Imperial College London

What are the new approaches to vaccines?

BSc Global Health Lecture

Dr Jamie Mann

30th October 2012

What is a vaccine?

"*A vaccine is a suspension or preparation of killed bacteria that is used to stimulate the production of antibodies in the animal to which it is administered.*"

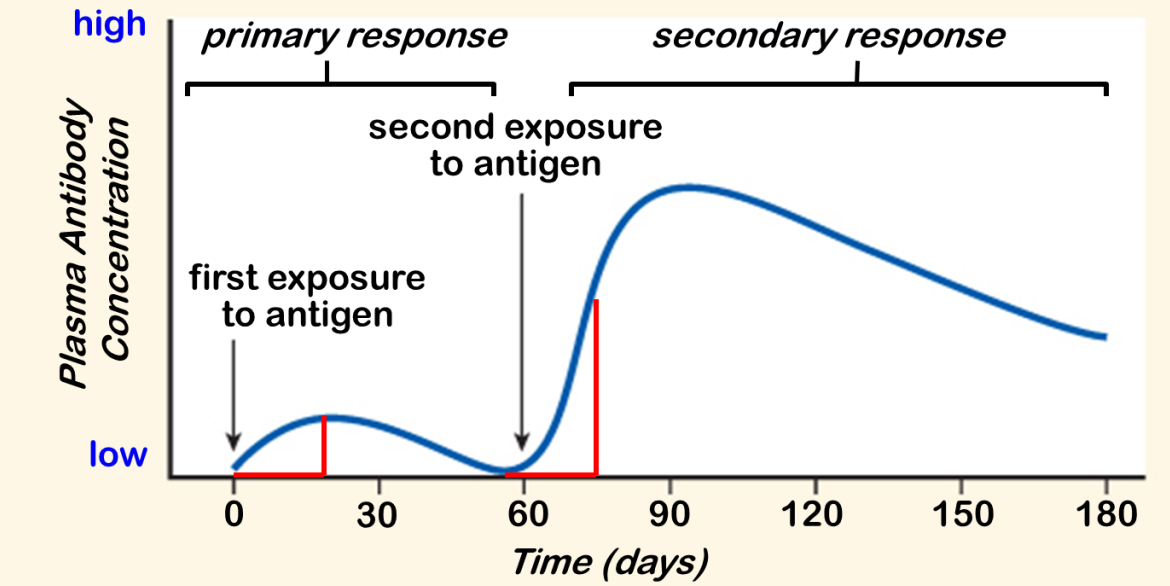
Alexander Fleming, BMJ. 1939.

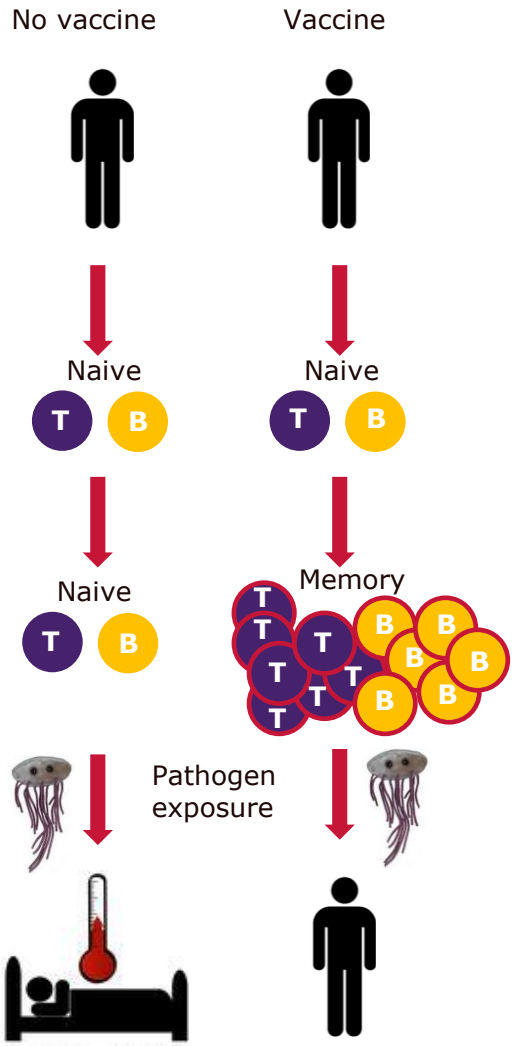
What is our understanding of a vaccine in 2012?

A modified form of the natural immunogen (vaccine) is administered so that the host develops B and T memory cells that can act against the natural immunogen.

What is the purpose of a vaccine?

Adaptive Immunity has Memory



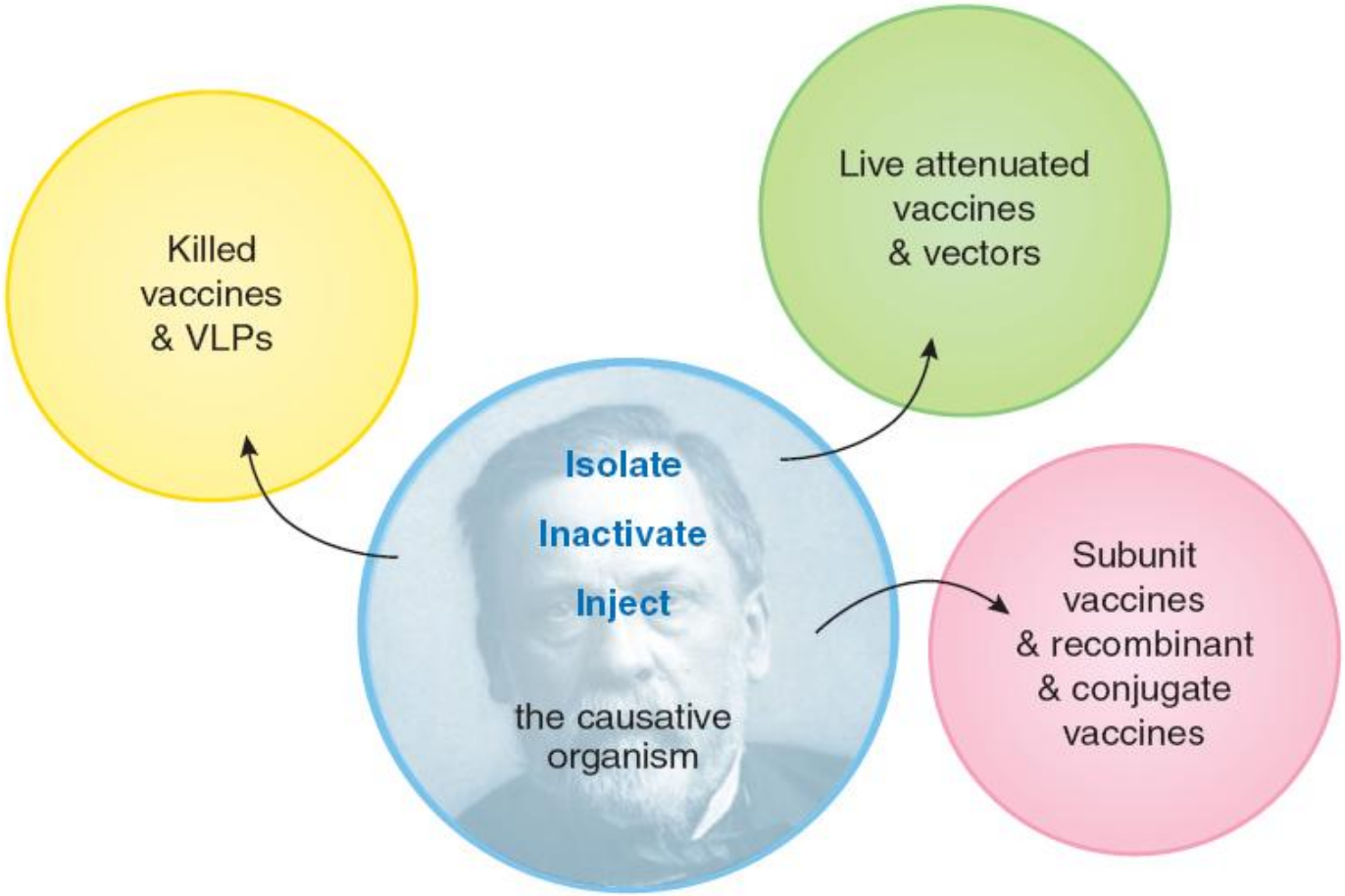


A historical perspective on vaccination.

