Biotechnologie Moléculaire

3ème année

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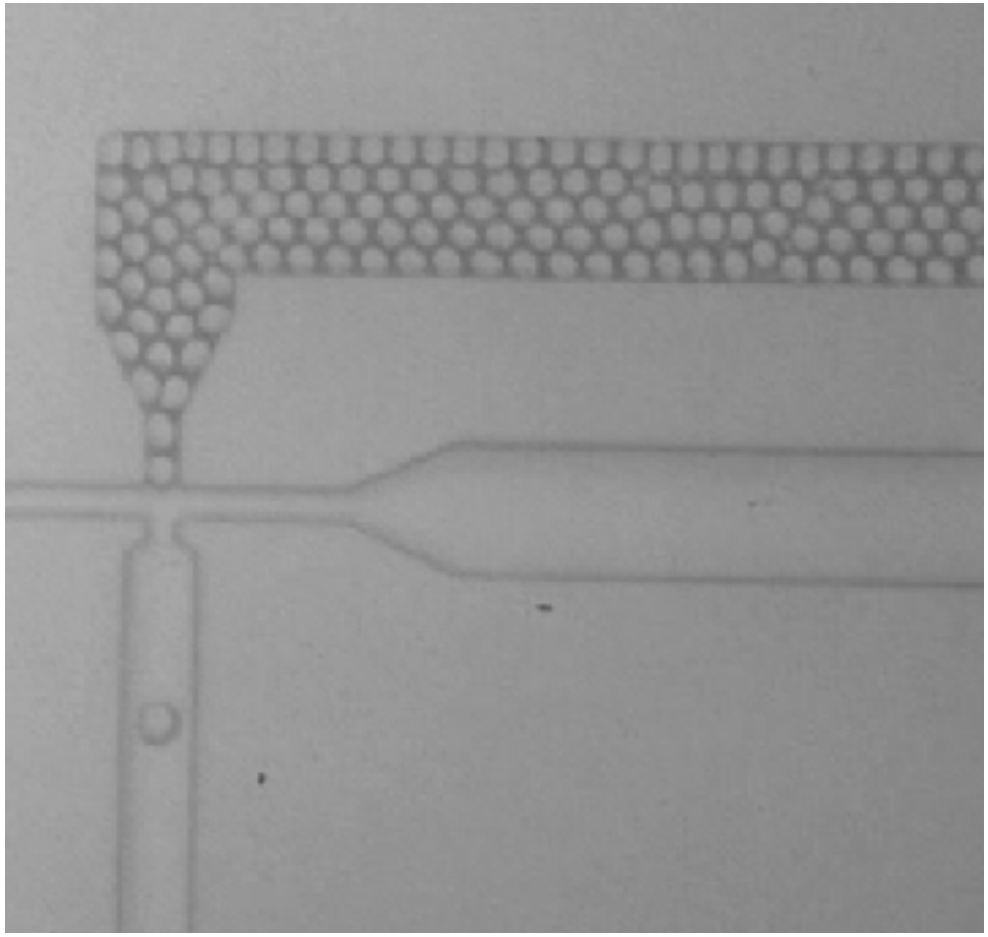


Vaccines

Vaccines
History

For many years there had been a folklore-based notion that cowpox (a mild cattle disease) could protect against human smallpox (an extremely virulent human disease with a high mortality rate) as it seemed that people working closely with cattle rarely caught Smallpox

In 1796, Dr. Edward Jenner inoculated James Phipps, an 8-year old boy, with an exudate from a Cowpox pustule. In two seperate trials after vaccination, the boy was completely protected against human smallpox.

Hence, the origin of the term Vaccination (Latin *vacca - cow*)

Vaccination has been extremely effective against many infectious diseases that have in the past been a scourge for mankind:

Smallpox, for example, has been completely erradicated

Modern Vaccines

Successes

Modern vaccines typically consist of either:

- A killed (inactivated) form of the infectious agent
- A live (attenuated) form of the infectious agent

Such vaccines have had considerable success in treating diseases such as:

German measles

Diphtheria

Whooping cough

Tetanus

Smallpox

Poliomyelitis

Modern Vaccines

Limitations to current modes of vaccine production

- Not all infectious agents can be grown in culture - so no vaccines are available for a number of diseases
- Production of viruses requires animal cell culture - this is expensive
- Yield and rate of production of viruses is often low - this makes it expensive
- Personnel making the vaccine need to be protected from the pathogenic agent
- Batches of vaccine may not be killed, or may be insufficiently attenuated - this introduces virulent organisms into the vaccine spreading the disease
- Attenuated strains may revert - this requires continual testing
- Not all diseases (e.g. AIDS) are preventable through the use of tradiational vaccines
- Most vaccines have a limited shelf-life and aften require refrigeration

Recombinant Vaccines

Recombinant Vaccines

Using recombinant DNA technology

Many limitations of traditional vaccines can be overcome using recombinant DNA technology. Gene cloning enables various novel strategies for vaccince development:

- Virulence genes can be deleted from an infectious agent - the agent can then be used as a live vaccine with no risk of reversion to vurulence (a whole gene cannot be re-acquired during culture)

- Live nonpathogenic carriers can carry discrete antigenic determinants from a pathogen and generate a strong immunological response against the pathogen

- For infectious agents which cannot be cultured - vaccines can be made from cloned proteins from pathogens which are expressed in bacterial or mammalian systems

Recombinant Vaccines

Human disease agents against which recombinant vaccines are being developed

Pathogenic agent	Disease(s)
Viruses	
Varicella-zoster virus	Chicken pox
Cytomegalovirus	Infection in infants and immunocompromised 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 virus	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

Recombinant Vaccines

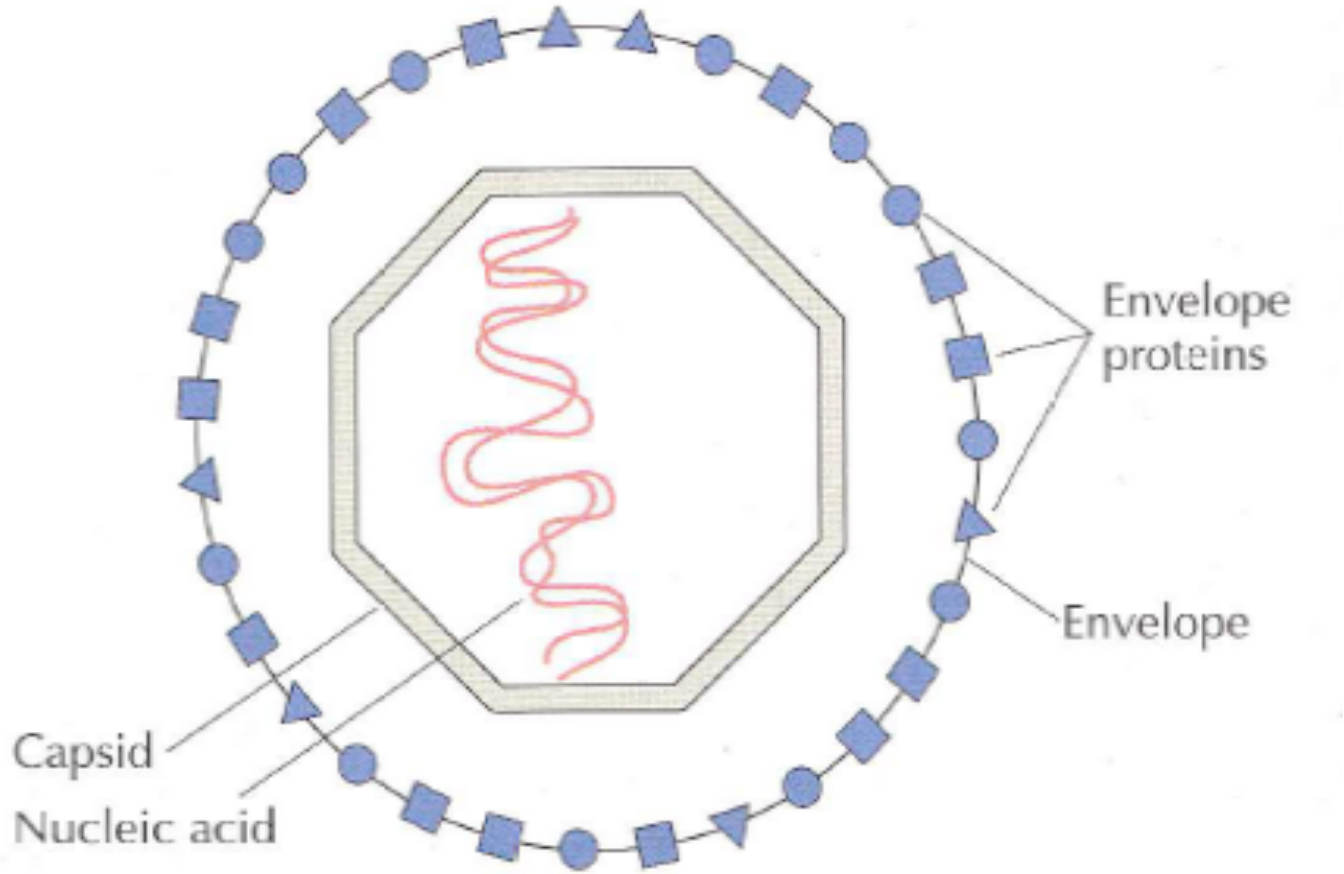
Human disease agents against which recombinant vaccines are being developed

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 typhi	Typhoid fever
Parasites	
Onchocerca volvulus	River blindness
Leishmania spp.	Internal and external lesions
Plasmodium spp.	Malaria
Schistosoma mansoni	Schistosomiasis
Trypanosoma spp.	Sleeping sickness
Wuchereria bancrofti	Filariasis

