective
Pseudomonas aeruginosa	Capsular antigen	Protective
Corynebacterium diphtheriae	Toxin	Protective
Legionela pneumoniae	Cytolysin	Nonprotective
Reovirus type 3	Hemagglutinin	Neutralizing
Poliovirus type II	?	Neutralizing
Influenzavirus	Hemagglutinin	Neutralizing
Rabies virus	Glycoprotein	Neutralizing
SV40 virus	T-antigen	Suppressive
Coxsackievirus B4	Binding receptor	Nonneutralizing
Coronavirus	A59 epitope	?
Blue tongue virus	?	Neutralizing
Foot and mouth disease virus	Surface antigen	Nonneutralizing
Hepatitis B virus	S antigen	?
Schisostoma mansoni	Glycoprotein	Protective
Trypanosoma organisms	Variable antigen type (VAT)	Protective
Trichothecene	Mycotoxin T2	Protective

Adapted from ref. 9.

foreign gene permits homologous recombination between plasmid and the viral genome, and double reciprocal recombination results in transfer of plasmid DNA into the viral genome. Permissive cells infected with virus will drive the synthesis of recombinant protein. The production of recombinant protein in mammalian cells has a lower yield, but such proteins are correctly glycosylated. Whatever the system, the production of recombinant protein requires purification procedures from the culture medium.

Immunity

By virtue of their protein nature, recombinant proteins require a B-CD4 T-cell collaboration.

Advantages

Recombinant protein vaccines are safe and can induce a strong humoral response. They can be immunogenic in adults as well as in infants.

Disadvantages

The stability of recombinant protein is high but costly procedures are required to prevent alteration of proteins. They cannot induce mucosal immunity except when they are administered intranasally or orally. They are unable to stimulate CTL activity. There are only a few recombinant proteins licensed with proven efficacy: recombinant hepatitis B protein produced in yeast, Osp A protein produced in yeast (recently approved as vaccine to prevent Lyme disease), and a protein used as a vaccine against Japanese encephalitis virus.

Recombinant Vectors Vaccines

Recombinant viruses or bacteria may act as vectors of a foreign gene, bearing protective epitopes that would be transcribed, translated, and capable of inducing an immune response. The preparation of recombinant microbial vaccines is carried out in two steps: first, the selection or engineering of a live attenuated virus or bacterium and second, expression of foreign gene in the vector. It is possible to express several genes in a single vector and therefore to prepare polyvalent vaccines. In recent years, poxvirus, adenovirus, Bacille Calmette-Guérin (BCG), *Salmonella* and recently *B. anthracis* have been used as vectors in attempts to develop recombinant vectors.

Vaccinia vectors

Since vaccinia displays reactogenicity, sometimes causing postvaccinal encephalitis or even generalized and fatal infection in immunodeficient subjects, new poxviruses were developed. One new vector called NYVAC has 18 complete open reading frames (ORFs) deleted, including two genes contributing to the ability of virus to replicate in vitro in various cells. It can replicate in Vero cells only in the presence of wild-type virus *(10)*. The second is ALVAC, which is an avipox virus that can infect mammalian cells but does not replicate *(11)*.

There are two methods to insert the foreign DNA in poxviruses:

Homologous recombination. Recombinant vaccinia vectors are prepared by infection of permissive cells with vaccinia virus and transfection with a plasmid expressing an antigen gene. Since the rate of homologous recombination is high, about 0.1% of virions incorporate the foreign gene. The recombinants are easily selected by common techniques. The genes of more than 20 RNA and more than 10 DNA viruses, bacteria, or parasites have been expressed in vaccinia *(12)*.

Genetic engineering. A foreign gene can also be introduced into the vaccinia genome by cutting the DNA at a unique endonuclease site, after which the foreign gene can be ligated at compatible ends in vitro.

