ANTIGENS

Antigens are substances which are able to induce detectable immune responses when introduced into an animal host. Immune responses could be cellular or humoral

Requirement for antigenicity

- Molecular size: molecules with high molecular weight are capable of eliciting a better immune response than those with low molecular weight. Proteins>carbohydrates>lipids>nucleic acids. Molecules with molecular weight less than 10,000 dalton are weakly antigenic or non-antigenic.
- Chemical complexity: molecules with high complexity are good antigens
  - Polymers are more antigenic than monomer
- Genetic make-up of the animal host
  - The response of an animal to an antigen is regulated by genes
  - The ability to mount an immune response to a antigen varies with genetic composition of the animal
- Method of antigen administration
  - Immune response may differ according to the route of administration
  - Level of immune response is dose-dependent
  - Excessively high dose may induce a state of specific unresponsiveness

EPITOPES

- Most foreign particles are composed of complex mixture of proteins, polysaccharides, lipopolysaccharides, lipids and nucleoproteins
- Such large molecules have specific regions responsible for antigenicity
- Epitopes are regions on the surface of molecules that specifically trigger immune reactions
- Epitopes are also called antigenic determinants
- An antigen mau possess more than one antigenic determinant
- The antigenic determinants on an antigen vary in immunogenicity
- Animal host respond better to an immunodominant epitope on an antigen
- An antigen may possess similar epitopes to those present on the host's self antigen

- However, the cell of the immune system only recognize and respond to foreign epitopes
- The number of epitopes on an antigen is related to its size
- Usually about one epitope is present for each five kDa of protein
- Immunopotency describes the capacity of a region of an antigen molecule to serve as an antigenic determinant and induce the formation of specific antibody
- Immunopotency is determined by:
    - Accesibility: exposure to the aqueous environment
    - Charge: electrical charges are dominant factor in specificity
    - Genetic factor: ability to induce immune response is under genetic control

HAPTENS

- Small molecules (e.g. drugs, hormones), or chemical groups with molecular wight of less than 1000Da which when bound to other larger molecules can function as epitopes
- Haptens are too small to be appropriately processed and presented to the immune system and are therefore not antigenic
- When haptens are linked to a larger molecule, a new epitope is formed on the larger molecule
- When this is injected into an animal host, immune response develops with antibody formation
- The antibody can react with the hapten in the larger molecule
- Haptens are non-immunogenic substances but can react with antibody in a specific manner
- Antigens are capable of inducing cellular immunity mediated by T-lymphocytes but haptens are unable to do so.
- The reactions of drugs which serve as haptens with body proteins may lead to allergies
- Examples of haptens: dinitrophenols, penicillin

ADJUVANTS

- Substances that enhance the immune response to an antigen when administered along with that particular antigen

- Mechanism of action:
  - Depot adjuvants: serve to protect antigen from rapid degradation and thereby prolong immune responses
  - Particulate adjuvants: effectively deliver antigens to antigen presenting cells, enhance cytokine production by antigen presenting cells, enhance T-helper cell responses and enhance cell mediated immunity
  - Immunostimulatory adjuvants: enhance cytokines production, T-helper cell response and enhance cell mediated immunity
- Examples:
  - Depot adjuvants: Aluminium phosphate, Aluminium hydroxide, Treund's incomplete adjuvants (water-in-oil emulsion)
  - Particulate adjuvants: liposomes, microparticles, immunestimulatory complex
  - Immunostimulatory a djuvants: glucose, dextran sulphate, detergents, saponins, lipopolysaccharides, anaerobic corynebacteria, bacillus calmette-Guerin (BCG m. boris), Borditella pertussis etc.
  - Mixed adjuvant: treund's complete adjuvant (water-in-oil emulsion plus mycobacterium)

Tutorial Questions

Define the following terms

i. Antigen
ii. Autoimmunity
iii. Haptens
iv. Adjuvants
v. Epitopes (*4 marks each = 20marks)*

Tutorial Questions2 (10 marks)

i. Describe lupus erythematosus cells
ii. Give the examples of systemic autoimmune diseases
iii. Outline three features of lymphocytic thyroiditis
iv. In equine polyneuritis, what acts as autoantigen?

v. What is the distinct clinical feature of reproductive autoimmune diseases resulting from the injection of terticular extract along with freund's complex adjuvants in male animals? *(10 marks)*

ANTIGEN-ANTIBODY REACTION

When an antibody comes in contact with its homologous antigen, it becomes attached to it by one of its of its combining sites which reacts with a determinant area on the antigen. This reaction leads into formation of an antigen-antibody complex

Ag + Ab ----------→ Ag-Ab complex

The forces that hold these together are at their strongest under physiological conditions of ionic strength and pH. If the pH is lowered, the antigen-antibody complex will dissociate.

