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Immune Responses in Fishes

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The term immunity means exemption. The meaning originated from exemption granted to certain citizens in the case of compulsory military services. This term was further extended to the disease resistance seen in people recovered from epidemic diseases. This knowledge led to the development of small pox vaccine by Edward Jenner in1798. In animals, the mechanism for self-recognition and non-self discrimination evolved some 400 million years ago and continues as the basic immune response mechanisms. The basic pattern of protein molecules involved in the self/nonself-recognition remained without much alteration, while the diversification of these molecules to suit the emerging challenges was super imposed. We have several examples of self recognition in invertebrates. Aggregation of dispersed colonies of sponges is regulated by species-specific surface glycoproteins. Failure of adhesion of unrelated species amounts to a primitive graft rejection. The cells lining the cavity of sponges are able to capture microorganisms. In the insect larval metamorphosis, phagocytosis of dead and disintegrated cells occurs by the same mechanism. Sea stars and corals reject grafts from unrelated forms. The same mechanism enables fertilization of ova in marine fishes and other organism by the spermatozoa of same species. Hence, the self-recognition mechanism enables the millions of species to maintain their integrity, while excluding invasion of their body from align cells and changed self- constituents (mutated cells including neoplastic cells).

The immune system, vested with the role of defense is composed of various cell types, tissues and organs. The mechanisms of defense executed by the immune system are of non-specific and specific types. The former is encountered in almost all living organisms including fish. It is non-specific because the same immune response can be elicited by a number of unrelated foreign particles. Specific or acquired mechanism of immunity is found only in vertebrates and the reactions are directed against specific molecules that stimulate such reactions. Though these two mechanisms appear as distinct, in fact they function in conjunction with each other, making, the study of immunology a complex and vast one.

Basic pattern of Molecules

The cell surface molecules that are markers of self/non-self recognition are either carbohydrates or carbohydrate terminal groups on glycoproteins. The recognition factors themselves are proteins. The marker proteins and recognition proteins have evolved from $\hat{a}_2$ micro globulin or Thy-1 protein. The basic structures of immunoglobulin, histocompatibility antigens and phagocyte receptors indicate this. Each $\hat{a}_2$ micro globulin consists of a polypeptide chain with 110 amino acids of molecular weight

12000 (approximate). Vertebrates including fish have improved upon this primitive recognition mechanism and evolved a highly efficient system to deal with potential invasion from the co-existing biological world as well as aberrant or rogue cells evolved through spontaneous mutations (eg. nepolastic cells). This system has retained all the primitive mechanisms such as, phagocytosis, agglutination of heterologus cells, lysis etc., while developing specific molecules and specific cellular mechanisms against foreign molecules and cells.

Innate Immunity: Non-specific Immunity

The innate immune mechanisms are non-specific. The main determinants of innate immunity are genetically controlled, varying widely with species, strain and to a lesser extent between individuals

Surface barriers:

- **Mucus**: a layer of mucus forms the interface between body and environment. It is rich in glycoproteins, proteoglycans proteins and humoral factors. Mucus entraps microorganisms and mucus is continually replenished by mucus secreting cells, which inhibits the colonization of integument. The rate of secetion of mucus increases in response to infections or due to action of irritants. Lyszyme, bacteriolysin and complement cascade present in the fish are anti-microbial.
- **Skin:** The skin surface of fish differs from that of higher vertebrates in that the epidemis is composed of non-keratinized living cells, which are continuously sloughed off preventing colonization of microbes. Epidermal integrity is vital to fish in maintaining osmotic balance and excluding microorganisms. Epidermal healing response in fish is extraordinarily rapid, even at low temperatures. It involves a migration of Malpighian cells from the periphery of wound surface rapidly closing the lesion, and is quite different from the scab formation, which occurs in mammals. Epidermis has resident migratory phagocytes. Malpighian cells are also capable of phagocytosis.
- **Gills:** The gill is a route entry for pathogens and has a large surface area of delicate epithelium. The epithelial layer secretes copious amount of mucus, which protect the gill. The epithelium responds to infections by hyperplasia. The pillar cells lining the brachial blood sinuses are also phagocytic.
- **Gastro-intestinal tract**: The mucus membrane of the tract secretes abundant mucus. The digestive tract environment is hostile to many pathogens. (1) Acidic pH in stomach. (2) Action of lytic enzymes (Pepsin, trypsin amylase peptidase etc.). In teleost M cells and Peyer's patches are absent. However, intra epithelial lymphocytes are seen. Macrophages lymphocytes and eosinophilic granular cells are found in lamina propria.

Non-specific humoral factors: These include (1) growth inhibitors (2) inhibitors of enzymes or toxins produced by pathogen (3) lysins (4) precipitins and (5) agglutinins.

- **Growth inhibitors**: These substances either deprive essential nutrients to microbes or interfere with their metabolism.
- **Metal ion binding proteins**: These occur in the serum of all vertebrates including fish. Iro binding proteins (siderophilins) such as apotransferrins, ceruloplasmin and metallothionein have been identified in fish. They deprive essential metal ions and inhibit growth of microbes.

