Manipulation of Immune Response - Vaccination

We start with a brief history of vaccinology.

① Chinese / Turks – variolation treatment (small pox vaccine derived from dried crust)

② Edward Jenner – milkmaids immunity to small-pox / fluid from cowpox pustules

③ Luis Pasteur – Fowl cholera vaccine and rabies vaccine

Necessary characteristics for vaccine –

① Reasonable cost
② Efficient delivery to at risks people
③ Long lasting immunity
④ Higher shelf life

Protective Immunity can be acheived by active / passive immunisation

Immunisation – process of eliciting a long lived state of protective immunity against a disease causing pathogen.

Vaccination – intentional exposure to forms of a pathogen that do not cause disease

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Passive Immunisation by Delivery of Preformed Antibody

- immunity elicited in one animal can be transferred to another by injecting serum from the first
- preformed antibodies are transformed to a recipient
- occurs naturally when maternal antibodies are passed down through breastmilk – antibodies for diphtheria, tetanus, streptococci, rubeola in the form of maternal IgA.

- can also be achieved by injecting a recipient with preformed antibodies (antiserum) from immune individuals
- does not activate the host's immune response, generates memory response & protection is transient
- generates no memory response
- risks associated — if from a foreign species, host can mount a strong response against the isotypic determinants of the foreign antibody or the part that is unique to the other species
- anti-allotype response < anti-isotype response

Active immunisation to induce immunity and memory

- trigger adaptive immune response to elicit protective immunity & immunologic memory
- subsequent exposure elicits secondary immune response
- can be achieved by natural infection and vaccines
- depends on proliferation of T cells and B cells & formation of memory cells

Why are repeated innoculations of some vaccines required (booster dose)?

- required specially in infants because of interference of passively aquired maternal antibodies
- sometimes maternal antibodies don't allow vaccines to trigger adaptive immune system
- sometimes each dose deals with different strains of the same virus

Active Immunity	Passive Immunity
• direct contact with pathogen	• direct contact not required
• time period reqd. for immunity	• immunity develops immediately
• antibodies produced	• antibodies supplied
• lasts for a long period	• lasts for a few days
• immunologic memory	• no memory triggered
• not much side effects	• often, body reacts to antisera (serum sickness)
• herd immunity	• only transient & personal

Vaccine Development Stages

① Exploratory stage – basic laboratory research (2-4 years)

② Pre-Clinical stage – tissue or cell-culture systems and animal testing to assess safety, immunogenicity (mice, chicken, rabbits, rats, mice)

③ IND Application – sponsor supplies application for Investigational New Drug

④ Clinical Studies with Humans –
- Phase I – small group of adults (20-80 sub)
- Phase II – randomised, well controlled trials with 100s of participants
- Phase III – randomised and double blind experiment testing 1000–10,000

safety, type, extent of immune resp.
immunogenicity, proposed dose, schedule, method of delivery
vaccine safety assessing

⑤ Approval and licensure – after phase III trial, Biologics License Application

⑥ Post-licensure monitoring of vaccines – Phase IV trials, after release of vaccine to check safety, efficacy & potential

Single-blind – subjects don't know if they are testers or control group

Double blind – subjects & testers are blinded

Triple blind – supervisor, tester & subjects are all blinded

Characteristics of an ideal vaccine:

① effective in preventing/reducing severity of infectious disease
② durable long-term protection against disease
③ achieve immunity with a minimal dose
④ provide the broadest possible protection against infection
⑤ cause mild or no adverse events
⑥ are stable at extremes of storage conditions
⑦ available for general use and affordable

Vaccine Strategies

- Live vaccines
- Live, attenuated vaccines
- Inactivated (killed) vaccines
- Toxoids
- Polysaccharide & polypeptide vaccines
- surface antigen (recombinant) vaccine

Major factor to be considered

- developing a measurable immune response
- activation of humoral & cellular wing
- immunological memory

Live, attenuated vaccines

- microorganisms can be attenuated or disabled to lose their pathogenicity, but retain their capacity for transient growth within an innoculated host
- acheived by culturing pathogenic bacteria under abnormal culture conditions
- can be naturally attenuated – cow pox (vaccinia virus)

Advantages

provide prolonged immune system exposure to individual epitopes on attenuated organism and more closely mimics the growth pattern of real pathogen — increased immunogenicity & efficient production of memory cells

- requires only a single immunisation (advantageous in developing countries)

Disadvantage

- incomplete attenuation - need booster doses to impart immunity from all strains of vaccines
- live forms may mutate and revert to virulent forms in vivo - resulting in paralytic disease & serve as a source of pathogen transmission
- post vaccine complications

eg., MMR, Polio (Sabin), TB, varicella, yellow fever

Sabin Polio Vaccine

- consists of a mixture of live, attenuated polioviruses of all 3 strains
- passive immunisation – booster doses required to enforce immunity