Features of antigen-antibody reaction:

- Close proximity: non-covalent binding forces are involved in antigen-antibody combination. The shape of each of the combining site on an immunoglobulin molecule is an accurate mould of the shape of the antigenic determinant and the two must be brought into very close contact to fit into each other.
- Specificity: the union of an antigen with its antibody is specific. The antigen react with its corresponding antibody and with no other. Specificity is dictated by the presence of determinant groups on the antigen and the type and pattern of amino acids present in the antigen-binding region of immunoglobulins.
- pH range: physiological range of pH (7.2-8.2) is required for a firm union. The optimal temperature for an antigen-antibody reaction depends on the type of antibody. IgM reacts best at 4^0C (cryoglobulin) while IgG reacts best at 37^0C.
- Optimal proportion: there is an optimum concentration where antigen-antibody reaction occurs. This optimum concentration is referred to as equivalence zone. The occurrence of an antigen-antibody reaction can be detected by the presence of some secondary phenomenon such as precipitation or agglutination complex. The presence of cisible agglutination of precipitation reaction will be inhibited by an excess of antibody and this is termed 'prozone phenomenon'.

Forces Responsible for the Union of Antigen and Antibody

The forces of interaction responsible for antigen-antibody reaction are the same as those seen in other proteins such as enzymes and transport proteins. The final strength of the bond is a summation of the various binding or repelling forces present on both antigen and antibody molecules. Covalent chemical bonding is not important and there is no obligatory requirement for charged groups on antigens. However, there can be strong attraction or repulsion between negatively charged ions and positively charged ions on these molecules at physiologic pH. The forces invoved in antigen-antibody union include the followings:

1. Electrostatic forces
2. Hydrogen bonding
3. Hydrophobic attraction
4. Van der waal forces

Electrostatic forces: these are due to the attraction between oppositely charged ionic groups on proteins side chains. An example is the interaction between an ionized amino group ($-NH_3^+$) on a lysine of one protein and an ionized carboxyl group ($-coo^-$) on a glutamate of another protein.

Hydrogen bonding: if molecules carrying hydrophilic groups such as –OH, $-NH_2$ and –COOH approach closely, they form hydrogen bridges which are relatively weak and reversible. The interaction between threonine and tyrosine is an example of hydrogen boding.

Hydrophobic attraction: non-polar hydrophobic groups such as those of the side chains of valine, leucine and phenylalanine tend to associate in an aquepus environment, just like oil droplets in water merge to form a single large drop. It has been estimated that hydrophobic forces may contribute up to 50% of the total strength of the antigen-antibody bond.

Van der waals forces: these are very weak forces which depend on interaction between the external “electron cloud” of molecules. Complimentary electron cloud shapes on the combining site of an antibody and on the surface determinant of an antigen fit the two molecules strongly together like a lock and key.

Antibody Affinity and Avidity

The antibodies that are first produced by the body after it has been stimulated with an antigen do not mate with so large an area of the antigenic seterminant as do those which are synthesized later and especially those which appear after repeated immunization have been carried out. Thus, antibodies produced soon after a first stimulation are very specific and have high affinity for a

particular area of the antigenic determinant. They are termed non-avid (i.e. the complexes they formed with the antigen are easily broken down). The strength of the interaction of an antibody with a monovalent hapten or a single antigenic determinant is referred to as affinity. Antibodies produced later or after repeated immunization are avid. The strength of the interaction of an antiserum with a fall antigen with its multiple determinants is termed avidity. The force binding two determinant groups by antibody is usually many fold greater that the arithmetic sum of the forces binding each separate antigenic determinant. Avidity makes for stronger bonds with the antigen and often able to cross-react with other related antigens.

a. Early non-avid antibody molecules only combine with a small area of the antigenic determinant
b. Later antibody, and antibody produced after repeated restimulation is very avid. It combines strongly with a larger portion of the antigenic determinant than does non-avid antibody.
c. Avid antibody is also able to combine with related antigenic determinants. The fit however is not very close and the binding is weak.

Mechanism of Protection by Antigen-Antibody Reaction

Antibody can protect the body from infection or its effect by neutralizing soluble toxins, coating organisms and thus promote phagocytosis, by direct lysis of organisms in the presence of the compliment proteins and by preventing the spread of intracellular organisms.

