Vaccines

Traditional vs. rDNA vaccines

Subunit vaccines

Peptide vaccines

Genetic immunization: DNA vaccines

Attenuated vaccines

Vector vaccines

Traditional vaccines and their drawbacks

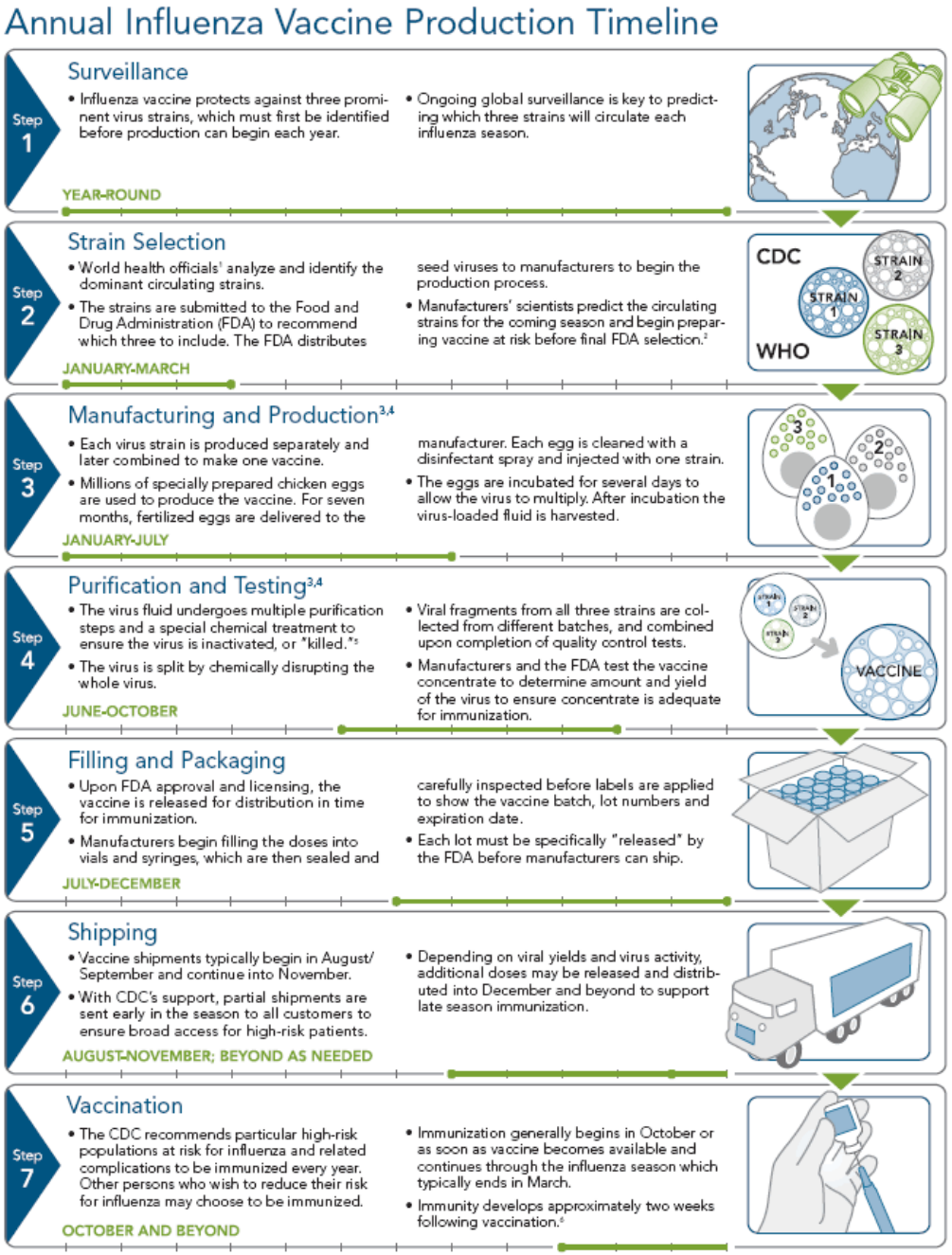
- **Traditional vaccines are either inactivated or attenuated infectious agents (bacteria or viruses) injected into an antibody-producing organism to produce immunity**

- **Drawbacks include: inability to grow enough agent, safety concerns, reversion of attenuated strains, incomplete inactivation, shelf life may require refrigeration**

How do you make a traditional vaccine?

See:http://www.influenza.com/Index.cfm?FA=Science_History_6

For information about H1N1 Flu (Swine Flu), see: http://www.cdc.gov/H1N1FLU/



Do vaccines need to contain the whole organism, OR specific portions of pathogenic organism are enough?

- **For disease causing viruses, it has been shown that purified outer surface viral proteins, either capsid or envelope proteins are sufficient for eliciting neutralizing antibodies in the host organism rather than the whole organism**
- **Therefore, vaccines that use components of pathogenic organism rather than whole organism are called "Subunit" vaccines**
- **Recombinant DNA technology is very well suited for developing new subunit vaccines**

Recombinant DNA technology can create better, safer, reliable vaccines

- **Immunologically active, non-infectious agents can be produced by deleting virulence genes**
- **A gene(s) encoding a major antigenic determinant(s) can be cloned into a benign carrier organisms (virus or bacteria)**
- **Genes or portions of genes encoding major antigenic determinants can be cloned in expression vectors and large amounts of the product purified and used as a subunit or peptide vaccine, respectively**

Advantages and Disadvantages

Advantages:

Using purified protein(s) as an immunogen ensures that the preparation is -

- **stable and safe**
- **precisely defined chemically and**
- **free of extraneous proteins and nucleic acids that can initiate undesirable side effects in the host organism**

Disadvantages:

- **Purifications of specific proteins costly, and in some cases**
- **an isolated protein molecule may have not the same conformation as it does in situ (within the viral capsid or envelope), with the result that its Antigenicity is altered**
- **Obviously, the decision to produce a subunit depends on an assessment of several biological and economic factors**

Table 12.2 Some human disease agents for which rDNA vaccines are being developed

Pathogenic agent	Disease
Varicella-zoster virus	Chicken pox
Hepatitis A and B viruses	High fever, liver damage
Herpes simplex virus type 2	Genital ulcers
Influenza A and B viruses	Acute respiratory disease
Rabies virus	Encephalitis
Human immunodeficiency virus	AIDS
Vibrio cholerae	Cholera
Neisseria gonorrhoeae	Gonorrhea
Mycobacterium tuberculosis	Tuberculosis
Plasmodium spp.	Malaria
Trypanosoma spp.	Sleeping sickness

TABLE 12.2 Human disease agents for which recombinant vaccines are currently being developed

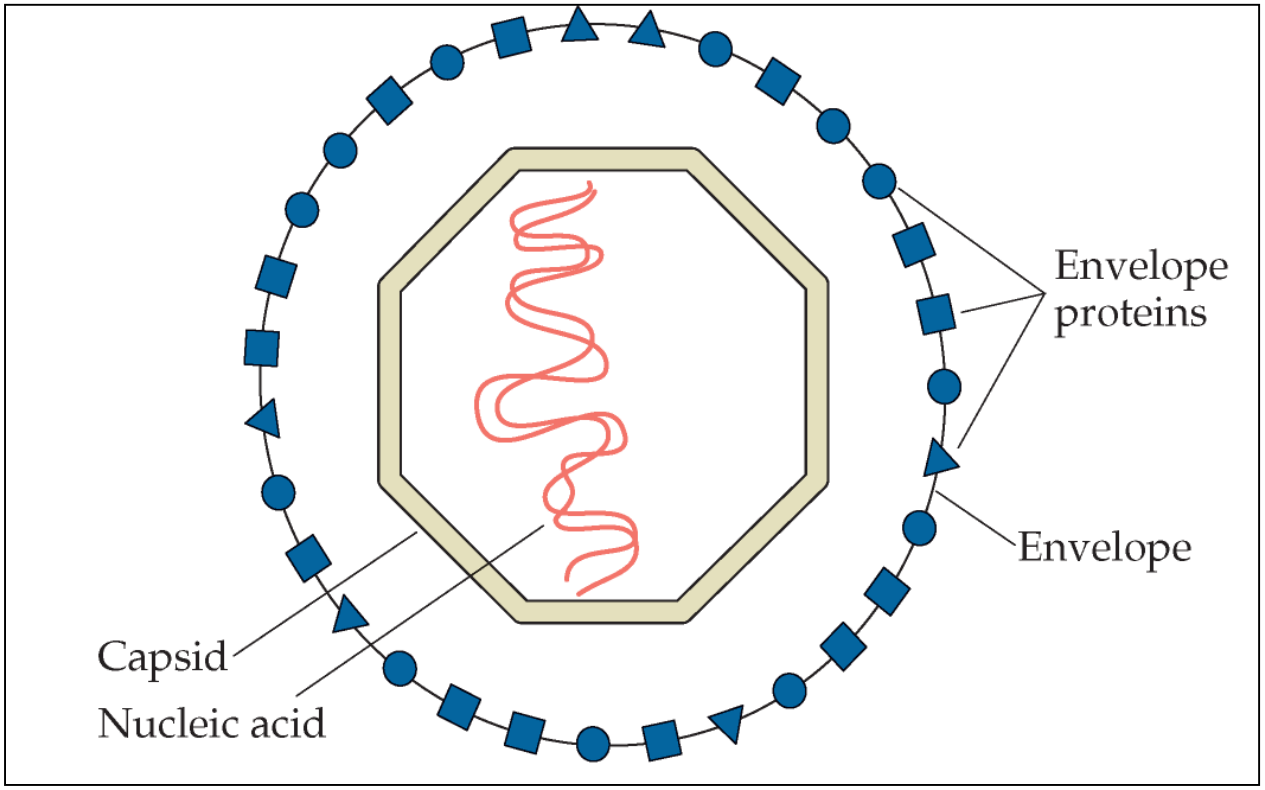
Pathogenic agent	Disease
Viruses	
Varicella-zoster viruses	Chicken pox
Cytomegalovirus	Infection in infants and immuno-compromised patients
Dengue virus	Hemorrhagic fever
Hepatitis A virus	High fever, liver damage
Hepatitis B virus	Long-term liver damage
Herpes simplex virus type 2	Genital ulcers
Influenza A and B viruses	Acute respiratory disease
Japanese encephalitis	Encephalitis
Parainfluenza virus	Inflammation of the upper respiratory tract
Rabies virus	Encephalitis
Respiratory syncytial virus	Upper and lower respiratory tract lesions
Rotavirus	Acute infantile gastroenteritis
Yellow fever virus	Lesions of heart, kidney, and liver
Human immunodeficiency virus	AIDS