Subunit Vaccines

Animal Viruses

A schematic representation

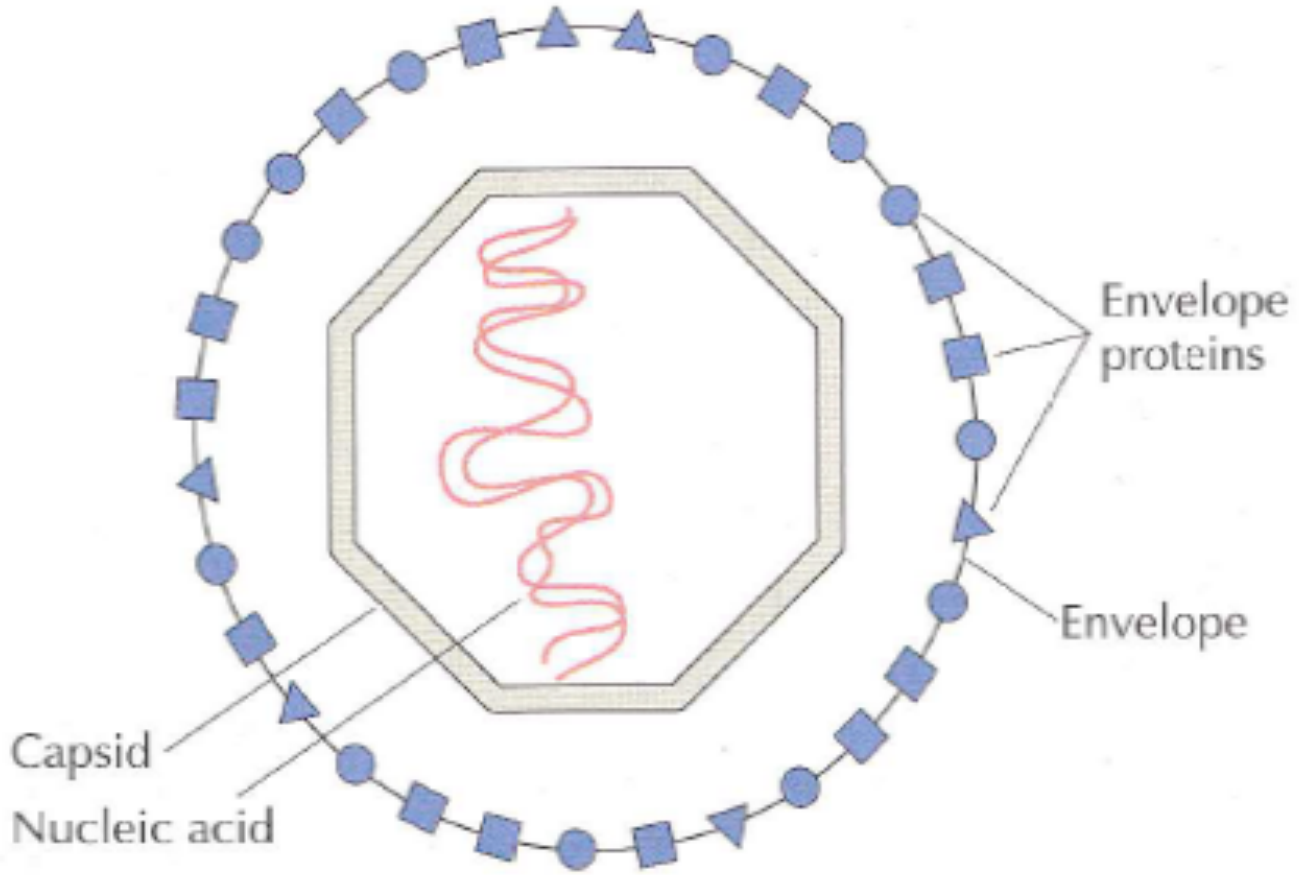


Generally have relatively small genome (3 to 200 kb of either double- or single-stranded DNA or RNA

The viral capsid is sometimes surrounded by a protein-containing viral envelope (membrane)

Subunit Vaccines

Vaccine composed of just certain viral proteins

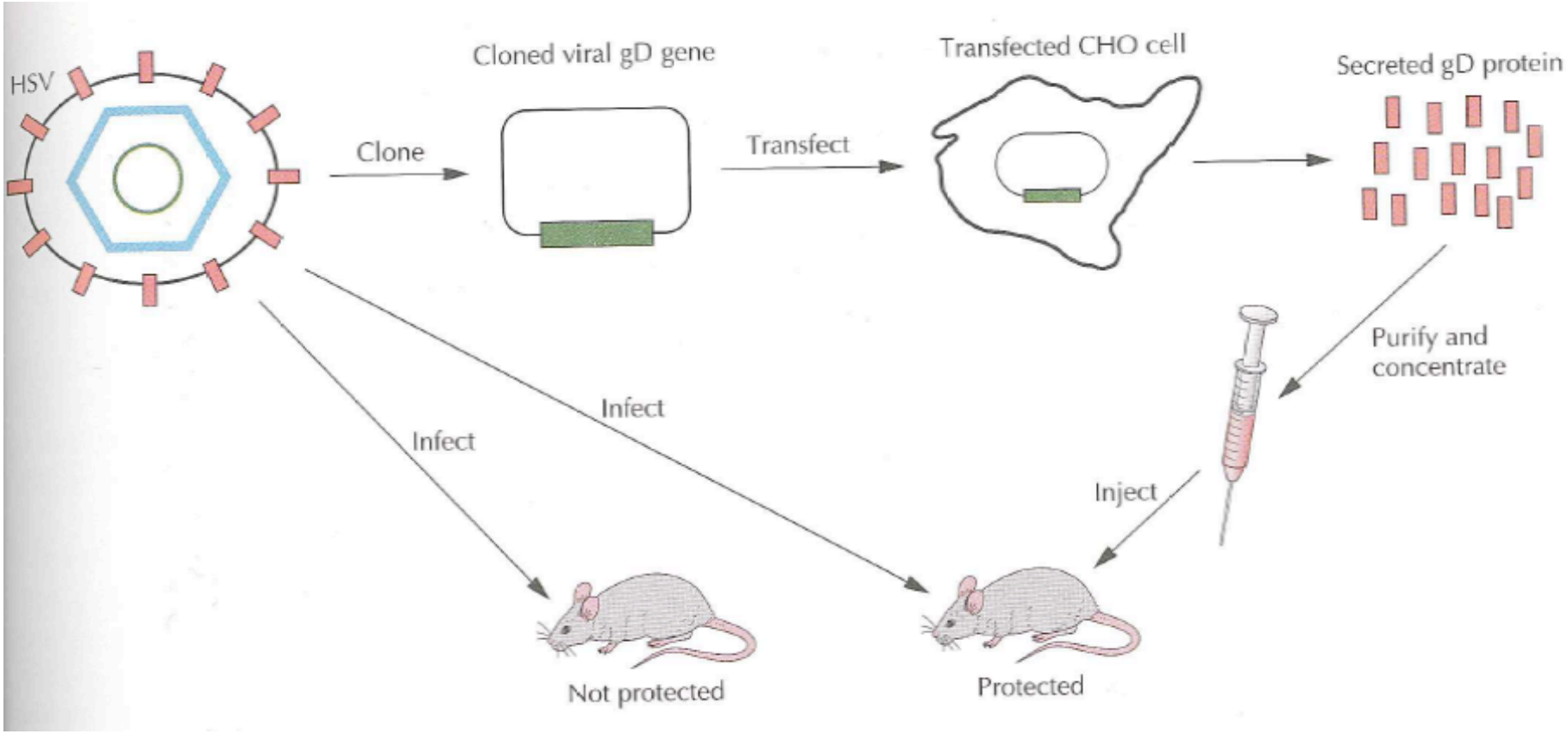


Generally, vaccines use either a killed or attenuated form of the whole virus

However, using recombinant DNA technology, subunit vaccines containing only viral capsid or envelope proteins can be created since the purified capsid or envelope proteins are sufficient for eliciting neutralizing antibodies in the host organsm

Development of a subunit vaccine

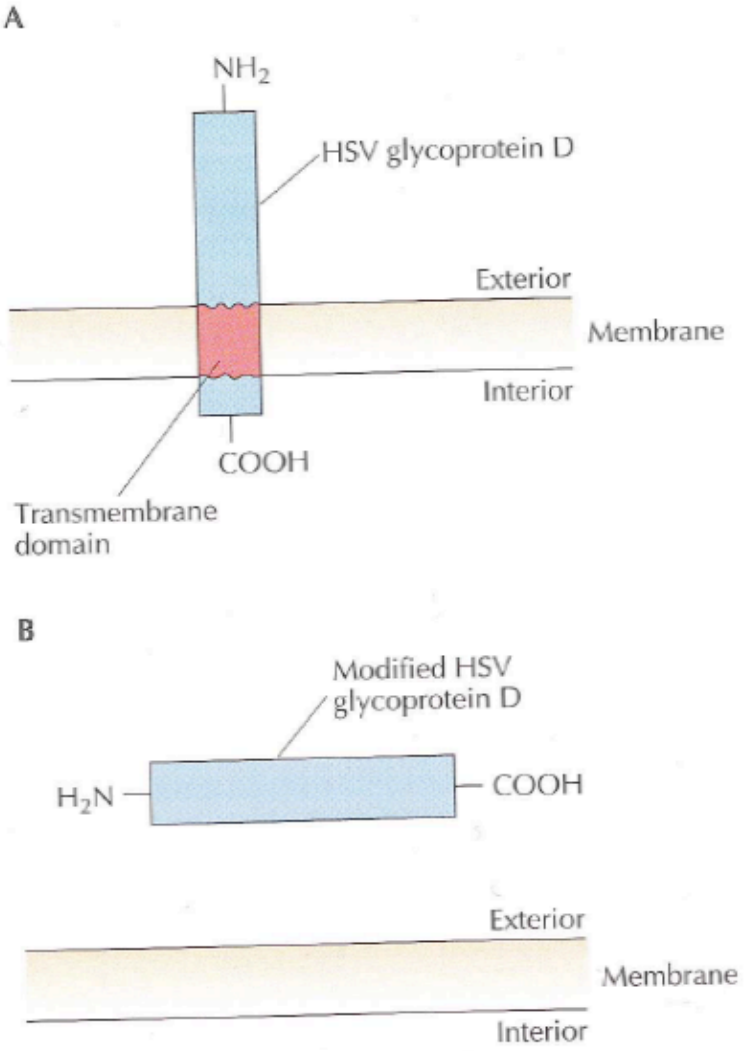
Development of a subunit vaccine against herpes simplex virus (HSV)



The HSV type 1 envelope glycoprotein D (gD) elicits antibodies that neutralize intact HSV confering protection against infection by HSV

Development of a subunit vaccine against HSV

Modification of the gD gene to remove produce soluble glycoprotein D



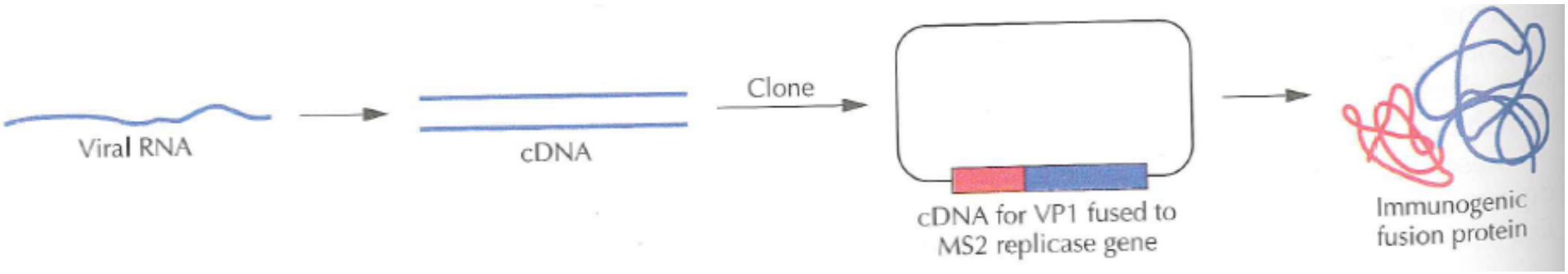
The HSV glycoprotein D is normally membrane bound which makes purification difficult

The gD gene was therefore modified to remove the C-terminal transmembrane-binding domain.

The modified gene was transformed into CHO cells, where the protein was glycosylated and secreted into the external medium.

