Immunization – basic principles and new developments

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Introductory remarks

Rapid historical view of immunization

The birth of immunization/vaccination and immunology in the broadest sense, stems from two key events

Edward Jenner in 1796
    Cowpox virus / Vaccinia virus

Louis Pasteur in 1879
    Microbial attenuation

Rapid historical view (cont.)

Viral vaccines that make up the bulk of vaccines used for animal health only became available in the 20th century

Loeffler and Frosch showed the filterable nature of foot-and-mouth disease virus in 1898

Frenkel's passage of FMD virus in bovine tongue epithelium in Amsterdam in the 1950s, laid the basis for controlled virus growth for the industrial production of viral antigens in cell cultures

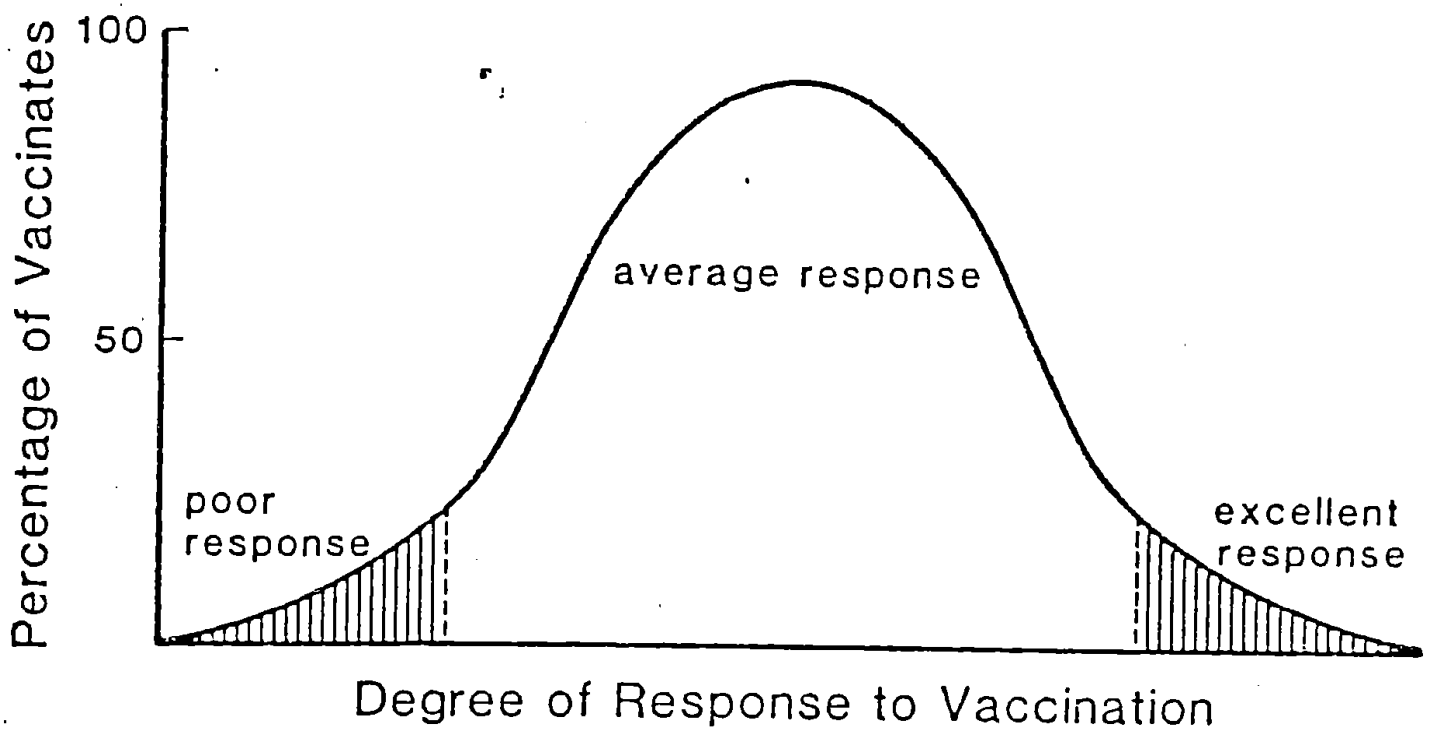
Vaccines – a philosophical view

Vaccination is the most successful medical and veterinary measure: More lives have been saved by immunization, more animal production safeguarded than through all other medical and veterinary activities combined

