

**VPM 403: INTRODUCTORY VETERINARY IMMUNOLOGY
LECTURE NOTES**

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VPM 403: INTRODUCTORY VETERINARY IMMUNOLOGY COURSE OUTLINE

Topics	Lecturer
History of Immunology	M. A. Oyekunle
Immunological concepts/terminologies	M. A. Oyekunle
Types of immunity Organs and cells involved in immune responses	M. A. Oyekunle
Immunoglobulin structures, classifications and functions	M. A. Oyekunle
The major histocompatibility complex	M. A. Oyekunle
Gene regulation of immune responses	M. A. Oyekunle
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Immune response to fungal and parasitic infections	M. Agbaje
Hypersensitivity reactions	M. Agbaje

IMMUNOLOGY

DR. M. A. OYEKUNLE

Immunology is an area of science which helps in understanding the way by which animals gained protection from disease causing agents. It also includes the use of antibody-antigen reaction or other laboratory work i.e. serology and immunochemistry.

History of Immunology

The Nobel Prize in Physiology or medicine (1908) was awarded to Ilya Ilyich Metchnikov (1845-1916) with Paul Ehrlich in recognition of their work in immunity.

Late 18th century

Jenner, Edward introduces cowpox vaccine for protection against smallpox (1798).

Late 19th century

- Pasteur; germ theory, attenuated & killed vaccines i.e. anthrax vaccine, developed rabies vaccine.
- Kock (1882) described tubercle bacillus and produced killed vaccine.
- Metchnikoff (1884) described phagocytosis.
- Pasteur (1885) developed rabies vaccine.
- Von Behring & Kitasato (1890) prepared killed vaccine.
- Bordet, Pfeiffer (1895) discovered complement activity.
- Ehrlich (1891) standardized diphtheria toxin so that its potency can be assessed and antitoxin measured against it.
- Durhan- bacterial agglutination.

Mid 20th Century to date

1902 –Landsteiner discovered blood group.

1903 - Wright and others discovered antibody in the blood of immunized animals.

1903 –Antigenic determinant – Landsteiner, Heidegger, Murrack

1903 –Electrophoretic separation of gammaglobulin by Kabat & Tiselius

1903 – Antiglobulin test – Coombs, Mourant and Race

1903 – Recognition of immunity.

1955 –Clonal selection theory of immunity – Burnet & Jerne

1953 –Medawar – discovered immune tolerance

1962 – Porter – propose basic structure for immunoglobulin G molecule

Transplant immunology, tumor immunology, Rhesus immunization, Deficiency states and role of thymus

Relationship between structure and biological activities of immunoglobulin Molecules and genetic control mechanism

-Determinant of immunogenicity of antigen molecule

-Immunogenetic and evolution of immune system

-Lymphocyte activation and cell cooperation.

-Role of macrophages – antibacterial and cytotoxic effects.

1975 – Monoclonal antibody production technique by Kohler & Milstein

1983-1984 –Mullis developed Polymerase Chain Reaction (PCR)

1986 -First vaccine (Hepatitis B vaccine) produced by genetic Engineering approved for human use.

1986 -Chickenpox vaccine approved for use in U. S

IMMUNOLOGY CONCEPT

Immunology is the study of host immune system from the moment of birth and sometimes even before that, the body exists in an environment filled with potentially harmful organisms and agents. Over the course of thousand of years of evolution, protective mechanism have developed in human – animal immune system reflects many aspect of this evolution ranging from the innate immunity afforded by the skin and mucous membranes to the highly complex specific response of T -cells and antibodies which recognizes invading pathogens if they are encountered again.

TERMINOLOGIES

Antibody (AB): A protein produced as a result of interaction with an antigen. The protein has the ability to combine with the antigen that stimulated its production.

Antigen (Ag): A substance that can react with an antibody. Not all antigens can induce antibody production; those that can are also called immunology.

B cell (also B lymphocyte): Strictly, a bursa–derived cell in avian species and, by analogy, a cell derived from the equivalent of the bursa in nonavian species. B cells are the precursors of plasma cells that produce antibody.

Cell – mediated (cellular) immunity: Immunity in which the participation of lymphocytes and macrophages is predominant. Cell–mediated immunity is a term generally applied to the type IV hypersensitivity reaction (see below).

Chemokines: low–molecular–weight protein that stimulate leukocyte movement.

Chemotaxis: A process whereby phagocytic cells are attracted to the vicinity of invading pathogens.

Complement: A set of plasma proteins that is the primary mediator of antigen-antibody reactions.

Cytolysis: The lysis of bacteria or of cells such as tumor or red blood cells by insertion of the membrane attack complex derived from complement activation.

Cytotoxic T cell: T cells that can kill other cells infected with intracellular pathogens.

Endotoxins: Bacterial toxins released from damaged cells.

Epitope: Site on an antigen recognized by an antibody. Also known as an antigenic determinant

Hapten: A molecule that is not immunogenic by itself but can react with specific antibody.

Histocompatible: Sharing transplantation antigens.

Humoral immunity: Pertaining to immunity in a body fluid and used to denote immunity mediated by antibody and complement.

Immune response: Development of resistance (immunity) to a foreign substance (e.g., infectious agent). It can be antibody-mediated (humoral) ,cell-mediated (cellular), or both.

Innate immunity: Nonspecific resistance not acquired through contact with an antigen. It includes skin and mucous membrane barriers to infectious agent and a variety of non specific immunologic factors, and it may vary with age and hormonal or metabolic activity.

Adaptive immunity: Protection acquired by deliberate introduction of an antigen into a responsive host. Active immunity is specific and is mediated by either antibody or lymphoid cells (or both)

Immunoglobulin: A glycoprotein, composed of H and L chain, that functions as antibody. All antibodies are immunoglobulin, but not all immunoglobulin have antibody function.

Inflammation: Local accumulation of fluid and cells after injury or infection.

Interferon: One of a heterogeneous group of low-molecular-weight proteins elaborated by infected host cells that protect noninfected cells from viral infection. Interferons, which are cytokines, also have immunomodulating functions.

Leukocyte: General term for a white cell.

Lymphocyte: A molecule cell 7-12pm in diameter containing a nucleus with densely packed chromatin and a small rim of cytoplasm, lymphocytes include the T cells and B cells, which have primary roles in immunity.

Macrophage: A phagocytic mononuclear cell derived from bone marrow monocyte and found in tissues and at the site of inflammation. Macrophages serve accessory roles in immunity, particularly as antigen presenting cells (APCs).