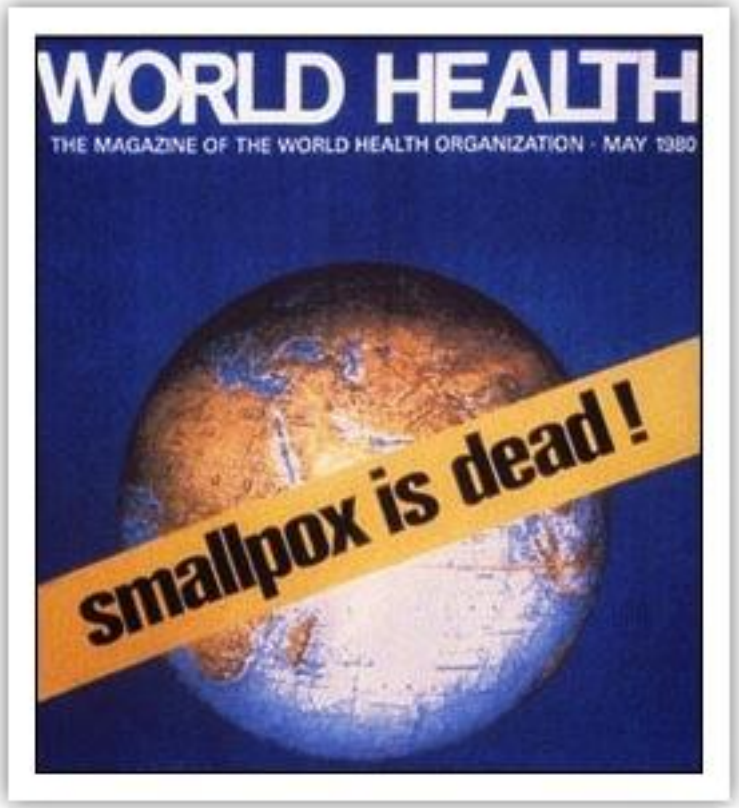
Edward Jenner

Louis Pasteur

Vaccines licensed so far have all been developed by following Pasteur's principles : "isolate, inactivate and inject" the causative agent.



Eradication of small pox - 1980



WHO polio vaccination campaign - 1988

95% reduction

Eradicated in 175 countries

467 cases last year, 171 this year

The requirement for new vaccine concepts.

1) Despite the success of current vaccines, there is a clear need for the development of vaccines against a number of infectious agents for which vaccines are - not available
- inadequate.

Human immunodeficiency virus (HIV),
hepatitis C virus (HCV),
RSV,
Neisseria Meningitides serotype B,
Group A and B streptococcus,
tuberculosis (TB)
malaria.

Unfortunately these pathogens have proven difficult to control with traditional; vaccine technologies, so novel approaches will be required.

The requirement for new vaccine concepts.

2) New vaccines may be needed to protect against a number of emerging or re-emerging infectious diseases.

severe acute respiratory syndrome (SARS),

Ebola,

Hanta,

Dengue

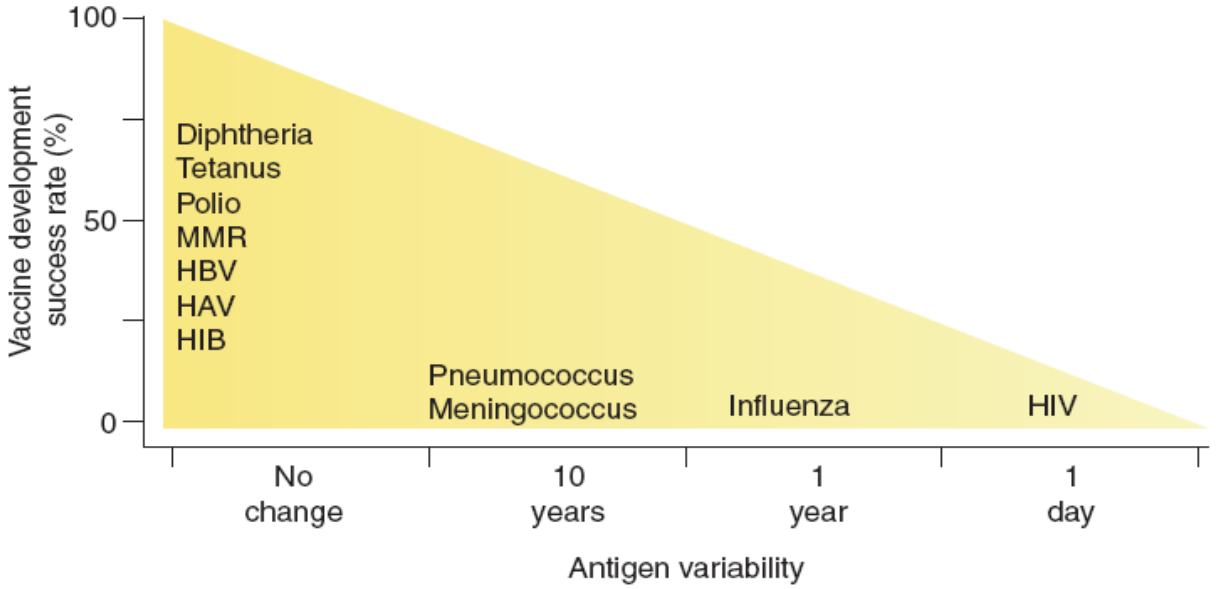
The requirement for new vaccine concepts.

3) ***Improved vaccines are necessary to protect against the continued threat of pandemic strains of influenza and drug resistant organisms.***

4) ***Vaccines may also be required to protect against the threat of bioterrorism.***

5) ***Vaccines may also be used as a therapeutic intervention against cancers or chronic diseases.***