Prof M. Horzinek, 1998


RINDERPEST ERADICATION PROGRAMME
GLOBAL 2010
The World Without Rinderpest
2011

Host Response to Vaccination



Inactivated vaccines

Advantages	Disadvantages
No reversion to virulence	Can result in hypersensitivities
No contamination with live organisms	Not as immunogenic as live vaccines
Less likely to cause abortion	Costly - require more frequent vaccination and high amounts of antigen are required
More stable during handling and storage	Require adjuvants to enhance the immune response
	Not as efficient in stimulating cell-mediated immunity ***

Inactivated (dead) vaccines

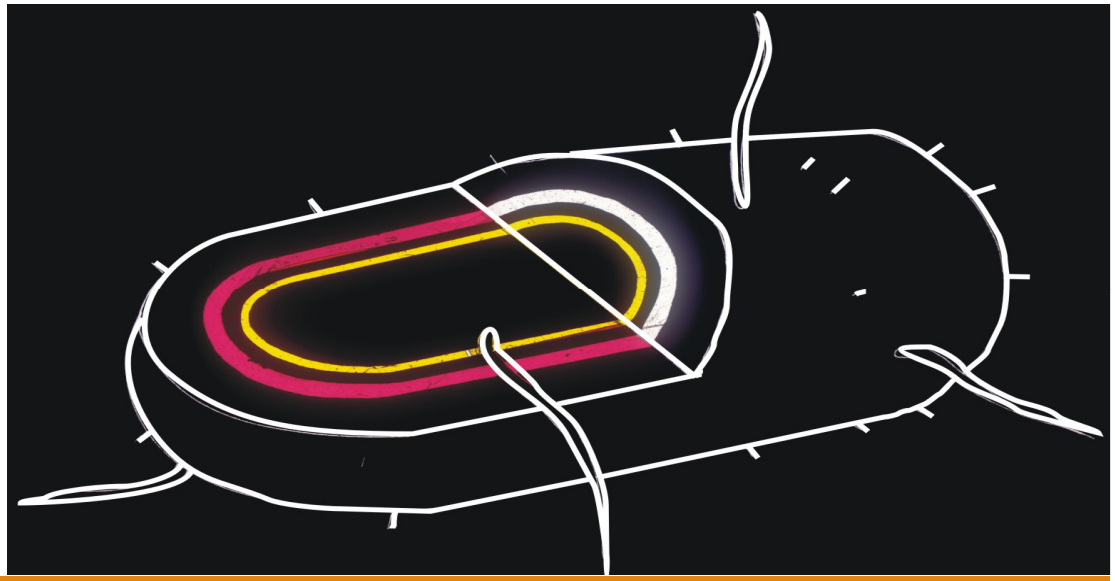
Killed whole bacteria – bacterins

Killed whole viruses

Bacterial toxins rendered avirulent – toxoids

Subunit vaccines –

purified proteins



Modified-live (attenuated) vaccines

Serial passage in an unnatural host

Modified-live (attenuated vaccines)

Alternative methods of production

- Chemical mutagenesis
- Temperature-sensitive mutants
- Genetically engineered site-directed mutations

Modified-live vaccines

Advantages	Disadvantages
More rapid protection	Have the potential to revert to virulence
Longer-lasting immunity - immunogenic proteins are amplified through replication	May be virulent in a non-target or immunosuppressed animal
Sometimes only require one dose	May cause abortion or teratogenesis
With rare exceptions require no adjuvants	May be contaminated with other live viruses
Less expensive	Require correct handling and storage to maintain viability
More efficient in stimulating cell-mediated immune responses	