Major histocompatibility complex(MHC): A cluster of genes located in close proximity eg, on human chromosomes 6, that encoded the histocompatibility antigens (MHC molecules)

Membrane attack complex: The end product of activation of the complement cascade, which contains C5, C6, C7, and C8 (and C9). The membrane attack complex makes holes in the membrane of gram-negative bacteria killing them and, in red blood or other cells, resulting in lysis.

Monoclonal antibodies: Each B lymphocyte produces antibody of a single specificity. However, normal B cells do not grow indefinitely. If B cells hybridization and fused cells that secrete the desired antibody-producing cell line, known as a hybridoma, is contained, and these hybrid cells produce monoclonal antibodies.

Monocyte: A circulating phagocytic blood cell that develops into tissue macrophages.

Natural killer (NK) cells: Large lymphoid cells with no known antigen-specific receptors. They are able to recognize and kill certain abnormal cells, e.g. tumor cells.

Oponin: A substance capable of enhancing phagocytosis. Antibodies and complement are the two main opsonins

Oponization: The coatings of an antigen or particle (e.g., infectious agent) by substances, such as antibodies, complement components, fibronectin, and so forth, that facilitate uptake of the foreign particle into a phagocytic cell.

Immunology

Immunology is the study of immunity or protein against infectious or other agents and conditions arising from the mechanisms involved in immunity. Immunity is the protection against infectious agents and other substance. There are two types of immunity,

1. Non adaptive immune response or Innate immunity. This is the immunity that is not affected by prior contact with the infectious agent or other material involved and is not mediated by lymphocytes.
2. Adaptive immune response/ specific immune response/Acquired immunity. This is the immune response that depends on the recognition and the elimination of antigens specific lymphocytes.

Adaptive/acquired Immunity can be natural or artificial, active or passive

	Active	Passive
Natural	Exposure to antigen induces an immune response s immunity that follows attacks of measles or canine distemper	Transfer of antibodies or cells produced by others as temporary immunity from antibodies of the mother transferred to infant across the placenta or in milk.
Artificial	Deliberate exposure to antigen induces an immune response e.g. immunization of children or young animals.	Antibodies in immune serum are introduced into body e.g. injection of rabies immune globulin after dog bite.

The Innate Defenses

1. The innate defense system is composed o first-line defenses, sensor systems such as toll-like receptors and complement, and phagocytes. Inflammation is a coordinated response that involves many aspects of the innate defenses.

First-Line Defenses

Physical Barriers:

1. The skin provides the most difficult barrier for microbes to penetrate, it is composed of two main layers- the dermis and the epidermis.
2. The cells of the mucous membrane are constantly bathed with mucous and other secretion that help wash microbe from the surfaces. Some mucous membranes have mechanism that propel microbes, directing them towards areas where hey can be eliminated more easily.

Antimicrobial Substances:

1. Lysozyme, peeroxidase, enzymes, lactoferrin, and defensins re antimicrobial substances that inhibit or kill microorganisms

Normal Flora:

1. Members of the normal floral competitively exclude pathogens and stimulate the host defenses.

The Cell of the Immune System

1. There are three types of granulocytes- neutrophils, basophila and consinophilis.

Mononuclear Phagocytes:

1. Monocytes differentiate into either macrophages or dendritic cells.

Lymphocytes

1. Lymphocytes, which include B cells, T cells and Natural Killer (NK) cells, are involved in adaptive immunity.

Cell Communication

Surface Receptors bind ligands that are on the outside of the cell, enabling the cell to detect that the ligand is present.

Cytokines:

1. Cyrokines include interleukins (LLs), colony-stimulating factors (CSFs), tumor necrosis factors (TNFs), chemokines, and interferons.

Adhesion Molecules

1. Adhesion molecules allow cells to adhere to other cells.

Sensor Systems

Toll-Like Receptors

1. Toll-like receptor enables cells to detect molecules that signify the presence of microbe.

The Complement System

1. Complement proteins circulate in the blood and the fluid that bathe tissues, in response to certain stimuli that indicate the presence of foreign material, they become activated.
2. The major protective outcomes of complement activation include opsonization, lysis of foreign cells, and initiation of inflammation.

Phagocytosis

The Process of Phagocytosis

1. The steps of phagocytosis include chemotaxis, recognition and attachment, engulfment, destruction and digestion, and exocytosis.

Specialized Attributes of Macrophages:

1. Macrophages are always present in tissues to some extent, but are able to call in reinforcements when needed.
2. A macrophage can increase its killing power, becoming an activated macrophage.
3. Macrophage, giant cells, and T-helper cells form concentrated groups called granulomas that wall off and retain organisms or other material that cannot be destroyed by macrophages.

Specialized Attributes of Neutrophils

1. Neutrophils play a critical role during the early stages of inflammation, being the first cell type recruited from the blood stream to the site of damage.

Inflammation-A Coordinated Response to Invasion or Damage

1. Swelling, redness, heat, and pain are the signs of inflammation, the attempt by the body to contain a site of damage, localize the response, and restore tissue function.

Factors that Initiate the Inflammatory Response:

1. Inflammation is initiated when pro-inflammatory cytokines or other inflammatory mediators are released as a result of the engagement of toll-like receptors or activation complement by invading microbes, or when tissue damage occurs.

The Inflammatory Process

1. The inflammatory process leads to a cascade that results in dilation of small blood vessels, leakage of fluids from those vessels, and the migration of leukocytes out of the bloodstream and into the tissues.
2. Acute inflammation is marked by a preponderance of neutrophils, chronic inflammation is characterized by the prevalence of macrophages, giant cells, and granulomas.

Outcomes of Inflammation

1. Inflammation can contain an infection, but the process itself can cause damage, a systemic response can be life threatening.

Apoptosis –Controlled Cell Death that Circumvent the Inflammatory Process.

1. Apoptosis is a mechanism of eliminating self-cells without evoking an inflammatory response.

Interferons

1. One of the roles of interferons is to induce cells in the vicinity of a virally infected cell to prepare to cease protein synthesis in the event they become infected with a virus, double, stranded RNA signifies to the cell that it has been infected.

Fever

1. Fever occurs as a result of certain pro-inflammatory cytokines released by macrophages when their toll-like receptors bind microbial products.
2. Fever inhibits the growth of many pathogens and increases the rate of various body defenses.

Strategy of the Adaptive Immune Response

The Humoral Immunity

Humoral immunity is mediated by B.cells; in response to extracellular antigens, these may be triggered to proliferate and then differentiate into plasma cells that function as antibody producing factories.