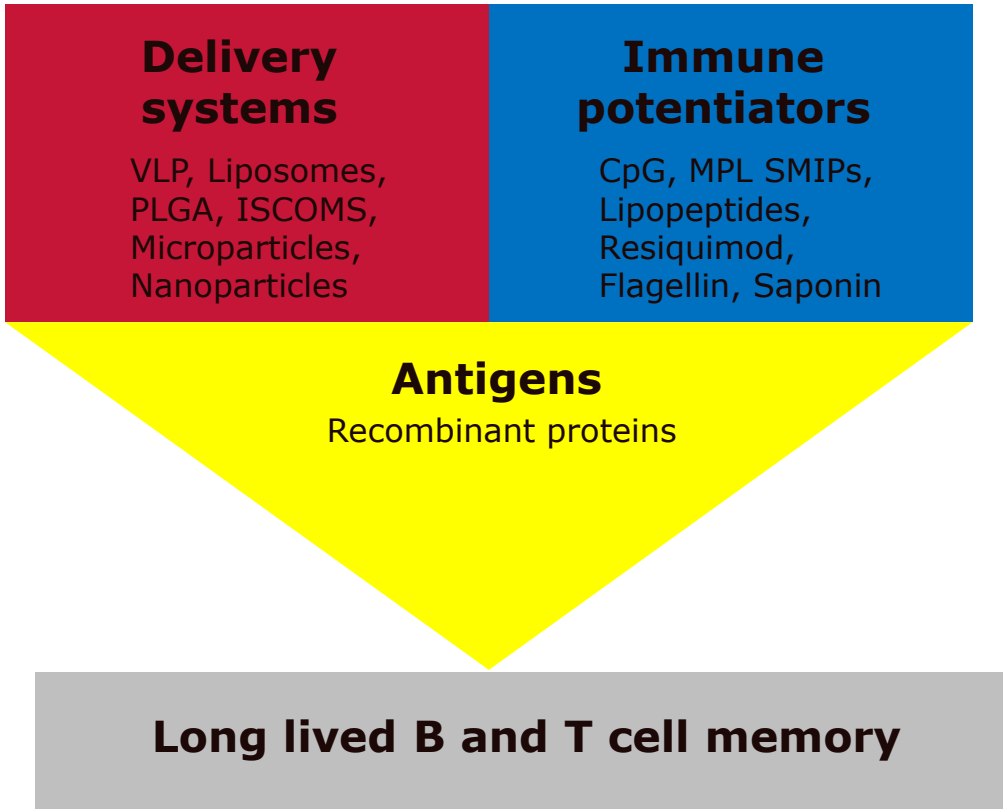
Traditional vaccines are effective at eliciting humoral responses.



And are protective against invariant agents……

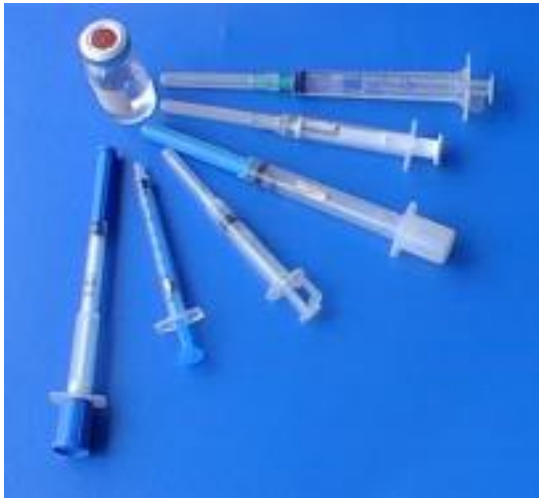
The more diversity, the less protective….

Optimal composition of new generation vaccines



Current delivery strategies

With few exceptions, vaccinations are delivered by injection intramuscularly, subcutaneously, or intradermally



1) Advances in vaccine delivery systems

Microparticles

Nanoparticles

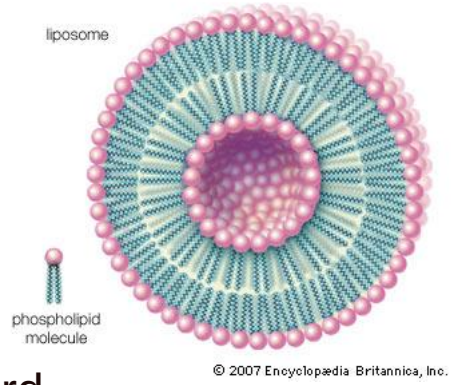
Liposomes

Bacterial vectors

Viral vectors

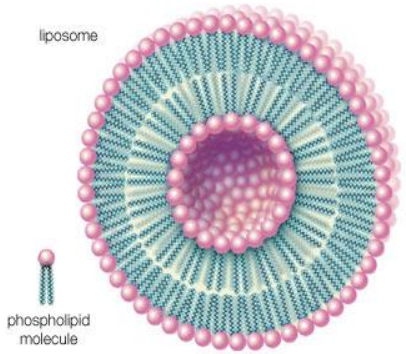
Virus like particles

Cell penetrating peptides

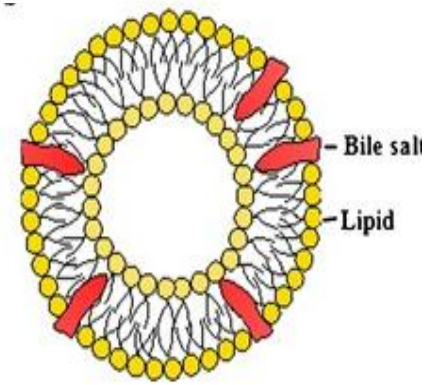


Liposomes:

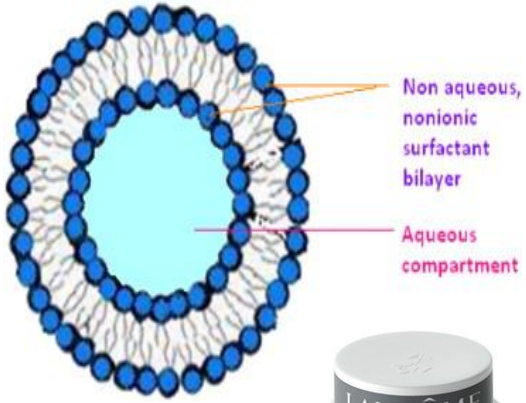
- Invented by Alex Bangham between 1961-1963 and are bilayerd membranes surrounding an aqueous centre.
- Liposomes, can be composed of varying ratios of lipids and cholesterol.
- Liposomes of varying size and charge can be formulated depending on its potential use.
- They can be formulated with antigens to increase the effectiveness of mucosally delivered antigens.
- Some formulations show enhanced stability in acidic solutions, bile and pancreatin – suggesting their suitability for oral delivery.
- Intranasal immunisation with liposome formulated whole cell *Yersinia pestis* enhanced local IgA and IgG and protected against respiratory challenge.
- Nasal administration of *S. mutans*, antigen formulated into liposomes enhanced local secretory IgA in volunteers.
- Despite this, liposomes probably have the greatest potential for success when used with immunomodulatory or targeting molecules co-formulated, entrapped or expressed on the surface.



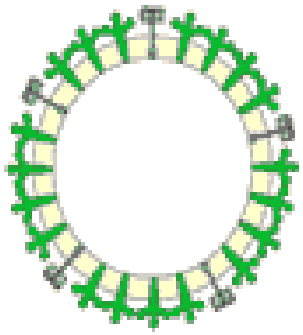
Liposomes



Bilosomes



Niosomes



Virosomes

Viral vectors:

• 28 years since the first published use of recombinant viruses to deliver antigens for vaccine purposes.