Development of a subunit vaccine

Development of a subunit vaccine against foot-and-mouth disease virus (FMDV)



FMDV is an extremely virulent disease of cattle and pigs - current vaccines use formalin-killed whole virus

A recombinant vaccine based on capsid viral protein 1 (VP1) is being developed

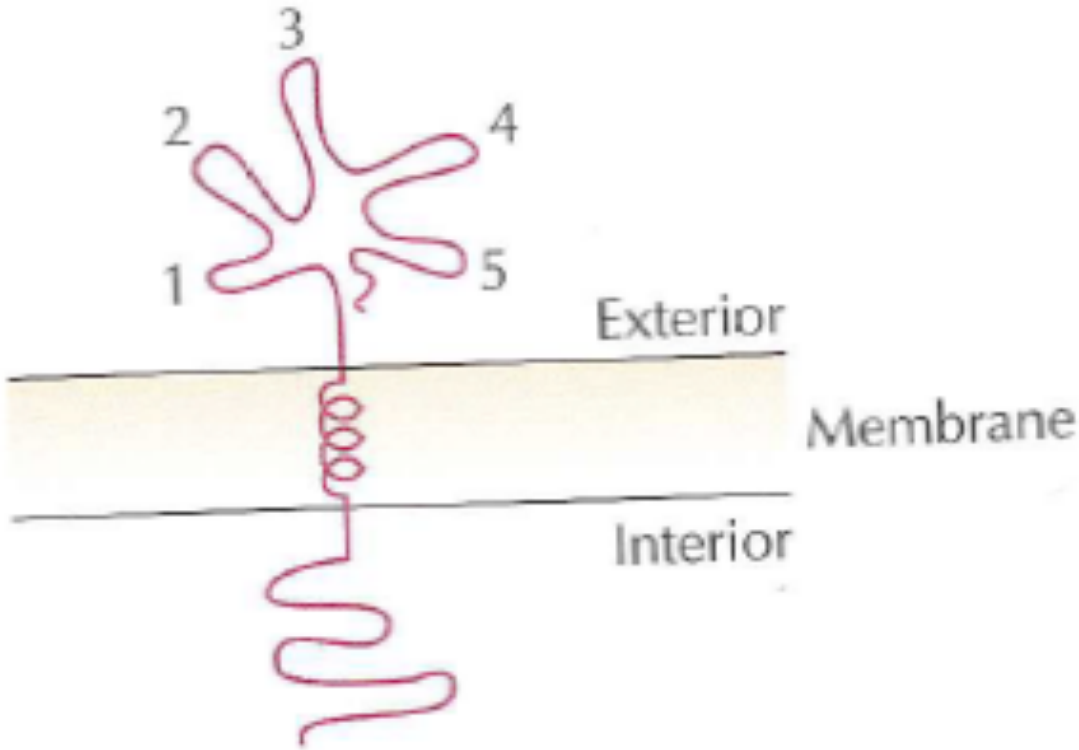
The entire viral RNA is made into cDNA

The cDNA is digested with restriction enzymes and cloned into an expression vector in frame with the *E. coli* phage MS2 replicative protein

The plasmid is transformed into *E. coli* and the stable fusion protein isolated and used to immunise animals

Development of a subunit vaccine

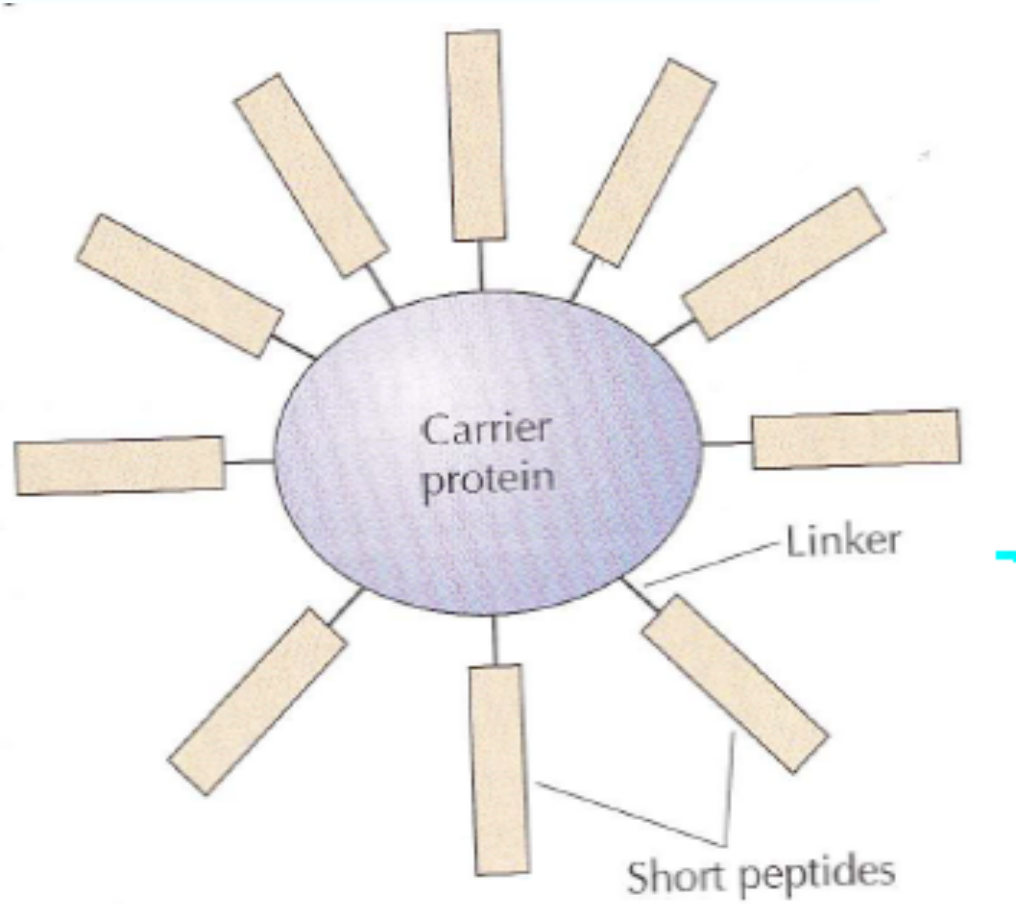
Development of a peptide vaccine against foot-and-mouth disease virus (FMDV)



Vaccine against FMDV are also being developed using chemically synthesized peptides derived from FMDV VP1

Development of a subunit vaccine

Development of a peptide vaccine against foot-and-mouth disease virus (FMDV)



The peptides from FMDV VP1 were bound to an inert carrier protein (keyhole limpet hemocyanin) and injected into guinea pigs

A single inoculation using peptide 141-160 from VP1 protected against subsequent challenge with FMDV

Development of a subunit vaccine

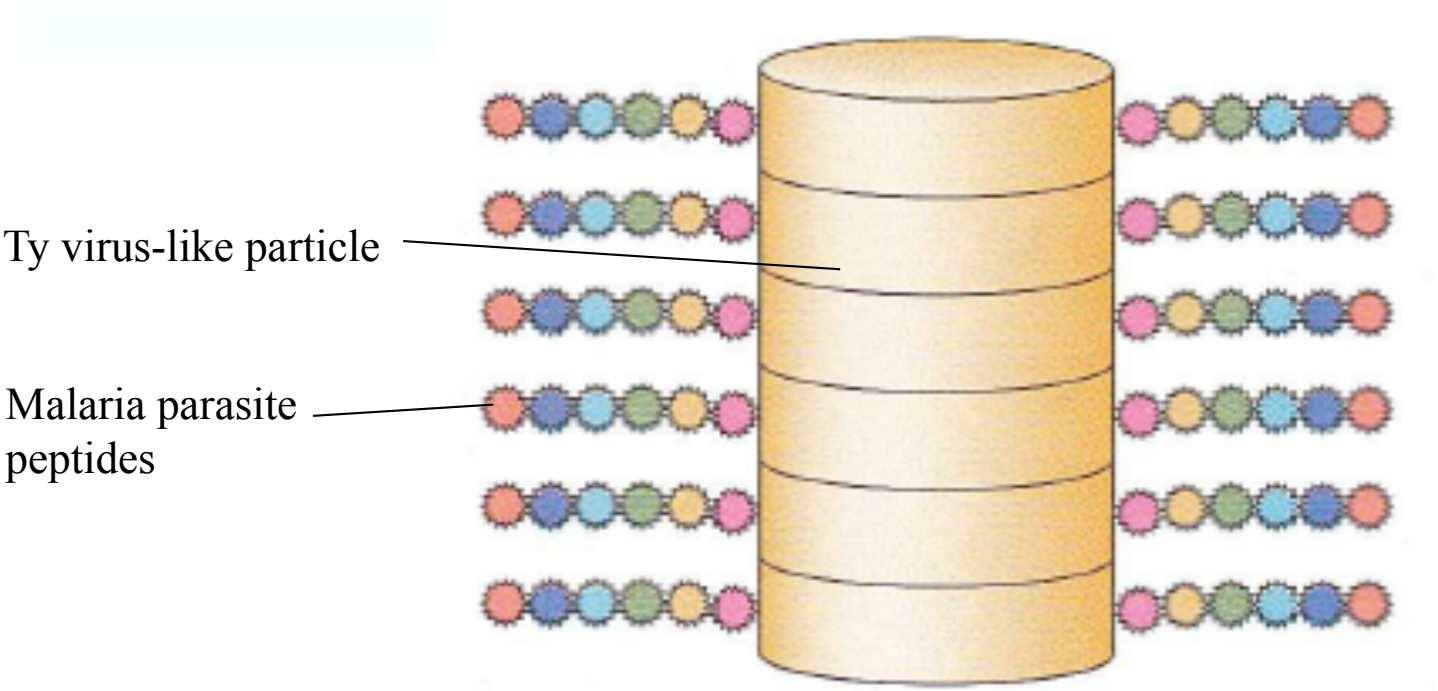
Limitations of using short peptides as vaccines

Limitations of using short peptides as vaccines are:

- To be effective, an epitope must consist of a short stretch of contiguous amino acids, which does not always occur naturally
- The peptide must be able to assume the same configuration as the epitope in the intact viral particle
- A single epitope may not be sufficient to be immunogenic
- Antibodies generated against soluble proteins or individual peptides, even when coupled to a carrier, are often insufficient to completely protect against an infective agent

Development of a subunit vaccine

Virus-like self-assembling particles



Short peptides can be expressed fused to proteins which spontaneously self-assemble into nanoparticles which are frequently highly immunogenic

This strategy has been used with:

- FMDV VP1 peptide 142-160 fused to the highly immunogenic hepatitis B core protein (HBcAg) which self-assembles into “27-nm particles”

- Plasmodium falciparum (malaria parasite) epitopes fused to pI protein of retrotransposon Ty1 of yeast

DNA Vaccines:
Genetic Immunization

DNA Vaccines

Genetic immunization with DNA

DNA can be used directly for immunization - the DNA is incorporated into the host cells where the antigen is synthesized.