Consequences of Antigen-Antibody Reactions in-vitro

Following the primary union of antigen to antibody in the laboratory, a number of events occur which produce visible effects. This primary interaction gives rise to a number of secondary phenomena such as precipitation, agglutination, flocculation, phagocytosis, cytolysis and neutralization. These secondary reactions are the basis of a number of standard immunological techniques. The primary reaction can simply be viewed as the specific recognition and combination of the antigenic determinant with the binding site of its corresponding antibody. Generally, primary tests are more sensitive than secondary tests. The quantitative tests that employ the primary reaction include immunoflourescence, radioimmunoassay and immunoenzymatic assays.

Harmful Effects of Antigen-Antibody Reaction in the Body

Antibody-antigen reactions in the body are not only helpful but can equally be harmful. In some situations the immune attack on the invading organisms also damage host tissues. Autoimmune reactions and hypersensitivity reaction and graft rejection are examples of harmful reactions.

AUTOIMMUNITY

- The body produces self-antigens
- Lymphocytes capable of binding and responding to self antigens in the body are suppressed
- Self-antigens to which the immune system is exposed during foetal life are recognized as self and the body develop tolerance to them
- Autoimmunity is a state in which the natural unresponsiveness of the lymphocytes (tolerance) to self antigens is lost
- In autoimmunity, autoantibodies are produced which react with self components. This may lead to disease condition and tissue damage
- Not all autoimmune responses are harmful. Infact, some are beneficial and crucial to survival. Some autoantibodies serve physiological functions e.g. destruction of senescent red blood cells
- The exact cause and mechanisms of autoimmunity are not well understood
- Autoimmunity could be mediated by either B cells or T cells (auto antibodies or T cells)
- **Mechanism of autoimmune diseases**
    - Normal immune response to an unusual or abnormal antigen
    - Abnormal immune response to a normal antigen: a situation in which regulations preventing development of self-responsive T-cells fails
    - Aberrant response to a single specific antigen
    - General defect in the regulation of B- or T- cells functions
- **Normal immune response**
    - Normal immune response to a previously hidden antigen
    - Cross reactivity between an infectious agent and a normal body component
    - Abnormal antigen processing
- **Abnormal immune response**

- Sustained immune response to hidden epitopes
- Lymphoid tumour cells producing autoantibody
- Defective destruction of self-reactive lymphocytes

- **Virus-induced autoimmunity**
  - Vaccine-induced autoimmunity: vaccines with adjuvants, especially excessive use
  - Example:
    - Endocrine diseases like lymphocytic thyroiditis, hyperthyroidism,
    - Neurological diseases: equine polyneuritis, canine polyneuritis, degenerative myelopathy
    - Eye diseases: equine recurrent ureitis
    - Muscle diseases: myasthenia gravis, canine cardiomyopathy, polymyositis
    - Skin diseases: perphigus complex, epidermolysis bullosa

AUTOIMMUNE DISEASES

Systemic autoimmune diseases

- Associated with the presence of circulating immune complexes and complement in tissues
- The deposition of immune complexes lead to chronic inflammation
- The initiating antigens are unknown but may well be infectious agents
- There is genetic predisposition linked with MHC
- Examples:
  - Systemic Lupus Erythematosus
- There is impaired clearance of apoptotic cells by macrophage phagocytosis
- Apoptotic cells accumulate in the tissue
- Nuclear fragments of apoptotic cells are processed by dendritic cells (antigen-presenting cells)
- There is formation of autoantibodies (antinuclear antibodies, ANA)
- This leads to formation and deposition of immune complex and tissue damage

- There is dermatitis (skin lesions), polyarthritis, heamolytic anaemia, thrombocytopaenia, proteinuria, positive ANA test, and positive LE cell test
- LE cells: cells that have phagocytosed opsonised nuclei oftern present in the bone marrow of SLE patients
- Seen in humans, other primates, dogs, rats, horses, mice

Sjogrens Syndrome: (Horses, dogs)

- Characterized by keratoconjuctivitis sicca (conjuctival dryness), xerostomia (mouth dyness) and rheumatoid factors
- Autoimmunity against salivary and lacrimal glands
- There is gingivitis, dental caries, excessive thirst, corneal dyness and abrasion leading to kretitis and conjunctivitis as well as other ocular lesions\there is also rheumatoid arthritis and polimuositis
- Autoimmune polyarthritis
- Deposition of immunoglobulins and immune complex within joints leading to joint diseases
- Could be erosive polyarthritis (e.g reumathoid arthritis) or non-erosive (e.g. equine and canine polyarthritis)