A new method of attenuation - Codon bias

- polio virus with hundreds of mutations in the genes encoding its capsid protein
- one "silent mutation" — changes codon into another coding for same amino acid
- new viruses were less infectious

Killed Vaccines

- compared to live vaccine, killed vacine require repeated dose
- only induce humoral response - cellular response often weak
- low IgA response
- use chemical for inactivation - formaldehyde fails to kill vaccines
- safe, stability and easy storage and transport

e.g., Flu, HepA and Cholera vaccine

Subunit Vaccines

- risk associated with killed or attenuated can be overcome by subunit vaccine
  - specific, purified macromolecules

- non-replicating vaccines
- major antigenic epitopes are identified and highly purified vaccines are produced - increased purification may lead to loss of immunogenicity
- may necessitate coupling to an immunogenic carrier protein or adjuvant ↑

- examples of purified subunit vaccines include the HA vaccines for influenza A & B

① Toxoids ② Capsular Polysaccharides ③ Recombinant protein

Toxoids

① C. diphtheriae and C. tetani are grown
② Tetanus and diphtheria toxins produced during growth
③ cultures are detoxified with formaldehyde
④ detoxified materials are separately purified by aluminum sulfate fractionation
⑤ further purified by column chromatography
⑥ tetanus and diphtheria toxoids are individually adsorbed onto aluminium phosphate
⑦ toxoid dose is determined in guinea pig potency test

Polysaccharide Vaccines

- protective immunity to encapsulated bacteria involves an antibody response to a polysaccharide antigen
- involves interaction with T and B lymphocytes and host defense mechanisms
- e.g., Neisseria meningitidis, Streptococcus pneumoniae (13 antigenic subtypes)

Limitations:

1. do not activate T_H cells – only B cells and IgM
2. no class switching – activation via thymus – independent manner
3. no affinity maturation

Conjugate vaccines – PS antigens are conjugated/coupled with protein carrier
– increases immunogenicity

Recombinant Subunit Vaccines

- limitation of synthetic peptide vaccine – poorly immunogenic → tend to induce only humoral immuno response
- ideal synthetic vaccine → contains both immunodominant B & T cell epitopes
- if CTL response is needed, Ag must be delivered intracellularly and presented with MHC

Making of a recombinant subunit vaccine:

- vector (oriR, selection marker gene, MCS)
- restriction enzymes digestion
- DNA ligase – will form a covalent phospho-diester bonds between DNA molecules

Transformation → Selection → Positive transformants hosted on expression host → grow and harvest protein → use them as vaccine

Downside of recombinant subunit:

1. development is time consuming and more expensive
2. requires highly skilled (expensive) R & D teams
3. some regulatory agencies are slow to accept rDNA products
4. multiple antigens may be required

- accummulation of liposaccharides (generally referred to as endotoxins)

Upside of recombinant subunit vaccines:

1. safety — pathogens can be entirely excluded from production
2. lower COGs — multi-epitope vaccines engineered to a single strain
3. Detoxified — suppresson genes can be eliminated
4. Efficient - better antigens can be further engineered
5. longer half-lives and can be frozen prior to formulations
6. genome sequencing - more antigens

Multivalent subunit vaccines

- to increase antigenicity and ability to involve both T and B cell
- SMAA - solid matrix - antigen - antibody complex
  - solid matrix attached to MAb
  - T cell & B cell epitope then added to saturate the Ab
  - used as vaccine
  - results activation of both arms
  - particulate form also increase antigenicity
  - facilitating phagocytosis by phagocytic cells

Immunostimulating Complexes

- vaccine adjuvants made of Quillaja saponins and cholesterol and phospholipids
- encapsulate antigens → fuse with antigen presenting cell → peptide transport into ER & presentation by MHC-I peptide complexes

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Virosomes

- virus-like particles used as vaccine delivery system and adjuvants
- have viral envelope proteins, phospholipid bilayer and antigens

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Liposomes

- lipid bilayer surrounding aqueous inner compartment that contains antigen

Disadvantages

- aggregation on storage
- rapid uptake by RES
- interaction with HDL
- difficulties in scaling up
- cost factor
- structural components induce immunological complications
- poor efficiency against some intracellular pathogens

DNA Vaccines

- DNA, encoding protein antigen is directly injected into muscle
- DNA integrates into host cell chromosomal DNA and maintained
- foreign pathogenic protein expressed by muscle cells
- local DC also get integrated
- muscle cell – low level MHC I expression, major involvement of DC cells

Advantages

1. DNA is relatively inexpensive and easier to produce
2. no denaturation, multiple gene maybe included
3. can result in more long-term production of an antigen protein
4. stimulates protective immunological memory

Disadvantages

1. delivery is not optimal
2. cancer problem
3. only protein can be included