Cellular Immunity

Effector T- cytotoxic cells are able to induce apoptosis in 'self' cells that present abnormal protein that signify danger. Effector T-helper orchestrates the various response of cellular and humoral immunity.

Anatomy of the lymphoid system

Lymphatic Vessels.

Lymph, which may contain antigens that have entered tissues, flows in the lymphatic vessels to the lymph nodes.

Secondary lymphoid organs

Secondary lymphoid organs are the sites at which lymphocytes gather to contact antigens; they facilitate the interactions and transfer of cytokines between the various cells of the immune system.

Primary Lymphoid Organs

1. Primary lymphoid organs are the sites where B.cells and T.cells mature.

The Nature of Antigens

1. Antigens are molecules that react specifically with an antibody or lymphocyte, immunogen refers specifically to an antigen that elicits an immune response.
2. The immune response is directed to antigenic determinant, or epitopes, on the antigen.

The Nature of Antibodies

Structures and Properties of Antibodies

1. Antibodies monomers have a Y shape with an antigen-binding site at the end of each arm of the Y. The tail of the Y is the Fc region.
2. The antibody monomer is composed of two identical heavy chains and two identical light chains; each chain forms several domains. The variable region contains the antigen binding site; the constant region encompasses the entire Fc region as well as part of the Fab regions.

Protective Outcomes of Antibody-Antigens Binding

1. Antibody-antigens binding result in neutralization, immobilization and prevention of adherence, agglutination and precipitation, opsonization, complement activation, and antibody-dependent cytotoxicity.

Immunoglobulin Classes

1. There are five major antibody classes, IgM, IgG, IgA, IgD, and IgE, and each has distinct functions.

Clonal Selection of Lymphocytes

1. When antigens enter a secondary lymphoid organ, only the lymphocytes that specifically recognize the antigen will respond; the antigen receptor they carry on their surface governs this recognition.
2. Lymphocytes may be immature, naïve, activated, effector, or memory cells.

B.Lymphocytes and the Antibody Response

The Response to T-Dependent Antigens

1. B.cells present antigen to effector T-helper cells for inspection. If an effector T-helper cell recognizes the antigen, it will deliver cytokines to the cell, initiating the process of clonal expansion, which ultimately forms plasma cells that produce antibody.
2. Under the direction of effector T-helper cells, the expanding B-cell population will undergo affinity maturation and class switching, and form memory cells.
3. In the primary response, a lag period occurs before antibodies can be detected; memory cells are responsible for the swift and effective secondary response, eliminating invaders before they cause noticeable harm.

The Response to T-Independent Antigens

1. T-independent antigens include polysaccharides that have multiple identical evenly spaced epitopes and LPS.

T Lymphocytes: Antigen Recognition and Response

1. The T-cell receptor recognizes antigen presented by major histocompatibility (MHC) molecules.
2. T-cytotoxic cells are referred to as CD8 T cell; T-helper are referred to as CD4 T-cells.

Functions of Effector T-Cytotoxic (CD8) Cells

1. T-cytotoxic cells induce apoptosis in cell that produce proteins associated with danger, they also produce cytokines that allow neighboring cells to become more vigilant against intracellular invaders.
2. All nucleated cells present peptides from endogenous protein in the groove of MHC class molecules.

Functions of Effector T-helper (CD4) Cells

1. T-helper cells respond to exogenous antigen, which are presented to MHC class II molecules.
2. ThI cells judge antigens presented by macrophages, a responding ThI cell activates that particular macrophage and secrete cytokines that help orchestrate the immune response.
3. Th2 cells judge antigen presented by B.cells; a responding Th2 cell activates that particular B. cell and supports actions that enhance its effectiveness

Activation of T Cells

1. Naïve T-cells require signals to become activated, upon activation the cell stimulates ti own proliferation and ten gain its effector functions.

2. Dendritic cell sample material in tissues and the travel to the secondary lymphoid organs to present the antigen to naïve T-cells. Those that detect molecules associated with danger produce co stimulatory molecules and are able to activate both subsets of T-helper-cells

Natural Killer (NK) Cells

1. NK cells mediate antibody-dependent cellular-cytotoxicity (ADCC).
2. NK cells kill host that are not bearing MHC class I molecules on their surface.

Lymphocyte Development

Generation of Diversity

1. Mechanisms used to generate the diversity of antigen specificity in lymphocytes include rearrangement of gene segments, imprecise joining of those segments, and combinatorial associations of heavy and light chains.

Negative Selection of Self-Reactions B-cells

Negative selection occurs as B cells develop in the bone marrow, cells to which material binds to their B-cell receptor are induced to undergo apoptosis.

References

1. Nester, EW, Anderson, D.Ce, Roberts (Jr), C. E Pearsal, N.N. Nester, M.T and Hurley. D (2004). Microbiology, A Human perspective 4th ed., published by McGraw Hill Higher Education
2. Brooks, G.F., Butel, J.S and Morse, S.A. (2004), Jawetz, Meinicte and Adelberg's Medical Microbiology, 23rd ed. Published by McGrawHill Education

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LECTURE NOTES

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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 may 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, Freund's incomplete adjuvants (water-in-oil emulsion)
 - ▶ Particulate adjuvants: liposomes, microparticles, immunostimulatory complex
 - ▶ Immunostimulatory adjuvants: glucose, dextran sulphate, detergents, saponins, lipopolysaccharides, anaerobic corynebacteria, bacillus calmette-Guerin (BCG m. boris), Bordetella pertussis etc.
 - ▶ Mixed adjuvant: Freund'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 tertiolar 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 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 reacts 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°C (cryoglobulin) while IgG reacts best at 37°C.
- ▶ 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 visible 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 involved 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 ($-\text{NH}_3^+$) on a lysine of one protein and an ionized carboxyl group ($-\text{COO}^-$) on a glutamate of another protein.

Hydrogen bonding: if molecules carrying hydrophilic groups such as $-\text{OH}$, $-\text{NH}_2$ and $-\text{COOH}$ approach closely, they form hydrogen bridges which are relatively weak and reversible. The interaction between threonine and tyrosine is an example of hydrogen bonding.

