BP 605 T. Pharmaceutical Biotechnology (Theory)

General method of the preparation of bacterial vaccines, toxoids, viral vaccine, antitoxins, serum-immune blood derivatives and other products relative to immunity

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Overview

- Introduction to vaccines
- General method of the preparation of vaccines
- Serum-immune blood derivatives and other products relative to immunity

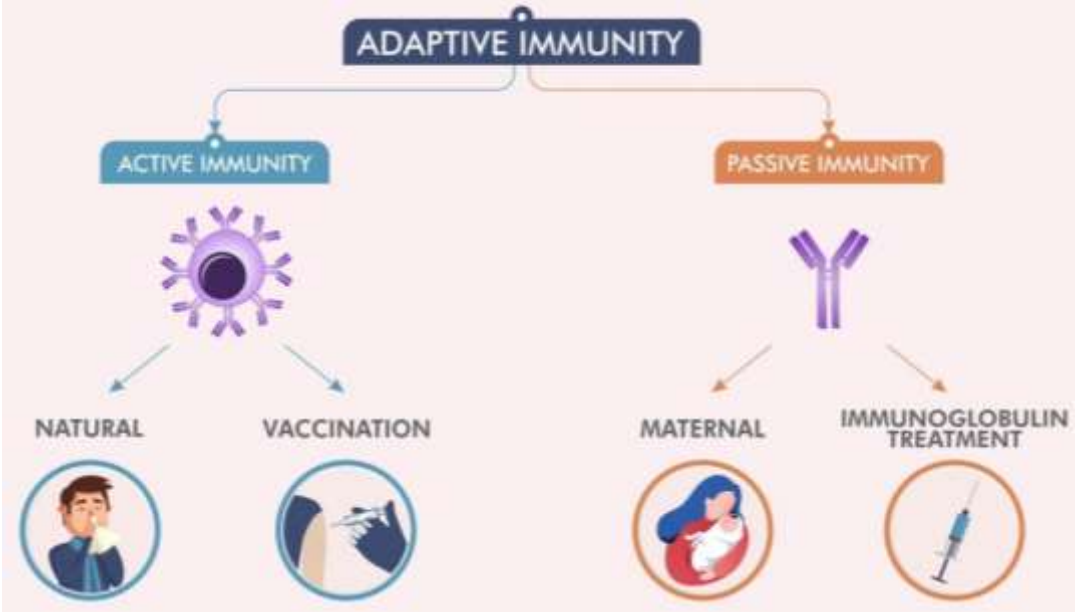
Types of Immunization

Passive Immunization

- ✓ Methods of acquisition include natural maternal antibodies, antitoxins, and immunoglobulins
- ✓ Protection transferred from another person or animal
- ✓ Provision of temporary immunity by the administration of preformed antibodies
  - Pooled human IG or IGIV
  - Specific immune globulin preparations
  - antitoxins

Active Immunization

- ✓ Methods of acquisition include natural infection, vaccines (many types), and toxoids
- ✓ Relatively permanent



Community immunity or "herd immunity" is an important part of protecting the community against disease

Acquisition of Passive and Active Immunity

Passive immunity

- **Natural maternal antibody**
- **Immune globulin** - An antibody-containing solution **derived from human blood**, obtained by **cold ethanol fractionation of large pools of plasma**; available in **intramuscular and intravenous** preparations
- **Humanized monoclonal antibody**
- **Antitoxin** - An antibody **derived from the serum of animals** that have been stimulated with specific antigens.