Organ-specific/Tissue-specific Autoimmune Diseases

Endocrine:

- Lymphocytic thyroditis
- Lymphocytic parathyroditis
- Insulin-dependent diabetes mellitus
- Atropic lymphocityx pancreatitis
- Sutoimmune immune adrenatitis
- Hyperthyroidism

Neurological

- Degenerative neuropathy
- Cerebellar degeneration

- Equine polyneuritis
- Steroid meningitis-arteritis
- Canine polyneuritis

Eye diseases

- Equine recurrent ureitis
- Ureodermatological syndrome

Reproductive

Skin diseases

- The pemphigus complex
- Skin basement membrane disease
- Alopecia areata
- Relapsing poluchondritis

Nephritis

- Autoimmune immune nephritis
- Autoimmune haemolytic anaemia
- Autoimmune immune thrombocytopenia

Muscle

- Myasthenia Gravis
- Polymyositis
- Autoimmune masticatoryy myopathy
- Canine cardinmyopathy

Organ-Specific Autoimmune Diseases

- Autoimmune diseases that affect a single organ or tissue
- Arises as a result of abnormal reponse to a small number of self- or foreign antigen but not necessarily a major loss of control of the entire immune system
- Examples:
  A Autoimmune endocrine diseases

I. Lymphocytic thyroditis

- Described in human, dogs and chicken
- Production of autoantibody against throglobulin which may also react with triialothyronine (T_3) or thyroxine (T_4)
- There is dull, dry, coarse coat, scaling, hypotrichosis, hyperpigmentation, pyoderma. Affected animals are fat sluggish and have area of in the skin

II. Lymphocytic parathyroiditis

i. Affects dogs and cats
ii. History of neurological or neuromuscular disorder like seizures
iii. There is marked lymphocalcaemia and low level of serum parathormones
iv. At histology, the normal parathyroid tissue is replaced by infiltrating lymphocutes and some plasma cells

III. Insulin-dependent diabetes mellitus

i. There is development of autoantibodies against islet cells enzyme called glutamic acid carboxylase
ii. There is atrophy of pancreatic islet and loss of β cells. Lymphocytes infiltrate the islets.

B. Autoimmune neurological diseases e.g. development of autoantibody to brain tissue following administration of rabies vaccines prepared in brain tissue

I. Equine polyneuritis

II. Peripheral myelin protein P_2 acts as autoantigen stimulating the formation of autoantibodies: There is a chronic granulomatous inflammation in the region of the extradural nerve roots. The nerves affected are thickened and discoloured. There is loss of myelinated axon, macrophage, lymphocyte, giant cells and plasma cells and plasma cells infiltration and deposition of fibrous material in the perineurium

C. Autoimmune reproductive diseases

– Damage to the testes may release hidden antigens and consequently autoimmunity

- Injection of testicular extract in Treund's complete adjuvant may produce autoimmune orchitis in male animals
- The presence of sperm antigens in the circulation stimulates the production of IgE or IgA autoantibodies
- The autoantibodies can agglutinate and immobilize sperm cells leading to infertility
- Autoimmune dermatitis may occur in intact female dogs as a result of hypersensitivity to endogenous progesterone or oestrogen
- This autoimmune dermatitis may coincide with oestrus or pseudopregnancy and it is characterized by bilateral erythema and popular eruption with intense pruritus

D. Autoimmune Muscle Diseases

a. Myasthenia Gravis
    i. Seen in humans, dogs and cats
    ii. Disease of skeletal muscle characterized by abnormal fatique and weakness following mild exercise
    iii. There is degradation of acetylcholine receptors by IgG autoantibodies
    iv. Autoantibodies also block acetylcholine binding sites and trigger compliment-mediated damage
    v. The deficiency of acetylcholine receptor: this leads to failure of transmission of nerve impulses across the motor end-plate of striated muscle

E. Autoimmune Haemolytic Anaemia

i. Destruction of red blood cells mediated by autoantibodies to red blood cells antigens
ii. Red blood cells destruction could be intravascular haemolysis mediated by complement or phagocytosis of antibody coated RBC in spleen and liver by macrophages (extravascular)

iii. Autoimmune haemolytic anaemia has been attributed to alteration in red blood cell surface antigen induce by drugs or viruses
iv. The condition is characterized by anaemia, weakness, lethargy, fever, uterus and hapto- splebomegaly. There could be tarchycardia, anorexia, vomiting or diarrhea
v. It has been described in human, dogs, horses, cats, mice, cattle and rabbits