Hydrophobic attraction: non-polar hydrophobic groups such as those of the side chains of valine, leucine and phenylalanine tend to associate in an aqueous 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 determinant 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 full antigen with its multiple determinants is termed avidity. The force binding two determinant groups by antibody is usually many fold greater than 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 complement 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 immunofluorescence, 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, hemolytic anaemia, thrombocytopenia, proteinuria, positive ANA test, and positive LE cell test

- LE cells: cells that have phagocytosed opsonised nuclei often present in the bone marrow of SLE patients
- Seen in humans, other primates, dogs, rats, horses, mice

Sjogrens Syndrome: (Horses, dogs)

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

Organ-specific/Tissue-specific Autoimmune Diseases

Endocrine:

- Lymphocytic thyroiditis
- Lymphocytic parathyroiditis
- Insulin-dependent diabetes mellitus
- Atrophic lymphocytic pancreatitis
- Autoimmune immune adrenitis
- Hyperthyroidism

Neurological

- Degenerative neuropathy
- Cerebellar degeneration
- Equine polyneuritis
- Steroid meningitis-arteritis

- Canine polyneuritis

Eye diseases

- Equine recurrent urethritis
- Ureodermatological syndrome

Reproductive

Skin diseases

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

Nephritis

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

Muscle

- Myasthenia Gravis
- Polymyositis
- Autoimmune masticatory myopathy
- Canine cardiomyopathy

Organ-Specific Autoimmune Diseases

- Autoimmune diseases that affect a single organ or tissue
- Arises as a result of abnormal response 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 thyroiditis
 - Described in human, dogs and chicken

- Production of autoantibody against thyroglobulin which may also react with triiodothyronine (T₃) or thyroxine (T₄)
- There is dull, dry, coarse coat, scaling, hypotrichosis, hyperpigmentation, pyoderma. Affected animals are fat sluggish and have areas of alopecia 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 lymphocytes 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 P₂ 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 Freund'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 papular 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 fatigue and weakness following mild exercise
- iii. There is degradation of acetylcholine receptors by IgG autoantibodies
- iv. Autoantibodies also block acetylcholine binding sites and trigger complement-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 induced by drugs or viruses

- iv. The condition is characterized by anaemia, weakness, lethargy, fever, uterus and hauto- splebomegaly. There could be tachycardia, anorexia, vomiting or diarrhea
- v. It has been described in human, dogs, horses, cats, mice, cattle and rabbits

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

- Cytokines are produced by antigenic stimuli acting through the T-cell and B-cell receptors
- Antigen-antibody complex acting through Fc receptors
- Super antigens acting through the T-cell receptors
- 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 is 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 poorly understood
- Tumour arises as a result of:
 1. Infection with a tumourigenic 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 histologic 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

Tumour-associated antigens

- 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 hepatoma cells is an onco-fetal 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

Humoral response

- 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

Cell-mediated responses

- 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 *glycobacterium 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 was 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 for 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°C. *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 from 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 laryngotracheitis (ILT) vaccines rubbed into the mucus membranes of cloaca
- Subcutaneous
 - ▶ *B. pertussis* vaccine, *Brucella S₁₉*, *T₁* 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. perfringens* 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
- ▶ Live 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 glutanaldehyde 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 42⁰C 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 immunosuppression

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 stringent
- ▶ 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 I 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 (*Borrelia 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.

VETERINARY VIROLOGY (VPM 402) LECTURE NOTES
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THE COMPLEMENT SYSTEM

Introduction

Complement is a collective noun that describes a series of approximately 30 plasma proteins. When activated they interact sequentially, forming a self-assembling enzymatic cascade and generating biologically active molecules mediating a range of end processes that are significant in the immune and inflammatory response. The basic principle of this cascade system is depicted in general terms; this is similar to the coagulation pathways involved in secondary haemostasis. Intrinsic in such pathways is the presence of a regulatory system that can switch off the cascade when no longer required in order to avoid inappropriate damage to normal tissue.

There are four complement pathways, known as the classical, lectin, alternative and terminal pathways. The first three of these pathways share a common end point, which in turn is the start of the shared terminal pathways. Complement components generated by the activations of these pathways will be described below.

Complements recognized in all animal species and the constituent components are relatively conserved. These components are mostly described using the abbreviation “C” (for complement), with a number to indicate the specific component (e.g. C4) and a lower case letter to indicate a subfraction of that component (e.g. C4a and C4b). The numbering of the components does not always follow a logical sequence, as each component was numbered in the order in which it was discovered. Rather than by the position it holds in the hierarchy of the system. Some components and regulatory proteins do not conform to the “C” nomenclature. To add further confusion, there is traditionally some minor difference in nomenclature used in North America and Europe (for example the classical pathway C3 convertase is C4bC2b in Europe and C4bC2a in North America). Readers should be aware of this, as it is the European system that is presented here.

The Classical and Lectin Pathways

The classical pathway, so called because it was the first discovered, is triggered by the binding of the first component of complement (C1) to the surface of an antigen (e.g a pathogen). In the case of some bacteria, this binding might be directly to a cell wall structural component (e.g lipoteichoic acid of the wall of gram-positive bacteria), or C1 may bind C-reactive protein that is in turn attached to a bacteria polysaccharide. Most often, C1 attached to an immune complex of antigen and antibody (IgG or IgM) by binding to a specific area of the immunoglobulin component of the complex (e.g to the CH2 domain of IgG). It is also possible for C1 to bind to an aggregate of antibody molecules in the absence of antigen. The antigen in these situations is generally relative large (e.g a cell or microbe) and provides a surface area onto which the complement molecules deposit as they are activated. C1 is comprised of three enzymatic subunits, C1q, C1r and C1s, which are activated in that sequence following attachment to the immunoglobulin molecule.

C1s then acts on the next component in the sequence, which is C4, and splits this into two subunits, C4a and C4b. the C4b fraction attaches to the surface of the antigen and binds C2, which is also cleaved by C1s to C2a and C2b. The C2b fragment remains associated with the C4b fraction and this complex has now become the classical pathway 'C3 convertase'. As this name suggests, the next stage of the sequence is that C4bC2b (C3 convertase) acts on C3 to split this molecule into C3a and C3b. C3a has a major biological role, but it does not affix to the surface of the antigen. In contrast, C3b deposit adjacent to the C4bC2b complex to form a new complex of C4bC2bC3b, which become a 'C5 convertase,. The generation of this C5 convertase is the final stage in the classical pathway.