DNA carrying an antigen gene under the control of a suitable (usually viral) promoter can be introduced into cells in the host by:

- Biolistic delivery of gold microparticles coated with DNA into the ears of mice (10-100 ng per mouse)
- Direct intramuscular or intradermal injection of large quantities of DNA (100 µg per mouse)
- Mucosal immunity can be achieved by delivery of DNA to mucosal surfaces - cationic liposomes have been used to deliver DNA to the respiratory tract

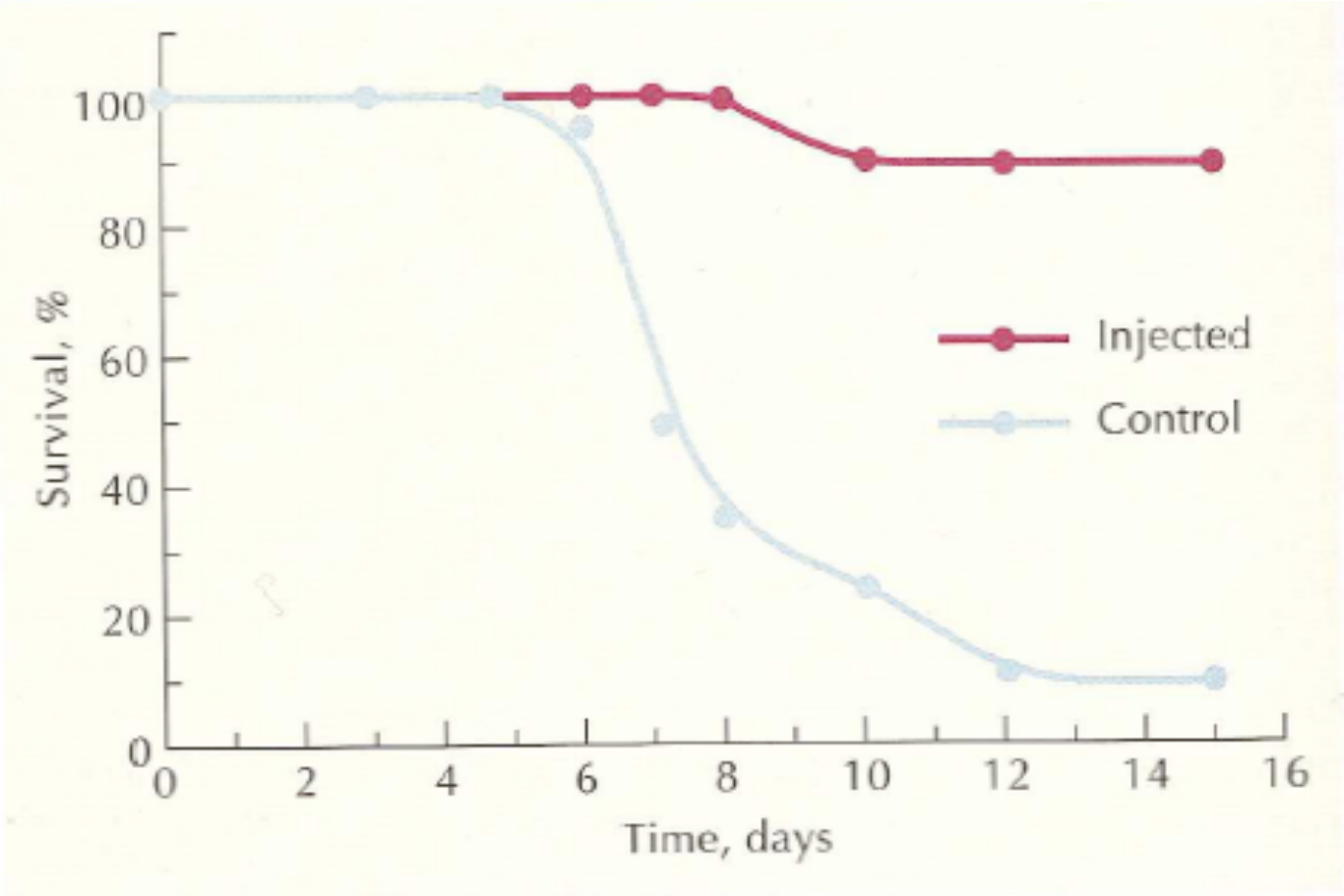
DNA Vaccines

Advantages of genetic immunization over conventional vaccines

- Cultivation of dangerous infectious 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 may cause disease in young or immunocompromised animals will not be 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.

DNA Vaccines

Survival of DNA-immunized mice

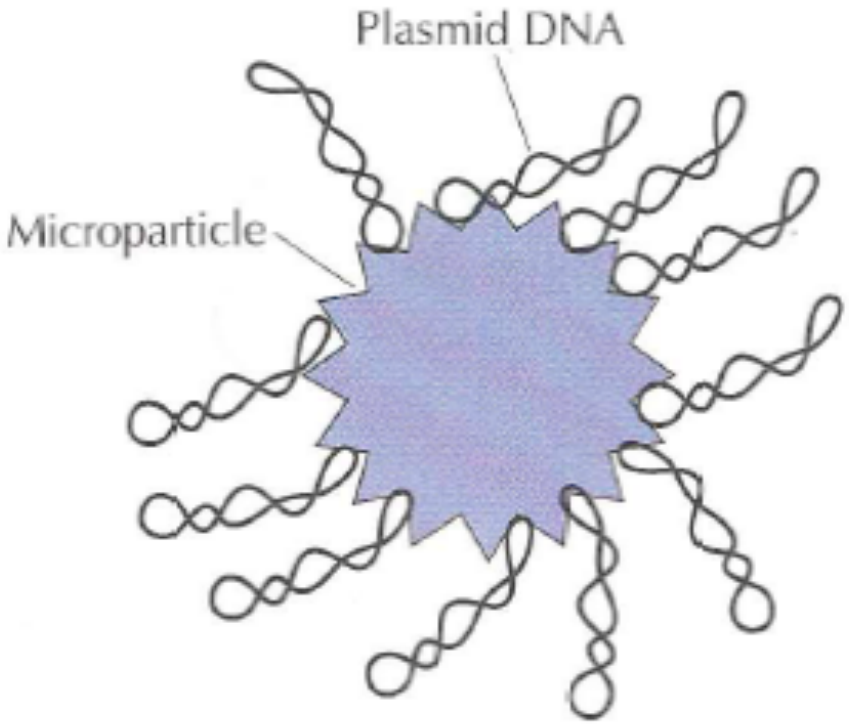


Mice were immunized by injecting DNA containing the influenza A virus nucleoprotein gene under the control of the Rous sarcoma virus promoter on an *E. coli* plasmid into the leg muscle

Control mice were injected with plasmid DNA only

DNA Vaccines

Reducing the amount of DNA required using microparticles



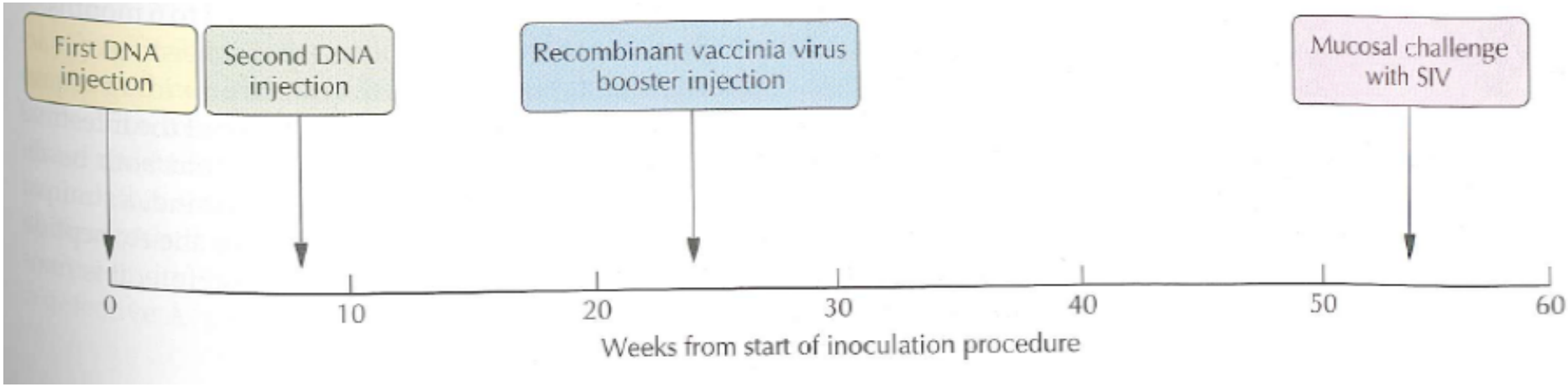
Milligrams of DNA is required for genetic immunization of large mammals (e.g. humans) which can be prohibitive

The amount of DNA required can be reduced ~250-fold by using biodegradable microscopic (0.3 to 1.5 μm) polymeric particles with a cationic surface that binds DNA

Plasmid DNA bound to these particles is slowly released after inoculation (75% released by day 14)

DNA Vaccines

Vaccination of rhesus macaques against simian immunodeficiency virus (SIV)



At 0 and 8 weeks the monkeys were injected with DNA expressing SIV proteins Gag, Pol, Vif, Vpx and Vpr

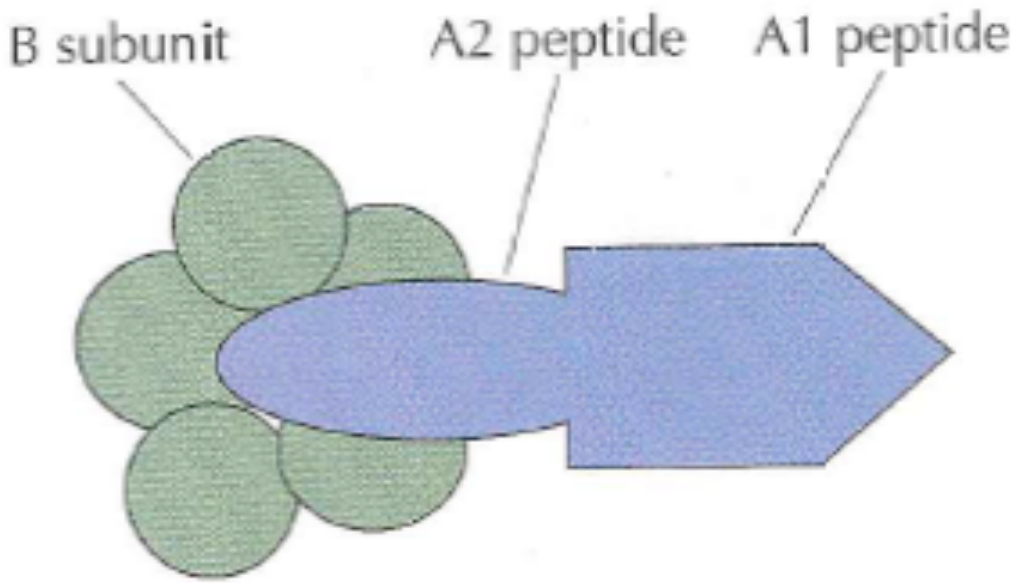
At 24 weeks the monkeys were injected with recombinant vaccinia virus, expressing SIV proteins Gag and Pol

This regimen protected against SIV infection at 7 months and confers immunity against a mucosal viral challenge, effectively blocking entry of the virus into the host