Apotransferrin binds two ferric ions. Ceruloplasmin oxidizes ferrous ions to ferric ions and metallothionein binds to copper, zinc, cadmium and mercury ions. Metallothionein specifically binds to macrophage plasma membrane, initiating respiratory burst activity and signal transduction. Apotransferins, which are also acute phase proteins, display anti-microbial properties by limiting the amount of endogenous iron available to pathogens including intracellular bacteria/protozoan.

- **Acute phase proteins**: Plasma proteins collectively termed as acute phase proteins increase in response to infections, and tissue injury. These include C-reactive proteins, serum amyloid A protein, á$_1$ antitrypsin, á$_2$ macroglobulin, ceuloplasmin, C$_9$ and factor B.
- **Cytokines:** Cytokine related molecules are detected in fish and invertebrates. These are interleukin-1 (IL-1), IL-2,IL-3, IL-4, IL-5, IL-6,tumor necrosis factor (TNF), Chemo tactic protein-1, macrophage migration inhibition facto (MIF) and other peptide factors, which are involved in modulation of immune response and inflammatory reactions. Cytokines mobilize the host immune response and inflammatory reactions. Cytokines mobilize the host immune response, activate inflammatory reactions, and mediate bi-directional communication among various organs/tissues and between cells. Colony-stimulating factors (glycoproteins and peptides) regulate haematopoiesis and haematopoietic cell function, and transforming growth factors profoundly affect wound healing and cellular differentiation.
- **Interferons**: are proteins that inhibit intracellua viral replication. The interferon has been reported from fish. They are classified into class I interferon (á and â interferons) and class II interferons (ã interferon). In each type there are several different forms. Á interferons are produced mainly by ly+
- **Lymphocytes and other nucleated cells:** Interferon â is produced by fibroblasts and interferon ã is produced by T-lymphocytes and natural kille cells (NK orNC cells). Interferon producing cells, when infected with virus (stimuli for interferon synthesis are nucleic acids, bacterial cell walls double stranded RNA and poly synthetic nucleotides) synthesize and secrete interferons into extra cellular fluid. The interferons bind to specific receptors of uninfected cells. The antiviral effect is produced by derepression of two genes leading to synthesis of two specific enzymes. One enzyme catalyses the phosphorylation of ribosomal protein and initiation factor elf-2, which is necessary for protein synthesis. This reduces the m-RNA translation in cells. The other enzyme catalyses the formation of short chain polymer of adenylytic acid, which activates a latent endonulease, thi in turn degrades viral and host cell m-RNA. This establishes a cordon of uninfected cells around the site of viral infection restraining its spread. In addition to these effects it has several other immunological functions such as major histocompatibility class II protein molecule (MHC II) expression on macrophages, increased phagocytosis by neutrophils and macrophages. It also enhances activity of natural kille cells, T-lymphocytes B-lymphocytes and other immune cells
- **Eicosanoids**: are important group of compounds derived from 20 carbon poly unsaturated fatty acids. In fish, eicosanoids are generally produced in organs rich in blood cells after ionophore stimulation. These eicosanoids include prostaglandins, thrombaxans lipoxins and leuktrines. They regulate blood clotting, MHC II expression, inflammation and phagocytosis.

Enzyme inhibitors: Many pathogens produce enzymes in order to gain access to host body. Host tissue fluid and blood contain many factors, which neutralize these lytic enzymes. The á$_2$ macro globulin is able to entrap and form covalent linkages with proteins such as transforming growth factor (TGF) B$_1$, Il-1B and platlet derived growth factor BB. The á$_2$ macro globulin thus regulates the action of coagulation cascades and complement cascade.

Lysins: There are several enzymes, which cause lysis of heterogenous cells. These include complement cascade, pro-phenol oxidase, lysozyme and trypsin.

Complement: is an enzyme cascade consisting of several protein components. In fish twelve proteins have been detected. In mammals about 26 proteins are involved. These proteins are heat labile and are inactivated at 45«%C in fish and at 55«%C in mammals. The function of complement include

- Making bacteria more susceptible to phagocytosis
- Directly lysing some bacteria and foreign cells
- Producing chemotactic substances
- Increasing vascular permeability
- Causing smooth muscle contraction and promoting mast cell degranulation.

Complement cascade is activated two ways; the classical pathway and alternate pathway. Once initiated, a cascade of events ensues, providing the functions listed above. Most of the components are numbered (C1, C2, C3, C4, C5, C6, C7, C8 AND C9). Some are referred as fators (factorB,D,P,etc.).