As for all complement pathways, a system of regulatory control is built into the classical pathway to inactivate the system when no longer required. The first means of control is relatively simple and relates to the fact that these complement components have a short half-life when generated. Moreover, complement molecules are highly susceptible to heat and, in vitro, the molecules may be inactivated by heating a sample of serum (containing the molecules) to 56 °C for a short period of time (a process known as 'heat-inactivation' of complement). Additional means of controlling the classical pathway relate to the presence of a series of specific inhibitory factors that act at different points of the pathway. The C1 inhibitor cleaves C1r from C1s, thereby disrupting the activity of this complex. The C4 binding protein displaces C2b from C4b

and work in combination with factor 1, which subsequently cleaves C4b into two active subfractions, C4c and C4d. factor 1 is also able to cleave C3b into the inactive subfractions C3c and C3d.

As these complement molecules have high biological potency, there must be a means of protecting normal cells that lie in the immediate vicinity of the activated classical pathway and may be potentially susceptible to complement fragments that diffuse away from the site of activation. In fact, normal cells have an in-built protective mechanism, as their cell membrane includes a series of constitutively expressed proteins that are able to disrupt the C3 convertase should it form on their surface. These proteins include the decay accelerating factor (DAF), complement receptor 1 (CR1) and membrane co-factor protein (MCP).

The lectin pathway of complement was the most recently discovered and shares elements of the classical pathway. Essentially, the serum mannan-binding lectin (MBL) initially associates with a bacterial surface carbohydrate and is then able to activate the MBL-associated serine protease-2 (MASP-2). Activated MASP-2 replicates the effects of C1 by activating C4 and C2 to form the C3 convertase complex.

The Alternative Pathway

The alternative pathway of the complement system is older in evolutionary terms than the classical pathway and as it does not require the presence of antibody to be activated, it may be considered part of the innate immune system. The alternative pathway has two distinct phases. The first of these continually cycles at low level in clinically normal animals and is often referred to as the 'tick over' phase. The key feature of the tick over phase is that it occurs within the extracellular fluid space and is not associated with the surface of cells. The second phase reflects full activation of the system and requires the presence of an appropriate trigger factor, which in the case of the alternative pathway is the presence of a 'trigger surface' that permits deposition of molecules of the enzymatic cascade. Such trigger surfaces are provided by microbes (particularly bacteria or yeast), by abnormal tissue cells (e.g virally infected or neoplastic cells), by aggregates of immunoglobulin or by foreign material (e.g asbestos).

The tick over phase is initiated by C3 in the extracellular fluid, which undergoes spontaneous hydrolysis to form C3i. In the presence of Mg²⁺, some C3i is bound by Factor B to form a complex of C3iB. Bound Factor B is acted upon by Factor D, which fragments the

molecule to Bb and Ba. Bb remains associated with C3i to form a C3 convertase, which in turn splits further C3 molecules in the fluid phase to C3a and C3b. In the tick over pathway, the majority of the C3b that is generated undergoes spontaneous hydrolysis and inactivation. Should any C3b deposit onto the surface of adjacent normal cells, it will be inactivated by the combination of MCP, DAF, CR 1, Factor H and Factor I.

The Terminal Pathway

In contrast to the pathways described above, the terminal pathway is relatively straightforward and benefits from the fact that the constituent molecules are named in correct numerical sequence. Accordingly, the pathway is initiated by C5 convertase splitting C5 into the subfractions C5a and C5b, C5a has potent biological activity similar to that of C3a, but has its effect distant to the surface of the cell that is the target of complement activation.

Activated C5b recruits C6 and C7 to form a complex of C5bC6C7, which associates with the membrane of the target cell. This complex in turn binds C8 that penetrates the cell membrane and recruits a number of C9 molecules that insert into the membrane to form a 'donut-like' transmembrane pore. This is known as the membrane attack complex (MAC). The formation of the MAC represents the end point of the terminal pathway.

Biological consequences of Complement activation

Cytolysis

Once the terminal complement pathway is activated in reality not one, but thousands of MACs are generated within the membrane of the cell that is the target of complement activation. The surface of this target cell becomes riddled with holes and an osmotic imbalance between the cell cytoplasm and extracellular fluid is established such that there is a net influx of water into the cell. The cell swells and subsequently bursts in a phenomenon known as 'osmotic lysis'. If that target cell is a bacterium or an abnormal tissue cell, then clearly this mechanism of cellular lysis (cytolysis) is beneficial to the host and is a valuable part of the protective immune response. Antibody-mediated (classical pathway) complement cytolysis is the basis for the type II hypersensitivity reaction.

Generation of Bioactive Substances

The second major consequence of complement activation is the generation of those fragments of complement that do not associate with the membrane of target cells. The two most important of these are C3a and C5a. C5a is more potent than C3a, but much larger quantities of C3a are generated. These molecules are sometimes known as anaphylatoxins or chemoattractants after the fundamental effects that they mediate.

C3a and C5a have key roles in the tissues inflammatory response via a number of mechanisms. These molecules mediate vasodilation of small vessels within the tissue in which they are generated. Dilated vessels with leaky endothelial cell junctions permit the egress of fluid, protein and cells from the circulation into the tissue. If the generation of C3a and C5a has resulted from infection or damage within that tissue, this process has clear benefit to the tissue. Vascular fluid loss leads to tissue oedema, which may be important in diluting locally produced toxins. Leakage of blood proteins (immunoglobulins and complement molecules) may be beneficial if these molecules participate in a local immune response. Migration of leucocytes, particularly phagocytic cells such as neutrophils and macrophages, is likely to be of benefit in the removal of infectious agents or tissue debris. C3a and C5a may additionally have direct activating effects on neutrophils in order to enhance their phagocytic function and release of further inflammatory mediators. The chemotactic role of C3a and C5a may further enhance the value of local tissue recruitment of leucocytes. After migrating from the circulation into tissue, phagocytic cells may be directed towards the location of pathogens or damaged cells by moving up a 'chemotactic gradient'. This gradient is formed by complement molecules, which are at their highest concentration at the point that they were generated and at progressively lower concentration the further away from that source they are within that tissue.

In some circumstances the action of C3a and C5a may cross the border between a useful role in local inflammation and induction of a pathological inflammatory response. Both molecules may act on tissue mast cells, causing them to degranulate and further expand the tissue inflammatory response. In the respiratory tract this may result in contraction of bronchiolar smooth muscle (bronchoconstriction).