Attenuated Vaccines

Attenuated Vaccines

Construction of genetically modified organisms for live vaccines - cholera



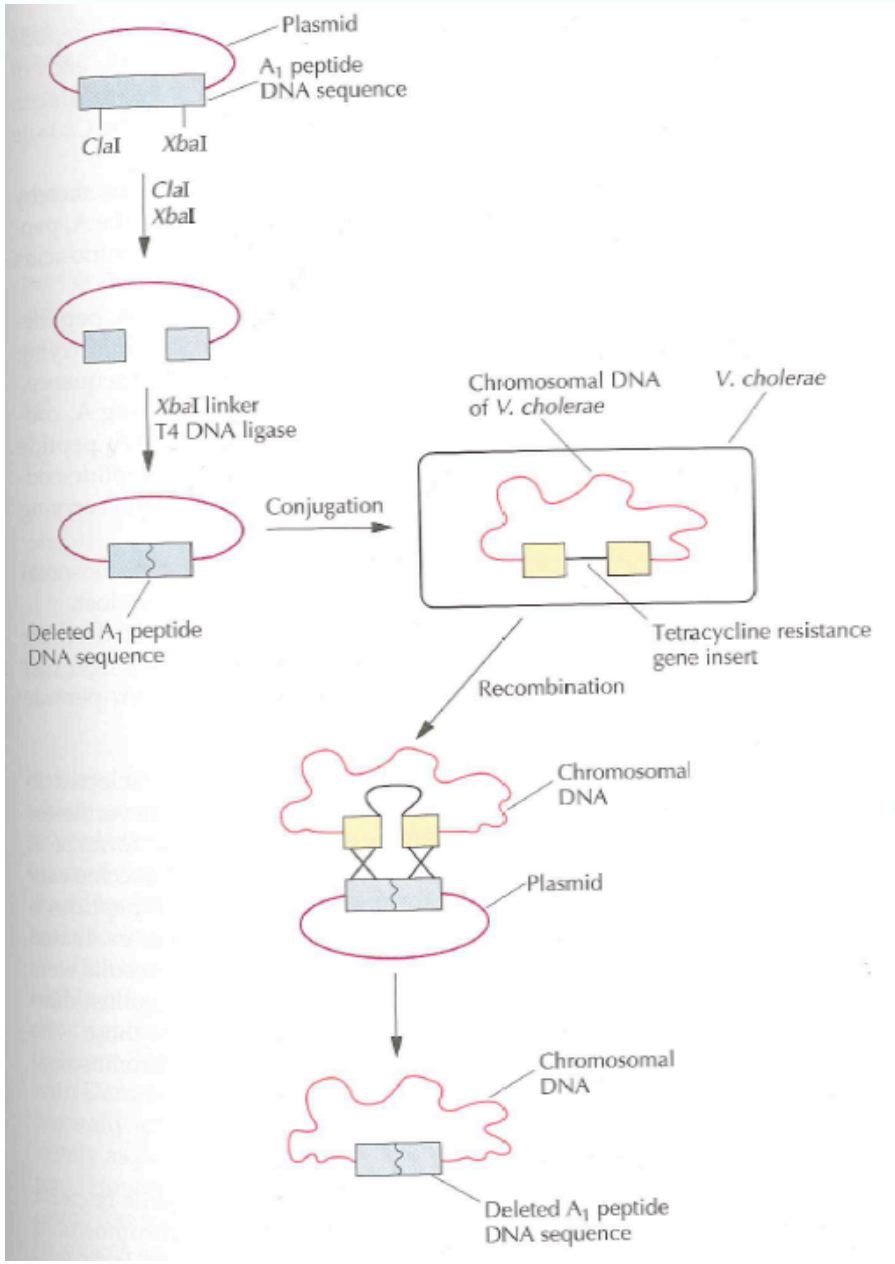
The pathogenic agent of cholera is the hexameric cholera toxin, which is secreted from the bacterium *Vibrio cholerae* in large quantities in the small intestine

The current vaccine uses phenol-killed *V. cholerae* but only generates moderate protection which normally lasts for only 3 to 6 months

Hence, a strain of *V. cholerae* was created with a deletion in the coding sequence for the A1 peptide

Attenuated Vaccines

Construction of genetically modified organisms for live vaccines - cholera



Strategy for deleting part of the coding sequence for cholera toxin A1 peptide

The A1 peptide gene in the V. cholerae genome was first replaced by a tetracycline resistance gene.

The tetracycline resistance gene was then replace by the gene for A1 peptide with an internal deletion

However

Results were equivocal - the strain conferred nearly 90% protection against diarrheal disease, but induced side effects in some of those tested

The strain may require modification at another chromosomal locus

Attenuated Vaccines

Construction of genetically modified organisms for live vaccines - *Salmonella*

Deleted gene	Gene function
galE	Synthesis of lipopolysaccharide; decreases toxicity from galactose
aroA, *aroC*, or *aroD*	Synthesis of chorismate, an aromatic amino acid, and PABA precursor. PABA is involved in the synthesis of iron chelators.
purA or *purE*	Synthesis of purines
asd	Peptidoglycan and lysine biosynthesis
phoP and *phoQ*	Regulation of acid phosphatases and genes necessary for survival in the microphage
cya	Encodes adenylate cyclase, which is involved in cAMP synthesis
crp	Encodes cAMP receptor. Regulates expression of proteins involved in transport and breakdown of carbohydrates and amino acids.
cdt	Involved in tissue colonization by the bacterium
dam	Encodes DNA methylase. Appears to be a master switch for 20–40 different virulence genes.
htrA	Encodes a stress-induced polypeptide. Results in significantly reduced persistence in human tissues.

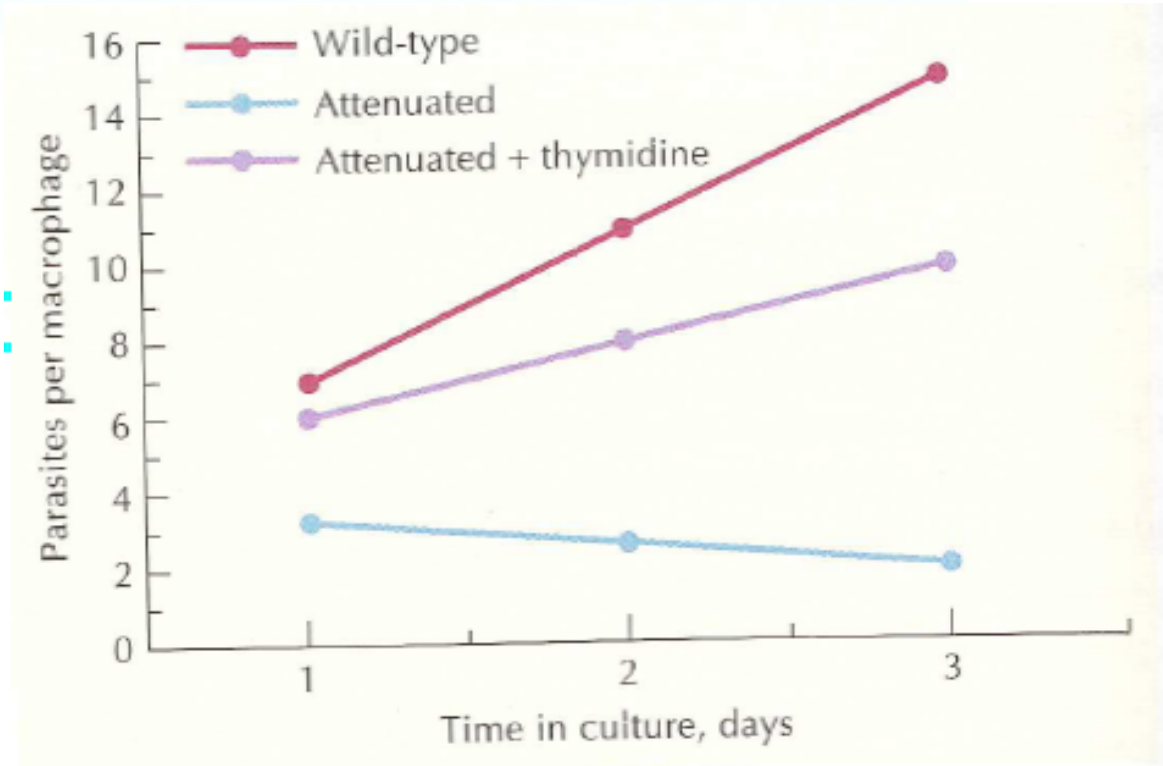
cAMP, cyclic AMP; PABA, *p*-aminobenzoic acid.

A variety of genes have been deleted to develop attenuated strains of *Salmonella* spp.

At least two deletions are preferred in attenuated strains to reduce the risk of reversion

Attenuated Vaccines

Construction of genetically modified organisms for live vaccines - *Leishmania*



Leishmania spp. are protozoan parasites. Attenuated strains of *Leishmania* often revert to virulence

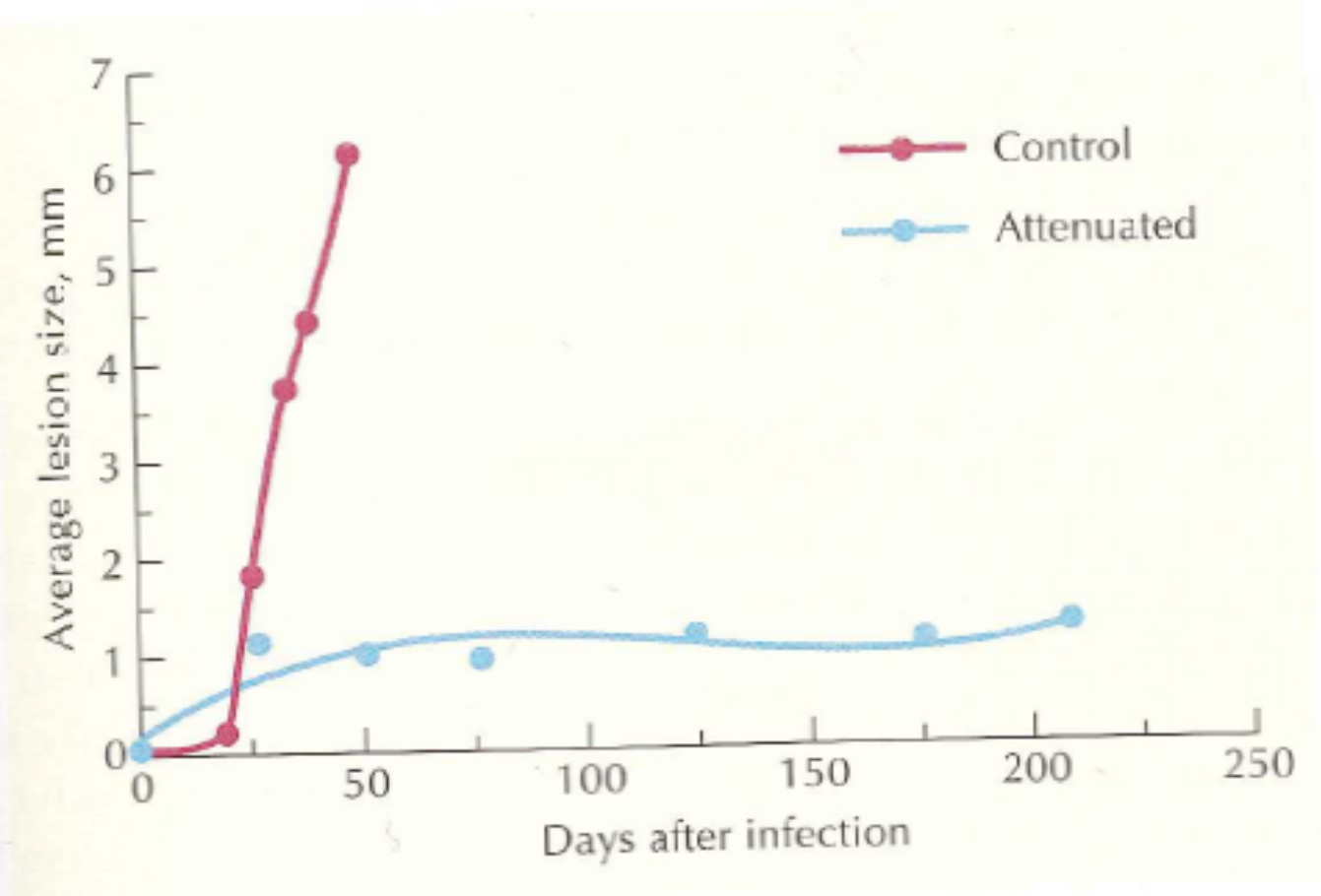
Hence, an attenuated strain was developed in which the two dihydrofolate reductase-thymidylate synthase genes were replaced by genes encoding resistance to the antibiotics G-418 and hygromycin