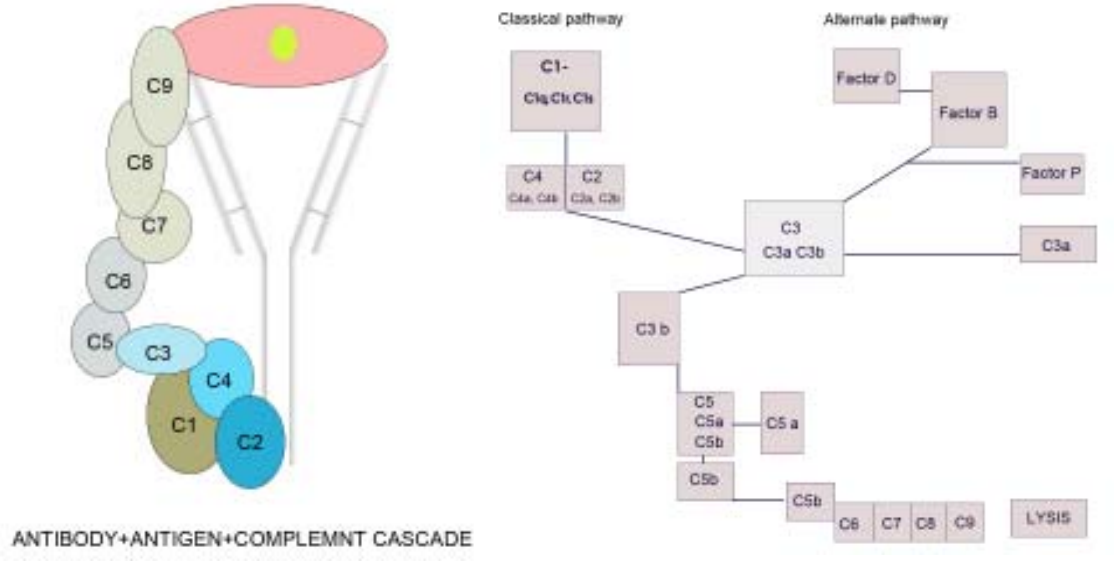
Components of Classical Pathway (Antigen Antibody Complex mediated pathway)

Native component	Active component	Functions
C1	C1q	Binds to antibody bound antigen, activate C1r
	C1r	Cleaves C1s to activate protease function
	C1s	Cleaves C2 and C4
C2	C2a	Unknown
	C2b	Active enzyme. Cleaves C3 and C5
C3	C3a	Mediates inflammation
	C3b	Activation of alternate pathway and binds cell surface-opsonization
C4	C4a	Mediates inflammation
	C4b	Binds C2 for cleavage by C1s. Binds cell surfaces for opsonization.
Components of the Membrane-Attack Complex (same for both classical and alternate pathways)		
C5	C5a	Mediates inflammation.
	C5b	Initiates the assembly of the membrane-attack complex (MAC)
C6	C6	Binds C5b, forms acceptor forC7
C7	C7	BindsC5b67, inserts into membrane forms acceptor forC8
C8	C8	bindsC5b67, initates C9 Polymerization
C9	C9n	Polymerizes around C5b678 to form channel that causes lysis

Components of the Alternate Pathway

Native component	Active component	Functions
C3	C3a	Mediates inflammation, anaphylotoxin
	C3b	Binds cell surfaces for activation of alternate pathway
FactorB	B	Binds membrane bound C3b cleaved by factor D
	Ba	Unknown
	Bb	Cleaved form stabilized by P produces C3 convertase
FactorD	D	Cleaves factor B when bound to C3b
Propedin	P	Binds and stabilizes membrane bound C3bBb

In fish complement is found in serum and mucus



In the alternate pathway, the bacterial endotoxins, polysaccharides like zymosan and inulin, which activate properdin or factor B that convert Factor C3 into C3b and C3a and thus setting the entire cascade into action.

Pro-phenol oxidase system: This system consists of an enzyme cascade leading to the activation of pro-phenol oxidase and other compounds with related activities. Pro- phenol oxidase on activation by â-1, 3 glucans and zymosan cleaves to phenol oxidase, which catalyses oxidation of tyrosine to quinones. Quinones polymerize to form melanin deposits. Melanin deposition is a common immune response seen in invertebrates. Conversion of pro- phenol oxidase to phenol oxidase is catalyzed by a protease named phenol oxidase activating enzyme. This enzyme is kept in check by a protease inhibitor.

Lysozyme: is a low molecul;ar protein found in the blood of vertebrates, haemocytes of invertebrates, serum, haemolymph and mucus. It functions as a mucolytic enzyme, splitting sugars off the glycopeptides of the cell wall of many gram-positive bacteria resulting in their lysis. Lysozyme also plays a role in the intracellular destruction of some gram- positive bacteria.

Trypsin: trypsin and trypsin containing cells are found in epidermis, gills and intestine indicating local production and its secretion into mucus.

Precipitin and agglutinins

C-reactive proteins (CRP) and Serum amyloid protein A: These are plasma proteins and forms part of acute phase proteins. CRP binds to phosphoryl ester groups of bacterial cell wall, which contain phosohoryl choline. This binding is Ca^{++} dependent and activates complement. It has structural analogy to inulin of horse shoe crab. CRP enhances the leukocyte migration, phagocytosis and respiratory burst of phagocytes. CRP can act as opsonin and cause precipitation of heterologus proteins/ carbohydrates in non immunized sera.

Serum amyloid protein A has got lectin binding property by which they bind to bacterial cell wall glycoproteins.

Agglutinins: These are a group of phylogenically conserved substances that are present in fish serum, mucus, bile and haemolymph of invertebrates. This group of substances includes lectins and other receptor specific substances. They act as opsonins and cause aggregation by binding to protein/ glycoproteins and or carbohydrate moieties that are free in solution or are constituent of microbes. Lectins are usually constitutive proteins or glycoproeins, which posses binding activity towards carbohydrate residues.