Removal of Particulate Antigen or Immune Complexes

The third biological consequence of complement activation is in enhancing the removal and destruction of particulate antigen or complexes of antigen and antibody by phagocytic cell

such as neutrophils and macrophages. A phagocytes might encounter such antigen within the tissue space by random encounter instance the phagocytic cell might internalize and destroy the particle and this process might involve the interaction of receptor molecules expressed by the phagocyte with ligands found on the target. Particulate antigen would be more likely to be phagocytosed were it aggregated together by antibody (particularly IgM antibody), making it a 'larger target' for the phagocytic cell. The interaction between target antigen and phagocyte can also be enhanced by the complement system by the processes of opsonization and immune adherence.

The phenomenon of opsonization arises because phagocytic cells express on their membrane receptors for IgG (the Fcγ Receptor) and receptors for the complement molecule C3b (C3bR). Simply put, if a particulate antigen is already coated with IgG, it is more likely to be phagocytosed due to the enhanced contact with that cell mediated through the IgG-FcR interaction. Even better is if the antigen is also coated with C3b, so that there is dual interaction involving IgG-FcR and C3b-C3bR binding. 'opsonization' comes from the Greek 'to make tasty for the table' and the analogy is often used that coating a target antigen with IgG and C3b is akin to covering dinner with gravy!.

A related event is that of immune adherence, a process that is of great importance in clearing particulate antigen from the bloodstream. Immune adherence arises from the fact that erythrocytes express a C3bR (but not an FcR) and this permits circulating antigen that is coated with C3b (with or without antibody) to attach to the surface membrane of red cells. As these antigen-laden red cells pass through the hepatic sinusoids and spleen, they encounter phagocytic cells resident in those tissues that bind the antigen via their own C3b receptors and remove the antigen from the red cells for internalization and destruction.

Interaction with other Inflammatory Pathways

As discussed above, the complement pathways have key roles in the inflammatory and immune response. During inflammation a series of biochemical pathways is activated in parallel within the same tissue, so it is not surprising that there may be molecular interactions between the mediators within the different pathways. Complement molecules may have a range of effects on the kinin, coagulation and fibrinolytic pathways, the fine details of which are beyond the scope of this discussion.

Tests of Complement Function

The complement pathways have been defined in most domestic animals species and, although rarely performed, it is possible to test for complement function. Complement molecules are very heat-labile (and may be destroyed by heating a serum sample to 56°C for 30 minutes), so such assays require freshly collected serum frozen rapidly to – 70°C to preserve the components. The total haemolytic complement assay (CH50) tests for the overall function of the classical and terminal pathways. Essentially, this test involves incubating patient serum with an indicator system of antibody-coated red blood cells. Complement present within the serum will cause lysis of these erythrocytes. A titration of the serum sample is performed and the CH50 value for the sample is defined as the reciprocal of the serum dilution that gives 50% haemolysis. An alternative to the tube-based CH50 assay is to perform the test by adding serum to wells cut in an agarose gel that contains the sensitized erythrocytes. After appropriate incubations, haemolysis in such plates is indicated by the formation of a clear zone around the well. The diameter of this cleared ring is proportional to the log sample. Finally, it is also possible to measure the using single radial immunodiffusion (SRID) or a haemolytic assay in which an excess of all complement components, except the one being tested, is provided, such that the missing component derives from the test sample.

HYPERSENSITIVITY

Occasionally, the immune system responds inappropriately to the presence of antigen. These responses are referred to as *hypersensitivities*. There are four different types of hypersensitivities that result from different alterations of the immune system. These types are classified as:

- Type I: Immediate Hypersensitivity
- Type II: Cytotoxic Hypersensitivity
- Type III: Immune Complex Hypersensitivity
- Type IV: Delayed Hypersensitivity

This section describes the four types of hypersensitivity, giving examples of diseases that may result.

TYPE I HYPERSENSITIVITY

Type I or Immediate Hypersensitivity can be illustrated by considering the following experiment:

1. First, a guinea pig is injected intravenously with an antigen. For this example, bovine serum albumin (BSA, a protein) will be used. After two weeks, the same antigen will be reinjected into the same animal. Within a few minutes, the animal begins to suffocate and dies by a process called *anaphylactic shock*.

2. Instead of reinjecting the immunized guinea pig, serum is transferred from this pig to a "naive" (unimmunized) pig. When this second guinea pig is now injected with BSA, it also dies of anaphylactic shock. However, if the second pig is injected with a different antigen (e.g. egg white albumin), the pig shows no reaction.
3. If immune cells (T-cells and macrophages instead of serum) are transferred from the immunized pig to a second pig, the result is very different; injection of the second pig with BSA has no effect.

These results tell us that:

- The reaction elicited by antigen occurs very rapidly (hence the name "immediate hypersensitivity").
- The hypersensitivity is mediated via serum-derived components (i.e. antibody).
- The hypersensitivity is antigen-specific (as one might expect for an antibody-mediated reaction).

The details of this reaction can be summarized as follows :

1. Initial introduction of antigen produces an antibody response. More specifically, the type of antigen and the way in which it is administered induce the synthesis of IgE antibody in particular.
2. Immunoglobulin IgE binds very specifically to receptors on the surface of mast cells, which remain circulating.
3. Reintroduced antigen interacts with IgE on mast cells causing the cells to degranulate and release large amounts of histamine, lipid mediators and chemotactic factors that cause smooth muscle contraction, vasodilation, increased vascular permeability, bronchoconstriction and edema. These reactions occur very suddenly, causing death.

Examples of Type I hypersensitivities include allergies to penicillin, insect bites, molds, etc. A person's sensitivity to these allergens can be tested by a cutaneous reaction. If the specific antigen in question is injected intradermally and the patient is sensitive, a specific reaction known as *wheal and flare* can be observed within 15 minutes. Individuals who are hypersensitive to such allergens must avoid contact with large inocula to prevent anaphylactic shock.

TYPE II HYPERSENSITIVITY

Type II or Cytotoxic Hypersensitivity also involves antibody-mediated reactions. However, the immunoglobulin class (isotype) is generally IgG. In addition, this process involves K-cells rather than mast cells. K-cells are, of course, involved in antibody-dependent cell-mediated cytotoxicity (ADCC). Type II hypersensitivity may also involve complement that binds to cell-bound antibody. The difference here is that the antibodies are specific for (or able to cross-react with) "self" antigens. When these circulating antibodies react with a host cell surface, tissue damage may result.

There are many examples of Type II hypersensitivity. These include:

- **Pemphigus:** IgG antibodies that react with the intracellular substance found between epidermal cells.
- **Autoimmune hemolytic anemia (AHA):** This disease is generally inspired by a drug such as penicillin that becomes attached to the surface of red blood cells (RBC) and acts as haptens for the production of antibody which then binds the RBC surface leading to lysis of RBCs.
- **Goodpasture's syndrome:** Generally manifested as a glomerulonephritis, IgG antibodies that react against glomerular basement membrane surfaces can lead to kidney destruction.