Active immunity

- **Natural infection**
- **Vaccines** - A **suspension of attenuated live or killed microorganisms**, or **antigenic portions of them**, presented to a potential host to induce immunity and prevent disease.
    - Attenuated organisms
    - Inactivated organisms
    - Purified microbial macromolecules
    - Cloned microbial antigens
    - Expressed as recombinant protein
    - As cloned DNA alone or in virus vectors
    - Multivalent complexes
- **Toxoid** - A **bacterial toxin** that has been **modified to be nontoxic** but **retains the capacity to stimulate the formation of antitoxin**

Passive Immunization

- ✓ Can occur naturally via transfer of maternal antibodies across placenta to fetus
- ✓ Injection with preformed antibodies
  - Human or animal antibodies can be used
  - Injection of animal Ab's prevalent before vaccines
- ✓ Effects are only temporary

TABLE 18-2 Common agents used for passive immunization

Disease	Agent
Black widow spider bite	Horse antivenin
Botulism	Horse antitoxin
Diphtheria	Horse antitoxin
Hepatitis A and B	Pooled human immune gamma globulin
Measles	Pooled human immune gamma globulin
Rabies	Pooled human immune gamma globulin
Respiratory disease	Monoclonal anti-RSV*
Snake bite	Horse antivenin
Tetanus	Pooled human immune gamma globulin or horse antitoxin

*Respiratory syncytial virus

Kuby, Immunology; Eighth edition, Table 18-2

The Immune System and Passive Immunization

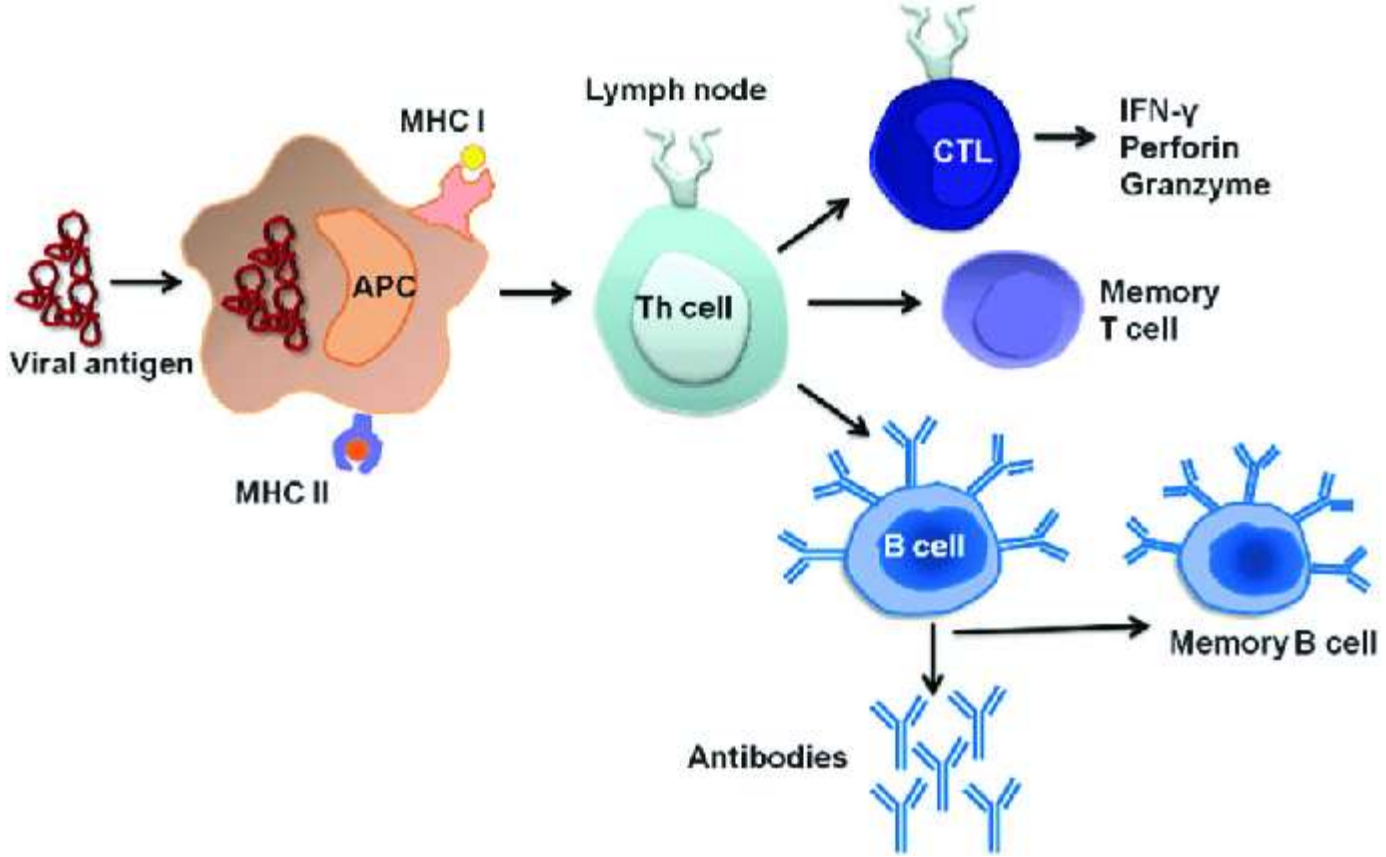
- ✓ The transfer of antibodies will not trigger the immune system
- ✓ There is NO presence of memory cells
- ✓ Risks are included
  - Recognition of the immunoglobulin epitope by self immunoglobluin paratopes
  - Some individuals produce IgE molecules specific for passive antibody, leading to mast cell degranulation
  - Some individuals produce IgG or IgM molecules specific for passive antibody, leading to hypersensitive reactions