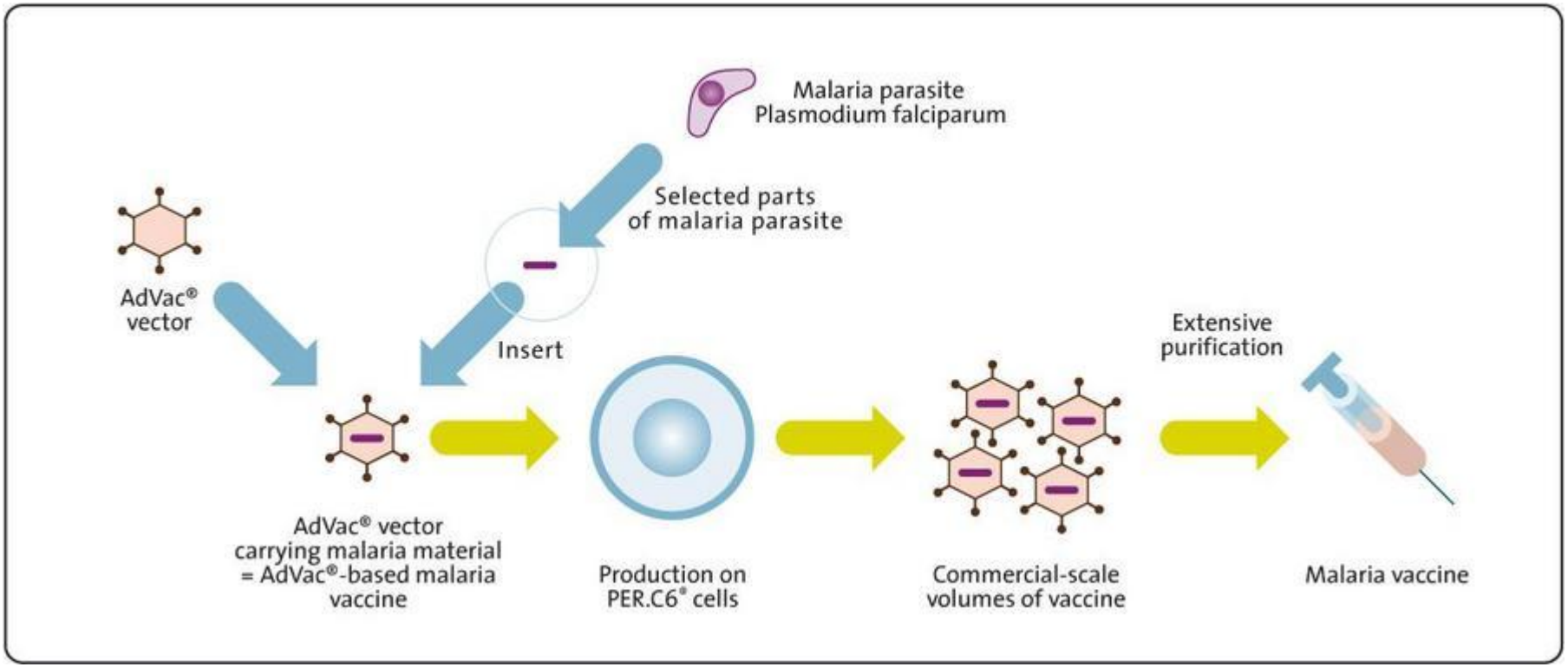
•Vaccinia virus recombinant for hepatitis B surface antigen induced immunity in chimpanzees that was sufficient for protection.

• However issues concerning large scale production, stability and safety have hindered progress to humans.

• A major breakthrough was the realization that replication competence and viral virulence do not always correlate with immunological robustness.

• modified vaccinia virus Ankara (MVA) is a very promising viral vector system because of its versatility for production of heterologous proteins.

- MVA was developed by serial passage in primary chicken embryo cells to serve as a safer vaccine against small pox.
- After 570 passages, MVA lost the broad cellular host range of VV.
- No problems in lab animals or 100,000 humans vaccinated for smallpox - demonstrated a good safety profile.
- Currently in trials for HIV-1, Influenza, measles and dengue fever vaccines.
- Macaque studies using SIV or HIV inserts have shown strong antibody levels and high cytotoxic T cell responses. Protection or reduced viral loads have been seen in challenge studies.
- It has been applied IM and intranasally with the latter generating virus specific immune responses in the genital and rectal tract.

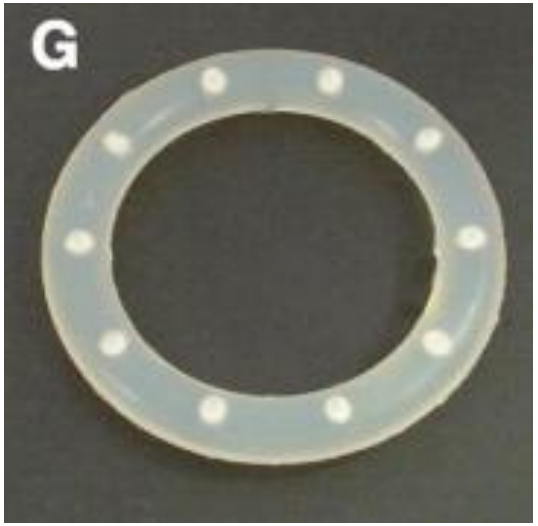

Malaria parasite
Plasmodium falciparum
Selected parts
of malaria parasite
AdVac®
vector
Insert
Extensive
purification
AdVac® vector
carrying malaria material
= AdVac®-based malaria
vaccine
Production on
PER.C6® cells
Commercial-scale
volumes of vaccine
Malaria vaccine

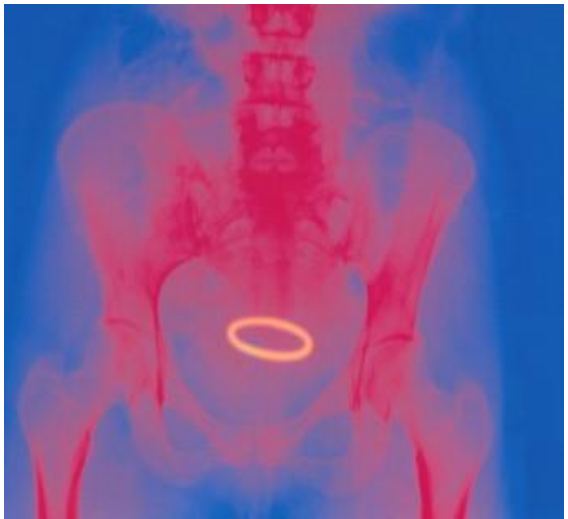
Viral vectors:

Recombinant viral vector	Target	
	Veterinary	Human
Adenovirus	Avian influenza virus, *Mycobacterium tuberculosis* and foot-and-mouth disease virus	*Plasmodium falciparum*, *M. tuberculosis*, influenza virus, HIV-1 and hepatitis C virus
Bacteriophage in *Shigella*	None	*M. tuberculosis*
Attenuated canarypox virus	Equine influenza virus, West Nile virus (WNV), rabies virus, feline leukaemia virus and canine distemper virus	HIV-1 and cancer
Fowlpox virus (FPV)	Avian influenza virus, FPV and Newcastle disease virus (NDV)	Cancer
NDV	Avian influenza virus and NDV	None
Turkey herpesvirus	Infectious bursal disease virus and Marek's disease virus	None
Flavivirus YFV-17D	WNV	WNV, dengue virus and Japanese encephalitis virus
Lentivirus	None	Melanoma and HIV-1
Measles virus	None	*P. falciparum* and human papilloma virus
Modified vaccinia virus Ankara	*Mycobacterium bovis*	*P. falciparum*, *M. tuberculosis*, influenza A virus, HIV-1 and colorectal, renal, lung and prostate cancer
New York attenuated vaccinia virus	None	*P. falciparum* and HIV-1
Sendai virus	None	HIV-1
Vaccinia virus	Rabies virus	Cancer

YFV-17D, attenuated yellow fever virus strain 17D.