Cellular factors

Phagocytosis and the inflammatory response: Various circulating and tissue fixed phagocytes rapidly engulf any foreign particles, which enter animal body. In vertebrates they are polymorphonulear leukocytes and macrophages; where as haemocytes constitute the phagocytes of crustaceans and mollusks. The phagocytes contain digestive enzymes that degrade the ingested material. The phagocytes recognize, bind and ingest particulate material. Recognition and binding take place though the interaction of cell surface glycoproteins and cell wall carbohydrates of microorganisms. More recently evolved mechanisms utilize receptors in the phagocyte cell membrane for a part of the antibody molecule (Fc portion of antibody) and for a component of the complement (C3b). Microorganisms coated with antibody and complement thus adhere to the phagocyte and can then be ingested. Antibodies that enhance phagocytosis are the opsonins.

Inflammation: Inflammation is the dynamic process occurring in a viable tissue. It is the reaction of tissues to irritants/ disease causing agents. Inflammation begins following sub lethal injury to tissues and ends with the repair or healing of injured/ damaged tissue. Following an injury the first sequence of changes occur in local vascular system. These vascular changes are the result of release of pharmacodynamic amines from injured mast cells. The eosinophilic granular cells found in fish are analogues to mast cells found in higher vertebrates. These cells are abundant in the connective tissue of blood vessels as well as the stromal connective tissue, which form the structural framework of many organs and tissues. The bacterial products, physical and chemical trauma, products of damaged cells and the complement factors released by immune response can produce injury to mast cells, which release the vaso-active amines like histamine and serotonin. These amines induce increased blood flow (*rubor*). Dilatation of capillaries causes stretching of capillary fenestration, which allows colloids of the plasma to escape into the interstitial space. These results in increase in

colloidal osmotic pressure that attracts fluid content of blood to tissue spaces, leading to swelling of the area (*tumor*) and the fibrinogen escaping with the plasma proteins will be initiated to form fibrin mesh work. Dilatation of arterioles and pre-capillary sphincter cause more capillaries to be opened. An increase in capillary and venule blood pressure is associated with dilation of vessels. Increased permeability of capillaries and venules leads to retardation of the flow and drop in blood pressure. The endothelial cells are activated to produce a lectin on their surface - selectin. The retardation of blood flow allows the heavy elements of blood to be distributed evenly. The leukocytes have on their surface the selectin receptor, which cause them to adhere to the endothelial layer. Then inside out signaling causes certain integrins on the leukocytes ($\hat{a}_1$ and $\hat{a}_2$ subunits) to gain affinity for molecules of immunoglobulin family: particularly those called **ICAM** (Inter Cellular Adhesion Molecules) on endothelial cells. These attachments help the leukocytes to stop, squeeze between endothelial cells and cross the blood vessel wall into the damaged or infected tissue.

Exudation of plasma / serum

The changes in blood flow and the dilatation of capillaries and venules following enlargement of of afferent arterioles lead to retardation of blood flow. This causes increased permeability and osmotic pressure change. Injury to cells leads to breakdown of macromolecules and they enter intercellular fluid resulting increased osmotic pressure. Loss of colloids into interstitial space through increased vascular permeability leads to fall in osmotic pressure of blood. Hydrostatic pressure at venules is increased due to vasodilatation of arterioles. Hence, there is increased accumulation of fluid at the tissue side, where as the re-absorption of fluid from tissue is retarded due to the fall in vascular osmotic pressure. The exudates formed will have plasma proteins including fibrinogen.

The exudates have following functions.

1. Dilute irritants
2. Globulins are brought in contact with irritants, which may neutralize them
3. Fibrinogen in the exudates forms fibrin scaffolding around the irritants, which will contain the spread of infection. The fibrin mesh will act as an anchor for leukocyte migration and phagocytosis.

Migration of leukocytes: Leukocytes emigrate to the tissues by amoeboid movement. The chemotaxis initiate this. Lipo-polysccharrides of bacterial cell wall released at the site are the major chemotactic agents. The cleavage products of complement, such as C3a, C5a C567 lymphokines produced from stimulated lymphocytes and the product of granulocytes and monocytes all act as chemotactic agents. Fatty acid derivatives derived from injured cell membranes such as leukotrienes 5 hydroxyeiosateraenoic acids (5HETE) are all chemotactic. White cells actively migrate through the fenestrae to enter the affected tissues. The cells penetrate junctions between endothelial cells and between basement membrane. They escape to the tissues at the points where basement membrane slits to accommodate pericyte. The collagenase enzyme of leukocytes digests collagen. Lymphocytes are pinocytosed at the basement membrane region. Erythrocytes also leave through the fenestrae. The cells, which leave blood, are 1) neutrophils, 2) monocytes, 3) eosinophils, 4) lymphocytes and 5) thrombocytes.

Neutrophils: These are the first cells to migrate. They contain numerous cytoplasmic granules, which are lysosomes containing number of enzymes capable of destroying the ingested organisms. They are hydrolytic enzymes, oxidative enzymes, proteolytic enzymes, phagocytin and lysozyme.