TYPE III HYPERSENSITIVITY

Type III or Immune Complex hypersensitivity involves circulating antibody that reacts with free antigen. These circulating complexes can then become deposited on tissues. Tissue deposition may lead to reaction with complement, causing tissue damage. This type of hypersensitivity develops as a result of systematic exposure to an antigen and is dependent on i) the type of antigen and antibody and ii) the size of the resulting complex. More specifically, complexes that are too small remain in circulation; complexes too large are removed by the glomerulus; intermediate complexes may become lodged in the glomerulus leading to kidney damage.

One example of a Type III hypersensitivity is **serum sickness**, a condition that may develop when a patient is injected with a large amount of e.g. antitoxin that was produced in an animal. After about 10 days, anti-antitoxin antibodies react with the antitoxin forming immune complexes that deposit in tissues. Type III hypersensitivities can be ascertained by intradermal injection of the antigen, followed by the observance of an "Arthus" reaction (swelling and redness at site of injection) after a few hours.

TYPE IV HYPERSENSITIVITY

Type IV or Delayed Hypersensitivity can be illustrated by considering the following experiment:

1. First, a guinea pig is injected with a sub-lethal dose of *Mycobacterium tuberculosis* (MT). Following recovery of the animal, injection of a lethal dose of MT under the skin produces only erythema (redness) and induration (hard spot) at the site of injection 1-2 days later.
2. Instead of reinjecting the immunized guinea pig, serum is transferred from this pig to a "naive" (unimmunized) pig. When this second guinea pig is now injected with MT, it dies of the infection.
3. If immune cells (T-cells and macrophages instead of serum) are transferred from the immunized pig to a second pig, the result is very different; injection of the second pig with MT causes only erythema and induration at the site of injection 1-2 days later.
4. In a separate experiment, if the immunized guinea pig is injected with a lethal dose of *Listeria monocytogenes* (LM) instead of MT, it dies of the infection. However, if the pig is simultaneously injected with both LM and MT, it survives.

These results tell us that:

- The reaction elicited by antigen occurs relatively slowly (hence the name "delayed hypersensitivity").
- The hypersensitivity is mediated via T-cells and macrophages.
- The hypersensitivity illustrates both antigen-specific (T-cell) and antigen non-specific (macrophage) characteristics.

The details of this reaction can be summarized as follows (*click the image to animate*):

1. Initial introduction of antigen produces a cell-mediated response. *Mycobacterium tuberculosis* is an intracellular pathogen and recovery requires induction of specific T-cell clones with subsequent activation of macrophages.
2. Memory T-cells respond upon secondary injection of the specific (i.e. MT) antigen, but not the non-specific (i.e. LM) antigen.
3. Induction of the memory T-cells causes activation of macrophages and destruction of both specific (MT) and non-specific (LM) microorganisms.

Immune responses to infectious agents

The immune Response to Viral Infection

Viruses are a highly successful class of pathogens responsible for very significant morbidity and mortality amongst both animal and human populations. This in part relates to the ability of these organisms to evolve a range of strategies to evade or inhibit the host immune response. Some viruses (e.g retroviruses such as feline leukaemia virus, Fel V). have been able to integrate their genetic material into the host genome, others are able to alter their antigenic appearance to produce repeated epidemics or pandemics of disease (e.g human and animal influenza virus) and yet other viruses have been able to capture host genes and express host-related proteins that interfere with development of the protective immune response (e.g the capture of the human IL-10 gene by Epstein-Barr virus).

As an example of a model immune response to virus infection, we shall consider how the immune system might deal with a viral infection of the enterocyte lining of the intestinal tract, as might occur with, for example, an intestinal rotavirus of domestic livestock. When infectious virus particles arrive at their target surface, they will encounter an array of innate immune defences relevant to that surface. In the intestinal mucosa these will include the enterocyte barrier, the secretions that coat the luminal surface of that barrier (including mucus, antimicrobial enzymes and defensins and polyreactive immunoglobulins) and the range of innate

immune cells that normally populate the epithelial compartment (e.g the TCR T cells) and the underlying lamina propria (e.g macrophages, dendritic cells and NK cells).

Virus particles generally infect host cells by binding to a receptor molecule expressed on the surface of the target cell. This is usually a normal surface protein that the virus uses as a receptor or co-receptor to gain entry to the target cell. In the present example, the virus interacts with receptor on the enterocyte surface to gain access to the host cell. Once within the cell, the aim of the virus is to replicate in order to produce new virions that might then leave that cell (and in the process destroy the cell) to infect new targets. The means by which this is achieved depends on the nature of the virus and its genomic materials. Fortunately, most virus-infected cells begin to secrete the antiviral cytokines IFN- α and IFN- β . These antiviral interferon's bind receptors on adjacent non-infected tissue cells and stimulate the uninfected cell to produce an array of other proteins that confer a measure of resistance to certain viral infections. The virus-infected cell may also display viral antigen on its surface membrane and if that expression is concurrent with down-regulation of MHC class I molecules, the infected cell becomes a target for innate NK cells in the vicinity. The antiviral interferons may also positively influence local NK cells. Alternatively, the infected cell may process and present virus antigen in the context to MHC class I and II molecules but this may only be of significance later.

At this stage of the viral infection it would be hoped that lamina propria dendritic cells may be able to sample virus antigen or even become infected by virus particles, allowing classic processing and presentation by these APCs. The interaction between virus and APCs involves viral Pathogen Associated Molecular Patterns (PAMPs) (often of nucleic acid origin) and dendritic cell PRRs (pattern recognition receptor) that are predominately cytoplasmic. These interaction presumptively lead to selective gene activation in the APC. In addition, the dendritic cell should enter lymphatics and migrate to the regional mesenteric lymph nodes. It is also possible that virus antigen breaches the intestinal barrier through the Peyer's patch lymphoid tissue.

Once the antigen-laden APC has entered the paracortex of the mesenteric lymph node, it will aim to locate and activate recirculating antigen-specific naïve peptides. The interaction between Th0 cell and APC will be guided by the range of co-stimulatory surface molecules and cytokines that have been activated within the APC following PRR-PAMP interaction. As the most 'relevant' type of adaptive immune response for this type of infection would be a Th1-regulated cytotoxic effector response, it would be hoped that the APC will activate clones of Th1

CD4- T cells and CD8 cytotoxic T cells. Recalling that Th1 cells may provide restricted help for those B cells committed to producing the subclass of IgG antibody able to opsonize or take part in ADCC, it would also be appropriate for virus-specific B cells to be activated and class switch to that IgG subclass.