Developments during the last thirty years

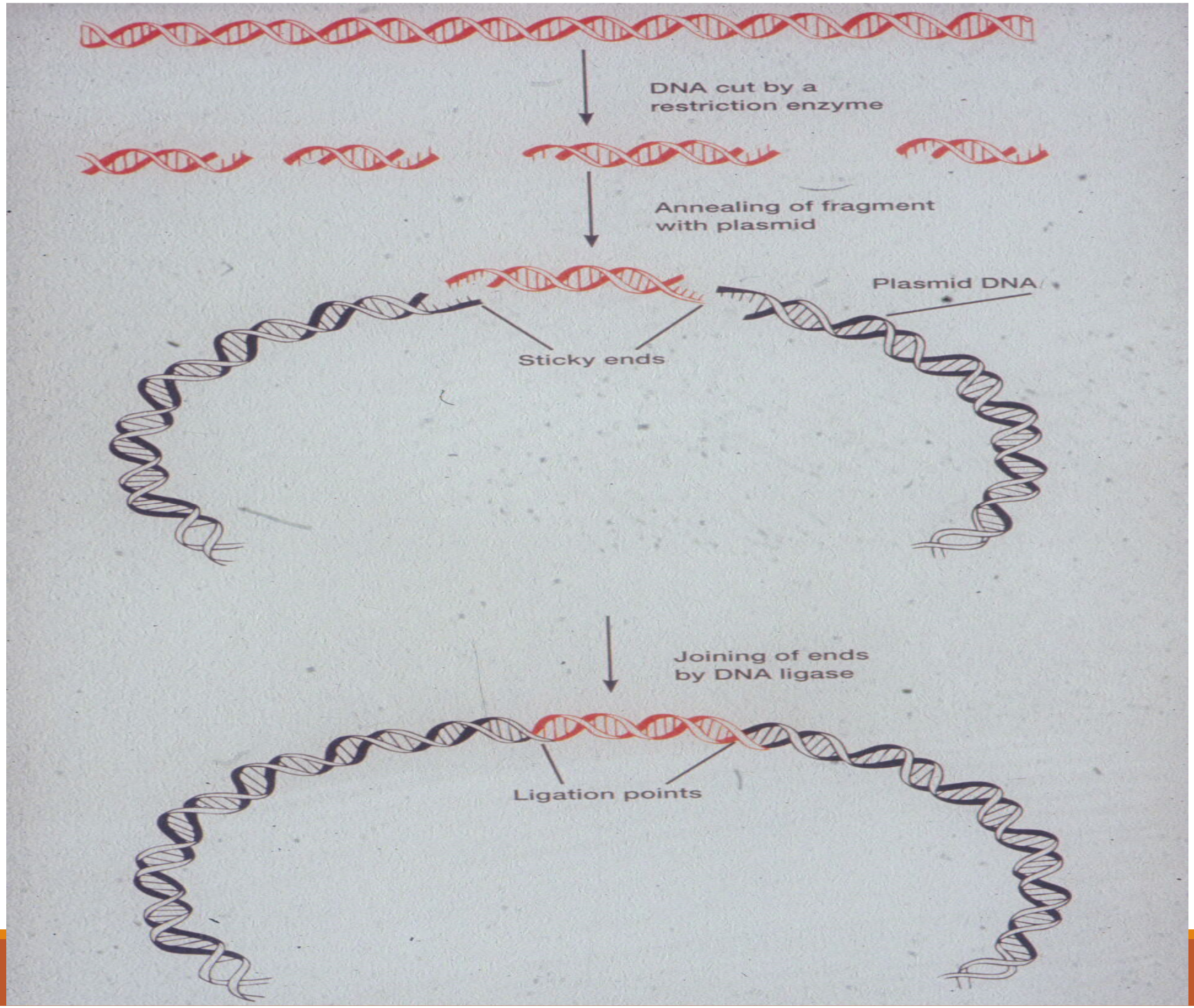
SECOND AND THIRD GENERATION VACCINES AND THE CONTRIBUTION OF ADVANCES IN IMMUNOLOGY TO VACCINE DEVELOPMENT