Conditions Warranting Passive Immunization

- ✓ Deficiency in synthesis of Ab as a result of congenital or acquired B-cell defects
- ✓ Susceptible person is exposed to a disease that will cause immediate complications (time is the biggest issue)
- ✓ Disease is already present

Active Immunization

- ✓ Natural Infection with microorganism or artificial acquisition (vaccine)
- ✓ Both stimulate the proliferation of T and B cells, resulting in the formation of effector and memory cells
- ✓ The formation of memory cells is the basis for the relatively permanent effects of vaccinations



History and Achievements of Vaccines

- ✓ During the 15th century, an **early form of smallpox vaccination** was practiced in **China and other parts of the world.** Healthy people were intentionally infected with substances from the **pustules of people suffering from smallpox, a technique called variolation.** A mild form of smallpox usually resulted from this practice.
- ✓ **An English doctor, Edward Jenner, improved the variolation technique** to create the first vaccine in 1796. **Dr. Jenner had heard that dairymaids** who had been **infected with cowpox**, a disease related to but **milder than smallpox**, were **not susceptible to smallpox**, and decided to test the idea. He **performed the first vaccination** on a boy with material taken from **lesions of cowpox.**
- ✓ In fact, **the word vaccination comes from the Latin word for cow, vacca.**

Principles Underlying Vaccination

Concept of Immunity

- ✓ Self vs. Non-self
- ✓ Antigen specificity
- ✓ Indicated by presence of effector cells
- ✓ Protection from infectious diseases using above methods

Effectiveness of Vaccinations

- ✓ Small percentage of recipients will respond poorly
  - Role of genetic determinants
- ✓ Herd Immunity
  - Majority of population is immune, so chance of susceptible individual contacting infected individual is low
  - Measles Epidemic