The attenuated strain requires thymidine in the growth medium to replicate in macrophages

The attenuated strain only survives for a few days in mice, does not cause disease, but does induce substantial immunity

Attenuated Vaccines

Construction of genetically modified organisms for live vaccines - *Leishmania*



Immunity to virulent *Leishmania major* induced in BALB/c mice inoculated with attenuated *L. major*

Size of parasite-induced lesions was measured at various times after challenge with virulent *L. major*

Therefore, the attenuated *L. major* protects against virulent *L. major*

Attenuated Vaccines

Construction of genetically modified organisms for live vaccines - *Herpes Simplex*

Developing a non-virulent Herpes Simplex Virus (HSV) is important as:

- Inactivated vaccines have been unsuccessful in inducing immunity
- Subunit vaccines have been unsuccessful in inducing immunity

A doubly deleted HSV has been created which is unable to proliferate in host cells, and the probability that both sets of functions can be simultaneously reacquired is very small

This replication-defective strain induces protective immunity that can reduce acute viral shedding and latent infection

Vector Vaccines
Viral antigen delivery systems

Vector Vaccines

Live viral vaccines expressing protein antigens from other pathogens

Live viral vaccines have proven to be very efficient vaccines

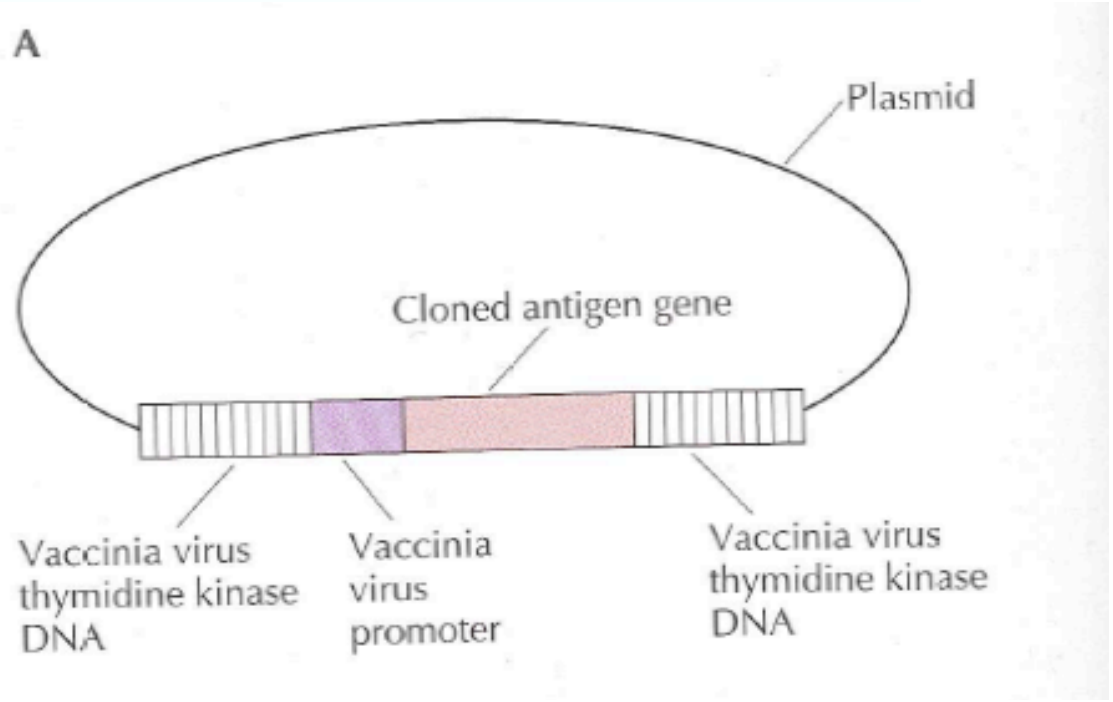
A range of viruses are being engineered to express foreign proteins from other pathogens.

Viruses being used include:

- Vaccinia virus
- Adenovirus
- Poliovirus
- Varicella-roster virus

Vector Vaccines

Integration of antigen genes into vaccinia virus

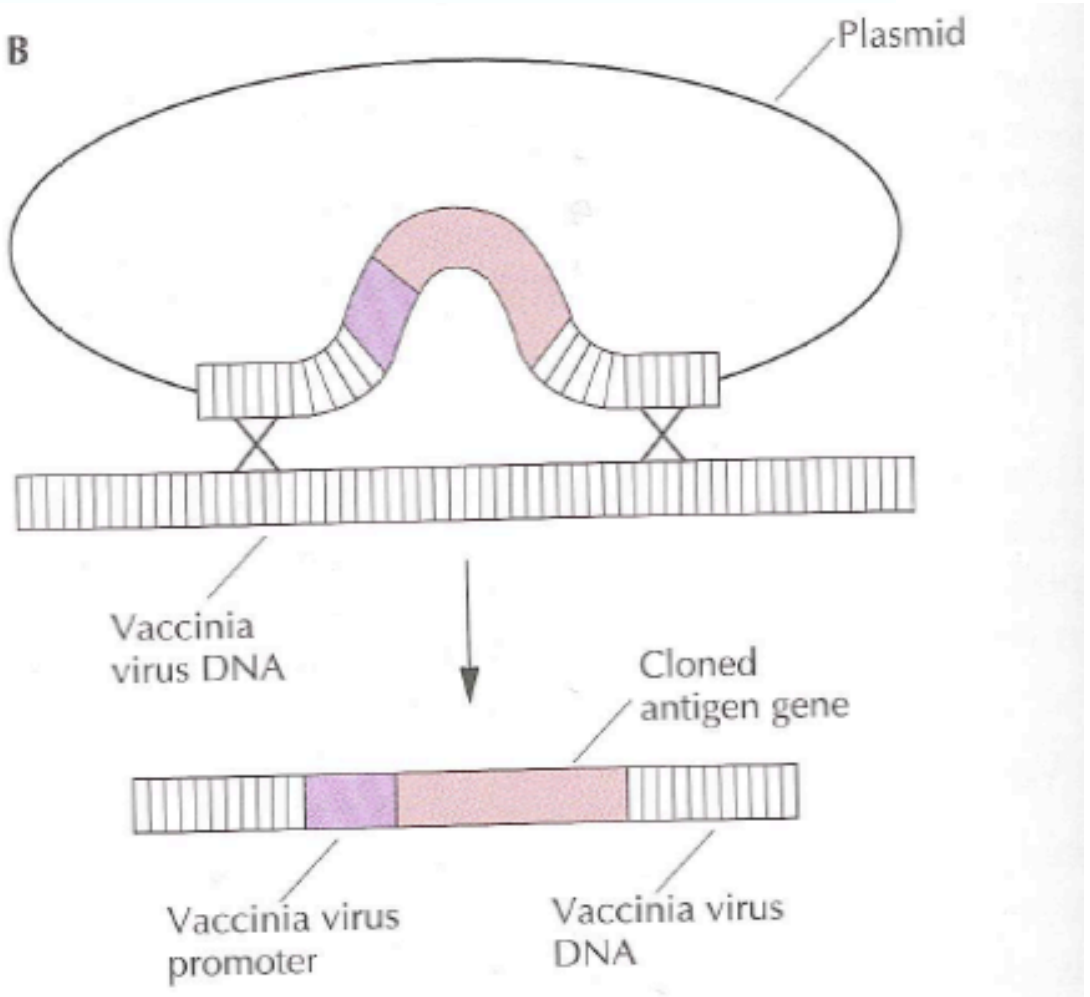


Vaccinia virus has proven a very efficient live vaccine, and it's use has resulted in smallpox being eradicated globally

Cloned antigen genes (from other pathogens) can be inserted into the vaccinia virus (via a double crossover event) genome in order to elicit an immunological response after infection with the recombinant virus