Pathogenic agent	Disease
Bacteria	
Vibrio cholerae	Cholera
E. coli enterotoxin strains	Diarrheal disease
Neisseria gonorrhoeae	Gonorrhea
Haemophilus influenzae	Meningitis, septicemic conditions
Mycobacterium leprae	Leprosy
Neisseria meningitidis	Meningitis
Bordetella pertussis	Whooping cough
Shigella strains	Dysentery
Streptococcus group A	Scarlet fever, rheumatic fever, throat infection
Streptococcus group B	Sepsis, urogenital tract infection
Streptococcus pneumoniae	Pneumonia, meningitis
Clostridium tetani	Tetanus
Mycobacterium tuberculosis	Tuberculosis
Salmonella enterica serovar Typhi	Typhoid fever

Pathogenic agent	Disease
Parasites	
Onchocerca volvulus	River blindness
Leishmania spp.	Internal and external lesions
Plasmodium spp.	Malaria
Schistosoma mansoni	Schistosomiasis
Trypanosoma spp.	Sleeping sickness
Wuchereria bancrofti	Filariasis

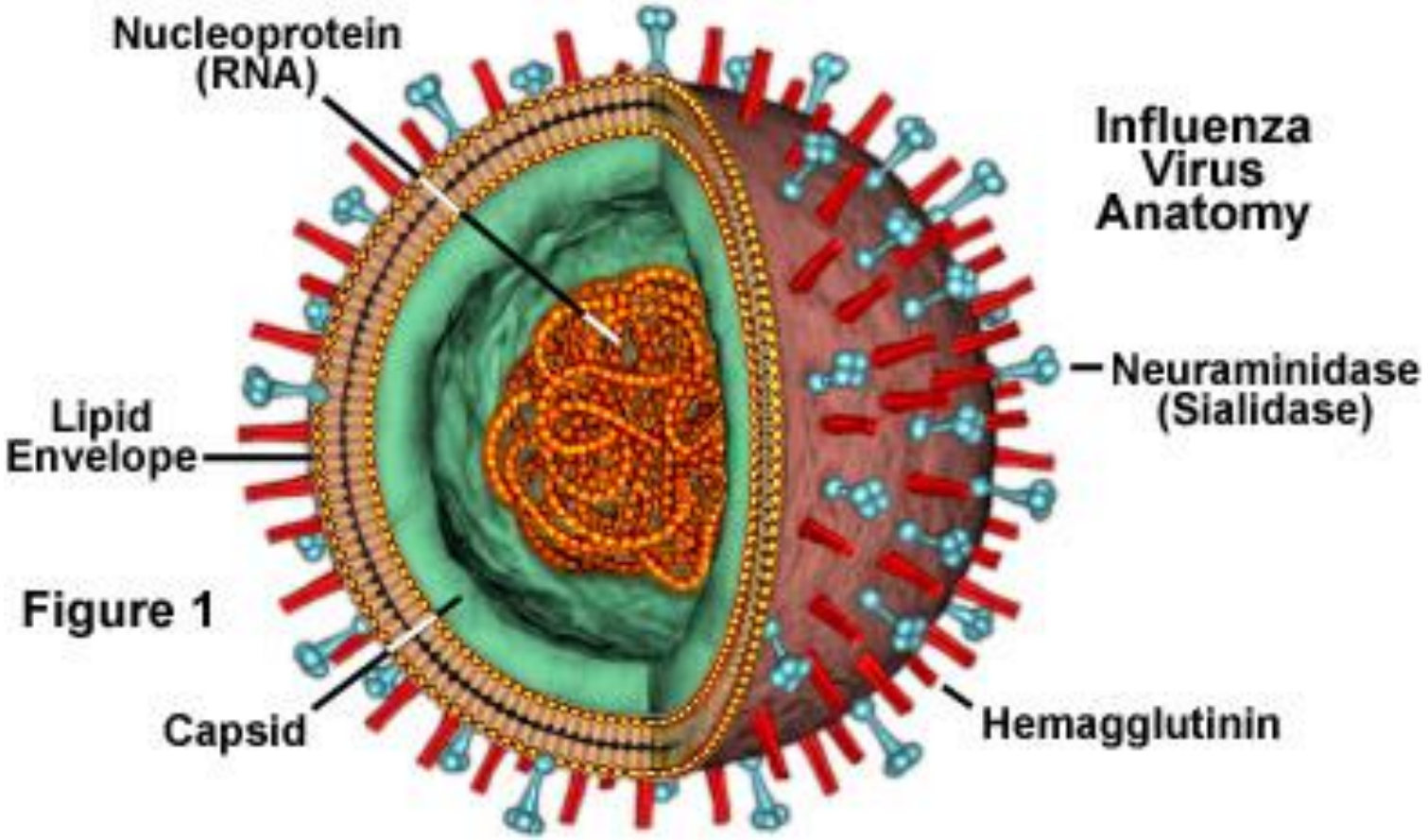
Typical animal virus structure

Figure 11.1 Schematic representation of an animal virus. Viruses generally consist of a relatively small nucleic acid genome (3 to 200 kb of either double- or single-stranded DNA or RNA) within a viral protein capsid that is sometimes, depending on the virus, surrounded by a protein-containing viral envelope (membrane).



Note: capsid and envelope proteins can elicit neutralizing antibodies

Influenza (Flu) virus structure



See: http://micro.magnet.fsu.edu/cells/viruses/influenzavirus.html

Herpes Simplex Virus (HSV)

