

C. Antigen-based vaccines

- In antigen-based vaccines, “**target**” antigens of a microorganism are produced and administered to the host. In this formulation, will generally induce a *humoral immune response*.
- There are three types of antigen based vaccines: **purified, recombinant, and synthetic**.

C.1 Purified antigen vaccines

- Vaccines composed of molecules purified directly from the pathogen. Sometimes called “**subunit vaccines**”.
- Identify molecules that generate a “**protective**” immune response: proteins, polysaccharides or **EXOTOXINS**. Exotoxins are bacterial proteins either chemically inactivated or attenuated (derived from mutated organisms) to prevent toxicity in the host.

Advantages of purified antigen vaccines

- These vaccines are composed of a limited number of molecules & are fairly simple to characterize.
- No danger of replication of the organism.

Disadvantages of purified antigen vaccines

- Purification procedures can be very expensive...
- Source organism may be either difficult to cultivate or limited in supply.
- Most purified antigen vaccines require the use of **adjuvants** to elicit a strong immune response and multiple immunizations must be given.
- For exotoxins, the conditions for inactivation must be carefully planned to avoid excessive modification of the epitope structure.

Examples of purified antigen vaccines

- Vaccines against causative agents of meningitis in children: Streptococcal pneumoniae consists of surface polysaccharides from 23 different serotypes; Neisseria meningitidis polysaccharide vaccine contains two serotypes; Hib polysaccharide vaccine is conjugated to protein carrier (T or D). S. pneumoniae & N. meningitidis vaccines used during local outbreaks of meningitis.
- Whooping cough is caused by Bordatella pertussis, and the vaccine consists of formalin-inactivated pertussis toxins, after removal of cells from culture (acellular pertussis, aP). Two other formalin-inactivated toxins are for tetanus (Clostridium tetani) and diphtheria (Corynebacterium diphtheriae). Together comprise DTaP vaccine. O

C.2 Recombinant Antigen Vaccines

- Production of immunogenic proteins by genetic engineering. DNA encoding for an immunogenic protein of a pathogen can be inserted into either bacteria, yeast, viruses which infect mammalian cells, or by transfection of mammalian cells. The cells will then produce the protein endogenously and the protein can be harvested. (also source of “naked DNA” vaccines, see part D)

Advantages of recombinant antigen vaccines

- Large amounts of antigen can be produced inexpensively.
- Once gene sequence of antigen is available, genetic manipulation of the antigen itself is possible. Exotoxins can now be genetically inactivated (e.g., tetanus, diphtheria) or antigens can be made to be more immunogenic.

Disadvantages of recombinant antigen vaccines

- Immune response generated by recombinant antigen vaccines is primarily humoral. The antigens are processed via the MHC Class II pathway, and therefore do not induce a CTL response. Naked DNA vaccines may solve.

Example of recombinant antigen vaccine

- The first recombinant vaccine produced consists of surface protein of Hepatitis B virus (HBsAg). The gene encoding this antigen is overexpressed in yeast.
- Antigen self-assembles, forming aggregates resembling viral particles which are then secreted. Antigen purified by conventional biochemical techniques.

C.3 Synthetic Antigen Vaccines

- Peptide antigens are synthesized by automated machines. Synthetic polynucleotide technology exists. Synthetic polysaccharide technology under development.
- Which sequences to choose requires knowledge of the conformational structures for B cell epitopes (sequential v. assembled) and of the anchor residues of MHC for T cell epitopes. Computer algorithms are available to assist in selection, but trial-and-error approach still required. Other aids include generation of "protective" monoclonal Abs (B cell epitopes & phage-display libraries) and peptide-dependent restimulation of T cells from convalescent subjects (T cell epitopes).