Recombinant Adenovirus Vector

Adenovirus vectors express antigen genes that are translated in replicas of native protein. The proteins do not exhibit posttranslational modifications and are capable of inducing neutralizing antibodies in both permissive and abortive animal models *(13)*. Several viral genes have been expressed in adenovirus vectors: hepatitis B, VSV, *env* and *gag* genes of HIV-1, HSV, CMV glycoprotein, rabies glycoprotein, F and HN of parainfluenza virus, and F and G of RSV viruses. The recombinant adenovirus vectors are able to elicit mucosal immunity.

Recombinant Salmonella *Vectors*

Attenuated *Salmonella* strains were obtained by deletion of genes encoding for virulence as toxins or invasin. The attenuated strains were then used to insert a foreign gene into a bacterial chromosome *(14)*. Since it was observed that synthesis of protein encoded by the foreign gene is low, an effort was made to increase the number of copies of foreign gene in the *Salmonella* genome. Several properties are required for an ideal *Salmonella* vector vaccine:

1. It should be complete avirulent and highly immunogenic.
2. It should be genotypically stable, with two or more deletions that do not revert and are not influenced by environmental factors. This is an important requirement since it was shown

that attenuated *Salmonella* organisms recovered from immunized animals lose the plasmid of avirulence or the foreign gene.

3. Finally, it should colonize to allow for a continuous synthesis of foreign protein.

Recombinant *Salmonella* vectors can be administered orally and therefore are able to induce mucosal immunity.

Recombinant BCG Vectors

Recombinant BCG vector vaccines were obtained by transfer of replicative or integrative plasmids by electroporation, gene replacement, plasmid conjugation, and phage lysogeny *(15)*. These vectors are able to induce a long-lasting humoral and cellular immunity conferred by the expression of foreign gene and by the nature of the BCG vector, respectively.

Bacillus anthracis (Stern strain)

B. anthracis (non-pathogenic Stern strain) was used to express foreign genes. This strain contains a pX01 gene coding for toxin but lacks pX02 plasmid coding for capsular polysaccharide, which is responsible for virulence. A vector expressing the *listeriolysine* gene was able to deliver Listeria protein to the cytoplasm and to induce a CTL response mediated by CD8 T-cells *(16)*.

DNA Vaccines

The utilization of DNA as a vaccine is based on the fact that the injection of a plasmid bearing a reporter gene leads to in vivo transfection of cells as well as to transcription of the foreign gene inserted into plasmid *(17)*. There has been an explosion of research in this area, leading to human trials of DNA vaccines.

DNA vaccines are constructed by insertion into plasmid of a foreign gene and a strong promoter, which ensures a high level of expression of the antigen gene, bearing protective epitopes. Recent studies have established the best conditions for constructing the plasmids used for vaccination. The spacing required between the regulatory and inserted genes, the stability of RNA transcripts, and the minimum number of copies required for a significant synthesis of foreign antigen able to induce immune responses have also been studied. Table 6 lists the systems in which DNA vaccination against viruses, bacteria, and parasites were assessed.

Immunity

The induction of a humoral immune response depends on the type of protein encoded by the foreign gene. Whereas a protein bearing epitopes recognized by a B-cell will induce the synthesis of antibodies, a protein expressing CD8 T-cell epitopes induces a CTL response.

In the case of the humoral immune response, the B-cells can recognize the conformational or linear epitopes on the surface of antigens secreted by transfected cells. In contrast, in the case of CD8 T-cells, the peptides required for activation of CTL precursors are generated via endogenous pathways, and the class I-peptide complexes translocated on the membrane are recognized by T-cells.

