CYTOKINES

- Proteins secreted by the cells of the immune system that regulate the immune response by communicating among cells
- **Characteristics:**
  - Cell rarely secrete only one cytokine at a time e.g. macrophages secrete at least five: IL-1, IL-6, IL-12, IL-18, and TNF-α
  - They affect a wide variety of cells and organs
  - Many different cytokines may have similar effect (redundancy) e.g. IL-1, TNF-α, TNF-β, IL-6, all have pyrogenic effect
- Types and Groups:
  1. Interleukins: cytokines that regulate the interaction between lymphocytes and other leukocytes. They are numbered sequentially in order of their discovery, IL-1 – IL-30
  2. Interferons
     - Antiviral cytokines produced in response to immune stimulation and virus infection
     - Interferes with viral RNA and protein synthesis
     - There are 2types: type I and type II
     - Type I: interferon alpha (IFN-α) and interferon beta (IFN-β) (antiviral)
     - Type II: interferon gamma (IFN-γ) (immune activation)
     - Some interferon are important in maintenance of pregnancy (e.g. type I IFN-δ)
  3. Tumor Necrotic Factors (TNFs)
     - Derived from macrophages and T-cells
     - They destroy tumor cells
     - They are important in acute inflammatory reactions especially TNF-α
     - They play dominant role in immune regulation and inflammation
  4. Growth Factors
     - Colony stimulating factors

- Control leukocyte production by regulating stem cell growth
- Make immune cells available for body defence

5. Chemokines
    - Regulates leukocyte circulation and migration (chemotaxis) during inflammation
    - They also activate leukocytes
    - Example: Interleukin-8 (CXCL-8)

Functions of Cytokines

i. Cytokines are produced by antigenic stimuli acting through the T-cell and B-cell receptors
ii. Antigen-antibody complex acting through Fc receptors
iii. Super antigens acting through the T-cell receptors
iv. Pathogen-associated molecules such as lipopolysaccharides acting through toll-like receptors

Pattern of Cytokine activities

Autocrine: they bind to receptors on the cell that produced them.

Paracrine: they bind only to receptors on cells in close proximity to the cell of origin

Endocrine: they spread throughout the body thereby affecting cells in distant location from the source of production

Functions

- when bound to target cells, cytokines may induce the target cell to divide or differentiate
- They may stimulate the production of new proteins by the target cell
- They may inhibit cell division and differentiation
- They may inhibit the process of protein synthesis in the target cell
- Most cytokines act on different target cell types and initiate different responses in each. This phenomenon in termed PLEIOTROPY

- Many different cytokines may act on a single target cell. This is termed REDUNDANCY e.g. IL-3, IL-4, IL-5, IL-6, all affect B-cell function
- Some cytokines work optimally only when in association with other cytokines. This is called SYNERGY e.g.IL-4 combines with IL-5 to stimulate B-cell switching to IgE synthesis
- Some cytokines may prevent/inhibit the action of others. This is called ANTAGONISM e.g. IL-4 and IFN-γ are mutual antagonists.

IMMUNE RESPONSE TO TUMOUR

- Events leading to the development of tumour are pooly understood
- Tumour arises as a result of:
    1. Infection with a tumourgenic virus e.g. herpes virus, papilloma virus
    2. Mutation in gene controlling cell growth
    3. Expression of pre-existing oncogenes (tumour genes)
    4. Disturbance in normal growth control mechanisms so that a genetically normal cell no longer displays normal differentiation

Tumour antigens

1. Antigens expressed on chemically or physically induced tumours
2. Antigens expressed on virally induced tumours
3. Antigens associated with oncodevelopmental products
4. Antigens of spontaneous tumour

Types of Tumour Antigens

i. Antigens of chemically induced tumours
ii. Antigens of virally induced tumours
iii. Onco-developmental tumour antigens
iv. Antigen of spontaneous tumours

- The major difference between a normal cell and a tumour cell is a loss of regulated cell growth as a result of multiple mutation
- Mutation may make the tumour cells express abnormal proteins on their surfaces
- The abnormal proteins may be recognized by the body's defence mechanism as being foreign
- This recognition will induce immunological attack

Antigenic Features of Tumour Cells

Changes on the cell surface of tumour cells that make them different from the normal cells

- loss or gain of histocompatibility antigen
- loss of blood group carbohydrates
- appearance of virus-associated antigen (tumour associated viral antigen TAVA)
- tumour-associated transplantation antigens common for the tumour of the same hytologic type (TATA)
- Tumour-specific transplantation antigen present on only one tumour type (TSTA)
- Antigen detected only by serologic reaction unique for a given tumour (Tumour-associated serologic defined antigens TASA)
- Tumour-associated developmental antigens (TADA): markers shared by embryonic or developing tumours and established tumours