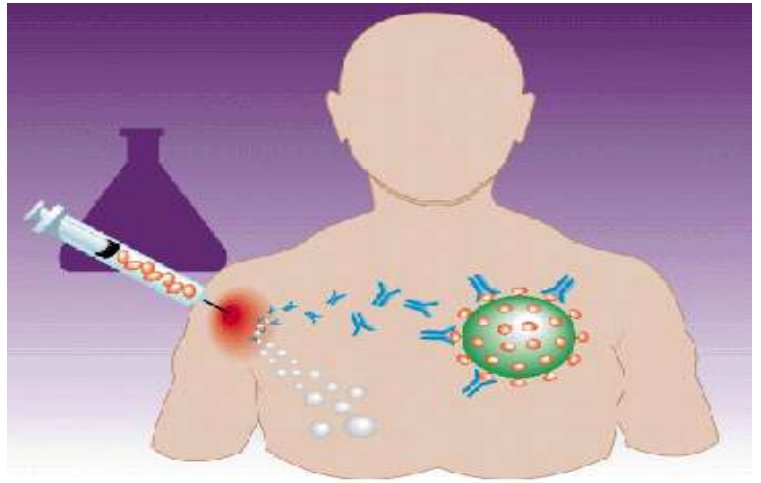
Herd Immunity

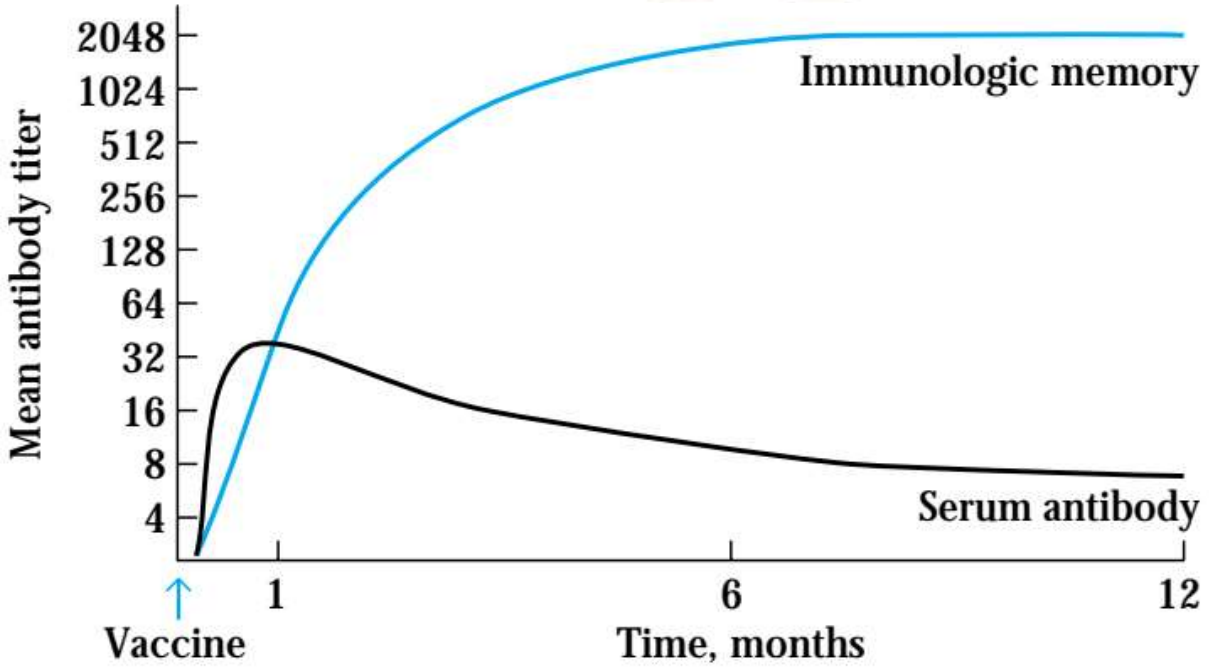
- ✓ Factors affecting herd immunity
  - Environmental Factors: crowded conditions, seasonal variations
  - Strength of Individual's Immune System
  - Infectiousness of Disease: greater the risk of infection, the higher percentage of people need vaccines to attain herd immunity
- ✓ When enough people are vaccinated, chance of germ infecting the non-immunized population is small
- ✓ Can lead to disappearance of diseases (smallpox)
  - Vaccination no longer necessary

Development of Vaccines

- ✓ Common misconception that activation of the immune system results in protective immunity
- ✓ Multiple factors affect decisions when making vaccines