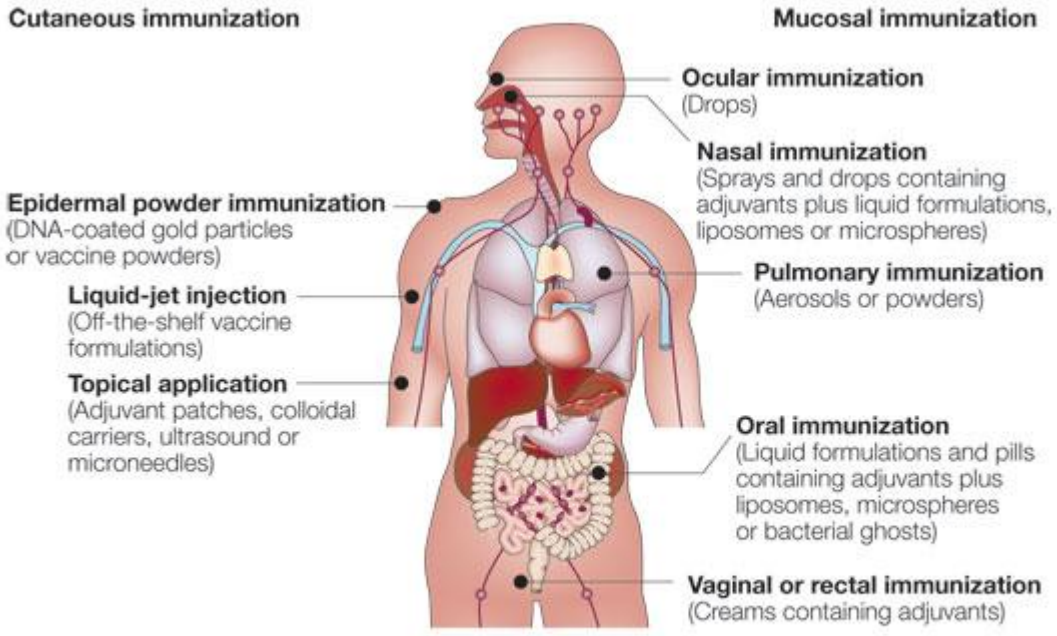
Vaginal rings





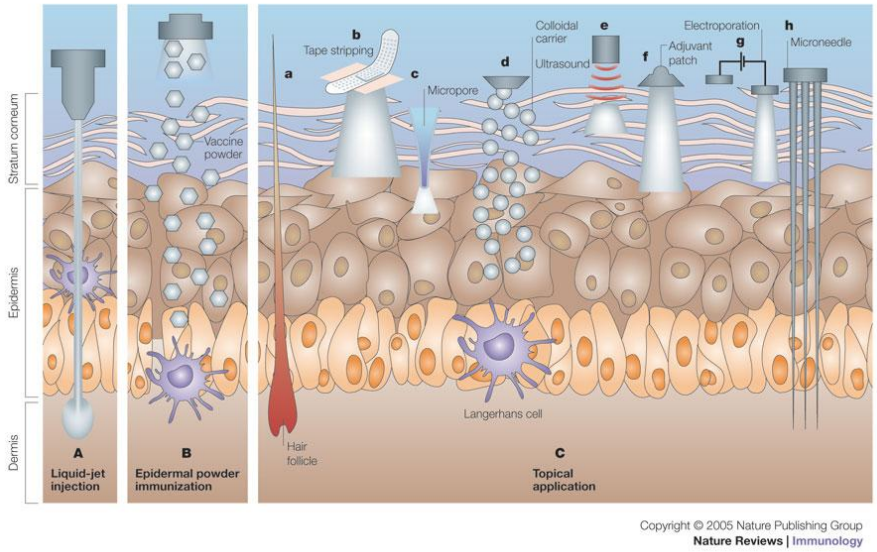
- Flexible o-ring shaped elastomer delivery devices.
- Provide lon-term sustained controlled release of substances to the vagina
- Designed to be self applied – normally adjacent to the cervix.
- contains solid drug (freeze dried rod).
- Pre-clinical studies in the HIV-1 field have shown them to elicit systemic and local mucosal immune responses.
- Human clinical trial are currently underway.

2) Advances in vaccine delivery routes



Nature Reviews | Immunology

Potential ways for cutaneous immunisation



A) Liquid injection: delivers vaccine to muscular, subcutaneous or dermal regions.

B) Epidermal Powders: delivers vaccine powders to the superficial layers of skin where antigen is recognised by Langerhans cells.

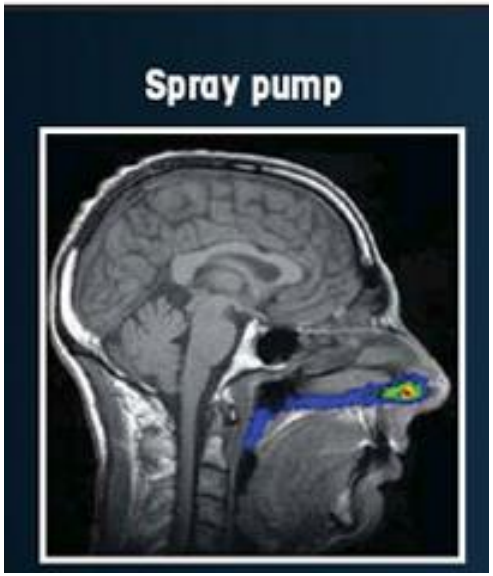
C) Topical application: delivers to the epidermis for recognition and processing by Langerhans cells.

Tape stripping to remove stratum corneum to facilitate vaccine absorption.

Thermal or radio-wave-mediated ablation of the stratum corneum creates micropores that increase vaccine delivery

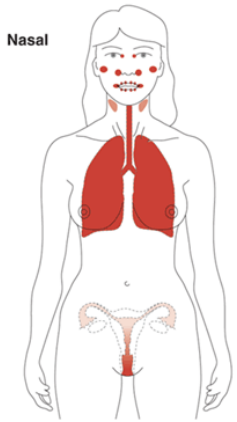
Colloidal carriers such as microemulsions and transfersomes increase dermal absorption of topically applied vaccines

Electroporation of the stratum corneum increases the delivery of DNA vaccines to the epidermis



Pulmonary/nasal immunisation

- Is a promising alternative to parenteral vaccination, as it is non-invasive and can elicit strong systemic and local immune responses.
- Vaccination stimulates the NALT and can generate immune responses in the GI tract and genital tract.
- Intranasal Flumist (MedImmune) provides significant protection against infection. It is licensed for use in Europe
- Antigens are taken up by the FAE overlying the NALT where DCs can prime CD4 or CD8 T cells.
- Flumist is a live attenuated vaccine (LAIV) and in Europe is called Fluenz.
- A volume of 100µl/nostril is optimal in adults but should be reduced for children to avoid dripping.
- Currently being utilised as a deliver route for a number of vaccines



3) Advances in adjuvant technology

• The role of an adjuvant is to improve the immunogenicity of antigens.

Generation 1:

• Canonical member of the first generation of vaccine adjuvants is represented by insoluble aluminium salts (Alum). Identified 1920's.

• Emulsion adjuvants were introduced a decade later by Freund.

• Both Alum and emulsion adjuvants are particulate dispersions (Alum aggregates or emulsion droplets), to which antigen is bound/associated.

• Both were designed to prolong the duration of antigen persistence at injection site (Depot effect).

• Induce inflammation – recruit APCs.

• Alternative particulate carriers were evaluated in the 1970's

  •Liposomes and polymeric particles.

3) Advances in adjuvant technology

Generation 2:

• Generation 2 adjuvants were initiated in the 1970's when additional components were added to generation 1 adjuvants to enhance potency.

• Derived from the discovery that synthetic components activated the immune system – muramyl dipeptide (MDP).

•MDP- was originally identified as the smallest water-soluble component of mycobacterial cell walls with adjuvant activity.

•As independent components, they were not optimally effective, hence they were coupled to or linked to existing generation 1 adjuvants.

•Hence generation 2 adjuvants comprise more than one adjuvant component.

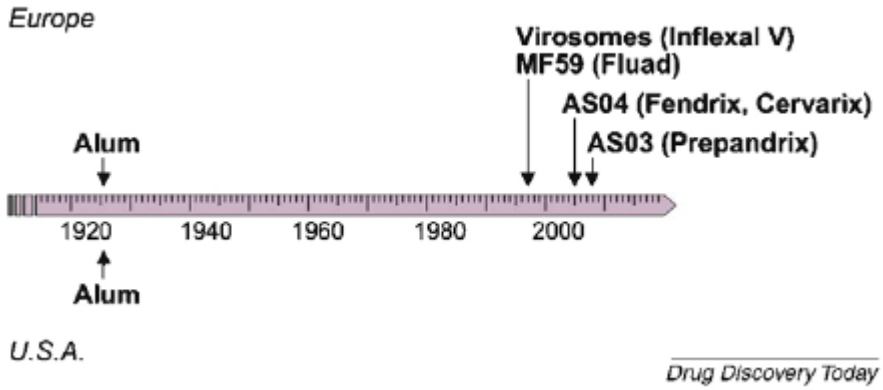
•Despite being around for more than 40 years, generation 2 adjuvants are only now gaining licensure in approved vaccine products.