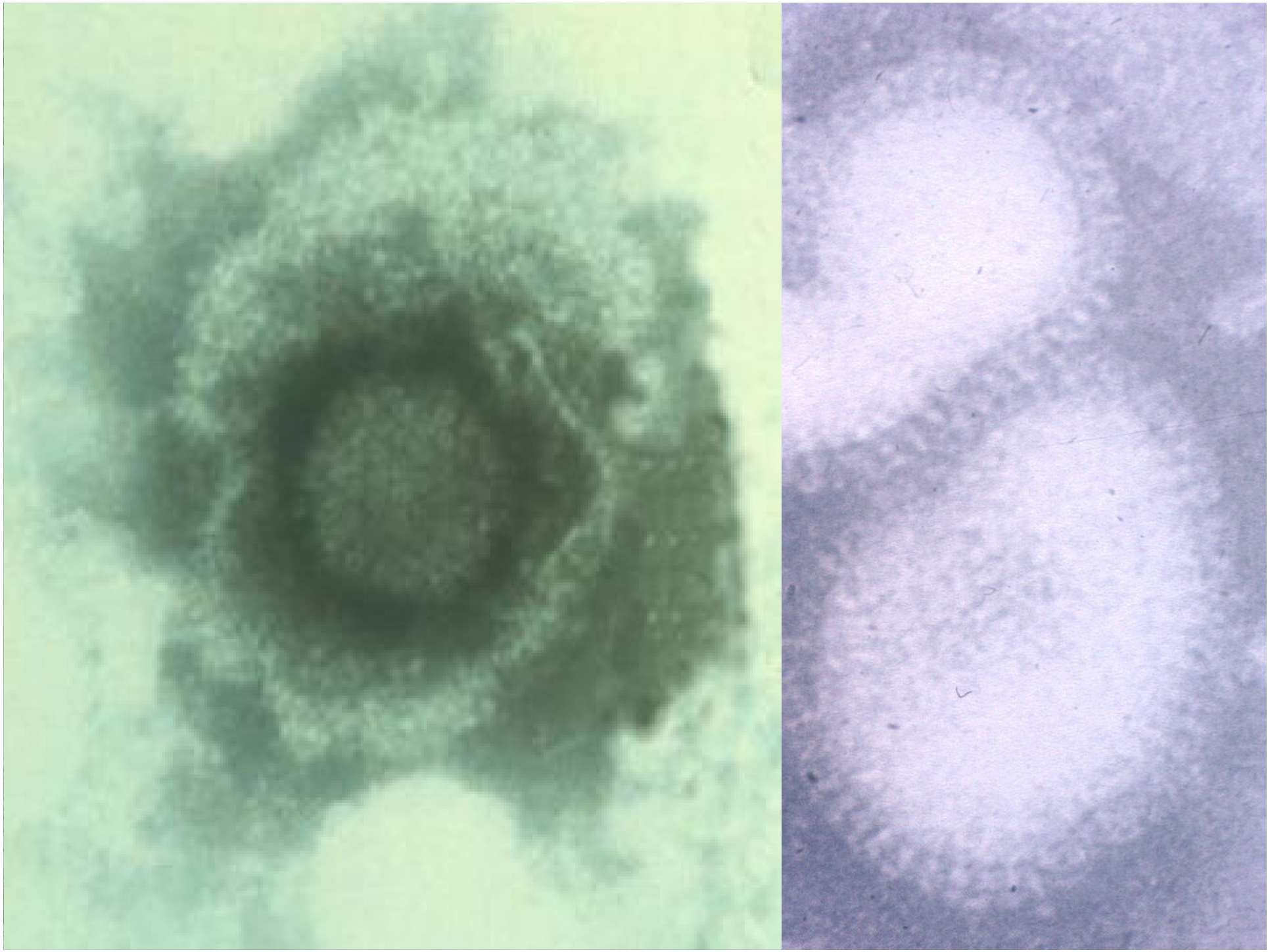
Inactivated recombinant vaccines

Immunogenic proteins are expressed in bacterial or mammalian cells *in vitro*

When expressed *in vitro*, it is purified and used as a purified protein antigen (type I recombinant vaccine), e.g. bovine herpesvirus 1 gB, gC & gD

Development of new adjuvants will have to parallel development of subunit recombinant vaccines


DNA cut by a
restriction enzyme
Annealing of fragment
with plasmid
Plasmid DNA
Sticky ends
Joining of ends
by DNA ligase
Ligation points



Live recombinant vaccines

Gene-deleted (type II recombinant) vaccines / marker vaccines / DIVA vaccines, e.g.