1. Activation of specific branch of immune system
2. Development of immunological memory





Types of Vaccines

- ✓ Whole-Organism
  - Attenuated Viral/Bacterial
  - Inactivated Viral/Bacterial
- ✓ Purified Macromolecules
  - Polysaccharide
  - Toxoid
  - Recombinant Antigen
  - Recombinant-Vector
- ✓ DNA
- ✓ Synthetic Peptide
- ✓ Multivalent Subunit

TABLE 18-4 Classification of common vaccines for humans

Disease or pathogen	Type of vaccine
WHOLE ORGANISMS	
Bacterial cells	
Anthrax	Inactivated
Cholera	Inactivated
Pertussis*	Inactivated
Plague	Inactivated
Tuberculosis	Live attenuated BCG†
Typhoid	Live attenuated
Viral particles	
Hepatitis A	Inactivated
Influenza	Inactivated
Measles	Live attenuated
Mumps	Live attenuated
Polio (Sabin)	Live attenuated
Polio (Salk)	Inactivated
Rabies	Inactivated
Rotavirus	Live attenuated
Rubella	Inactivated
Varicella zoster (chickenpox)	Live attenuated
Yellow fever	Live attenuated

TABLE 18-4 Classification of common vaccines for humans

Disease or pathogen	Type of vaccine
PURIFIED MACROMOLECULES	
Toxoids	
Diphtheria	Inactivated exotoxin
Tetanus	Inactivated exotoxin
Capsular polysaccharides	
Haemophilus influenzae type b	Polysaccharide + protein carrier
Neissera meningitidis	Polysaccharide
Streptococcus pneumoniae	23 distinct capsular polysaccharides
Surface antigen	
Hepatitis B	Recombinant surface antigen (HBsAg)

*There is an now also an acellular pertussis vaccine consisting of toxoids and inactivated bacteria components.

†Bacillus Calmette-Guerin (BCG) is an avirulent strain of *Mycobacterium bovis*.

Kuby, Immunology; Eighth edition, Table 18-4

Whole-Organism Vaccines

- ✓ Many common vaccines used consist of inactivated or attenuated bacterial cells or viral particles
- ✓ Includes attenuated and inactivated vaccines

Attenuated Viral or Bacterial Vaccines

- ✓ Attenuation – to reduce in force, value, amount, or degree; weaken
  - Achieved by growth under abnormal culture conditions
  - Bacillus Calmette-Guerin (BCG)
  - Act as a double edged sword, as they have distinct advantages and disadvantages.

Advantages of Attenuated Bacterial or Viral Vaccines

- ✓ Advantages stem from their capacity for transient growth
- ✓ Prolonged immune-system exposure
- ✓ Single immunizations
- ✓ Replication within host cells

Exception to the Rule…

- ✓ Sabin Polio vaccine consists of 3 attenuated strains of poliovirus
- ✓ Colonization of intestine results in immunity to all 3 strains
  - Production of secretory IgA and induction of IgM and IgG
- ✓ Result is the need for boosters
  - Individual strains interfere with one another
- ✓ First immunization - one strain predominates in growth
- ✓ Second Immunization - immunity generated by previous immunization limits growth of previously predominant strain
- ✓ Third Immunization - same principle as second immunization

Disadvantages of Attenuated Bacterial or Viral Vaccines

- ✓ MAJOR disadvantage is possible reversion
  - ex: Rate of reversion of Sabin Polio vaccine is one case in 4 million doses
- ✓ Presence of other viruses as contaminants
- ✓ Unforeseen post vaccine complications

The Future of Attenuation…

- ✓ Genetic engineering techniques provide new methods of attenuation
- ✓ Herpes virus vaccine for pigs
- ✓ Possible elimination of reversion?