Vector Vaccines

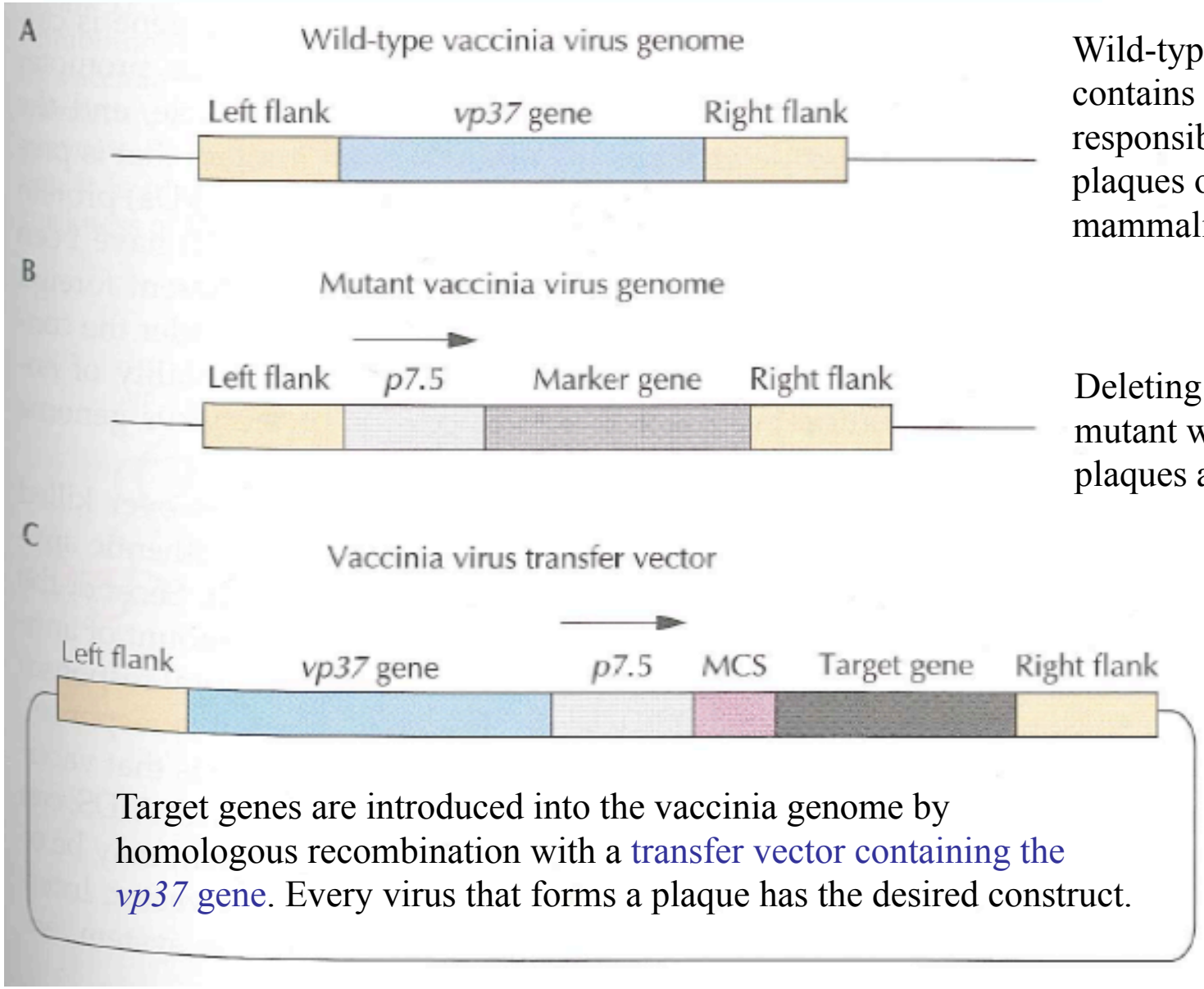
Integration of antigen genes into vaccinia virus



Cloned antigen genes (from other pathogens) can be inserted into the vaccinia virus (via a double crossover event) genome in order to elicit an immunological response after infection with the recombinant virus

Vector Vaccines

A vaccinia vector in which vectors containing the target gene can form plaques



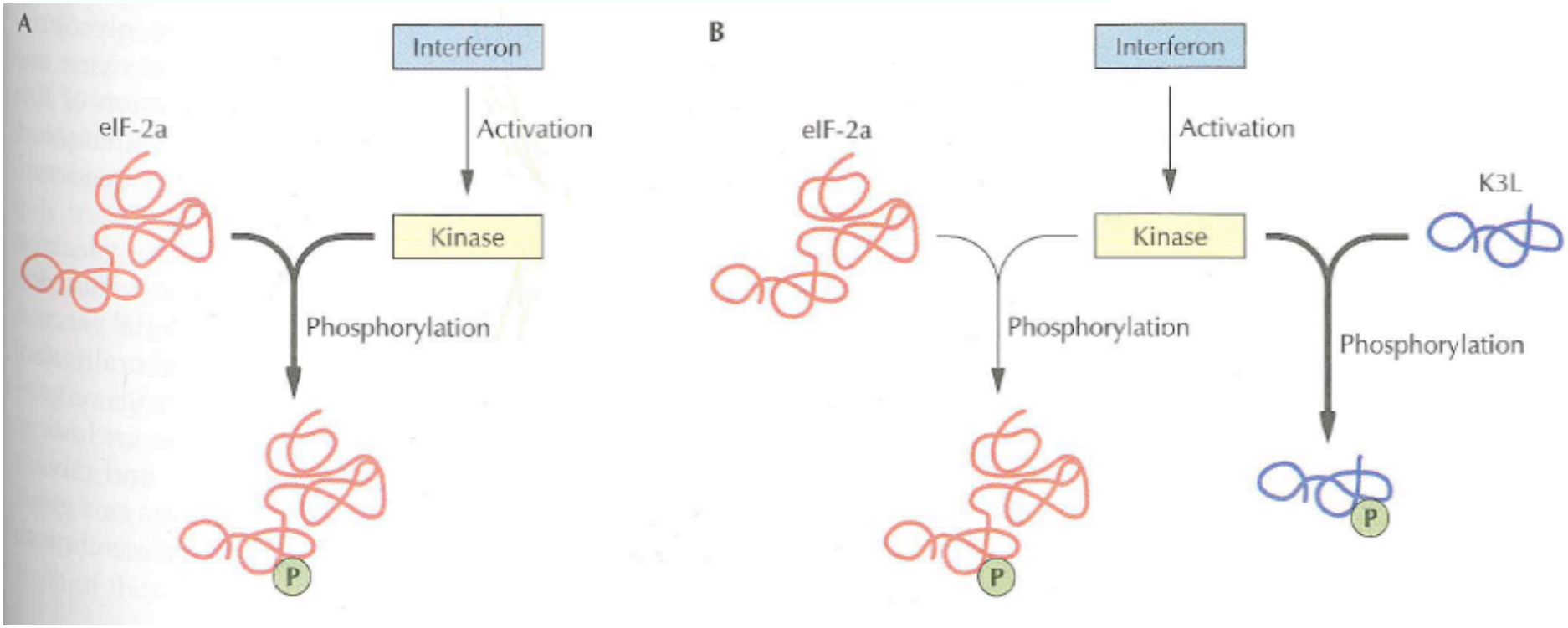
Wild-type vaccinia virus contains the *vp37* gene, which is responsible for the formation of plaques on monolayers of mammalian cells

Deleting the *vp37* gene creates a mutant which does not form plaques after 2 to 3 days

Target genes are introduced into the vaccinia genome by homologous recombination with a transfer vector containing the *vp37* gene. Every virus that forms a plaque has the desired construct.

Vector Vaccines

Interferon-sensitive vaccinia virus vectors



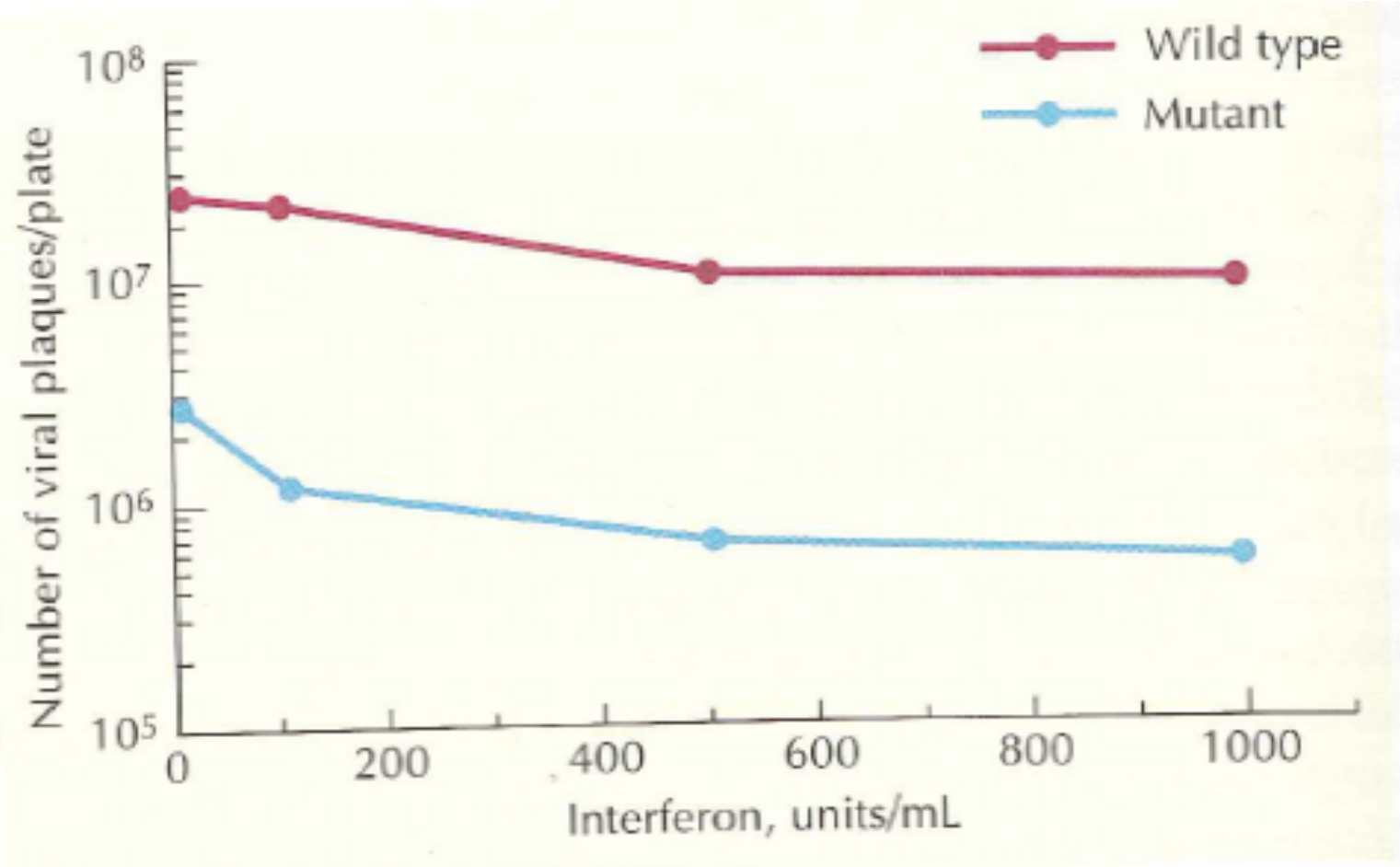
Construction of a vaccinia vector sensitive to interferon allows the virus to be removed (if necessary) by treatment with interferon

In uninfected cells, interferon activates a kinase, which phosphorylates eukaryotic initiation factor eIF-2a inhibiting protein synthesis

In vaccinia infected cells, the vaccinia protein K3L competes with eIF-2a for the kinase allowing protein synthesis to continue

Vector Vaccines

Interferon-sensitive vaccinia virus vectors



Vaccinia vectors in which the K3L gene is mutated are more sensitive to interferon than wild-type vaccinia virus

Vaccines Against Bacteria

Vaccines Directed Against Bacteria

Reasons to develop vaccines against bacteria

Since the discovery and widespread use of antibiotics there has been little work on developing vaccines for bacterial diseases

However, there are good reasons for developing bacterial vaccines:

- Not all bacteria are readily treated with antibiotics
- The use of antibiotics has resulted in the proliferation of strains resistant to several antibiotics
- Reliable refrigeration facilities are not available in many tropical countries
- It is often difficult to ensure that individuals receiving antibiotics undergo a full course of treatment

Vaccines Directed Against Bacteria

Vaccines against tuberculosis

Currently approx 2 billion people are infected with *M. tuberculosis* and 2 to 3 million deaths per year result from these infections.

Antibiotics have proven very effective, but multiple multidrug-resistant strains are now prevalent.