Adjuvant science is clearly slow and difficult with many failures along the way.

3) Advances in adjuvant technology

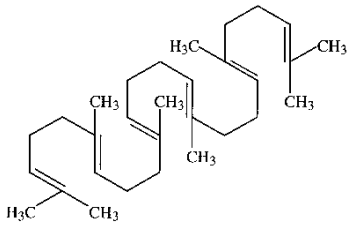
Adjuvant formulations tested in humans

Name	Company	Class	Indications	Stage
Generation 1 adjuvants				
Alum	Various	Mineral salt	Various	Licensed
MF59	Novartis	O/W emulsion	Influenza(Fluad)/pandemic flu	Licensed (EU)
Liposomes	Crucell	Lipid vesicles	HAV, Flu	Licensed (EU)
Montanide	Various	W/O emulsion	Malaria, cancer	Phase III
PLG	Novartis	Polymeric microparticle	DNA vaccine (HIV)	Phase I
Flagellin	Vaxinnate	Flagellin linked to antigen	Flu	Phase I
QS21	Antigenics	Saponin	Various	Phase I
Combination adjuvants – generation 2				
AS01	GSK	MPL + liposomes + QS21	Malaria, TB	Phase II
AS02	GSK	MPL + O/W emulsion + QS21	Malaria	Phase II
AS03	GSK	O/W emulsion + α tocopherol	Pandemic flu (Pandemrix)	Licensed (EU)
AS04	GSK	MPL + Alum	HBV (Fendrix), HPV (Cervarix)	Licensed (EU)
RC-529	Dynavax	Synthetic MPL + Alum	HBV	Phase II
Iscom	CSL, Isconova	Saponins + cholesterol + phospholipids	Various	Phase I
IC31	Intercell	Peptide + oligonucleotides	TB	Phase 1
CpG 7909	Coley/Pfitzer Novartis	Oligonucleotide + Alum, oligonucleotide + MF59	HBV, malaria, HCV	
ISS	Dynavax	Oligonucleotide Alum	HBV	Phase II
MF59 + MTP-PE	Chiron/Novartis	Lipidated MDP + O/W emulsion	HIV, Flu	Phase I



- Many adjuvants have been extensively evaluated in preclinical and clinical studies.
- Only Alum is available in the US
- MF59 was successfully introduced to the European market in 1997.
- AS03 and AS04 licensed in Europe.

3) Advances in adjuvant technology

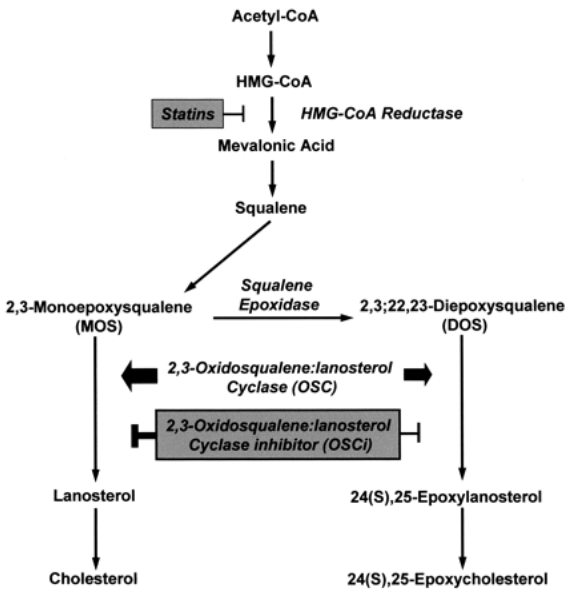


•MF59

• O/W emulsion of squalene oil.

• Squalene oil is a naturally occurring substance found in plants and a range of animal species , including humans.

• Squalene is an intermediate in the human steroid hormone biosynthetic pathway and is a direct synthetic precursor to cholesterol.



• Squalene is biodegradable and biocompatible.

• 80% of shark liver oil is squalene, and sharks provide the original natural source of squalene for the MF59 adjuvant.

• MF59 contains two non-ionic surfactants:

  •Polysorbate 80

  •Sorbitan trioleate 85

} System stabilisers

• MF59 is safe and efficacious in humans.

• MF59 induces high levels of antibody.

3) Advances in adjuvant technology

•**MF59**

• Largest clinical experience with MF59 was with Fluad™ (licensed in over 20 countries).

• 23 million doses of Fluad™ given to date.

• Result is significantly increase antibody titers against flu, compared to conventional non-adjuvanted flu vaccines.

• Particularly important in the elderly population, who are at most risk of for developing the most severe consequences of flu infection.

• MF59 induces immune responses against heterovariant flu strains.

• MF59 has also been evaluated as a potential adjuvant for inclusion in pandemic influenza vaccines.

• MF59 can significantly reduce the vaccine antigen concentration in the formulation.

• MF59 has been tested mainly in adults but has been shown to be safe in neonates and children.

•More potent than Alum – most vaccines tested.

3) Advances in adjuvant technology

- **AS04**

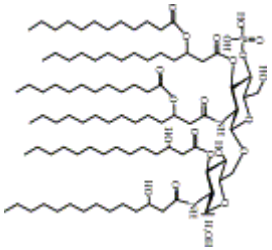
- Adjuvant system 04 (AS04) contains MPLA (3-O-desacyl-4'-monophosphoryl lipid A) adsorbed onto a particulate form of aluminium salt.

- Is currently a component in two licensed vaccines.

  - Human papilloma virus vaccine (Cervarix) which contains virus-like particles (VLPs) of the L1 protein from human papillomavirus (HPV)-16 and HPV-18 oncogenic strains.

  - Hepatitis B virus.

- A third vaccine against herpes simplex 2 virus is in phase III clinical trials.



2) Advances in adjuvant technology

- MPLA is a detoxified derivative of LPS isolated from the gram-negative bacterium *Salmonella minnestoa* R595 strain.
- LPS has been found to function as a specific agonist of TLR4
- MPL signals via TLR4 with reports suggesting TLR2 as well.
- TLR4 stimulation – NFκB – proinflammatory cytokines.
- Results in APC maturation and tolerance inhibition (Tregs suppression).
- MPLA promotes IFN-γ production by $CD4^+$ T cells - T_H1.
- Alum promotes Abs and a T_H2 response.
- Alum acts as a Depot due to its particular nature.
- Aluminium salt stimulates Nlrp3, a component of the inflammasome (intracellular multi-protein platform, for recruitment and activation of caspase-1 and processing of cytokines such as IL-1β and IL-18).

