Therapeutic Proteins

BIT 230
Blood Products

• CLOTTING
  – Haemophilia
  – Benefix

• ANTICOAGULANT

• THROMBOLYTIC AGENTS
  – tissue plasminogen activator
  – streptokinase
Coagulation pathway

Factor VIII (Haemophilia A)

Factor XI (Haemophilia B X linked)

Vit K deficiency cofactor for enzymes

Purify from Plasma
precipitate
immunoaffinity chromat filtration

Recombinant Blood Factors
no viral infections
abundant
use eukaryotic systems
Anticoagulants

Break/prevent clots

Treats: Heart attacks
stroke
deep vein thrombosis

Heparin
Warfarin - vit K antimetabolite
Hirudin
leeches
binds thrombin
Thrombolytic agents

tPA

Clot (post injury)

Plasminogen (inactive)

\[ \text{tPA (serine protease)} \]

Plasmin

Streptokinase
activates plasminogen
found in haemolytic streptococci
History - Vaccines

Edward Jenner

cowpox (vaccinia)

smallpox
Mechanism of Vaccination

Establish resistance to virus/pathological organism by evoking an immune response

1. Give host a foreign organism/protein in non-infectious form

2. Antibodies are generated
   Ab binds to surface proteins of organism

3. Memory B and T lymphocytes

Antibody Response Graph
I. Types
   A. Inactivated (Killed)
   B. Live
   C. Attenuated (Live, Non-infectious)

   LIVE MORE EFFECTIVE THAN KILLED

II. Pathogens
   A. Bacteria
   B. Virus
   C. Parasites
Limitations To Traditional Vaccines

1. can’t grow all organisms in culture
2. safety to lab personnel
3. Expense
4. insufficient attenuation
5. reversion to infectious state
6. need refrigeration
7. do not work for all infectious agents
Recombinant Vaccines

1. Subunit Vaccines
   - peptide vaccines
   - Genetic immunization

3. Attenuated Vaccines

4. Vector Vaccines

5. Bacterial Antigen Delivery Systems
Recombinant Vaccines

1. Delete Virulence Genes (can not revert)
   V/B as Vaccine

2. Clone gene for pathogenic antigen into non-pathogenic virus or bacteria
   V/B as Vaccine

3. Clone pathogenic antigen gene into expression vector

   A. Vaccinate with ‘protein’
      1. Subunit
      2. Peptide
Subunit vaccines

• Do NOT use entire virus or bacteria (pathogenic agent)

• Use components of pathogenic organism instead of whole organism

• Advantage: no extraneous pathogenic particles ie DNA

• Disadvantage: Is rprotein same as \textit{in situ}?

Cost
Examples of Subunit Vaccines

A. Hepatitis B
• Problem with Traditional vaccine- HSV is oncogenic
• envelope glycoprotein D (gD) elicits Ab response
• Clone gene into vector
• Express in yeast cells
• HBsAg - First Recombinant Vaccine (SB)
Examples of Subunit Vaccines

A. HSV
• Problem with Traditional vaccine- HSV is oncogenic
  • envelope glycoprotein D (gD) elicits Ab response
  • Clone gene for gD into vector
  • Express in mammalian cells
  • Transmembrane protein
    modify gene to remove TM portion
Other Subunit Vaccines

B. Tuberculosis

*Mycobacterium tuberculosis*

antibiotic resistant strains

use purified extracellular (secreted) proteins as Vaccine

C. *Bordetella pertussis*

whopping cough

express surface antigen in E coli

D. Tetanus

express toxin in E coli
Vector Vaccines: Virus as Antigen Gene Delivery System

Antigen Gene → Virus → Patient → Antigen Protein is Made
Vector vaccines

Vaccinia good candidate for a live recombinant viral vaccine
  • benign virus
  • replicate in cytoplasm (viral replication genes)
  • easy to store

A) Insert cloned gene encoding antigen
B) Interrupt thymidine kinase (non-essential gene)
C. Infect host cell with native virus
D) Transform these cells with recombinant plasmid
E) HOMOLOGOUS RECOMBINATION
F) Select cells which are resistant to BROMODEOXYURIDINE

**MODIFIED VIRUS USED AS VACCINE** ie. HIV
Peptide Vaccines

Use discrete portion (domain) of a surface protein as Vaccine

These domains are ‘epitopes’

antigenic determinants
are recognized by antibodies
HIV Vaccines

Mutates with high frequency
  r transcriptase

antibodies not enough
  need cell-mediated response

Traditional vaccines only stimulate humoral response

Poor animal models
Cancer vaccine

Target Tumor surface antigens (TSA)

  Use viral vectors to express TSAs
  Use TSA as vaccine
  Genetic Immunization
    Add DNA to TSA

Problem: TSA is also on non-cancerous cells
Monoclonal Antibody Production
Antibody

Fig 42.6
FIG 42.11
Less Immunogenecity

Chimeric Antibodies

Humanized antibodies
Examples

OKT3
  kidney rejection
  anit-CD3 (cluster of differentiation)

(2000) 18 antibodies approved  - diagnostic and therapeutic

90 in clinical trials
Magic Bullets

1. unconjugated antibodies  Fc attracts macrophages

2. Radioactively tagged antibodies

3. Toxin conjugated

4. Enzyme conjugated antibody
   enzyme converts prodrug into cytocidal drug