- BVDV – targeted deletions of the Npro and Erns genes in the genomes of vaccine strains
- Bovine herpesvirus 1 gE and gG deleted vaccines

Vectored (type III recombinant) vaccines

Genes coding for antigenic proteins are inserted through genetic engineering into an expression vector, e.g.

- Recombinant canarpoxvirus
- Herpesvirus of turkeys

RNA Particle Technology – strain-specific and herd-specific vaccines

The gene coding for the protective antigen is inserted into VEE virus

The viral RNA coding for the protective antigen is absorbed into eukaryotic cells

After incubation, RNA particles (RP) released from the production cells are then harvested, purified and formulated into a final vaccine

The RPs are able to enter antigen-presenting cells (Langerhans cells) and carry the gene of interest of the disease identified

The animal's immune system recognizes the protein encoded by the gene of interest and triggers an immune response

There is no shedding from the primary host, and no chance for reversion to virulence

Table 1

Comparison of the RNA particle vaccine to traditional vaccines

Variable	Traditional vaccine			RNA particle vaccine
	Killed virus	Modified live	Extract antigen	RNA particle
Humoral immunity	+	+	+	+
Cellular immunity	-	+	-	+
May cause disease	-	+	-	-
Grow agent	+	+	+	-
Adjuvant required	+	-	+	-
Emergency vaccine	-	-	-	+

Virus-like particles / empty capsids

The immunogenic antigen in the VLP vaccine can maintain the native antigenic conformation and mimic the whole structure of the wild virus but as they lack viral nucleic acids, they are not infectious. The VLP vaccine is a category of a subunit vaccine and can be produced using recombinant protein technology without a viral replication system

However, the most important advantage of the VLP vaccine is that it can stimulate B cells and proliferate CD4+ and cytotoxic T-lymphocyte responses

Recognition of antigens by T cells and antibodies

T lymphocytes recognize antigen that has been processed and presented in association with MHC molecules on the surface of antigen presenting cells (APCs)