The proteolytic enzymes are two categories; acid proteases and neutral proteases. Acid proteases act within phagosomes; where as neutral proteases degrade collagen, basement membrane, fibrin, elastin and cartilage. The neutral proteases are responsible for the tissue destruction and this may release kinin and split complement factors C3 and C5 that in turn induces chemotaxis

Opsonic serum factors coating on particles enable phagocytosis. They are complement fragments in fish. Immunoglobulin coated opsonization is weak in fish. The phagocytosis by neutrophils release some quantity of enzymes to the tissues because the fish neutrophils are not efficient phagocytes. The phagocytosis and subsequent digestion are energy dependent. There are two types of digestion, oxygen dependent and oxygen independent systems. In oxygen dependent system there are two types. The superoxide system is characterized by increase in hexose monophosphate shunt activity (This is called respiratory burst, which can be demonstrated by Nitroblue tetrazolium staining (NBT). This generates superoxide anions O_3^-, H_2O_2, ^-OH and O^-. These radicals affect (peroxidation) macromolecules of living organisms like bacteria. In the myeloperoxidase - peroxidase system, myeloperoxidase enzyme increases efficiency of H_2O_2 generating system by releasing halide ions (free halide). This system is more efficient in killing. In oxygen independent system -Hydrgen ions (H^+) reduce pH. Hydrolytic enzymes hydrolyze macromolecules; lysozyme slits off sugars of bacterial cell walls. Fish neutrophils have very similar morphological and histochemical properties to mammalian neutrophils. They are present in kidney, spleen, blood and inflammatory lesions.

Monocytes

These cells appear in an inflammation in later stages. They are actively mobile and have numerous pseudopodia. The monocytes nucleus is ovoid, kidney shaped or indented. Nucleus is usually eccentrically placed. Nucleoplasm is condensed near its membrane. Cytoplasm is abundant and contains mitochondria, Golgi apparatus, and rough and smooth endoplasmic reticulum. Once these cells reach tissues after leaving the blood stream they divide and mature. They are known as mononuclear macrophages. Their main function is phagocytosis by engulfing large particles. They can recognize complement coated cells and particles through specific receptors that assist in phagocytosis. They also recognize molecules that have altered or denatured membranes and engulf them. They can secrete hydrolytic enzymes. Some macrophages mature into secretary cells with abundant cytoplasm and become closer to each other with indistinct boundaries. They are called epithelioid cells. Some macrophages fuse their cytoplasm while attempting to ingest large particles and become multi-nucleated giant cells. Differentiation into epithelioid cells and giant cells occurs in chronic inflammation.

Macrophages are wide spread in tissues but their concentration is more in reticulo-endothelial system. Reticulo-endothelial cells are found in interstitial tissue of kidney, parenchyma of spleen and endocardial lining of heart. Many macrophages in fish contain melanosomes within lysosomes and are termed melano-macrophages. Melanin plays a role in bactericidal mechanism involving the release of free radicals. Melano-macrophages form aggregates in parenchymal organs, which are called melano-macrophage centres

Natural Killer Cells/ Natural Cytotoxic Cells (NK/ NC Cell)

They are large granular lymphocytes. They recognize structures on high molecular weight glyco-proteins, which appear on the surface of infected cells. This recognition occurs through receptors

on NK or NC cells' surface, which bring killer and target into close opposition. Activation of NK cells ensues and leads to release of granular contents into the space between the two cells. The important factor perforin or cytolysin insert into the membrane of the target cell and produce an annular pore, inducing cell death. The granules contain two serine esterases.

Eosinophils

Eosinophils are evolved in killing parasites. A major basic protein (MBP) is located in the core of the granules. Cationic protein and peroxides are present in the matrix of granules. Other enzymes are arylsulphatase B, Phospholipase D and histaminase. It also contains dopaminase. Eosinophils have receptors for C_{3b} which allows eosinophils to adhere and the activated eosinophils secrete MBP and cationic protein

Haemocytes

These are invertebrate blood (haemolymph) cells and have the same enzymes described for leukocytes and perform the phagocytic and degradation function seen in vertebrates.

Acquired Immunity

Acquired immunity has two wings. (1) humoral immunity (2) cellular immunity or cell mediated immunity (CMI). Fish has developed both these systems.

Cell mediated immunity (CMI)

Lymphoid cells may be induced, by prior exposure to antigen, to react subsequently directly with inducing antigen and bring about cytotoxic effects, as for example destruction of foreign cells from a graft. In both type of immunity the executors of the reaction are lymphocytes. Lymphocytes are found in the circulation, lymphoid organs and other tissues. In mammals and birds there are two distinct type of lymphocytes (1) originating from or primed in thymus - T lymphocytes, (2) bone marrow or bursa of *Fabricius* derived cells - B-lymphocytes. T lymphocytes are responsible for cell mediated immune responses (CMI), and providing assistance for antibody production (helper function) and suppression of immune reaction.

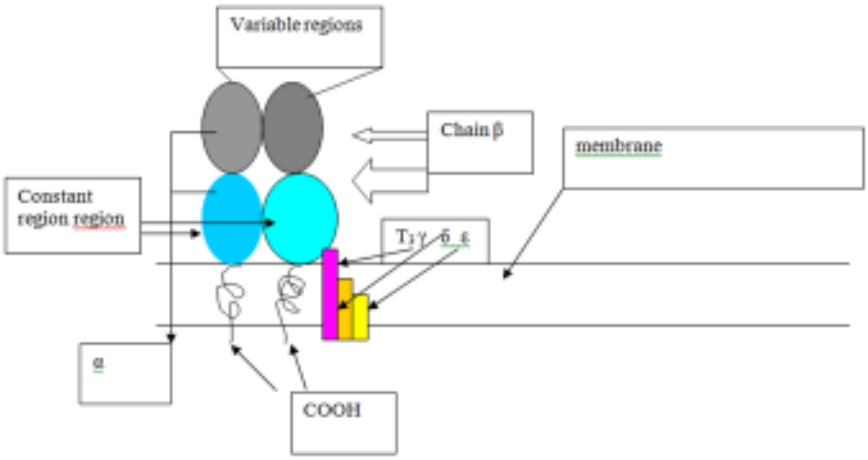
B-lymphocytes are executors of humoral immunity. They transform into antibody or immunoglobulin secreting cells on antigenic stimulation. Subsets of T&B cells perform another function i.e. memory of antigen encounter. In fishes dichotomy of immune system is also present but the details are not fully worked out. Evidence for T&B lymphocyte is also available.