The range of Th1, Tc and B cells generated must then leave the mesenteric lymph node in efferent lymph in order eventually to enter the bloodstream and 'home' to the anatomical site to viral infection (the intestinal mucosa). This will involve the interaction of homing receptors such as the $\alpha_4\text{-}\beta_7$ integrin with the vascular addressin MAdCAM (Mucosaladdressin cell adhesion molecule). Once these adaptive immune cells arrive in the mucosa, the full effector phase of adaptive immunity will come into play. Th1-derived IFN- γ will amplify the effects of NK cells and Tc cells, and such cell-mediated cytotoxicity is the major effector mechanism in the antiviral immune response. The Th1 cell will also stimulate B-Cell transformation to plasma cells secreting IgG subclass that contributes to the cytotoxic process. Antibody bound to infected cells may also mediate their lysis via activation of the classical pathway of complement. Although in a protective anti-viral immune response the Th1 arm of adaptive immunity is important, it is also likely that some Th2 effectors are generated within the mesenteric lymph node and also home back to the site of infection. These cells may have a relevant role in stimulating the local production of anti-viral IgA that could be secreted across the mucosal barrier to bind virus particles and block their interaction with receptors. Locally secreted IgG may act in a similar fashion. If this adaptive immune response is successful in containing the infection, then late stage immunosuppression (induced T-Cell receptors TCRs) and the development of T-and B-cell memory will occur.

The Immune Response to Bacterial Infection

To remain with the intestinal model, we will next consider the nature of the immune response that might be generated in response to an enteric bacterial pathogen such a *Escherichia coli* or *Salmonella spp.* On arrival in the intestinal tract these organisms will also immediately encounter the range of innate immune defences outlined above. However, of particular importance in this context would be the presence of the endogenous intestinal bacterial microflora, which will compete with the pathogen for space and nutrients, making colonization more challenging. Another element of innate immunity that may have greater relevance to this class of infection is

the $\gamma\delta$ T cell within the enterocyte layer. These cells are well situated for early interaction with bacterial pathogens and are thought to be primarily activated in response to this type of organism.

As for viruses, bacteria often require an initial receptor-mediated interaction with target host cells. For example, the K88 and K99 pili of *E.coli* permit attachment to receptors at the enterocyte interface between these bacteria and host tissue. Enteric pathogens, such as *E.coli* or *S. Typhimurium*, may utilize a variety of different mechanisms to induce disease, dependent on the genetic strain of the bacterium. Some may produce locally active enterotoxin that bind toxin receptors and lead to osmotic imbalance and metabolic diarrhea. Others may attach to and disrupt the epithelial surface or invade the intestinal mucosa and regional lymph nodes, leading to a local pyogranulomatous inflammatory response. Such gram-negative rods, are also characterized by the ability to produce severe generalized disease (endotoxaemia).

Once the mucosal surface is colonized, the adaptive immune response will be required to help resolve the infection. Again, mucosal dendritic cells should sample bacterial antigen and this process involves the interaction of PRRs with a range of bacterial PAMPs. Particulate bacterial antigen may be more likely to gain access to the lamina propria immune compartment via M cells within the dome epithelium overlying the Peyer's patch. The activated dendritic cells will migrate to the regional mesenteric lymph nodes in order to activate paracortical T cells and, in turn, follicular B cells. The desired effector immune response in this situation would be one dominated by the production of antigen-specific immunoglobulin, so APC signalling of the Th0 cell would generate Th2 effector. These, together with antigen-specific B cells, would then exit the mesenteric lymph node to home back to the mucosal surface.

The most beneficial effector immune response in this context will involve the synthesis of specific IgA and IgG antibodies. For those organisms mediating pathology via toxin production, IgG neutralization of toxin will be important, IgG antibodies may also opsonize invasive organisms for phagocytosis or permit the complement-mediated lysis of the bacteria. Bacterium-specific IgA antibodies will be secreted to the luminal surface, where they may interfere with the interaction of organism with receptor molecules. Again, in a successful immune response, final down-regulation of the effector populations will be required together with the generation of immunological memory.

The Immune response to fungal infection

As for nematodes, many fungal pathogens provide a challenging target for the immune system because of the relative size of the colonies of organisms. In this final example we shall consider the immune response of the dog to colonization of the nasal sinuses and nasal cavity by the organism *Aspergillus fumigatus*. This fungus form a large colony over the mucosa of these nasal tissues, but the organisms tend not to infiltrate into the lamina propria. The colonies comprise a tangled mass of fungal hyphae, with intermittent conidia that represent the sites of spore formation.

In order for such a colony to establish, the organism must broach the normal innate immune barriers of the upper respiratory tract, including the antimicrobial substances found within nasal secretions. Although innate phagocytic cells (neutrophils and macrophages) are capable of phagocytosing fungal spores, they appear unable effectively to kill these elements. The fungal hypae are simply too large a target for effective phagocytosis.

The adaptive immune response to this infection has recently been well characterized. It is likely that APCs carrying fungal antigen stimulate this response in regional lymphoid tissue such as the nasopharyngeal tonsil or retropharygeal lymph nodes. The effector phase of the mucosal immune response involves infiltration by CD4⁺ Th1 and probably Th17 cells, as determined by up-regulation of gene expression for IFN- γ and IL-23 in inflamed tissue. There is also a marked pyogranulomatous element to the reaction and infiltration of IgG-bearing plasma cells. Th1-derived IFN- γ most likely provides stimulation to macrophages to permit these cells to destroy any phagocytosed fungal spores. Antibody and complement molecules are likely to coat the hyphal elements and form a bridge to FcR-bearing granulocytes. Similar to helminth infection, these cells may degranulate locally and induce focal damage to the hyphae. Infected dogs generally mount a strong serum IgG antibody response to the organism. The inflammatory response itself it likely responsible for the extensive tissue and bone destruction that may occur in this disease. Similar to observations in leishmaniosis, there is an additional regulatory element to the response, as there is concurrent up-regulation of IL-10 gene expression. Again, this is interpreted as an attempt by the adaptive immune of systemic sequelae, but at the same time allows persistence of the infection and the development of chronic sinonasal disease.