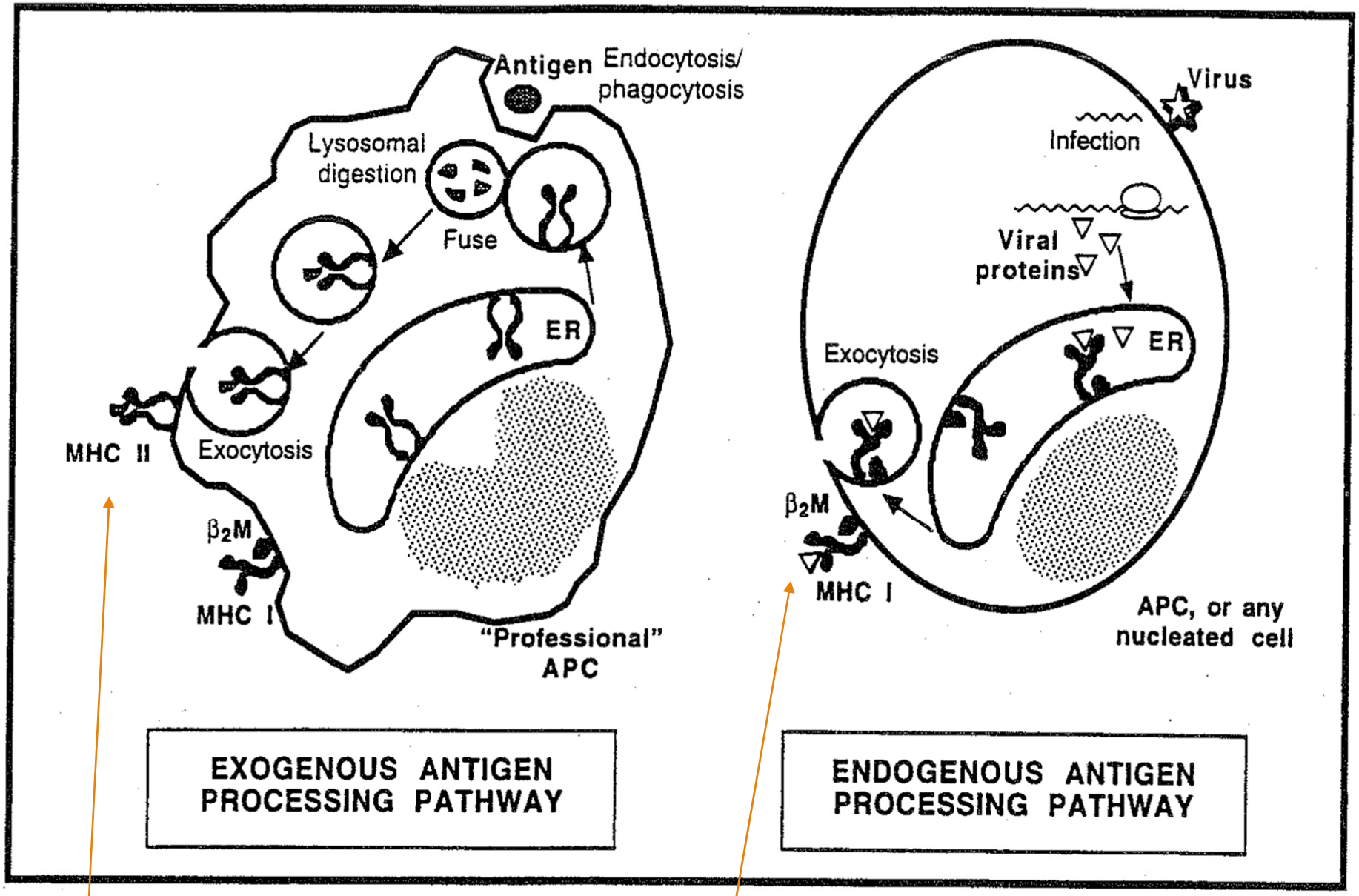
Antigenic determinants (epitopes) recognized by T cells are generated by denaturation and degradation of proteins

In contrast, epitopes recognized by antibodies are dependent on the native conformation of the protein

T-cell responses

Viruses and some bacteria and protozoa are degraded by proteases in host cells, and are then bound to MHC class I molecules that in turn induces a CD8+ T-cell response (endogenous or class I pathway)

Other organisms are taken up by host cells through phagocytosis, bind to MHC class II molecules and induces a CD4+ T-cell response (exogenous or class II pathway)



CD4+ (Th1 and Th2)

CD8+

T-helper cells

Cytokines can activate different compartments of the immune system

These responses can either lead to control of infection and immunity, or can potentiate disease

In 1986 it was discovered that 2 types of CD4+ cells exist, namely Th1 & Th2

Distinguished by the cytokines they produce

T-helper cells (cont.)

Th1 cells produce IL-2, IFN-gamma, TNF-beta

- Activate macrophages, IgG2 & DTH (***predominantly*** cell-mediated response)

Th2 cells produce IL-4, 5, 9, 10 & 13

- Activate eosinophils, IgG1 & IgE (***predominantly*** humoral response)

Implications for vaccine design

Important to know whether the protective responses against target pathogens involve mainly Th1 or Th2 responses

There is the potential to incorporate into subunit vaccines cytokines or other co-stimulatory molecules that have the capacity to push the T-cell response to a Th1 or Th2 cytokine profile

Part 2

The complexities of vaccines and vaccine development ***

Vaccine production has a broad quality goal, namely:

- Each and every dose is:
  - equivalent
  - Safe
  - effective

Has stringent regulatory considerations:

- A certain level of proof is necessary
- Documentation is required
  - to provide guarantees that the broad quality goal is achieved
    - *on an ongoing basis*

Why do these considerations apply to vaccines?