Lymphocyte subpopulation

The T lymphocytes have surface antigen receptors, which are á and â receptors in adult and ä ã receptors in embryonic stage. T lymphocyte receptor is a heterodimer composed of á and â chain each of molecular weight 40-50 kD. Each chain is folded into two domains, one having a relatively constant structure, the other exhibiting far more variability. The variable region has the job of binding to antigen and MHC.

Both á and â chains are required for antigen specificity. In all immuno competent T lymphocytes, the antigen receptor is non-covalently but still intimately linked in a complex with T_3, molecule composed of three peptide chains (ã ä å), which transduces the antigen recognition signal received

by the á â heterodimer to the inside of cell. The complete receptor is thus consisting five peptides. In fish, thymocytes have a portion of Immunoglobulin heavy chain. In mammals no heavy chain receptor is found in thymocytes, where as they bear light chain related molecule.



Structure of antigen receptor in T lymphocyte

T lymphocytes are differentiated in thymus. In higher vertebrates the priming of lymphocytes to T lymphocytes occurs in the thymus. Thymus is protected against the ingress of foreign antigens by specialized endothelial lining of the blood vessels. The role of thymus in fish is probably similar to that of mammals. The thymus consists of mainly lymphocytes in various stages of development and a few epithelial cells and macrophages. There is no differentiation into cortex and medulla. Thymus is a paired organ occupying dorsal region of the brachial cavity and is extremely superficial, being situated within epithelium, external to basement membrane. The blood vessels of thymus have specialized endothelium with tight junctions. In the embryo this is the first organ to become lymphoid. A single layer of epithelium covers the thymus up to post fingerling stage. There are fenestrations of 20 ìm diameter in the epithelial layer. In older fish these fenestrations close and epithelium become thickened or thymus becomes deeply embedded in underlying tissue. Involution of thymus starts at sexual maturity, but it is a slow process in fish. Even in older fish some amount of thymic tissue will be discernable. Thymus is a primary non-executive lymphoid organ. Foreign particulate matter and protein antigen present in circulation are not able to enter thymus. Lymphocytes also do not enter thymus. There is high mitotic activity in thymus and there is migration of thymocyte from thymus to spleen, anterior kidney and intestine. In adult fish, thymus is responsible for regulating antibody response to T dependent antigen and suppressor activity. The developing thymocytes exhibit membrane antigen, which decide their future roles. These antigens are extensively studied in mouse. The mouse T cell antigens are termed Thy (ô) TL and Ly antigens. TL antigen is lost and the amount of Thy antigen is reduced during differentiation. Ly antigen appear later in the development. Ly-1, 2, 3 antigens expressing cells are the immature T cells. Ly-1, 2, 3 cells give rise to Ly-1 cells, Ly2, 3 cells and Ly-1Qa^{+} cells (60% Ly-1 cells are bearing Qa^{+}). Ly-1 cells are having helper function and Ly-1Qa $^{+}$ cells control the generation of suppressor cells. Ly-2, 3 cells have suppressor function on helper cells and B-cells. Ly-2, 3 cells also function as T-killer cells or cytotoxic cells. In other vertebrates the helper function is associated with CD_4 receptor bearing cells and killer and suppressor function

is associated with CD_8 receptor. CD4, CD8 cells are immature T cells. Though clear-cut division based on T cell antigen has not been studied in fish thymus cells, helper, killer and suppressor function are noticed in sub population of lymphocytes of fish. Antigen difference between thymus lymphocytes and lymphocytes of anterior kidney are also evident. In mammals T lymphocyte antigen cross reacts with brain tissue antigen. In fish the lymphocytes, which are responsive to T cell mitogens have antigens cross-reacting with brain tissue antigens.

B-cells have antigen receptors as single heavy chain of ì (Ig M). The B cell is produced in lymphoid tissues like anterior kidney and spleen. The B lymphocyte on biding with respective antigen through the receptor is stimulated to undergo multiplication and many of these cells acquire immunoglobulin secretary function.

Response to mitogens: Certain plant lectins are found to specifically stimulate division of lymphocytes. Phyto-haemagglutinin (PHA) and Concavalin-A (Con.A) are T_cell mitogens, where as Lipo-polysccharrides specifically stimulate B-lymphocytes. In fishes it is found lymphocyte cross-reacting with brain tissue antigen are responsive to PHA and Con.A, where as they are not responsive to Lipo-polysaccharides. Lipo-polysaccharide responsive cells bear Ig M heavy chain molecule on their surface; where as PHA, and Con.A responsive cells bear a part of heavy chain Ig molecule.