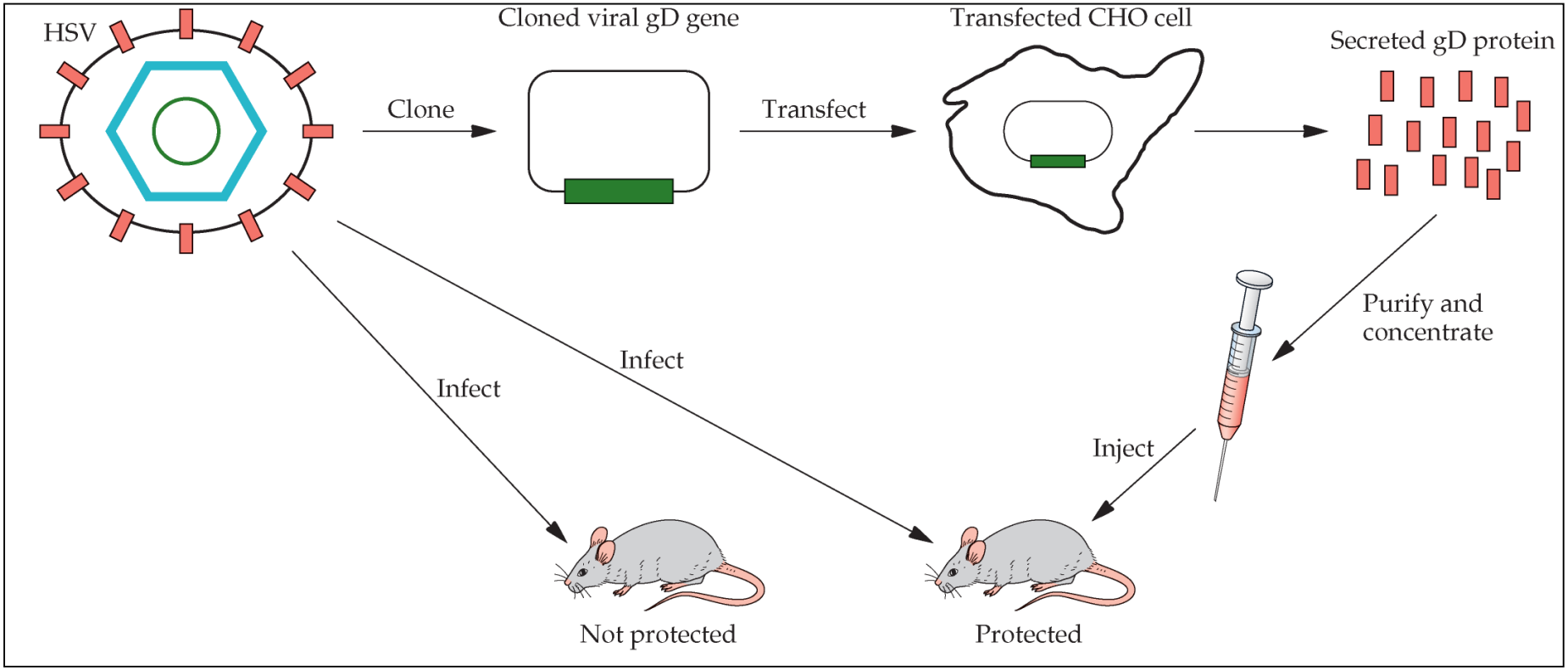
- HSV has been implicated as a cancer causing (oncogenic) agent
- In addition to its more common roles in causing sexually transmitted disease, severe eye infections and encephalitis (inflammation of brain)
- So that the prevention of HSV infection by vaccination either killed or attenuated virus may put the recipient at the risk of cancer
- Thus, protection against HSV would be best achieved by a subunit vaccines-which would not be oncogenic
- Primary requirements for creating any subunit vaccine is identification of the component(s) of the infectious agent that elicit antibodies which react against the intact form of the infectious agent

Herpes Simplex Virus (HSV)

- **HSV type 1 envelope glycoprotein D (gD) is such a component because after injection into mice, it elicits antibodies that neutralize intact HSV**
- **The HSV-1 gD gene was isolated and cloned into mammalian expression vector and expressed in Chinese hamster ovary cells (CHO)**
  {Why not in *E. coli* but in *CHO cells?*}
- **However, a membrane-bound protein is much more difficult to purify than a soluble one, therefore, modification was done in order to make it soluble**
- **In laboratory trials, the modified form of gD was effective against both HSV-1 and HSV-2**

A subunit vaccine against Herpes Simplex Virus (HSV)

Figure 11.2 Schematic representation of the development of a subunit vaccine against HSV. The isolated HSV gD protein gene is used to transfect CHO cells. Then the transfected cells are grown in culture and produce gD protein. Mice inoculated with the purified gD protein are protected against infection by HSV.



CHO cell = Chinese Hamster Ovary cell; gD = glycoprotein D

A subunit vaccine against Herpes Simplex Virus (HSV)

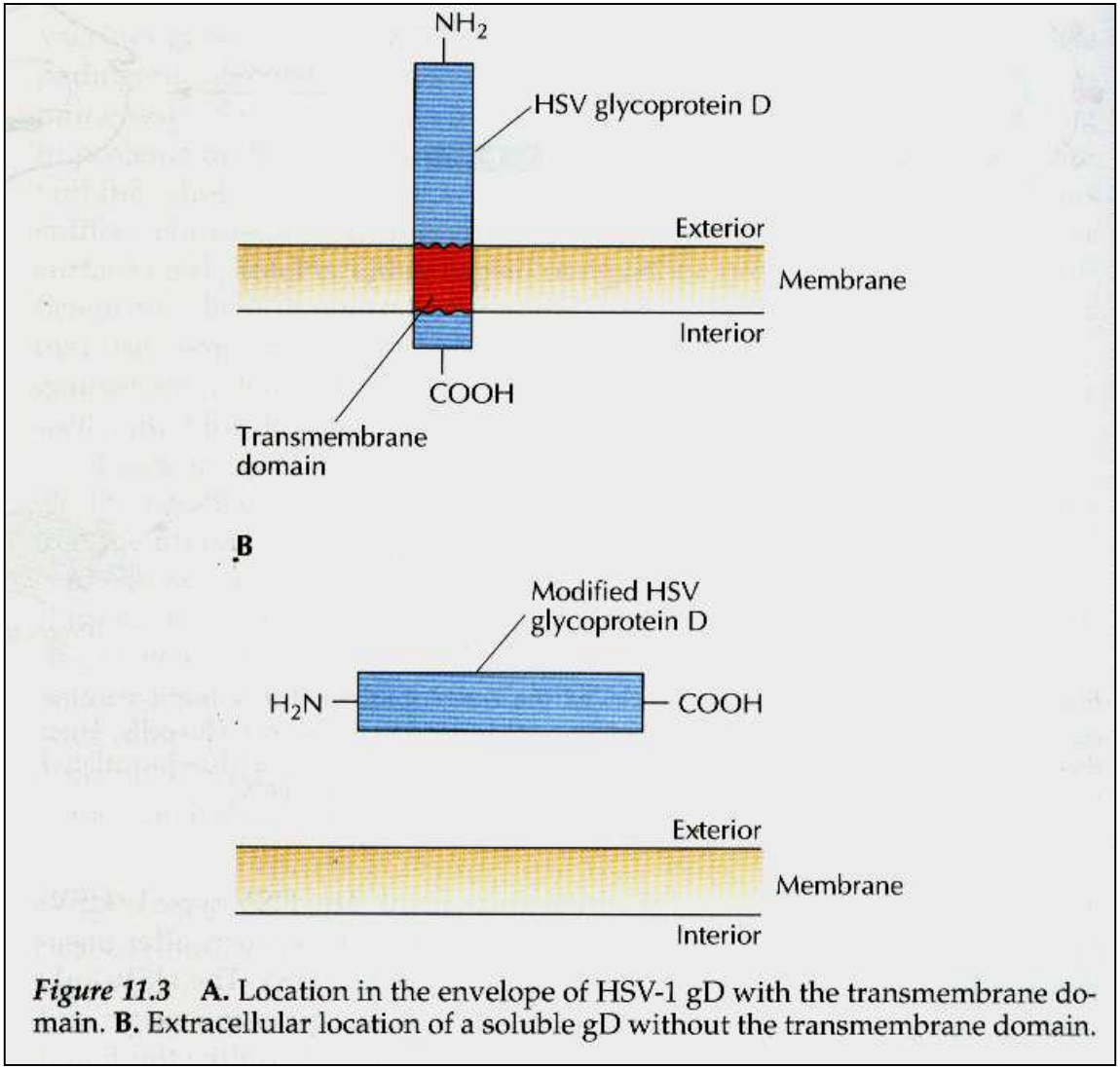


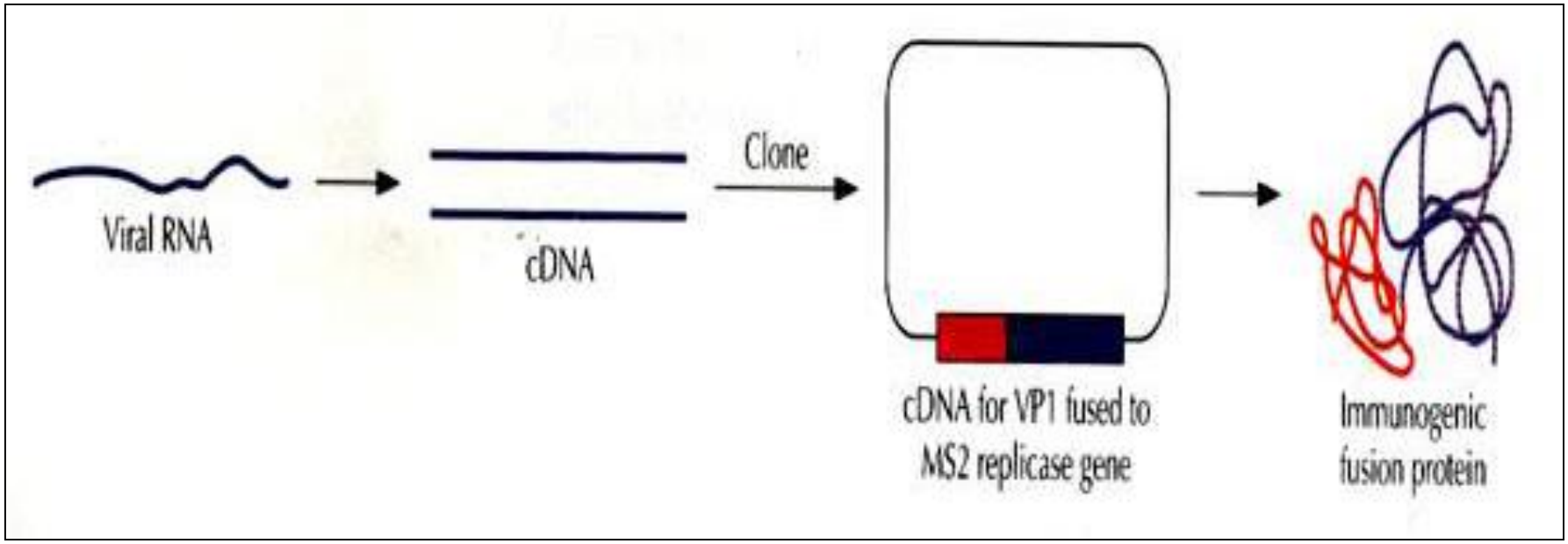
Figure 11.3 **A.** Location in the envelope of HSV-1 gD with the transmembrane domain. **B.** Extracellular location of a soluble gD without the transmembrane domain.