Justification for stringent registration requirements

A vaccine is a biological product produced in entirely different circumstances to a medicine and requiring totally different criteria for establishing efficacy

A vaccine has to be tested post production by means of a rigorous set of controlled tests

The antigen has to be qualified and quantified and the potency established

Vaccines vs pharmaceuticals

Vaccines are more difficult to characterize analytically than most pharmaceuticals

Pathogens, or the proteins making up subunit vaccines are:

- Large (complex) and delicate (unstable) molecules
- Are much larger than small molecule drugs (low molecular weight drugs)
- Easily destroyed (unstable)

Small molecule drugs *vs* proteins

Paracetamol has a molecular weight of 150

Monoclonal antibody has a molecular weight of

150 000

Proteins are *chemically* unstable:

deamidation, oxidation, proteolysis

Proteins are *physically* unstable:

denaturation, aggregation, precipitation, adsorption

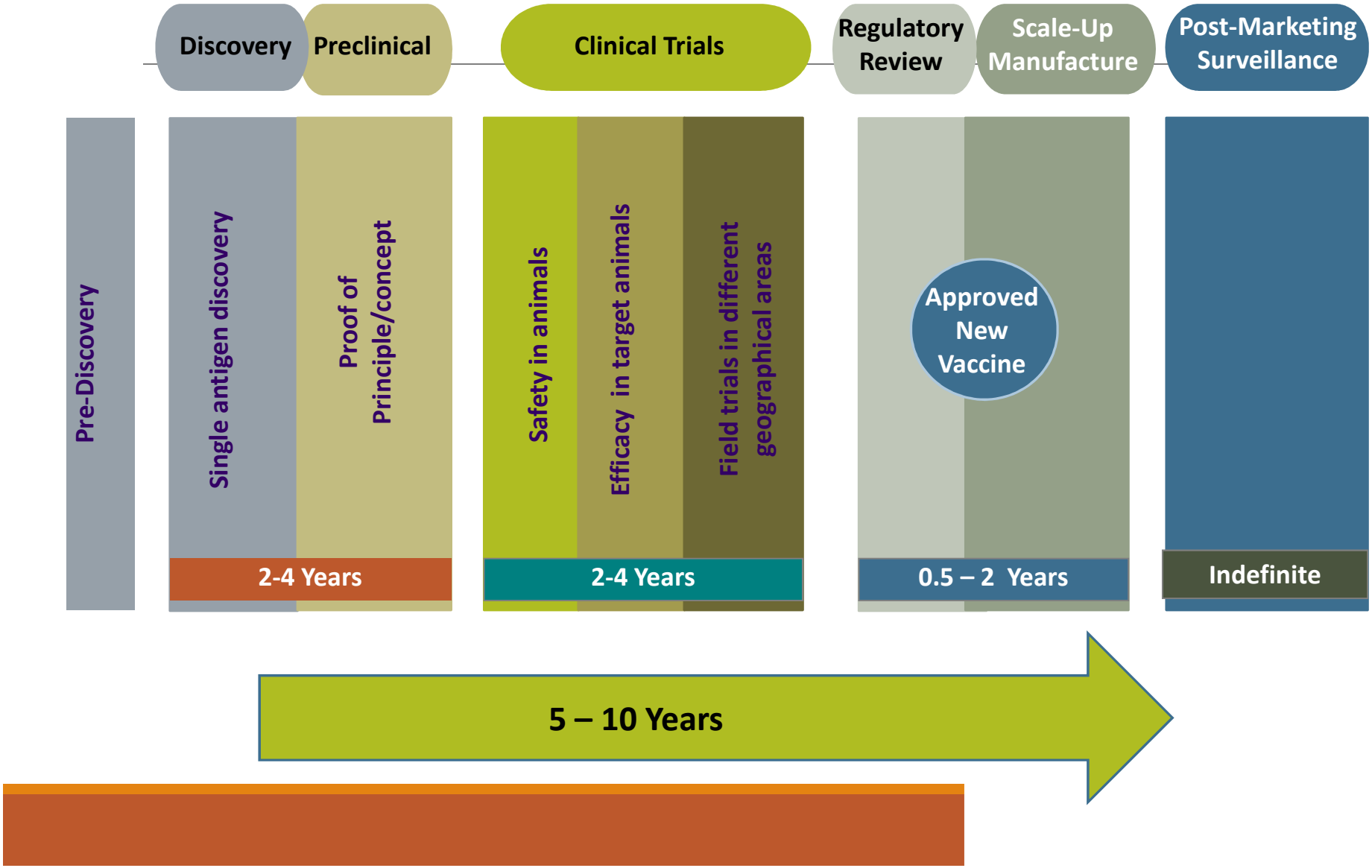
Essential differences between low MW and high MW molecules