Advantages/Disadvantages of Synthetic Antigen Vaccines

- (A) Manufacture is automated, yielding chemically definable product, usually easy to sterilize, store (freeze-dried), and ship. Like recombinant antigens, contamination with infectious agents not an issue.
- (D) Synthetic peptides are poorly immunogenic and require the use of adjuvants, although use of MAPs (multiple antigenic peptides) increases efficacy but also complicates manufacturing. Issues of conformation (B cell epitopes) & proper processing (T cell epitopes)..
- Currently experimental trials in schistosomiasis, malaria, and HIV show promise. ○

D. Recombinant vector and DNA vaccines

- This class of vaccines utilizes attenuated versions of certain microbes as recombinant vectors to express target antigens from other pathogens, minus disease-causing genes of the latter. Because there is an infection (mild) by the vector microbe, a more complete immune response (cellular + humoral, to "native" antigens) can be generated against the inserted antigens.
- In place of a vector organism, it is possible to vaccinate with "naked" recombinant DNA molecules and achieve a similar response to the gene products without infection, but by transfection of host cells instead.

D.1 Viral vector vaccines

- With recombinant viral vector vaccines, genes encoding antigens of pathogenic organisms are inserted into an attenuated live virus. The recombinant virus is then able to replicate in and display the inserted proteins to the host.
- Since the genes are encoded in a virus, the antigens can be processed via both the class I MHC pathway [in virus-infected APC, or APC which phagocytose virus-infected cells] and by class II MHC [in APC which phagocytose free virus particles].

Advantages of viral vector vaccines

- Elicit strong humoral and cell-mediated immune responses, resulting in immunological memory....
- Can be targeted by viral tropisms for particular cells, e.g. intestine, brain, etc., inducing desired immunity.
- Can also encode for several antigens from different pathogens, introducing the possibility of a single vaccine for several diseases.
- Viral vectors have been found not to interfere with the protection produced by other types of vaccines..
- Vaccines are relatively inexpensive and, for some, easily transportable.

Disadvantages of viral vector vaccines

- Since the live virus being used is an attenuated form of a human pathogen, there is always a risk of reversion to virulence..
- Some of the vectors under consideration, such as adenovirus, have the capability of transforming cells to a cancerous phenotype. While these oncogenes are removed, vector virus could recombine with naturally occurring, pathogenic strains in the environment and form a new hybrid virus with transforming properties.
- Immune response to virus-infected cells may cause pathology.

Examples of viral vector vaccines

- Much research has been done with attenuated vaccinia virus as a vector, which is the virus that immunizes against smallpox (now eradicated). Genes encoding for hepatitis B, herpes simplex, and influenza antigens were inserted into vaccinia vector. Exposed mice were protected against challenge from all three pathogens.
- Other vectors under consideration are poliovirus, herpes virus and adenovirus, as well as avipox vectors. Currently, a canarypox virus vector encoding surface antigens for HIV is being used in clinical trials. Canarypox is related to vaccinia but does not cause disease in humans. These are the first clinical trials to be conducted using a viral vector vaccine, so the results should prove very informative.

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D.2 Bacterial Vector Vaccines

- Bacteria infect humans in many different ways which can be exploited for vaccine development, e.g. to target the vaccine to certain host cells and to stimulate specific features of the immune response. Like viral vectors, DNA encoding for the antigenic determinants is inserted into the attenuated bacterial genome. The bacterial then expressed the antigen along with its own proteins. Another method is to create fusion proteins of endogenous bacterial proteins and of the antigens in question.
- Attenuated Salmonella typhi (Ty21a), the causative agent of food poisoning, is being used as a vector and is currently in human trials. Induces mucosal immunity.

Advantages of bacterial vector vaccines

- The safety of existing bacterial vaccines such as V. cholera, S. typhi, and BCG is already documented. Use of these vectors should generate immune responses against the vector as well as inserted gene products..
- Depending on the bacteria used, MHC class I and/or II antigen processing pathway can be targeted. Some bacteria induce both humoral and cell-mediated immunity, including mucosal immunity since some bacteria can survive in GI tract, making this technology attractive for oral immunizations.
- If there are reversions of attenuated bacterial vector, such mutations can be controlled by using antibiotic-sensitive strains.