A similar approach was used to create a subunit vaccine against Foot-and-Mouth Disease Virus (FMDV) and Human Papillomavirus (HPV)

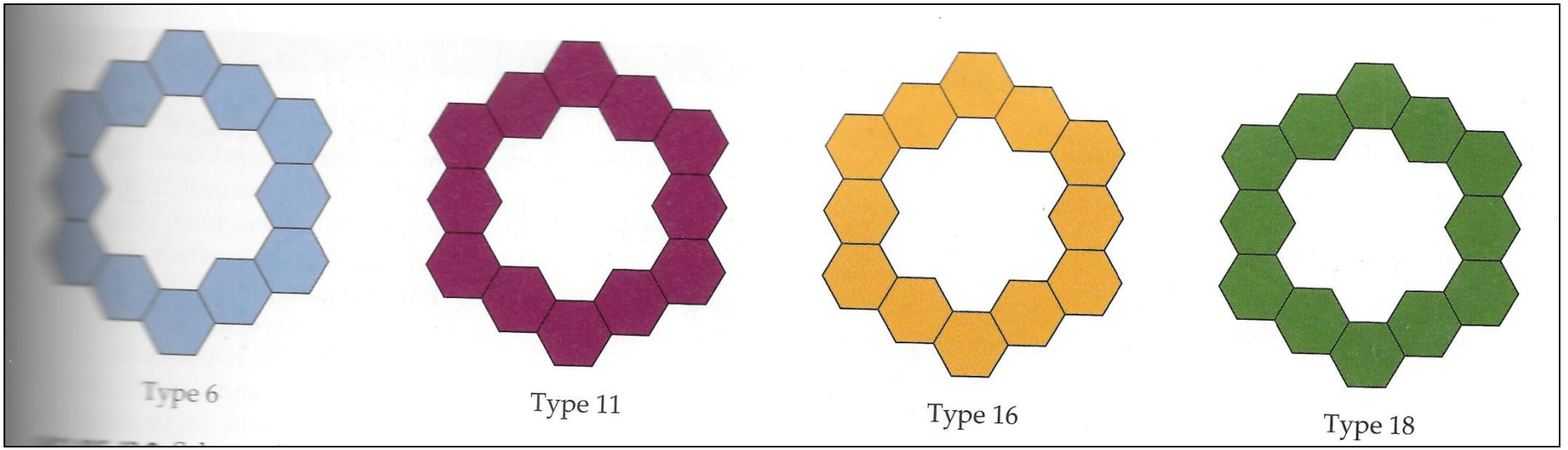
- **FMDV has a devastating effect on cattle and swine**
- **The successful subunit vaccine is based on the expression of the capsid viral protein 1 (VP1) as a fusion protein with the bacteriophage MS2 replicase protein in *E. coli***
- **The FMDV genome consists of a 8kb ssRNA; a cDNA was made to this genome and the VP1 region identified immunologically (see Fig. 12.4)**
- **A subunit vaccine (Gardasil) was developed against Human Papillomavirus; this virus causes genital warts and is associated with the development of cervical cancers; used the capsid proteins from four HPVs**

A subunit vaccine against Foot and Mouth Disease Virus (FMDV)

Figure 11.4 Schematic representation of the development of a subunit vaccine against foot-and-mouth disease. The entire viral RNA is made into cDNA, which is then digested with restriction enzymes. The DNA fragments are cloned into an expression vector in frame with the gene for the *E. coli* bacteriophage MS2 replicative protein. The plasmid constructs are used to transform *E. coli*, and then the stable fusion protein is isolated and used to inoculate animals.



Subunit vaccine against Human Papilloma Virus



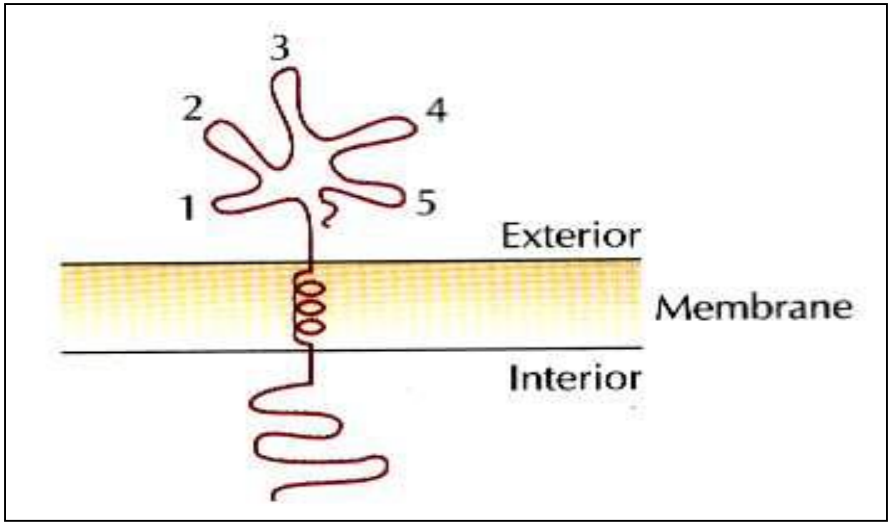
Schematic representation of the virus-like particles assembled from cloned and overproduced L1 proteins from the capsids of four different strains of human papillomavirus. These virus-like particles are the constituents of a commercial subunit vaccine against the virus.

Peptide Vaccines

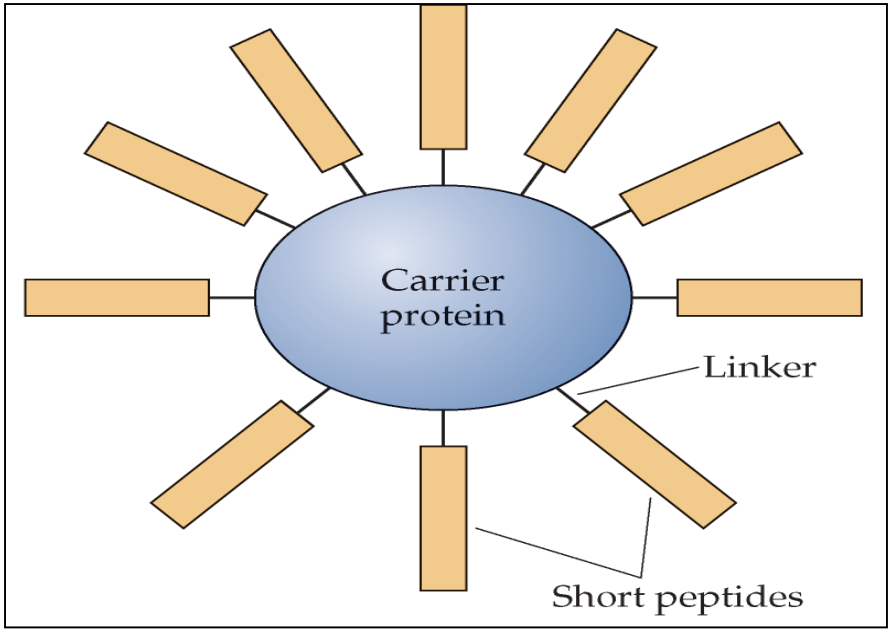
- **The question arises whether a small discrete portion (domain) of a protein can act as an effective subunit vaccine and can induce the production of neutralizing antibodies**
- **One would expect that only the portions or domains of a protein that are accessible to antibody binding would be immunologically important**
- **While those located in the inaccessible regions inside the virus particle can be ignored if they do not contribute to the conformation of the immunogenic domain**
- **If this argument has validity, it is possible that short peptides that mimic epitope (antigenic determinant) will be immunogenic and could be used as vaccines i.e. peptide vaccines**

Structure of a peptide vaccine

Generalized envelope bound protein with external epitopes (1 to 5) that might elicit an immune response.



Structure of a peptide vaccine composed of identical short peptides bound to a carrier protein.