The CD4 T-cells are stimulated by in vivo transfected APCs, which synthesize the protein, process it, and present the peptides in association with class II molecules to CD4 T-cells. Recent reports showed that both macrophages *(18)* and dendritic cells *(19)* are transfected in vivo and are able to activate the CD4 T-cells

Table 6
DNA Vaccines Used in Experimental Models

Microbe	Virus
Negative-strand RNA viruses	Influenza
	Measles
	Newcastle
	Sendai
	Bovine respiratory syncytial
	Rabies
	Lymphocytic choriomeningitis
	Ebola
Positive, single-strand RNA viruses	Hepatitis C
	St. Louis encephalitis
	Tick-borne encephalitis
	Japanese encephalitis
	Russian-spring encephalitis
	Bovine viral diarrhea
	Infectious bronchitis
	Foot and mouth disease
Double-strand RNA	Rotavirus
Retroviruses	Human, simian, and feline immunodeficiency
	Human T-cell leukemia/lymphoma
	Cas murine leukemia
DNA viruses	Hepatitis B
	Bovine herpes
	Herpes simplex
	Cytomegalovirus
	Pseudorabies
	Papilloma

Advantages

Several advantages have made genetic immunization appealing for vaccination:

1. The DNA vaccine is very stable and easy to manufacture; it is easy to construct new plasmids in the case of vaccines against microbes exhibiting natural genetic variation.
2. Long-lasting persistence of plasmid and sustained synthesis of low doses of antigen preclude induction of high-dose tolerance and favor the generation of memory cells.
3. Lack of contaminant proteins in plasmid preparation prevents side effects such as allergic reactions.
4. DNA immunization does not require adjuvants since the plasmids rich in CpG motifs are endowed with intrinsic adjuvanticity.
5. It can induce humoral and cellular immune responses.
6. It can prime neonates, which may lead to development of vaccines for neonates or infants otherwise unresponsive to inactivated or live attenuated vaccines.

Disadvantages

Various studies have demonstrated the safety of DNA vaccines. However, DNA vaccination has two possible drawbacks: first, the induction of anti-DNA antibodies and second, the possibility of integration of plasmid into the host genome by non-homolo-

gous recombination. Such phenomena can lead to the occurrence of mutated structural genes, inhibition of expression of suppressor genes, or mutation of protooncogenes favoring the development of cancers.

Peptide-Based Vaccines

In contrast to B-cells able to recognize the epitopes on the surface of native antigen, T-cells recognize peptides derived from the processing of proteins in association with MHC molecules. Thus, the peptide-based vaccines can be efficient only against protein antigens and can be used only against infectious agents for which the cellular immunity is the major arm of the immune responses

The peptide-based vaccines can be divided into two categories: CD4 T-cell vaccines having potential usage against obligatory intracellular microbes (*Mycobacterium, Salmonella, Brucella, Francisella, Listeria, Rickettsia, Candida, Nocardia, Histoplasma, Leishmania, Babesia, Trypanosoma,* and *Schistosma* organisms) and CD8 vaccines against all viruses *(20).*

Synthetic peptides corresponding to epitopes recognized by CD4 or CD8 T-cells represent ideal safe vaccines. However, the peptides themselves cannot be used as efficient vaccines because of a short half-life and poor immunogenicity. Because of these drawbacks, several approaches have been taken to present the peptides loaded in liposomes and adjuvants or on platforms in which oligonucleotide sequences coding for peptides are inserted by genetic engineering.

Synthetic Peptide as a Vaccine

Because of drawbacks of induction of an immune response by peptides, several artificial systems have been used to increase immunogenicity such as immunization with liposomes containing peptides, synthetic lipopeptides, or coadministration with immunostimulating complex (ISCOM). Whereas injected peptides can bind directly to surface MHC molecules on the surface of APCs, the peptides delivered within liposomes or trapped in ISCOM are released subsequent to processing by APCs. Figure 3 illustrates the mechanisms of activation of CD8 T-cells by peptides.

Viruses Expressing Foreign Peptide Epitopes

DNA or RNA viruses expressing foreign peptides are constructed by genetic engineering. Briefly, a minigene encoding a given peptide is inserted in a viral gene by PCR mutagenesis. These viruses produce chimeric protein made up of viral protein expressing the foreign epitope. This chimeric protein elicits an immune response against viral protein as well as against foreign peptide.