The carrier hapten effect: One method to detect T-helper function is to estimate carrier hapten effect. Certain low molecular weight simple substances when injected into an animal will not produce any antibody or immune response. This low molecular weight substance is chemically linked to large molecule. Then the Linked compound is injected to an animal; will induce antibody response against the low molecular substance. The molecule, which is attached to the large molecule, is the hapten and the molecule, which is carrying it, is the carrier. Dinitro-phenyl (DNP) molecule will not induce immune response, where as; if it is linked to bovine serum albumin (BSA), it will elicit antibody response against DNP. In carrier-hapten effect, T-cell co-operation with B cell is required. Carrier-hapten effect has been demonstrated in a number of fish. It is also noticed that T lymphocytes of fish are capable of forming rosettes with sheep erythrocytes.

Cell Mediated Immune Reactions in Fish

The markers of CMI are allograft versus host reaction and delayed hypersensitivity. Both these reactions are found in fish. In graft versus host reaction, an organism rejects organ/ tissue transplants from individuals of the same species as well as phylogenically different species. The rejection process will be faster, if donor and recipient are not genetically related. In this type of reaction no antibody is involved but only lymphocytes and macrophages. Once an animal reject a tissue transplant, it will reject another transplant from the same donor at a short duration of time, thus rejection reaction induces immunological memory in recipient.

Scale transplantation (both allograft and xenograft) have been attempted in fish. In all cases rejection and immunological memory have been noticed. The lymphocyte of the recipients have been shown to retain sensitivity to donor antigens

Delayed hypersensitivity reactions are lymphocyte mediated reactions and lymphokines play a major role. These are specifically provoked by slowly evolving mixed cellular reactions involving lymphocyte and macrophages. The reaction is not brought about by circulating antibody but by

sensitized lymphocytes. It can be transferred in experimental animals by means of such cells not by serum. The classical example is the tuberculin response. The animals infected with *Mycobacterium tuberculi* are given 0.1 ml of 1 in 100 dilution of protein extract of *M. tuberculi* intradermally. An indurated inflammatory reaction in the skin appears about 24 hours later and it may persist for weeks. The injection site is infiltrated with large number of lymphocytes and macrophages, most of these cells are seen around small blood vessels. Among circulating lymphocytes there are a few sensitized lymphocytes, which on contact with antigen produce lymphokines and influence other lymphocytes and monocytes to aggregate at the site of antigen concentrations; and lymphocyte multiply at the site.

Delayed hypersensitivity can be demonstrated in fish by injecting tuberculin or T dependent antigens like BSA and making lymphocytes sensitized. Later an intradermal injection of antigen will produce locsl inflammatory reaction like one described above. In chronic disease like bacterial kidney diseases (*Renibacterium* infection) of trout delayed hypersensitivity has been demonstrated

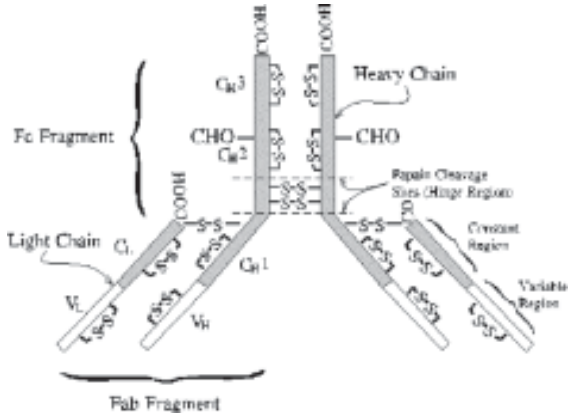
Thus it is clear fish has got a very good CMI response and cells analogues to T lymphocytes are present in fish. The production of lymphokines in fish can be demonstrated by test like macrophage migration inhibition test and demonstration of chemotaxis in special chambers. Macrophage activation by the lymphokines can also be demonstrated. *In vitro* tests like specific contact cytotoxicity, mixed leukocyte reaction and antigen induced blatogenesis of lymphocytes indicate fish has a strong CMI.

Humoral immunity

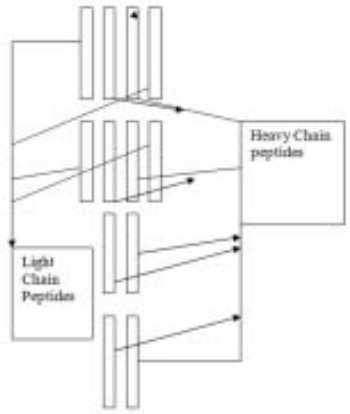
The characteristic form of this immunity is the appearance of globulins-immunoglobulin or antibodies in blood and lymph. These antibodies combine specifically with the antigen, which stimulate their production and lead to remarkable consequences. Induction of the humoral response begins with the recognition of antigen by specific B-cells. This process requires the intervention of T cells bearing CD4 B-cells which recognize the antigen via immunoglobulin receptors on their surface. T cells recognize the antigen through their receptors in association with CD4 and MHC II molecule. T-Cells Produce lymphokines on antigen recognition, resulting in the proliferation of B-Cell leading to antibody secretion. In addition to T helper cells, several other cells present antigens to B-cells and stimulate antibody production. They are macrophages, B cell itself and the spleen ellipsoids. The basic structure of immunoglobulin can be discerned from the structure of vertebrate Immunoglobulin G or Ig ã. It is formed of two heavy chains which acquire the form of 'Y' when unfolded and two light chain one on each side of the arm of 'Y'.