<u>Tumour-associated antigens</u>

- Tumour cells may produce new proteins
- Tumour cells may produce excessive amounts of normal proteins

1. Some tumour cells may express the products of developmental genes that are turned off in adult cells and are normally only expressed early in an individual's development. These proteins are called onco foetal antigens e.g. carcinoembryonic antigen (CEA, CD66e) is a glycoprotein produced by tumour cells of the gastrointestinal tract which should normally be found only in fetal intestine; α-fetoprotein produced by lepatoma cells is an onco-foetal antigen normally found only in the foetal liver
    - Onco-fetal antigens are poor immunogens and do not provoke protective immunology

- Measurement of their level in blood may be useful in diagnosis and in monitoring the progress of tumour

2. Antigens to spontaneous tumour
    - Rarely demonstrate tumour-specific antigens/new antigens
    - Normal antigens are expressed in unusual quantities
    - There may be abnormal proteins associated with cell division e.g. glycosylation of proteins
3. Antigens due to oncogenic viruses
    - Tumour cells gained new antigenic character of inducing virus
    - Antigens are coded in viral genome but not part of the virion
4. chemically induce tumour
    - chemical may induce mutation
    - tumour cells therefore expressed mutated surface antigens
    - carcinogenic chemicals may produce different mutation
    - Tumour induced by a particular chemical may be antigenically different
    - Resistant to one chemically induced tumour does not prevent the growth of another tumour induced by same chemical

- The ability of tumour cells to elicit immune reaction depends on their ability to cause/induce inflammation
- A tumour cell that does not invade the lymphoid organs may not elicit immune reaction
- Tumour cells that invade the lymphoid organs may elicit either a strong or a weak immune reaction
- Tumour cells that are processed by dendritic cells elicit a strong T-cell response
- Tumour cells that are walled off may not be processed enough and thus only a weak immune response
- Tumour cells that produce inflammation in tissue also trigger dendritic cell activation and processing

Effector Mechanism in Tumour Immunity

- Tumour cells express different antigens from normal cells
- However, tumour cells are not always recognized as foreign
- The normal molecules on tumour cells are not appropriately presented to the immune cells especially cytotoxic T-cells
- However, tumour cells may be attacked by natural killer cells, cytotoxic T-cells, activated macrophages and antibodies
- Natural killer cells are the most important in immunity to tumour

<u>Humoral response</u>

- Antibodies can be demonstrated in the body against tumour
- The presence of antibodies does not induce resistance to tumour
- Antibody detection are important in serological characterization and isolation of tumour-associated antigen
- Therefore, antibodies can mediate anti-tumour activities
    - Compliment-mediated lysis
    - Opsonization and phagocytosis
    - Loss of cell adhesion

<u>Cell-mediated responses</u>

- Direct lysis by T-lymohocytes
    - Immune T-lymphocytes can specifically recognize and kill target cells that share the same antigens as the immunizing tumour cells
    - Able to destroy solid tissue as well as dispersed tumours
- Antibody-dependent cell-mediated cytotoxicity (ADCC)
    - Tumour target cells coated with IgG can be destroyed by effector celss such as granulocytes, macrophages and killer cells
- Killing by activated macrophages
    - Activated macrophages have tumouricidal capabilities
- Lysis by natural killer cells
    - They can discriminate between normal and abnormal cells

Evasion of Immune Mechanism by Tumour Cells

- Tumour in privilege sites
    - Tumour in the central nervous system and eyes
    - Effector cells can not reach them
- Antigenic modulation
    - Loss of antigenicity or change in antigenic marker
    - Tumour cells avoid immunologic destruction
- Enhancement and blocking factors
    - Humoral factors enhance tumour survival by interfering with the cellular assault against tumour
    - Early production of antibodies may result in absorption to tumour surface and most tumour antigen
    - This prevent induction of T-killer cell-mediated immunity
- Immune capacity versus tumour mass
    - If tumour challenge is sufficiently larger, the animal may succumb to the growth of lethal cancer
- Suppressor of T-lymphocytes
    - Tumour-specific suppressor T-cels have been demonstrated in tumour-bearing mice and may play a role in the apparent ineffectiveness of the response in tumour-bearing mice
- Suppression mediated by the tumour
    - Some tumour synthesize various materials such as prostagladins which affect the activity of immune response

Immunodiagnosis

Based on:

1. Detection of tumour markers e.g. alpha fetoproteins, carcinoembryonic antigen (CEA), prostate-specific antigens (PSA)
2. detection of tumour-specific immunity using the presence of humoral or cellular antibodies autoimmune immunity for diagnosis