This approach may contribute to the preparation of polyvaccines, an example being an influenza HK strain expressing a CD8 epitope on its nucleoprotein and a different CD8 epitope inserted in hemagglutinin. This virus was able to induce a strong CTL response against nucleoprotein peptides recognized in association with class I K^d and D^b molecules *(21).* Similarly, a chimeric Sindbis virus expressing a minigene encoding two distance epitopes was able to prime CD8 cytotoxic T-cells *(22).*

The advantage of these vaccines lie in their ability to induce immune responses not only against proteins of host virus but also against foreign peptides. These vaccines induce immune responses subsequent to penetration and eventual replication of virus in APCs, followed by processing of protein in endogenous pathways and presentation

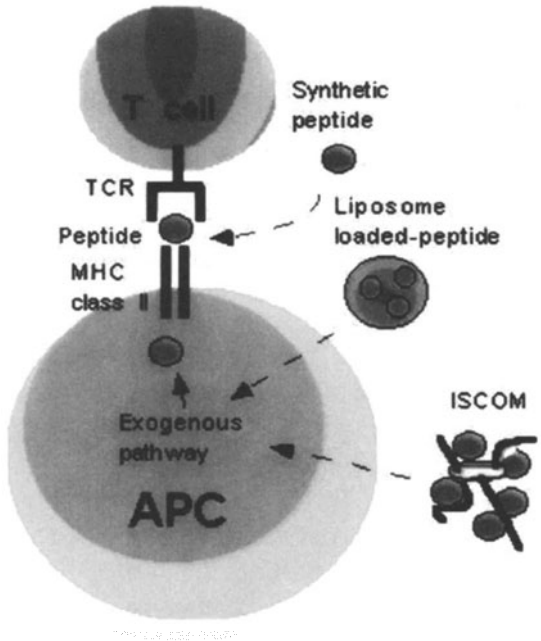

Fig. 3. Mechanisms of activation of CD4 T-cells by peptides. Soluble peptides can bind directly to MHC molecules by displaying the endogenous peptides. Once the complex is formed, it can activate the T-cells. The peptides trapped in liposomes or adjuvants are internalized and released in endosomes. APC, antigen-presenting cell; ISCOM, immunostimulating complex; TCR, T-cell receptor.

of peptide in association with class I and eventually class II molecules. Figure 4 illustrates the mechanism of activation of T-cells by chimeric viruses expressing foreign epitopes.

The disadvantages of this approach consist in the induction responses against viral proteins devoid of protective epitopes as well as fast clearing owing to the presence of antiviral antibodies, which precludes efficient boosting.

Delivery of T-Cell Peptides by Recombinant Proteins

Molecular engineering methods allowed for the in-frame insertion of oligonucleotides encoding a given peptide within coding regions of genes coding for otherwise unrelated proteins. The translation of this chimeric gene led to synthesis of a chimeric protein expressing the epitopes recognized by T-cells. In constructing such molecules several factors should be taken into consideration:

1. The insertion of foreign peptide should not alter the correct folding of carrier molecule nor preclude its secretion.
2. The carrier molecule should have permissive sites where the peptide is inserted.
3. The flanking sequences of carrier molecules at the site of insertion should be accessible to processing by APC proteolytic enzymes.

Various T-cell epitopes were expressed in bacterial organelles or in secreted proteins *(23,24).*