4) Advances in antigen technology

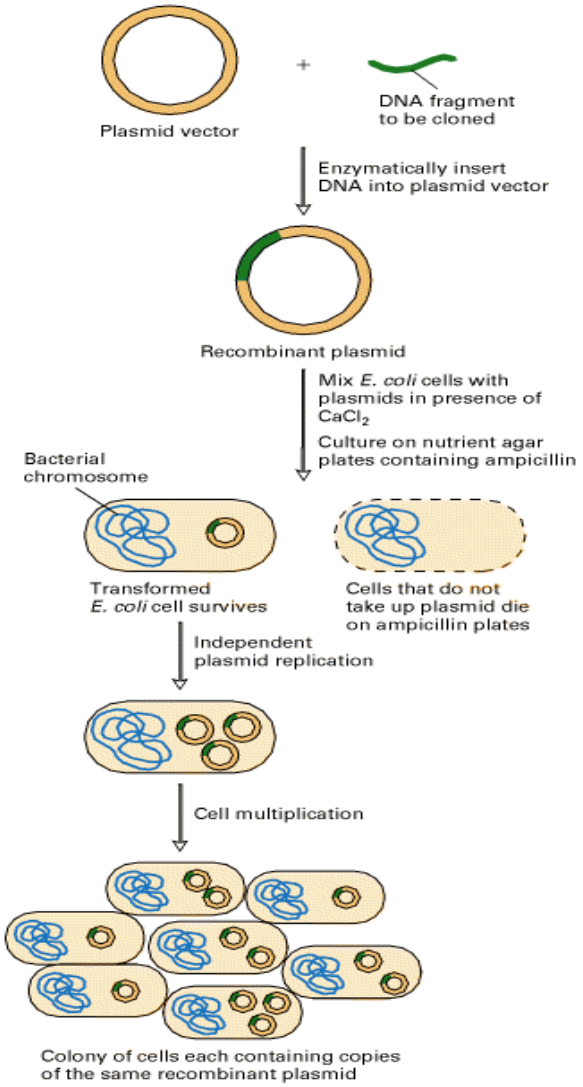
• Initially vaccine antigens were produced by purifying the specific antigens from cultures of the pathogenic virus or bacteria

– expensive, required large scale production facilities and costly downstream processing procedures.

-Risk of accidental release of dangerous organisms (level III) to external environment.

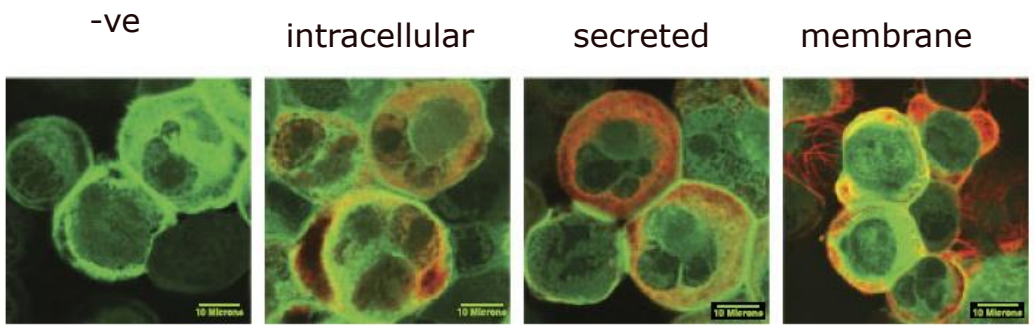
• Molecular biology and genetic engineering have provided the tools and techniques to produce proteins in prokaryotic and eukaryotic systems.

4) Advances in antigen technology

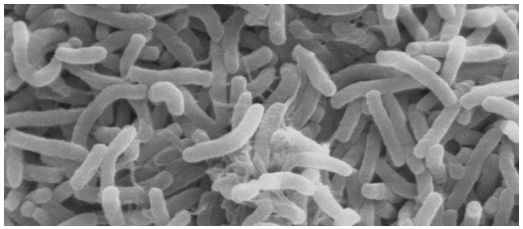


- Vaccine antigens can be tailored such that the antigen can be expressed on cell surfaces, within cells or secreted.

CHO cells transfected with vaccine antigen expressing plasmids

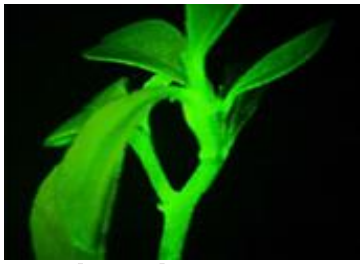


4) Advances in antigen technology



- Bacterial expression systems:
- Veterinary field.
- Production of non-glycosylated proteins.
- Escherichia coli and Salmonella typhimurium.
- protein G of Respiratory syncytial virus, antigens of feline leukaemia virus.
- Efficient and affordable.
- Production of many viral glycoproteins in prokaryotic systems does not result in immunologically protective antigens .
- LPS and other pyrogenic contaminants.
- Therefore for glycoproteins, other expression systems are better.

4) Advances in antigen technology



- Plant expression systems:
- Plants as biofactories has gained much attention.
- Ability to produce glycosylated proteins similar to higher eukaryotes.
- Can be delivered as oral preparations with minimal downstream processing.
- Cereal or oilseed crops also present the possibility of enhanced immungen stability – storage at ambient temperatures.
- Yield is variable.
- Subunit B of cholera toxin (CT) has been expressed in potato tubers. Targeting to endoplasmic reticulum – 0.3% total protein. Targeting to cholorplast can yield 4% total protein.

4) Advances in antigen technology

- Mamalian expression systems:
- Expression is expensive.
- For some viral glycoproteins it is critical.
- Especially for gp's where post-translational modification is important for proper folding and generation of specific epitopes.
- Develop an expression system where gp's are secreted into the media and not retained within the cell.

    1) Overexpression of many proteins leads to cell death due to toxic nature of viral proteins.

    2) Lysing of cells adds extra cost to production – remove cellular debris.

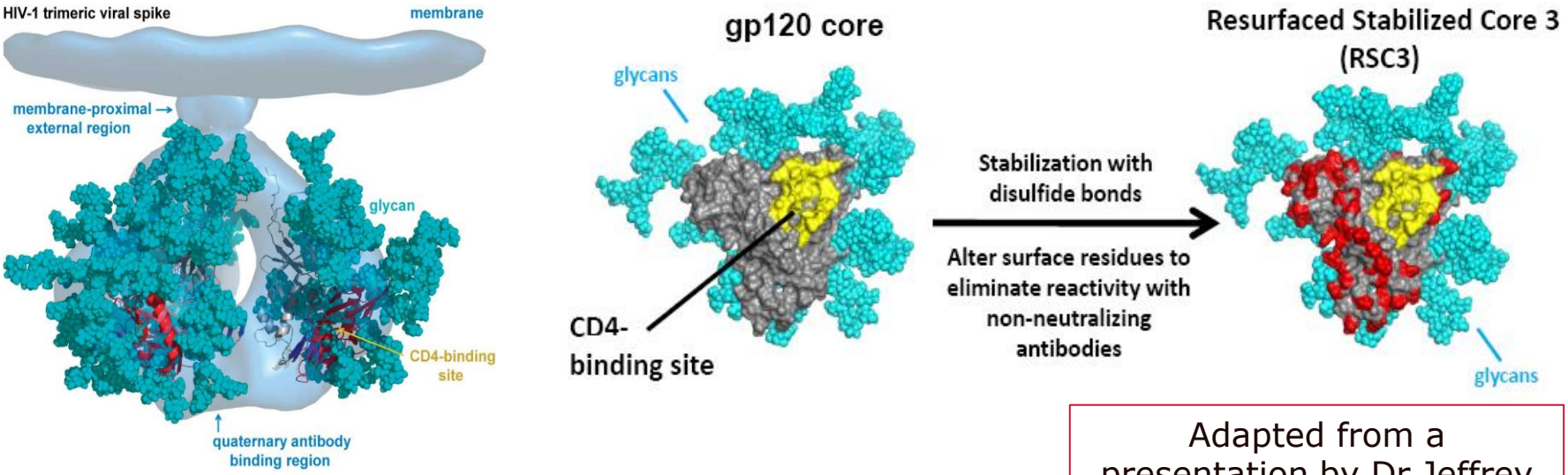
- Glycoproteins are normally membrane bound – remove the transmembrane anchor to allow secretion.

4) Advances in antigen technology

- non-anchored cellular protein antigens can have a secretion signal sequence added.
- Cells should be grown in serum free medium – again makes downstream processing cheaper.