Small molecule drugs are chemically pure synthetic molecules and can be readily formulated

Proteins are large complex molecules, heterogeneous mixtures that vary from batch to batch and are difficult to formulate

Any changes in either scale or process require reassurance that the product will remain unchanged in terms of its efficacy and safety

Vaccine Development



Registration dossier for veterinary biologicals

Part/Section/Chapter 1 – General information

Part 2 – Pharmaceutical/ Analytical/ Quality data

Part 3 – Safety data

Laboratory and field safety data

Residue data in food-producing animals for withdrawal periods

Part 4 – Efficacy data

Laboratory trials and field trials

Control tests on the final product – release specification/batch control

Release specifications do not only relate to quantitative parameters, but also biological activity

To assure quality and safety of the vaccine, each batch must be tested and released before being placed on the open market

This should include as appropriate:

- Identification assay for active ingredients
- Potency
- Identification assay for adjuvants
- Sterility
- Residual moisture content of freeze-dried vaccines
- Safety
- Extraneous agents including *Mycoplasma*

Part 3 - Safety of the vaccine

The demand for safety is unusually high for any vaccine – healthy vs sick recipients

The risk/benefit ratio of vaccines emphasizes very well characterized and very safe vaccines

The resulting strict regulatory oversight impacts every step in the production cycle

Part 4 - Efficacy studies – the basics

Trials are done as in-house/laboratory trials or clinical/field trials

- Number of animals and/or groups must be sufficient to be statistically significant
- Animals that are susceptible to the disease must be used
- Trial designs must consider the variables that may influence the results
- The vaccine used in the trials must have an end-of-shelf life titre or lower

Licensing procedures for genetically modified vaccines

Recombinant vaccines must in addition to evaluation for purity, potency, safety and efficacy, also be evaluated for any potential effects on the human environment that could result from release of a live recombinant micro-organism

Why it is not a good idea to use unregistered vaccines

Registration of vaccines involves a very complex evaluation of the safety, efficacy and quality of a vaccine by an independent expert or experts

No individual, no matter how brilliant can operate in a vacuum and produce vaccines without a control system in place

Autogenous vaccines

Autogenous vaccines are specific vaccines intended for emergency/quick and restricted use (time and/or locality) and are defined as safe, non-spreading products derived from specific pathogens isolated from a specific animal/bird or herd/flock of animals/birds and used under veterinary supervision in that specific animal/bird or herd/flock of animals/birds only

The fundamentals

Autogenous vaccines (also termed emergency vaccines):

- Are farm-specific vaccines
- Are able to stimulate a potent immune response to the specific pathogens that they are targeted against
- Are usually inactivated vaccines produced from bacteria or viruses involved with a particular infectious problem on a farm

Most basic autogenous vaccines e.g. orf and papillomatosis (warts)

- Thoroughly homogenize (mush up) the scabs or warts
- Add virus transport medium, or buffered saline
- Add a small amount (few drops) of broad spectrum antimicrobial drug
- Strain through muslin gauze or a bandage
- Leave overnight in a fridge
- To apply, make scrapings on a hairless area until you see some blood; paint a drop on to the wound (orf)
- Vaccinate subcutaneously e.g. 5ml (papillomavirus)

When are autogenous vaccines administered?

Autogenous vaccines are administered when:

No registered vaccine is available

No commercial/registered vaccine is available against specific disease-causing strains

Can a veterinarian produce an autogenous vaccine?

The prescribing or dispensing veterinarian is ultimately responsible for the efficacy and safety of the product

The client should be informed that the product is not registered and no proof of efficacy has been presented

Should an investigation be instituted, the veterinarian will be held accountable