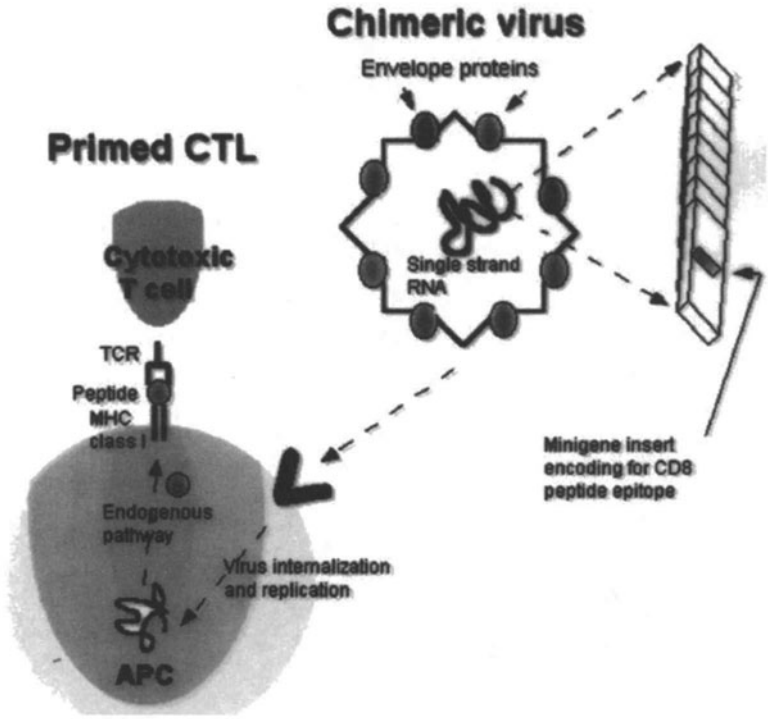

Fig. 4. Mechanism of activation of CD8 T-cells by chimeric viruses expressing T-cell epitopes. APC, antigen-presenting cell; CTL, cytotoxic T-lymphocyte; TCR, T-cell receptor.

The immune response elicited by recombinant proteins follows the uptake by APCs and their processing in the endosomal compartment. Figure 5 illustrates the mechanisms of induction of immune response by recombinant protein expressing T-cell epitopes, which are contained in various bacterial organelles.

Although the recombinant molecules are safe, they can induce strong responses against multiple antigenic determinants of carrier, and therefore the protective response might be diluted.

Receptor-Mediated Delivery of Peptides

The principle of this procedure is to artificially conjugate a peptide to a ligand interacting with a receptor or to a molecule expressed on the surface of APCs. Among the receptors able to internalize the conjugates are transferrin, ferritin, and α_2-macroglobulin receptors

The internalization of peptides can be achieved by conjugation of peptides with antibodies specific for a molecule expressed on APCs such as class I, class II, or Ig *(20).*

The T-cells are activated subsequent to internalization of conjugates and their processing within APCs (Fig. 6). Until now this approach has had only academic interest because it is difficult to optimize coupling conditions as well as to preclude the formation of aggregates.

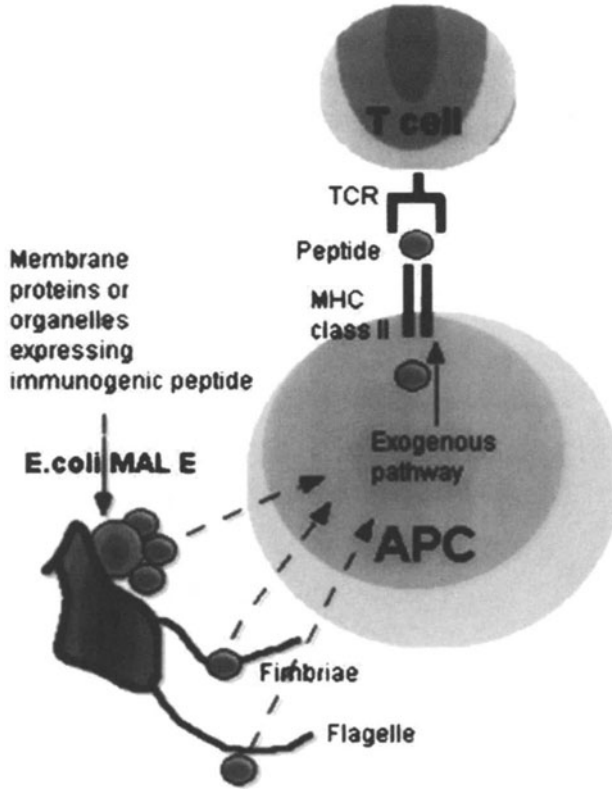

Fig. 5. Mechanism of generation of peptides by proteins expressing foreign epitopes. Chimeric viruses are internalized within the cell subsequent to binding to cellular receptors. Subsequent to replication, viral proteins are produced and processed in endogenous pathways leading to the release of foreign peptide from the viral protein in which it was inserted. APC, antigen-presenting cell; TCR, T-cell receptor.