4) Advances in antigen technology

- Antigen modifications to enhance immunogenicity:



Adapted from a presentation by Dr Jeffrey Boyington

- stabilized flexible regions with disulphide bonds.
- altered immunodominat regions.
- remove glycans near site of vulnerability.
- add glycans to cover immunodominant epitopes.
- Multimerized to improve immunogenicity and placed on VLP.

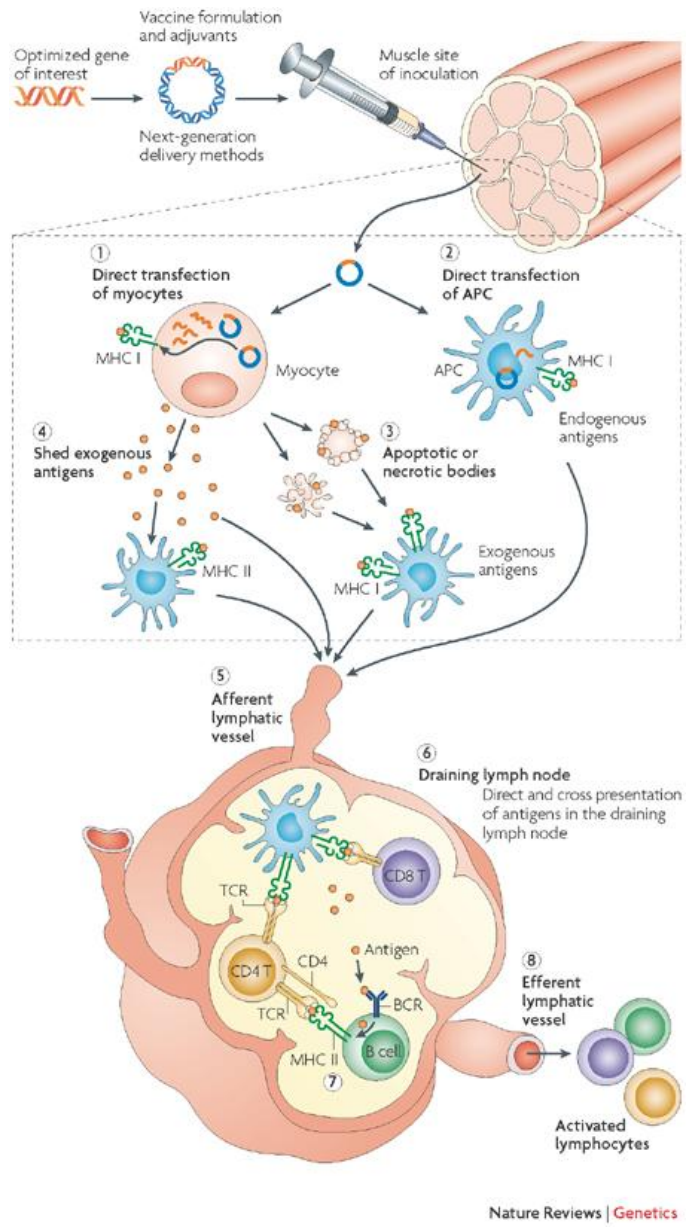
➡ Immuno-focusing

5) DNA vaccines

- Discovered over 14 years ago as a method to generate cellular and humoral immune responses to foreign antigen.
- Generate potent antibody, CD4+, CD8+ T cell responses in mice and afford protection in a variety of disease models.
- The success in rodent models has not translated in humans to date..

  - *A possibility is that amount of plasmid relative to body mass is markedly lower in humans than mice*.

  - *TLR expression levels differ in mice and humans* e.g. TLR 9 expression in lower in humans than mice. It is thought that TLR-9 stimulation by CpG sequences in plasmid backbone contributes to immunogenicity of DNA vaccines in mice.



5) DNA vaccines

1) Plasmid DNA vaccines are purified plasmids engineered to express an antigen of interest.

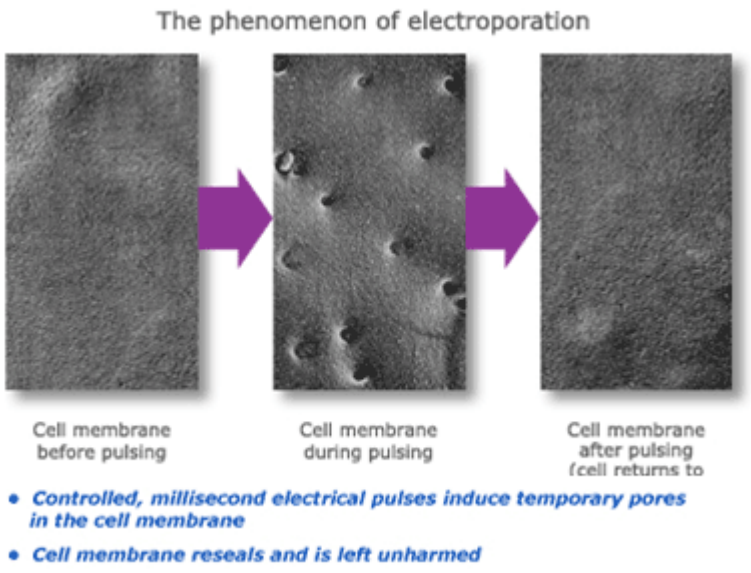
Injection of the plasmid results in transfection of the host cell, antigen expression, and priming of both humoral and cellular responses.

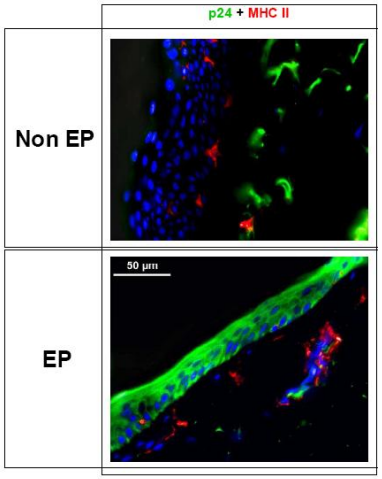
2) Live recombinant or **attenuated replication incompetent** viruses or bacteria have also been engineered to express antigens of interest.

Injection of live recombinant vectors results in infection of the host cell, antigen expression and generation of adaptive immune responses.

Both vaccine technologies results in MHC I antigen expression and efficient CD8 cytotoxic T cell priming

6) Electroporation





- **DNA electroporation has been used to transfect cells for almost 30 years.**
- **Recently it has been investigated for its ability to increase DNA vaccine transfection rates.**
- **Involves applying a small electric field across the site of injection in order to cause temporary membrane instability and produce an electric gradient.**
- **IM vaccination with EP has been extensively studied in macaques.**
- **10-40 fold increase in your B cell and T cell ELISpot results when using EP.**
- **Increase the quality of your immune response by increasing CD8 T cell proliferation and functionality.**
- **Currently being utilised in HIV-1 and influenza clinical trials.**

Summary and future trends

- Major technological advances have been made over the last couple of decades.
- Resulted in numerous vaccine delivery systems, adjuvants and antigenering concepts being developed
- Enabled the medical community to "pick and choose" the best options for any particular disease.
- Numerous vaccines are currently enrolled in clinical trials for a range of diseases
- Steady pipeline of novel vaccine components being made available.
- Expansion in target populations from children to adolescents.
- With a global increase in the aging population, increased focus will be placed on influenza, pneumococcal and RSV vaccines.
- The first therapeutic vaccine for prostate cancer has been licensed, ushering in a period of new therapeutic and preventative cancer vaccines.

However, as in the past, success will depend on our ability to successfully implement vaccination programmes that fulfil the potential of these approaches and programmes!

Thank you for your attention!

Questions.....?