Peptide Vaccine against FMDV

- **Therefore, based on the X-ray crystallographic structure of the soluble antigenic FMDV VP1, chemically synthesized domains of the protein were tested as potential peptide vaccines.**
- **Peptides corresponding to amino acids 141-160, 151-160 and 200-213 which were located to near C-terminal end of VP1 and amino acids 9-24, 17-32 and 25-41, located near N-terminal end of VP1 were each bound to separate inert carrier protein (keyhole limpet hemocyanin) and injected into guinea pigs.**
- **Very small peptides or molecules are usually degraded unless they are bound to the surface of a large carrier molecule.**
- **A single inoculation with peptide 141-160 elicited sufficient antibodies to protect the animal against subsequent challenges with FMDV.**
- **By contrast 9-24, 17-32 and 25-41 yielded a lower level of neutralizing antibodies**

Peptide Vaccine against FMDV

• **In an additional experiment, a longer peptide consisting of amino acids 141-158 joined to amino acids 200-213 by two proline residues high levels of neutralizing antibodies even when it was injected without any carrier protein.**

• **This "two peptide" molecule was more effective than either of the single peptides alone and prevented FMDV proliferation in cattle as well as in guinea pigs.**

Although these results are promising but there are certain limitations to using short peptides as vaccines:

✓ **To be effective, an epitope must consist of a short stretch of contiguous (nearby/adjacent) amino acids, which does not always occur naturally**

✓ **The peptide must be able to assume the same conformation as the epitope in the intact viral particle**

✓ **A single epitope may not be sufficiently immunogenic**

THERE IS CURRENTLY NO COMMERCIALLY AVAILABLE VACCINE AGAINST MALARIA. FOR MORE THAN 20 YEARS OF ACTIVE RESEARCH FOR ITS CREATION, TESTED SEVERAL CANDIDATES

Challenges to developing malaria vaccines

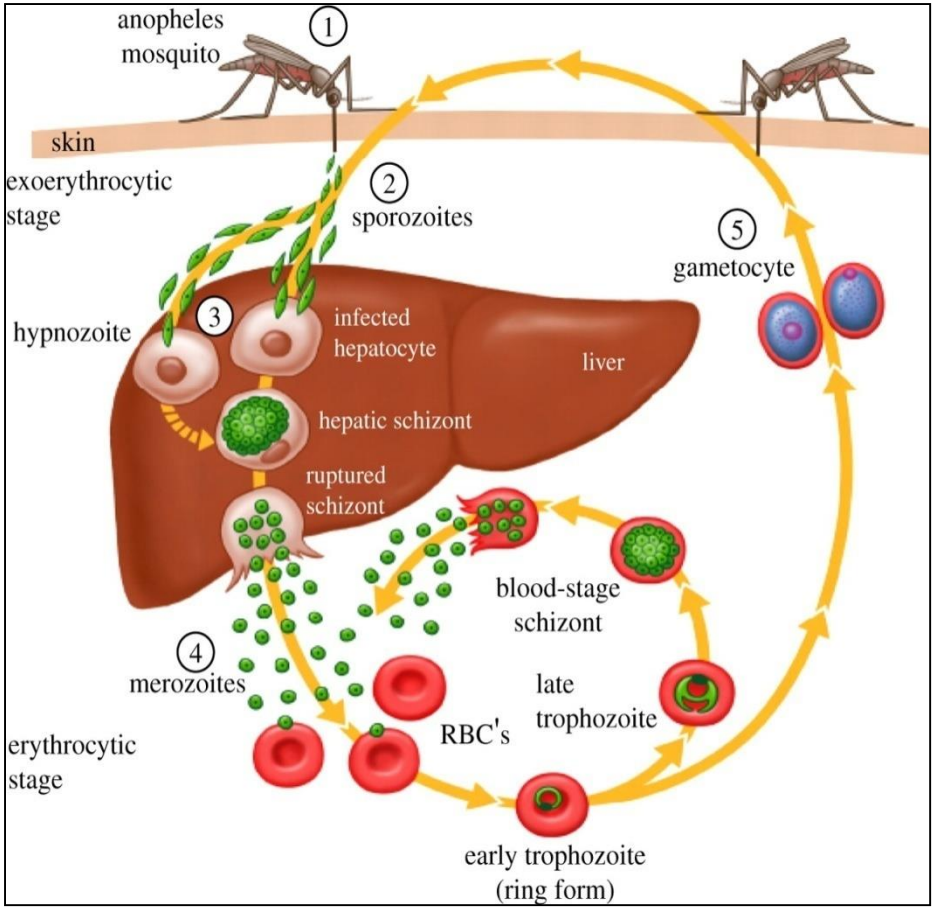
Scientific

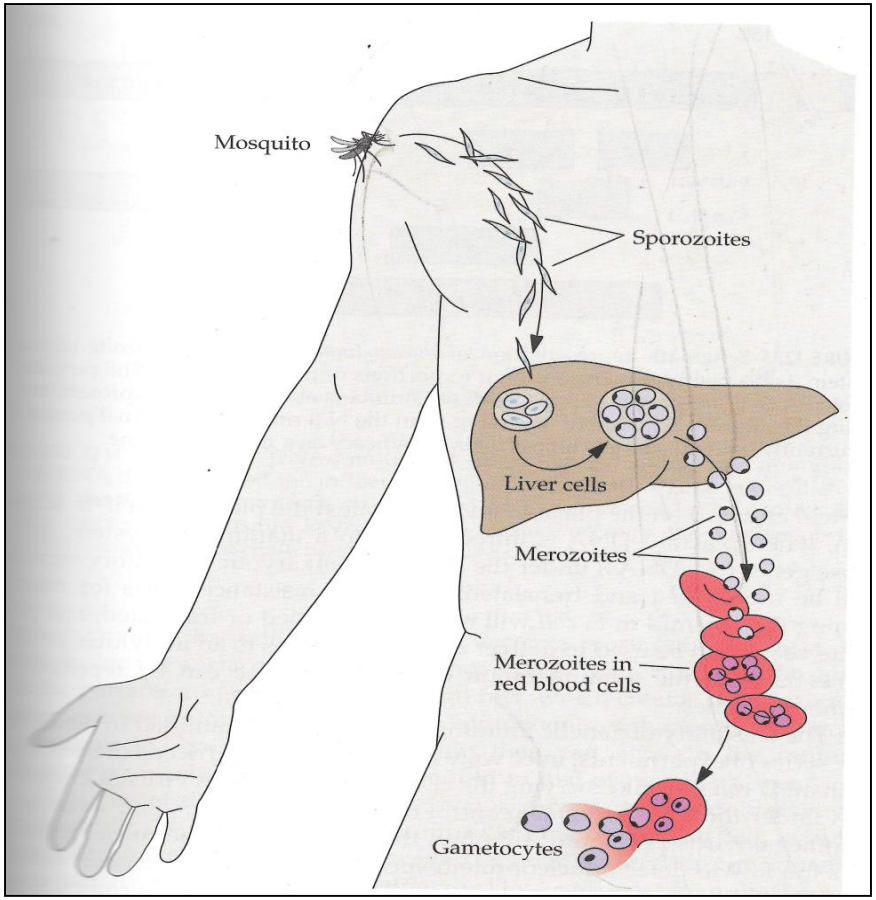
- No vaccine is in human use against a parasite
- Malaria parasite has ~6,000 genes, many more than a virus
- How to predict a vaccine candidate's success?

Commercial

- Limited market in developed countries
- Malaria-endemic countries are mostly poor
- Vaccine development is high-risk, high-cost

Peptide Vaccine against Malaria





Infection of an individual with *Plasmodium falciparum* (a malaria causing parasite) introduced by a mosquito.