Delivery of Peptides by Self Molecules

Self protein molecules are an ideal tool to deliver peptides since they are safe and do not elicit immune responses against carrier protein. Three major approaches have been undertaken to construct such molecules:

1. Genetically engineered replacement of a segment of the V_H gene (i.e., *CDR3*) with an oligonucleotide encoding a peptide recognized by T-cells. The resulting "antigenized" immunoglobulin molecules are taken up by APCs, which process chimeric Ig molecules and generate the peptide.
2. The peptide is attached to the sugar moiety of the Ig molecule by enzymatic engineering. This type of molecules can activate T-cells without the need for antigen processing since the molecule, by its Fc fragment, binds to the Fc receptor of APCs as well as to class II via the peptide attached to the sugar moiety *(25)*.
3. Generation by genetic engineering of soluble class I or class II molecules in which the peptide is covalently linked to the heavy chain of class I or to the β chain of class II molecules respectively *(26)*.

Depending on the dose used, these molecules can stimulate or anergize the T-cells. Figure 7 illustrates the structure of such molecules. In the future new approaches will develop toward safe and efficient delivery of peptides using various self molecules.

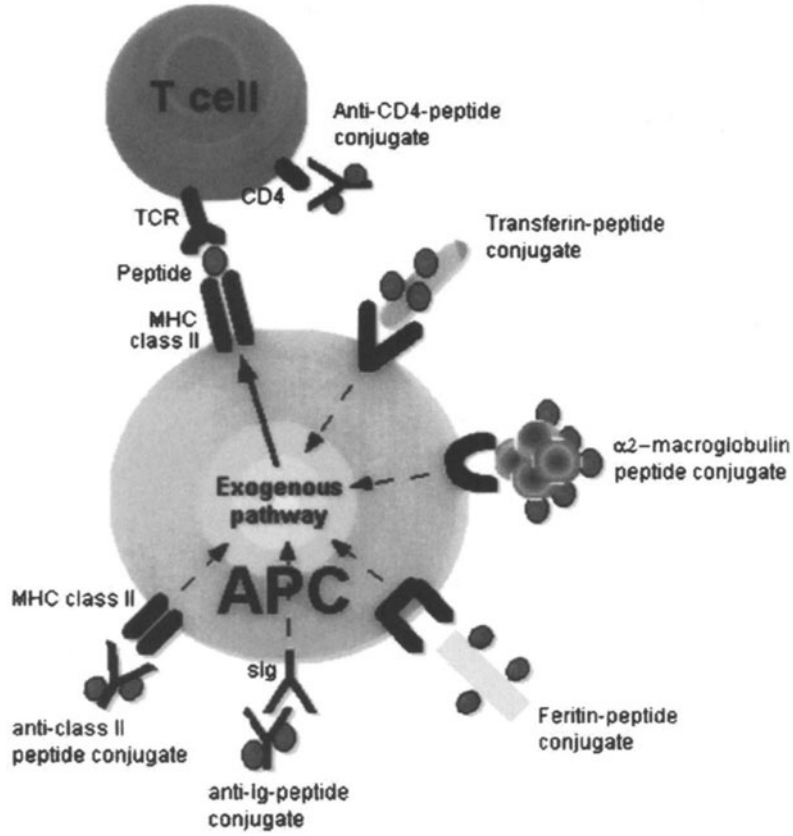

Fig. 6. Receptor delivery systems of peptides. Peptides chemically conjugated to ligands of cellular receptor or antibodies specific for membrane antigens are internalized by antigen-presenting cells (APC). After processing within the endosomal compartment, the peptides are released and then bind to MHC molecules. TCR, T-cell receptor.