Inactivated Viral or Bacterial Vaccines

- ✓ Methods of inactivation include heat or chemical agents
  - End result…. Loss of replication ability
- ✓ Difficult to inactivate due to potential for denaturation of epitopes
  - Dependence on higher order levels of protein structure

Inactivated Viral or Bacterial Vaccines

TABLE 18-5 Comparison of attenuated (live), inactivated (killed), and DNA vaccines

Characteristic	Attenuated vaccine	Inactivated vaccine	DNA vaccine
Production	Selection for avirulent organisms: virulent pathogen is grown under adverse culture conditions or prolonged passage of a virulent human pathogen through different hosts	Virulent pathogen is inactivated by chemicals or irradiation with γ-rays	Easily manufactured and purified
Booster requirement	Generally requires only a single booster	Requires multiple boosters	Single injection may suffice
Relative stability	Less stable	More stable	Highly stable
Type of immunity induced	Humoral and cell-mediated	Mainly humoral	Humoral and cell-mediated
Reversion tendency	May revert to virulent form	Cannot revert to virulent form	Cannot revert

Other forms of Vaccines

Subunit vaccines

- Vaccines made from well defined components of microorganisms are called a subunit vaccine

Recombinant vaccines

- A *subunit vaccine* that is produced using recombinant techniques is called a *recombinant vaccine*.

Newer vaccines – Still Experimental

- **DNA vaccine**
- **Peptide vaccine**
- **Anti-idiotype vaccine**

Advantages of DNA vaccines

- ✓ Plasmids are easily manufactured in large amounts
- ✓ DNA is very stable
- ✓ DNA resists temperature extremes so storage and transport are straight forward
- ✓ DNA sequence can be changed easily in the laboratory.
- ✓ By using the plasmid in the vaccinee to code for antigen synthesis,
- ✓ Mixtures of plasmids could be used that encode many protein fragments from a virus/viruses so that a broad spectrum vaccine could be produced
- ✓ The plasmid does not replicate and encodes only the proteins of interest
- ✓ There is no protein component and so there will be no immune response against the vector itself
- ✓ there is a CTL response

Possible Problems

- ✓ Potential integration of plasmid into host genome leading to insertional mutagenesis
- ✓ Induction of autoimmune responses (e.g. pathogenic anti-DNA antibodies)
- ✓ Induction of immunologic tolerance (e.g. where the expression of the antigen in the host may lead to specific non-responsiveness to that antigen)

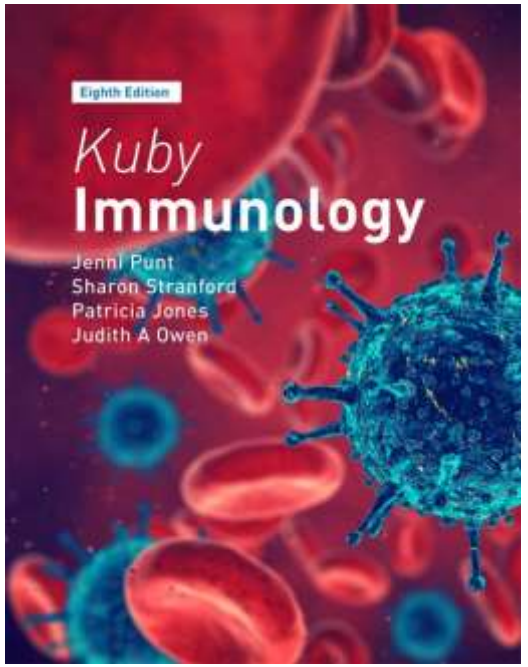
Adjuvants

- ✓ Adjuvants are CRITICAL for the use of inactivated vaccines
- ✓ Most widely used are aluminum salts (mainly hydroxide or phosphate)
- ✓ Effects include liberation of antigen, chemoattraction, and inflammation

ISCOMS

- ✓ Immunostimulating Complexes
- ✓ Multilmeric presentation of antigen/adjuvant
- ✓ Enhanced cell-mediated immune response, delayed-type hypersensitivity, cytotoxic T lymphocyte response, increased Ag expression associated with MHC II

Acknowledgement



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