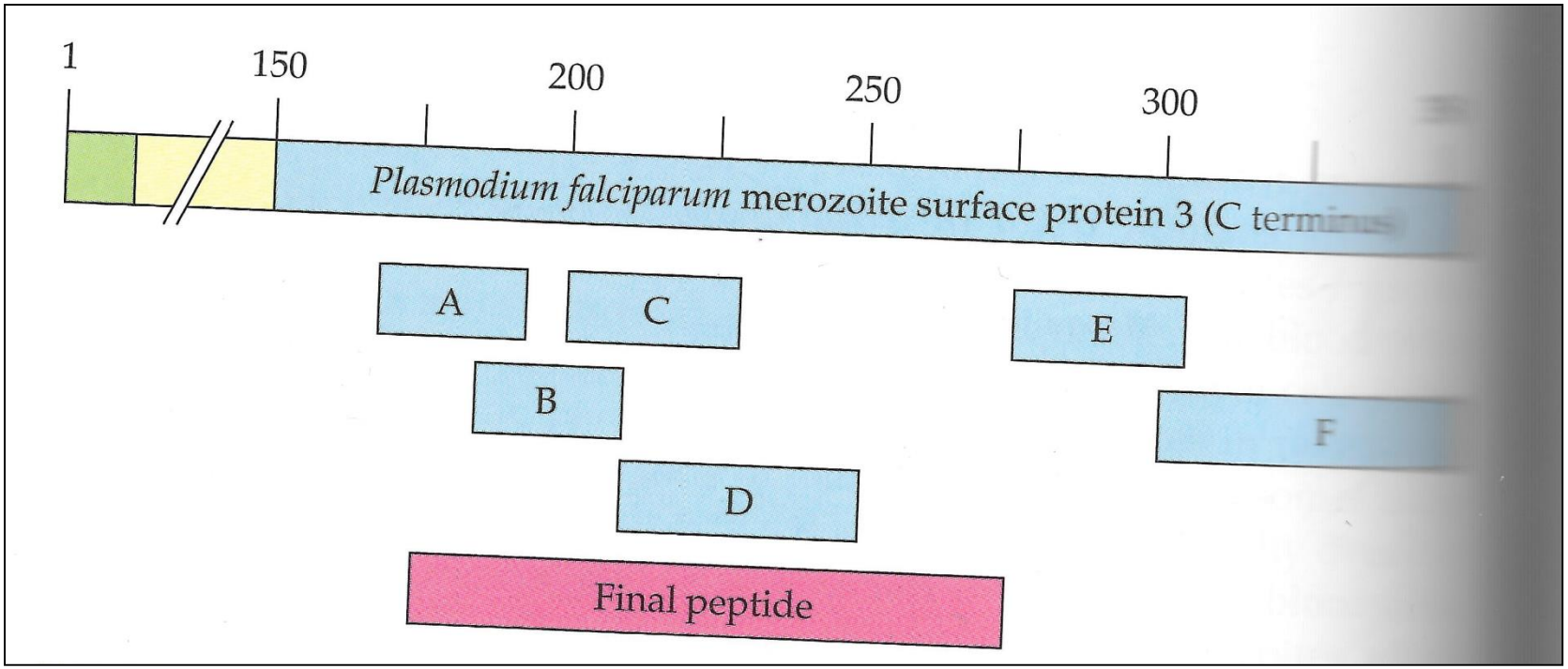
Peptide Vaccine against Malaria

- **In the life cycle of malaria parasite, it is the asexual blood stage multiplication that is responsible for most of the acute symptoms of the disease.**
- **In areas where malaria is endemic, some individuals show considerable resistance to the disease despite the fact when their blood is examined they are found to carry the parasite.**
- **This resistance to the worst symptoms of malaria was shown to be a result of an "antibody dependent cellular inhibition" mechanism that inhibits parasite development.**
- **In other words, some individuals who are infected with the malarial parasite made antibodies against a parasite protein that prevented the growth of the parasite.**
- **Following a detailed study, it was determined that the protective antibodies targeted merozoite surface protein 3.**
- **When this protein was examined in different strains of *Plasmodium*, it was observed that while the N-terminal part of the protein varied considerably from one strain to another, the C-terminal end of the protein was highly conserved among the various isolates of the parasite.**
- **It was therefore decided to chemically synthesize peptides that corresponded to small portions of the C-terminus of the merozoite surface protein 3.**

Peptide Vaccine against Malaria

- **Human antibodies from individuals who were resistant to the parasite were affinity purified based upon their interaction with one or more of these peptides.**
- **The antibodies that bound to the peptides were then tested in an antibody dependent cellular inhibition assay.**
- **Antibodies directed against peptides B, C, and D (figure) had a major inhibitory effect on parasite growth.**
- **Based on the ability of peptides B, C, and D to bind to and select protective antibodies, a peptide representing amino acid residues 181 to 276 of merozoite surface protein 3 was chemically synthesized.**
- **This peptide is currently being tested in clinical trials as a novel malaria vaccine.**
- **While more research needs to be done, in the future, synthetic peptide vaccines could become highly specific, relatively inexpensive, safe and effective alternatives to traditional vaccines.**

Peptide Vaccine against Malaria



Schematic representation of *Plasmodium falciparum* merozoite surface protein 3 and peptides corresponding to portions of the C-terminus. The peptides labeled A to F are drawn to scale, with the numbers above the whole protein indicating the amino acid number (counting from the N-terminus). The "final peptide" is currently being tested in clinical trials for efficacy as a malaria vaccine.

Genetic immunization: DNA vaccines

- **A novel strategy that elicits an antibody response without the introduction of an antigen has been developed**
- **In this case, the gene encoding an antigenic protein is incorporated into the cell of a target animal, where the antigen is synthesized**
- **In the initial experiments, gold microprojectiles were coated with *E.coli plasmid DNA carrying an antigen gene* under the transcriptional control of an animal viral promoter**
- **A biolistic method was used to deliver the micro-projectiles into cells in the ear of mice**
- **Subsequently, other workers introduced cloned cDNAs into mouse cells by injecting large amounts of the plasmid carrying the target DNA directly into the muscles of test animals**

Genetic immunization: DNA vaccines

- **Direct injection into muscles (100 µg per mouse) requires 3 to 4 orders of magnitude more DNA than the biolistic delivery system (10 to 100 ng per mouse)**
- **One distinctive feature of "genetic immunization" is that the costly and time consuming procedure of either purifying an antigen or creating a recombinant vaccine vehicle is bypassed**
- **Moreover protein produced by this procedure are more likely to be correctly posttranslationally modified than the proteins that are produced by different host organisms**
- **Genetic immunization may hold promise for the vaccination of domestic animal**
- **The feasibility of genetic immunization has been examined in detail**

Genetic immunization: DNA vaccines

- **Advantages of genetic immunization, besides bypassing the need for purified protein antigens, is that**

✓ **It can trigger response only against the protein encoded on the plasmid and not against the plasmid itself**

✓ **Thus the same vector can be used to deliver different proteins to an individual at the same time or**

✓ **administration of same gene can be repeated in a number of times**

Genetic immunization: DNA vaccines

- At this time, the fate of the introduced DNA is not known
- It could have the undesirable effect of integrating into the genome of the host cell, possibly disrupting an important gene
- However, this risk is currently considered to be extremely low
- It is more than likely that DNA will exist for a short period of time as a non-replicating extra chromosomal element before it is degraded
- To date, genetic immunization has been used primarily to induce immune responses in animals, and only to a limited extent in humans against a number of pathogenic organisms including: Influenza A virus, HIV type 1,Bovine Herpes Virus, Rabies Virus, Plasmodium species, Hepatitis B and C virus, Bovine rotavirus, Bovine respiratory syncytial virus, Pseudorabies virus, Foot and mouth disease, Newcastle disease virus, Clostridium tetani, Mycobacterium tuberculosis
- Several human clinical trials using DNA vaccines are currently ongoing

Genetic immunization: DNA vaccines

Delivery Methods

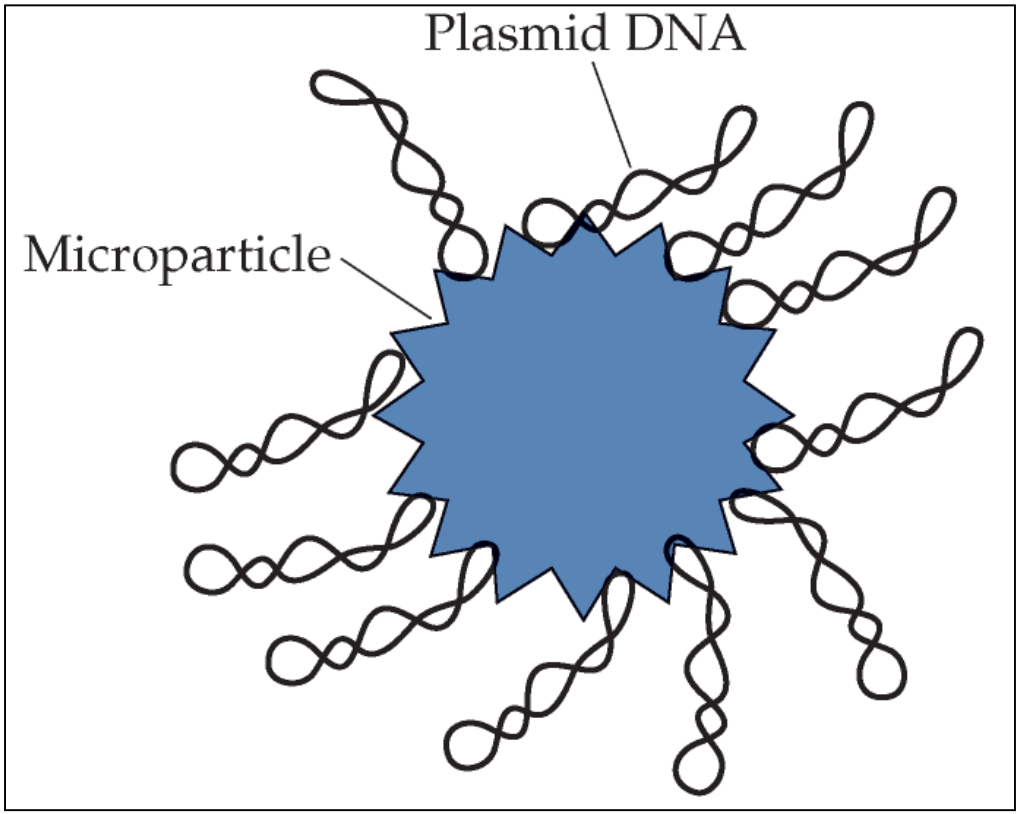
- **To avoid using mg amounts of DNA to immunize large animals or humans, it is necessary to develop a delivery system that is considerably more effective than the direct injection of naked plasmid DNA into muscle or other tissues**
- **One successful approach of this problem utilizes biodegradable microscopic (0.3 – 1.5 µm) polymeric particles with a cationic surface that binds the plasmid DNA (figure)**
- **Plasmid DNA is bound to the surface of these "microparticles" and is released slowly after inoculation of an animal ~35% released after 1 day and by 14 day 75%**
- **Thus DNA persists and is expressed over a relatively long period of time**
- **Using this method, it was possible to achieve the same biological effect as with naked DNA with about 250 fold less DNA, demonstrating the potential of this approach**
- **In addition, the level of antibodies induced by plasmid encoded genes bound to micro particles was significantly enhanced by:**