Immunotherapy

- Active immunotherapy
  - Stimulate the immune system non-specifically e.g. use of attenuated strain of *glycobaterium bovis* BCG which activate macrophages and stimulates cytokines release thereby promoting T-cells activity
  - Use of tumour cells/antigens to stimulate immune response X-irradiated, neuraminidase or glutaraldehyde-treated cells can be used in tumour vaccines
- Passive immunotherapy
  - Cytokine therapy: IFN-α, TFN-α, IL-2
  - Activated cytotoxic cell therapy: NK and NK-like cells activated
  - Antibody therapy: use of monoclonal antibodies

VACCINES AND VACCINATION

- The term vaccine eas coined from vacca (cow)
- Edward Jenner was the first to discover the use of vaccine to prevent infectious disease
- Jenner used vaccinia virus of cow to protect against smallpox in human in 1798
- Vaccines can be directed against infectious agents or its toxin

History of vaccination

Ancient time practices of vaccination fir disease protection:

- King Mithridates of Pontus protected himself from poison by drinking the blood of duck given the poison
- Pliny the Elder in Rome ate liver of 'mad dogs' to protect against rabies
- Edward Jenner inoculated James Philip on the arm with material from a typical cowpox on the hand of a milk maid
- Pasteur produced different vaccines against livestock diseases: *Fowl cholera* (using dead bacteria to protect chicken in 1880). *Anthrax vaccine* for cattle and sheep in 1881 by growing *B. anthracis* at 42^0C. *Rabbis vaccine* in 1885

Types of vaccines

- Homologous vaccines
  - Developed from the pathogen or from its virulent mutant e.g. *Salmonella typhi* vaccine for the protection of typhoid in human, *E. Dublin* vaccine to protect animals from virulent strains.
- Heterologous vaccines
  - Developed from different organisms to protect against another sharing close antigenic properties e.g. rinderpest vaccine (TCRV) used for the protection of goats from PPR
- Autogenous vaccine
  - Vaccine developed from organism recovered/isolated form an infected animal and the vaccine administered to the same animal for protection. Used in case of chronic diarrhea of animals

VACCINATION

- Active immunization
- Artificially acquired
- Long lasting protection against infectious agents

Advantages:

- Better and cheaper than chemotherapy; some diseases can only be prevented
- Prevention is better than cure; prevention of zoonotic disease
- Decreases morbidity
- Decreases mortality

- Duration of protection is influenced by:
  - Age
  - Immune complexes
  - Nutritional status
  - Nature of the antigen
  - Presence of adjuvants

- Presence of maternal antibodies
- Modified Live vaccine confers more prolonged immunity than killed, inactivated vaccines

Routes of administration

- Aphthization
  - A crude method produced by Fulani herdsmen
  - In an outbreak of foot-and-mouth disease, cattle rearer obtained saliva from clinically-ill cattle and rub it on the tongue of healthy cattle in the flock.
  - Infection is in the head and recovery is synchronized
- Mucus membranes
  - Newcastle disease vaccines given intravenously to day-old chicks
  - Infectious laryngotracheotis (ILT) vaccines rubbed into the mucus membranes of cloaca
- Subcutaneous
  - *B. pertusis* vaccine, *Brucella* S_{19}, T_1 *vaccine* of CBPP, typhoid vaccine (TAB)
- Intramuscular
  - Yellow fever vaccine, tetanus toxoid
- Intradermal
  - Pox vaccines, tuberculosis (BCG) vaccine
- Oral
  - *E. coli* vaccine
  - Poliomyelitis vaccine

Time of vaccination

- Depends on the disease to be prevented
- Influence by government policies
- Age susceptibility of host
- Examples: BCG, polio, PPR, cumboro, rabies
- Pregnant animals may be vaccinated for passive protection of offspring

- *C. perfrigens* type B and type D infection in lamb prevented by vaccinating pregnant ewes 4 weeks and 2 weeks before lambing
- *Brucella* vaccine given to calves 4-8 months old. *M. paratuberculosis* given to 30-day old calves

Advantage of vaccination over chemotherapy

- Some diseases can not be treated but can only be prevented e.g. viral diseases
- Vaccination is cheaper than chemotherapy
- Production of organic meat

Danger of vaccination

- Accidental self-innoculation
- Precipitation of the disease to be prevented
- Vaccine failure
- Hypersensitivity
- Contamination of vaccine by extraneous organism

Vaccine production

- Capital intensive
- Require skill personnels

Process of vaccine preparation

- Killed viral or bacterial vaccine
- Inactivated toxin or toxoids
- Line attenuated vaccines
- Recombinant vaccines