IMMUNOTHERAPEUTIC VACCINES

Vaccines were initially conceived to prevent the infectious diseases associated with morbidity and mortality. The vaccine concept was extended to therapeutic reagents to cure chronic infection caused by persistent viruses or bacteria, autoimmune diseases, or cancers. The concept of therapeutic vaccines derives from the understanding of T-cell biology and pathophysiology: T-cells are not simply good soldiers fighting microbes or tumor cells but also vicious mercenaries contributing to the destruction of tissues that leads to autoimmune diseases.

Therapeutic vaccines against chronic infectious diseases (*Mycobacterium leprae,* HSV virus hepatitis B virus) are aimed at harnessing the immune response in carriers or cancer patients who are otherwise tolerant or unresponsive to microbial or tumor-associated antigens. In the case of autoimmune disease, therapeutic vaccines are used to eliminate autoreactive lymphocytes.

Most of the approaches used to develop the vaccines discussed in this chapter have been undertaken to prepare therapeutic vaccines.

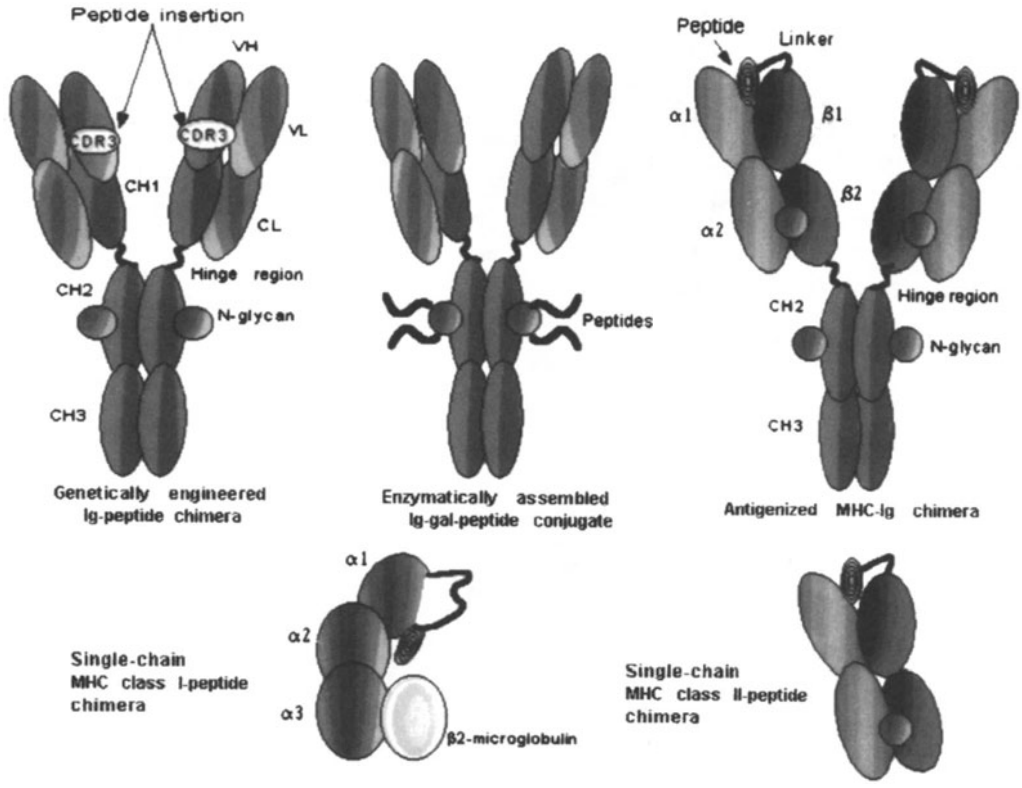

Fig. 7. Structure of self molecules expressing peptides recognized by CD4 or CD8 T-cells.

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