✓ **the addition of the vaccine adjuvant aluminum phosphate and**

✓ **The use of nano-particles 0.05 µm in diameter that were coated with poly-lysine**

Genetic immunization: DNA vaccines

Figure 11.10 Schematic representation of the binding of plasmid DNA to the cationic surface of a polymeric microparticle.



(with gene encoding the antigenic protein under the control of an animal virus promoter)

A biolistic system or direct injection is used to introduce this DNA microparticle into animals

Genetic immunization: DNA vaccines represent

Delivery Methods

- **In contrast to naked DNA, DNA bound to micro particles induced potent cytotoxic T lymphocyte response at a low dose**
- **To date, most of DNA vaccines have been delivered by either intramuscular or intradermal injection**
- **Although these vaccines can induce a potent immune response, they do not induce mucosal immunity**
- **Mucosal immunity can prevent pathogens from entering the body, while systemic immunity only deals with pathogens once they are inside the body**
- **This is an important consideration because mucosal surfaces are the primary sites of transmission of most infectious diseases**
- **However, because of protective barriers of the mucosal surfaces, traditional antigen-based vaccines are largely ineffective unless they are administered with specific agents that penetrate or bind to the mucosa i.e. mucosal adjuvants**

Genetic immunization: DNA vaccines

Delivery Methods

- **Immune system has two distinct compartments:**

✓ **Systemic Immune System: Includes bone marrow, spleen and lymph nodes**

✓ **Mucosal Immune System: Includes lymphoid tissue associated with mucosal surfaces and external secretary glands**

- **Mucosal immunity induces a separate and distinct response from systemic immunity**
- **The antibodies produced as part of the mucosal immune response restrict not only mucosal pathogens but also microorganisms causing systemic disease that initially colonize mucosal surfaces**
- **Many of current mucosal vaccines are live attenuated organisms that infect mucosal surfaces and are effective at inducing mucosal responses**
- **Of these vaccines, oral polio and both attenuated *Salmonella enterica* serovar Typhi Ty 21 and *Vibrio chlolerae* are licensed for use in humans**
- **DNA vaccines are designed for delivery to mucosal surfaces are similar in principle to those used for intramuscular or intradermal delivery**

Genetic immunization: DNA vaccines

- **To ↑ plasmid uptake and ↓ subsequent degradation, various methods of formulating DNA have been tried, For e.g.**

✓ **Cationic (positive charged) liposomes used to deliver DNA to the respiratory tract, (here the lipid composition greatly affects the level of expression of the introduced DNA)**

✓ **In addition, DNA entrapped in biodegradable micro/nano particles has been used for the oral delivery of foreign DNA**

✓ **To improve the potency of DNA vaccines, especially for humans, a number of strategies have been devised**

✓ **For example, increased immune responses have been observed when plasmids also expressed a cytokine(s) such as IL-2, IL-10 or IL-12 in addition to antigen**

- **Different systems including:**

✓ **Liposomes**

✓ **Live vectors like bacteria and viruses and**

✓ **Adjuvants including bacterial toxins, carboxy methyl cellulose, lipid derivative, aluminum salts and saponins have been tried for delivery of DNA to a variety of cell types**

Genetic immunization: DNA vaccines

TABLE 12.3 Advantages of genetic immunization over conventional vaccines

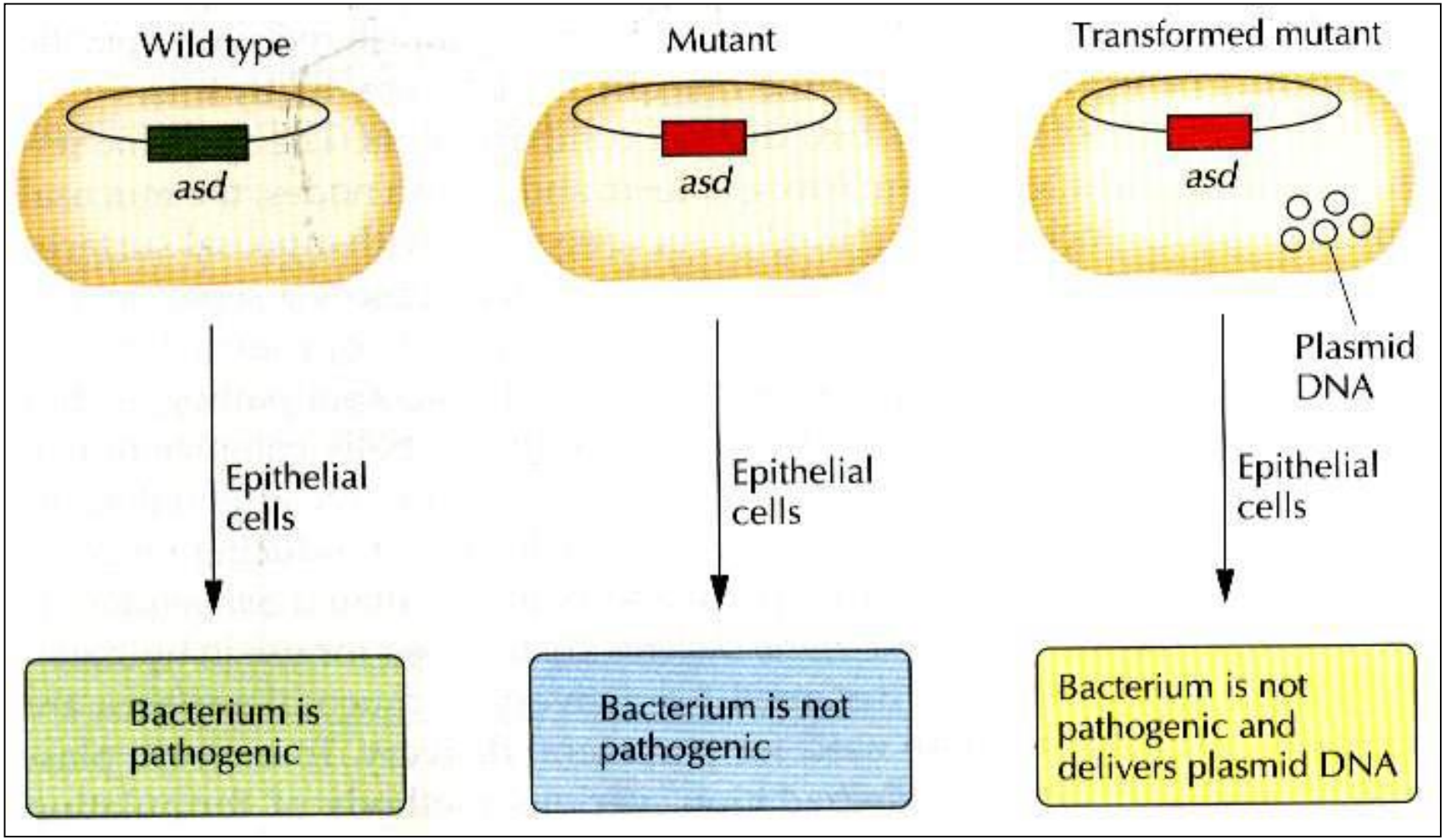
Cultivation of dangerous agents is not required.
Since genetic immunization does not utilize any viral or bacterial strains, there is no chance that an attenuated strain will revert to virulence.
Since no organisms are used, attenuated organisms that many cause disease in young or immunocompromised animals are not a problem.
Approach is independent of whether the microorganism is difficult to grow or attenuate.
Production is inexpensive because protein does not need to be produced or purified.
Storage is inexpensive because of the stability of DNA.
One plasmid could encode several antigens/vaccines, or several plasmids could be mixed together and administered at the same time.