- Killed vaccines
  - Chemical killing e.g. formalin, beta-propiolactone
  - Heat killing, high temperature
  - Radiation killing e.g. UV light, ultrasonic wave, x-rays

- Viability may be destroyed i.e. decreased immunogenicity
- Beta-propiolactone destroys nucleic acid and preserve antigenicity

- Toxoids
  - Detoxified toxin
  - Use formalin or glutenaldehyde for detoxification
  - Antigenicity increased by adsorption on mineral carrier
- Live attenuated vaccines
  - Passages/several subculturing in monolayer tissue culture e.g. viral vaccine
  - Cultivation at abnormal temperature e.g. *B. anthracis* at 42^0C for anthrax vaccine
  - Culture on unusual media e.g. *B. abortus* S19 on potato medium or ox bile medium for BCG
  - Use of avirulent strain of poor growth e.g. streptomycin-dependent mutants of blingis spp
  - Biochemically-deficient *S. typhimurium*
- Recombinant vaccine
  - Mutant hybrids
  - Safe and effective
  - Genetic modification

Live attenuated:

- a number of route of administration because they have relevant antigens for protective immunity
- high level of cell-mediated and humoral and mucosal surface protection
- no need for adjuvants; they can replicate in the recipients
- booster dose can be spaced widely. Spaced interval if needed because of good immunological memory
- Live attenuated vaccines can produce adverse reactions such as immunosupression

Inactivated:

- Can induce high level of antibodies but less cell-mediated and mucosal immunity.
- Inactivated vaccines often contain many irrelevant antigenic substances some with undesirable biological activity

Advantages of Live vaccines

- Good antigen with good antibody production
- Excretion of vaccine strains may protect those infected with the strain
- Back mutation extremely rare. When present, it is due to deletion rather than spontaneous mutation
- Early non-specific protection is initiated within 1-2 days of administration in cases of viral

Disadvantages of Live vaccine

- Residual virulence may produce clinical signs e.g. S19 in bulls may produce orchitis
- Cannot withstand rough handling; storage condition is very strigent
- Limited shelf-life or danger of contamination with other organism found on tissue culture
- Mutation of vaccine organism
- Immunosuppression especially in young

Advantages of Killed Vaccine

- Can withstand rough handling and ambient temperature
- No overt diseases produced
- Long shelf-life

Disadvantages of Killed Vaccine

- Killing destroys essential antigens
- Poor immunogens, therefore requires several inoculation
- Adjuvants may be required with possible adverse reaction
- Repeated vaccination may lead to hypersensitivity

Note: many disease agents still don't have vaccines for their prevention

Recombinant Vaccine/Biotechnology: subunit or genetically engineered live vaccines

- Increased efficacy
- Increases safety

RECOMBINANT VACCINES

There are three categories:

1. Type 1 recombinant vaccine: composed of antigens produced by genetic engineering
2. Type II recombinant vaccine: genetically attenuated microorganism
3. Type III recombinant vaccine: composed of modified live viruses or bacteria into which DNA encoding a particular antigen is introduced

Type I: subunit proteins produced by recombinant bacteria or other microorganisms. DNA encoding the required antigen is isolated and introduced into a suitable bacterium or yeast in which the recombinant gene/antigen is expressed. There is need for adjuvants to enhance their immunogenicity. Have been used for FMD, feline leukemia and Lyne diseases (*Borrlia burgdoferi)*

Type II: virulent microorganisms are rendered less virulent by gene deletion or site directed mutagenesis. The genome of large DNA viruses (e.g.) contains many genes not required for in vitro replication. With DNA technology, a pseudorabies vaccine lacking the gene for thymidine kinase has been produced. Thymidine kinase is required by this herpes virus to replicate in non-dividing cells such as neurons. The vaccine virus with deleted gene can infect neurons but unable to replicate in their cells. The deleted mutants induce a protective immune response in pigs.

Deletion of the gene encoding for the glycoprotein gI on the pseudorabies virus prevent differentiation of infected pigs which permit differentiation of infected pigs which produce antibodies against gI from vaccinated pigs which lack the antibodies. Thus vaccination can be done in countries where the disease is being eradicated without interfering with serological recognition and removal of the infected pigs.

Type III: Necessitated because vaccine failure often result from delivery system.

Type III: modified live organism called vectors into which a gene is inserted and this organism also serves as a delivery system in the recipient. Vector must not pose any threat to the host.

A vaccinia virus vector carrying the rabine G glycoprotein gene has been successfully used as an oral vaccine administered to wild carnivores in baits.