I some countries, bacillus Calmette-Guérin (BCG), an attenuated strain of *M. tuberculosis* developed between 1906 and 1919 is used as a vaccine. However BCG has some drawbacks:

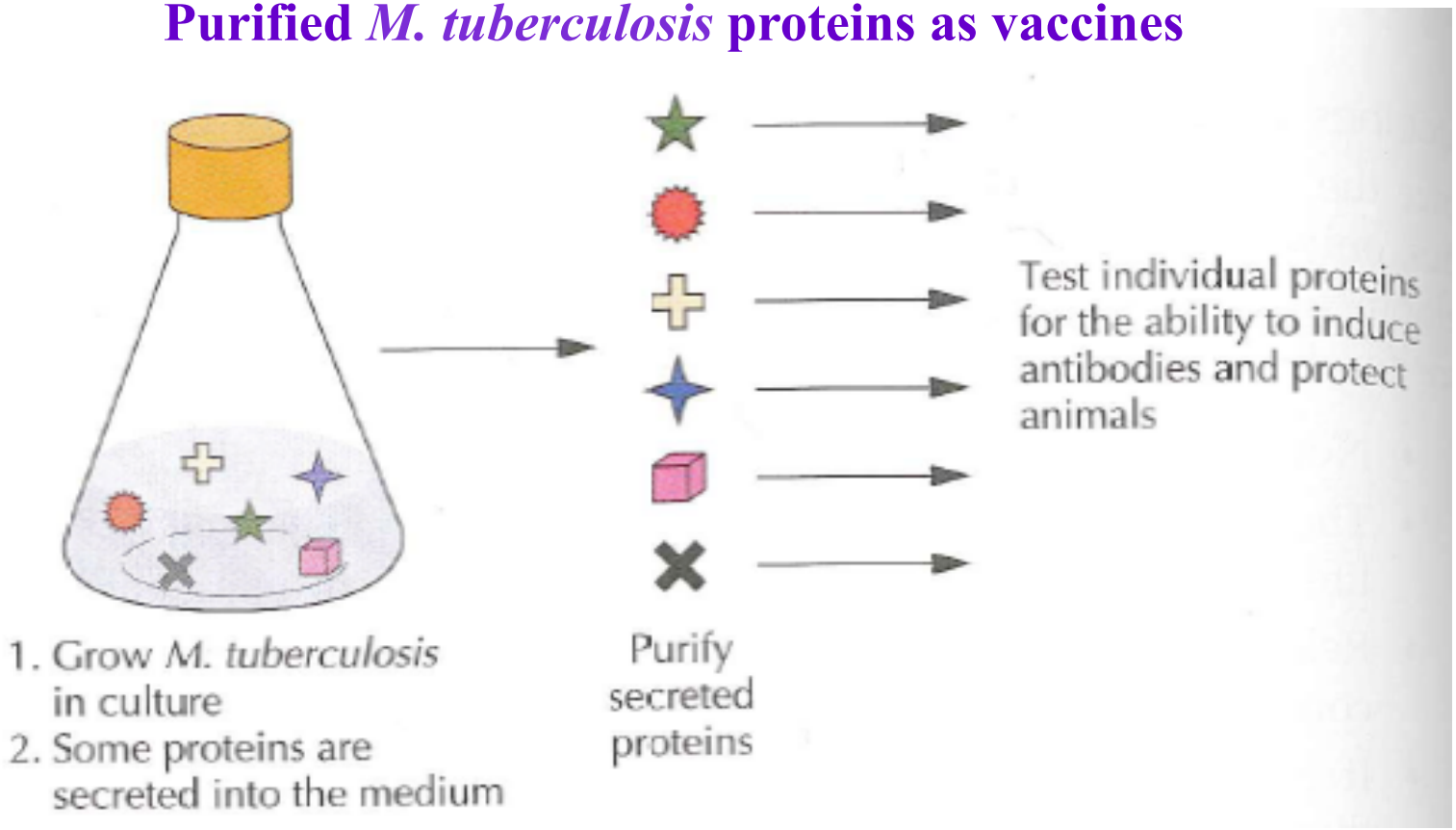
- Live BCG cells can cause tuberculosis in immunocompromised individuals, such as AIDS patients

- Individuals immunised with BCG react positive to the common tuberculosis diagnosis test, making it impossible to see if they are infected or not

For this reason, the use of BCG is not approved in several countries, including the USA

Vaccines Directed Against Bacteria

Purified *M. tuberculosis* proteins as vaccines



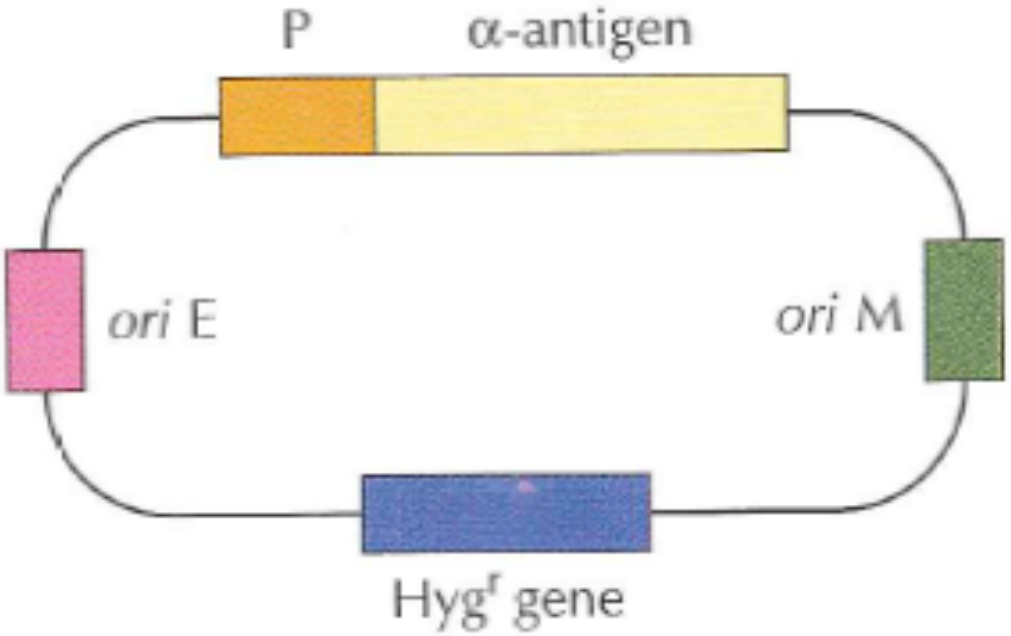
The six most abundant proteins secreted from *M. tuberculosis* in culture were purified and tested individually to determine their ability to produce antibodies and protect guinea pigs against *M. tuberculosis*

Some combinations of these proteins were nearly (but not quite) as effective in protecting the animals as BCG

One of these proteins was the 30-kDa mycolyltransferase, also known as the α-antigen

Vaccines Directed Against Bacteria

Making BCG a more effective vaccine



The α-antigen of *M. tuberculosis* was cloned into an *E. coli* plasmid, the plasmid (above) was purified, and introduced into BCG by electroporation.

P = the α-antigen promoter
ori E = *E. coli* origin of replication
ori M = *Mycobacterium* origin of replication
Hyg^r gene = hygromycin resistance gene

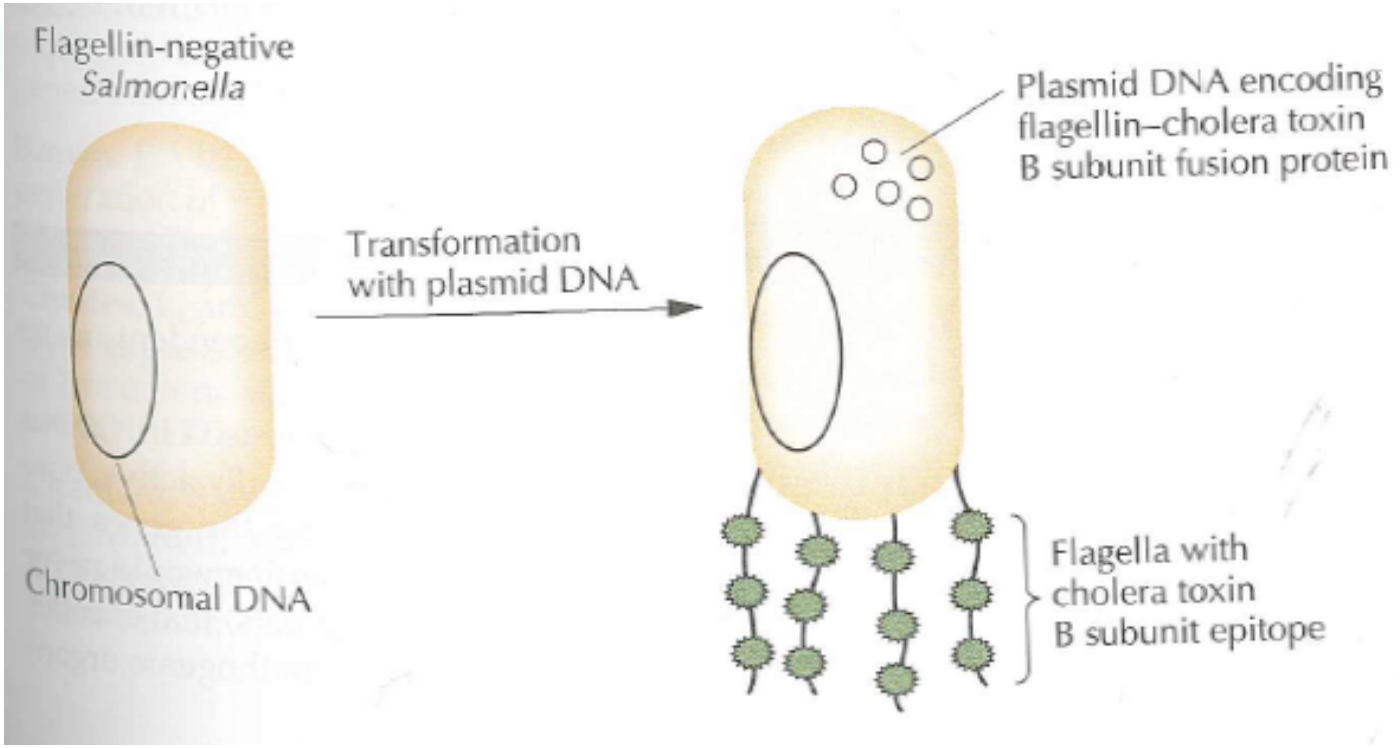
The vaccine results in high levels of expressed α-antigen and is more potent than the currently available commercial vaccine (in guinea pigs)

Vector Vaccines

Bacteria as antigen delivery systems

Bacteria as Antigen Delivery Systems

Expressing antigens from pathogenic bacteria on non-pathogenic bacteria



A cholera vaccine was engineered by inserting a synthetic oligonucleotide coding for residues 50 to 64 of cholera toxin B into a proportion of the *Salmonella* flagellin gene that varies considerably from one strain to another (the hypervariable region).

The construct was introduced into a flagellin-negative strain of Salmonella resulting in bacteria with functional flagella displaying the cholera toxin B epitope

Intraperitoneal injection of live ot formalin-killed bacteria elicited high levels of antibody against the peptide and intact cholera toxin B. Two or three different epitopes can be inserted into a single flagellin gene