Genetic immunization: DNA vaccines

Delivery Methods

- **A modified strain of the bacterium *Shigella flexneri* has been developed to facilitate the delivery into animal cells of DNA for genetic immunization**
- ***Shigella flexneri* can enter animal epithelial cells, escaping the phagocytic vacuole and directed plasmid to the cytoplasm of the host cell, Where, if the introduced gene(s) contains a eukaryotic promoter, it is transcribed and translated**
- ***Shigella* is normally a pathogenic organism and would not be an acceptable DNA delivery system**
- **Therefore, to use *Shigella*, it was first necessary to construct a nonpathogenic version of the wild type organism (figure)**
- **Determination of the safety of using Shigella flexneri as a vector for delivery of DNA to animal cells must await the results of human trials, however, the results of experiments with guinea pigs are promising**
- **The greatest potential advantage of this approach is that with the *Shigella flexneri* system, DNA for vaccination may be delivered orally, greatly simplifying the delivery of a variety of vaccines**

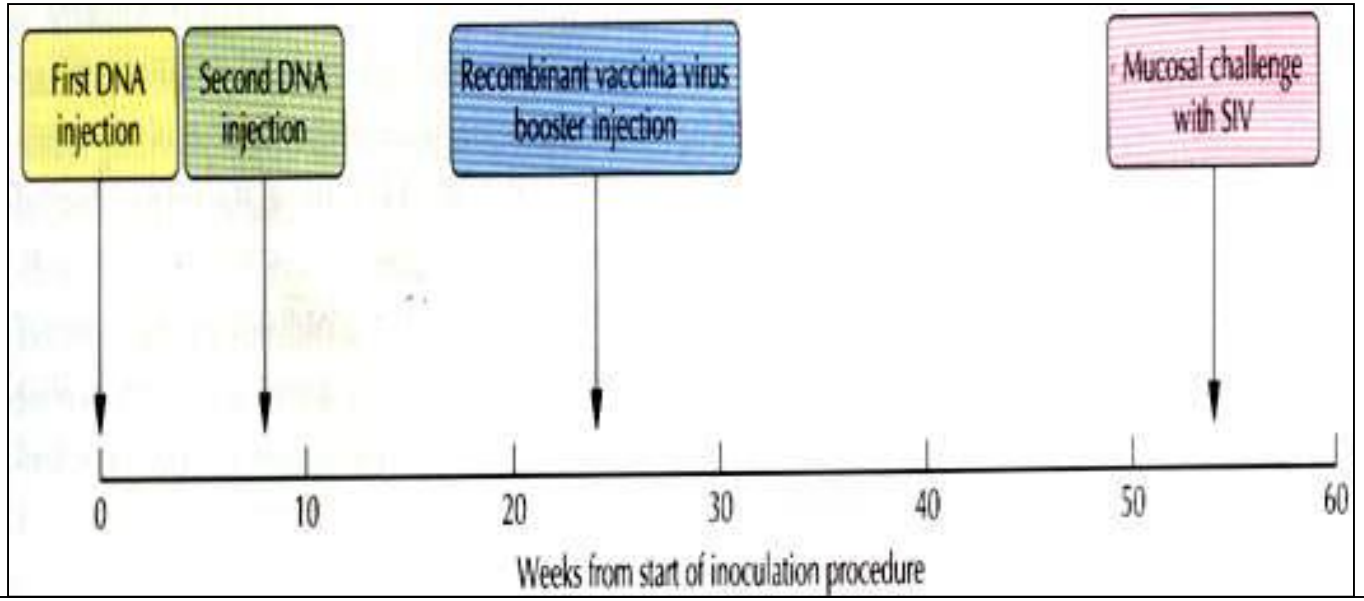
Use of nonpathogenic *Shigella flexneri* to deliver foreign DNA to mammalian epithelial cells. A strain of *Shigella* with a deletion mutation in the *asd* gene, which encodes the enzyme *aspartate betasemialdehyde dehydrogenase*, is unable to proliferate and can be used as a live vector.



Note: *aspartate betasemialdehyde dehydrogenase* is normally involved in the synthesis of the bacterial cell wall constituent diaminopimelic acid, therefore, mutants cannot grow unless diaminopimelic acid is added to the medium.

Genetic immunization: DNA vaccines

- **Of optimization strategies need to be tested in mice before they are tried on larger animals and then on humans and**
- **There is no guarantee that an approach that works best in mice will also be the best strategy in humans**
- **Vaccination of rhesus macaques (monkeys) with DNA encoding simian immunodeficiency virus proteins, followed by a booster with a modified vaccinia virus that encoded many of the same proteins, protected the monkeys against infection by simian immunodeficiency virus**



Vaccination regimen of Rhesus monkeys with DNA containing *simian immunodeficiency virus* (SIV) genes and vaccinia virus carrying the same genes

Attenuated vaccines

- Genetic manipulation may be used to construct modified organism (bacteria or viruses) that can be used as live recombinant vaccines
- These vaccines are either non-pathogenic organisms that have been engineered to carry and express antigenic determinants from a target pathogenic agent or
- Engineered strains of pathogenic organisms in which virulence genes have been modified / deleted
- It is advantageous to develop a live vaccine, because they are generally much more effective than killed or subunit vaccines
- The major requirement of live vaccines is that no virulent forms be present in the inoculation material
- With this object in mind, a live cholera vaccine has been developed
- Cholera is fast acting intestinal disease characterized by fever, dehydration, abdominal pain and diarrhea
- It is transmitted by drinking water contaminated with fecal matter
- In developing countries, the threat of cholera is a real and significant health concern whenever water purification and sewage disposal system inadequate

Attenuated vaccines

- **The bacterium *V. cholerae,* the causative agent of cholera, colonizes the small intestine and secretes large amounts of a hexamer enterotoxin, which is the actual pathogenic agent (figure)**
- **The cholera vaccine that is currently used consists of phenol-killed *V. cholerae***
- **This vaccine generates only moderate protection which normally last for 3 to 6 months**
- **Since *V. cholerae* colonizes the surface of the intestinal mucosa, it is thought that an effective cholera vaccine should both be directed to this structure and administered orally**
- **With this in mind, a strain of *V. cholerae* was created with part of the coding sequence for the A1 peptide deleted, the enterotoxin gene for the A1 peptide of *V. cholerae*, the causative agent of cholera, was deleted; the resulting bacteria was non-pathogenic and yet elicits a good immunoprotection (some side effects noted however) and is good candidate for a live vaccine**

A vaccine against Cholera

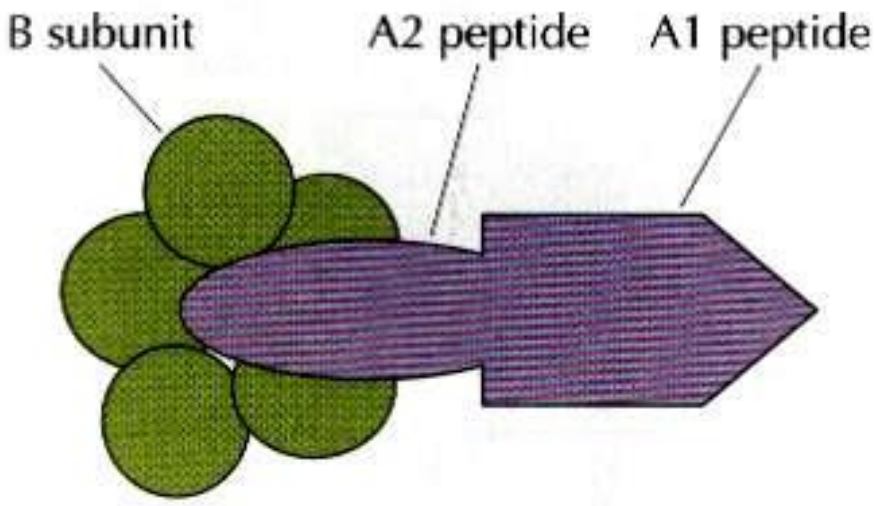
The protein is hexamer that consists of two subunits:

- **A subunit that has ADP ribosylation activity and stimulates adenyl cyclase and has two functional domains;**

A1 peptide contains toxic activity and A2 peptide joins the A subunit to B subunits

- **B subunits contain five identical units that bind specifically to an intestinal mucosal cell receptor**

Figure 11.12 Schematic representation of hexameric cholera toxin. The A peptide is shown in blue, and the B peptide is shown in green.



Vector vaccines

- **Here the idea is to use a benign virus as a vector to carry your favorite antigen gene from some pathogenic agent**
- **The vaccinia virus is one such benign virus and has been used to express such antigens**
- **Properties of the vaccinia virus: 187kb dsDNA genome, encodes ~200 different proteins, replicates in the cytoplasm with its own replication machinery, broad host range, stable for years after drying**
- **However, the virus genome is very large and lacks unique RE sites, so gene encoding specific antigens must be introduced into the viral genome by homologous recombination (see Fig. 11.16)**

Edward Jenner used the cowpox virus to vaccinate individuals against smallpox virus in 1796

Smallpox

See http://www.youtube.com/watch?v=jJwGNPRmyTI