Disadvantages of bacterial vector vaccines

- Much of the population has already been exposed to and/or vaccinated against the bacteria being used as vector. Possibly these individuals will clear the vaccine before it taking effect...
- Danger of reversion and the emergence of a pathogenic form.
- Since foreign DNA is being inserted, this part of the genome tends to be unstable and may not be expressed at high levels.
- The foreign antigen also may be subject to processing and proteolysis by endogenous bacterial enzymes.

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D.3 DNA vaccines

- Genes encoding antigens of an infectious organism are expressed by the host's own cells.

Genes are inserted into a bacterial plasmid under the control of a mammalian promoter. The chimeric plasmid is either directly injected into muscle or the DNA is conjugated to a solid matrix such as gold particles. The plasmid-gold particles can be injected intracutaneously via a "gene gun." These particles are taken up by skin cells and the genes are expressed, as in a viral vector.

Advantages/Disadvantages of DNA vaccines

- (A) DNA vaccines stimulate both humoral and cell mediated responses. Dendritic cells may play a role. In animal studies, the vaccination produced long-term protection. Such vaccines are heat stable and nonvirulent. DNA vaccines are easily constructed with available tools of molecular biology.
- (D) Concerns over foreign DNA integrating into the cell's DNA. Such integration could cause the cell to transform and become cancerous. Also thought that DNA vaccines might produce anti-DNA Abs, causing auto-immunity in host, but naked DNA is not very immunogenic in animal studies. It is not know exactly how long the DNA persists in the host cells.

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Other approaches follow

E. Other vaccine delivery systems

- Besides viral and bacterial systems, or naked DNA, other types of antigen delivery systems are being developed for vaccines. Since there is a diversity of systems being researched, the different types will be grouped into two categories and examples given of each class.
- E.1 Encapsulation Systems
- E.2 Antigen-Display Delivery Systems

E.1 Encapsulation Systems

- In encapsulation systems, the antigens are placed inside a matrix, which releases antigen inside the body. Several antigens as well as adjuvants can be encapsulated..
- **E.1a - Polymer capsules** of lactic & glycolic acid (polyLG) are biodegradable and used many years for sutures & drug-release vehicles, so their safety is documented. Since they remain intact in the GI tract, poly LG capsules are an attractive vehicle for oral and mucosal vaccines. Capsules can be designed to release antigens in controlled phases, which could eliminate the need for booster immunizations. The capsules can be phagocytosed by APC and thus target antigens to the lymphoid system. The process of polymerization requires denaturing organic solvents that may change the alter the conformation of the antigen. Also, since the interior of the capsule is water-based, the antigens must be stable in a soluble environment.

Liposomes & Micelles

- **E.1b - Liposome encapsulation:** antigen is captured in tiny spheres of naturally occurring lipids which mimic the body's own lipid bilayer membrane. The liposome interior is hydrophilic and soluble antigens can be enclosed, while insoluble antigens can be inserted into the membrane. Liposomes fuse with plasma membranes of cells. Internalized antigens are processed via both MHC pathways, generating humoral and cell-mediated responses. Liposomes containing hepatitis A antigens were found to elicit high titers of antibody with no side effects..
- **E.1c - Micelles** (also called proteosomes and virosomes): protein antigens are mixed with detergent and the detergent slowly removed by dialysis. The antigenic proteins orient with the hydrophilic residues facing outside and the hydrophobic residues inward.

ISCOMS

- **E.1d - Immune stimulating complexes (ISCOMS) :** cage-like particles consisting of a combination of naturally occurring lipids mixed with a detergent and a glycoside such as Quil A. These substances form a lipid membrane, which encases the antigens inside. ISCOMS are similar to liposomes in immuno-stimulatory properties. Long-lasting protection is generated, lasting up to several years and ISCOMS are able to survive the degrading environment of the GI tract, making it suitable for oral and mucosal vaccine.

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E.2 Antigen-display delivery systems

- **E.2a - Solid matrix-antibody-antigen complexes (SMAA):** monoclonal Abs to infectious organism are complexed to a solid particulate matrix. This structure is mixed with antigens, which complex with the Abs. A variety of antigens can be presented by using a mixture of monoclonal Abs. Complexes are large, easily phagocytosed, and stimulate humoral and cell-mediated immunity..
- **E.2b - Antigen co-chleates:** large continuous solid sheets consisting of a phospholipid-calcium mixture. Antigen dispersed throughout the bilayer, and entire sheet folds into a spiral, similar to a jellyroll. Not all antigen is displayed externally, some hidden inside, protecting it from degradation. When co-chleate enters body, matrix slowly breaks down, exposing hidden antigen. No toxicity in humans. Generates both humoral and cell-mediated immunity. Can encapsulate proteins, DNA. Oral delivery possible, since they are not easily broken down. o

F. Adjuvants

- An adjuvant is any substance that can accentuate, enhance, or prolong the immune response generated against an antigen. It helps the antigen to be more immunogenic, in the following 5 ways (for different adjuvants):
- (1) prolonging presentation of the antigen by forming a protective biodegradable matrix and acting like an antigen depot.
- (2) delivering antigens directly to immune cells for processing, such as M cells for mucosal immunity or professional APCs.
- (3) inducing complement, enhancing antigen display to B cells.
- (4) amplifying nonspecific cellular responses to the antigen by generating danger signals to recruit APCs.
- (5) aiding lymphocyte proliferation by inducing secretion of certain cytokines.

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G. Antibody technology and related topics

- Antibodies (Abs) raised against pathogenic epitopes are useful in passive immunization. Properties of Abs in combination with genetic engineering are being exploited to create new immunotherapies..
- G.1 - Human monoclonal antibodies grafted with mouse antigen-specific variable regions (see Kuby p. 136) [Anti-isotypic humoral responses against nonhuman species leads to a condition known as serum sickness.]

G.2 Targeted toxin delivery

- **G.2a - Immunotoxins:** conjugates composed of target-antigen specific monoclonal Abs coupled to toxins. Abs selectively bind target cells. Toxins are activated and cause cell death. This is a very important part of the immunotherapy available to leukemia and lymphoma patients today and several clinical trials have shown significant responses in most treated individuals. The disadvantage to this approach is that it is not easy to identify tumor-specific antigens..
- **G.2b - Bacterial toxin "vaccines":** manipulation and exploitation of particular properties of bacterial toxin proteins (specific target cell binding, internalization) to produce an attenuated form (replace toxic domain with another function) that can be used as a vaccine vector. Related to use of monoclonal Abs to direct an activity to target cells.

G.3 Ig gene libraries

- **G.3 - Immunoglobulin gene libraries:** A different method of generating monoclonal Abs involves the use of polymerase chain reaction (PCR) to amplify DNA encoding human antibody heavy and light chain Fab fragments from hybridoma cells or plasma cells. The PCR products are then cloned into a bacteriophage (see part C.3) to yield separate heavy and light chain libraries. Random joining of these two libraries yields numerous novel Ab constructs. Such libraries can then be screened for reactivity against pathogenic antigens.

G.4 Abzymes

- **G.4 - Abzymes:** Abs with the ability to catalyze the chemical modification of an antigen upon specific binding can be generated. Mimic enzymes, hence are called abzymes. Such a property could be exploited for cleaving specific viral antigens and thus blocking viral infectivity. This novel use of antibody technology in a vaccine preparation has not yet been evaluated in humans but has shows great promise in animal models. It has been proposed that such Abs could be selected by screening the above mentioned immunoglobulin gene libraries with the appropriate antigens.

Identification of peptides using “phage display” library

- Through genetic manipulation, filamentous bacteriophage (viruses that infect bacteria) are engineered to express random peptide sequences in its "tail" (attachment structure). A library of peptide sequences can be expressed, one peptide sequence/bacteriophage, within the tail gene..
- Neutralizing Abs isolated from an immune donor are immobilized. Affinity selection methods are used to screen these libraries for phage that bind specifically to these antibodies. The different phage are each purified. Once isolated, the peptide sequences of the epitopes can be readily determined and used in a synthetic or recombinant antigen approach for production of vaccine antigens against the disease of interest.