The tips of the Y and light chains enclose the hyper variable areas, (V_H and V_L respectively) which are antigen binding site and designated as Fab fragment while the other area is called Fc region , which is responsible for complement binding, attaching to macrophages and other activities (C_3b mediated inflammatory reactions) resulting from antigen antibody complexes. Each heavy chain is formed by four peptides and light chain formed by two peptides. These peptides are derived from â$_2$ micro-globulins of 110 amino-acids. This is depicted below.

Presence of antibodies can be demonstrated in fish sera by agglutination, precipitation and complement fixation tests. In mammals and birds there are five classes (isotypes) of immunoglobulins based on the antigenic difference in heavy chains. The heavy chain classes are ì, á, ã, å and ä (IgM, IgA, IgG, IgE andIgD). The light chains are two types' ë (lambda) and ê (kappa). In mammals, serum IgM is a pentamer, which consists five basic units linked in the form of ring attached with a protein



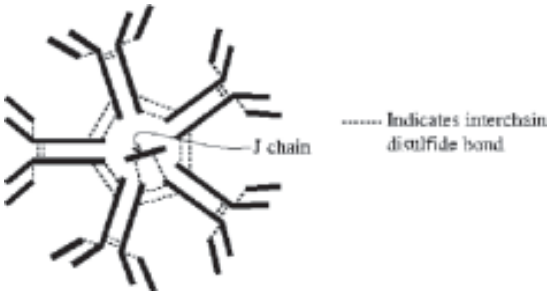
Structure of antigen receptor in T lymphocyte



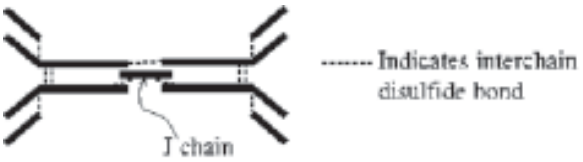
Basic Structure of Immunoglobulion

called J protein segment. In teleosts only IgM isotype is found. The serum IgM is tetrameric. However monomeric, and dimeric forms are found in mucus, bile, skin and eggs.

In mammals and birds functional specialization is associated with heavy chain classes. In teleosts, antibodies can execute most of the reactions observed in mammals and birds, indicating heterogeneity. There is also increasing evidence of local synthesis of secretary antibodies in bile, cutaneous and gastrointestinal mucus. Though immunological methods demonstrated only one type of antibody in fish, there is difference in amino-acid composition of heavy chains. The difference is not amplified enough to recognize them as separate epitopes in immunological reactions to classify them into different idiotypes.



IgM Pentameric structre



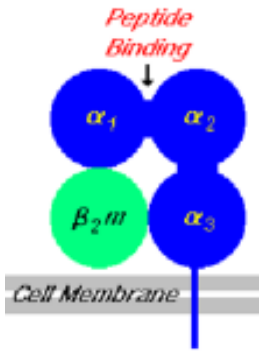
Ig Dimeric form

Cell co-operation and major histocompatibility complex

The precise mechanism by which immuno-competent cells co-operate involves cell surface antigens. These antigens are glyco-proteins, involved in the rejection of grafts to unrelated recipients and are recognized as foreign. The cell surface antigens are known as histocompatibility antigens or **Major Histocompatibility Complex (MHC)**. The genes controlling MHC are closely related to immune response genes (Ir genes). They are situated very close to Ir genes in the same chromosome. The MHC genes have been studied in many animals including trout and carps.

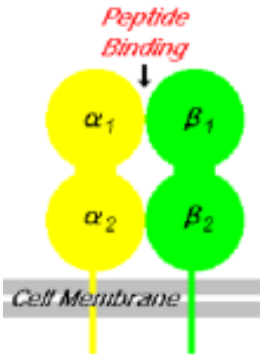
The histocompatibility genes/antigens belong to two classes class I and class II. The genes coding for C2, C4 and factor B, have made their way into the MHC region and are referred to as

class III genes. Both class I and class II molecules are membrane bound heterodimers. Class I molecule consist of a heavy chain of 43 kD non covalently linked to a 11kD peptide, â2 micro globulin. The heavy chain has the globular domains á$_1$ á$_2$ á$_3$, which protrude from cell surface. The hydrophobic section anchors the molecule into cell membrane and short hydrophilic end, which is C terminus, enters cytoplasm.



MHC Class I Molecule

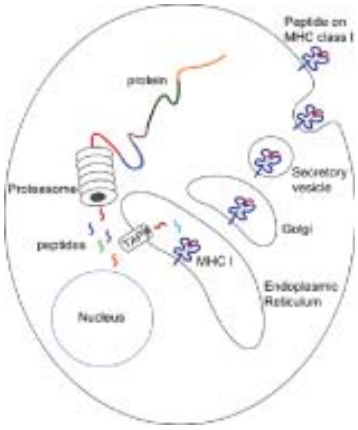
The class II MHC is also trans-membrane glycoprotein having á and â polypeptide chains with molecular weight of 34 kD an 28 kD respectively. Both chains are folded to give two domains, the ones nearest to the membrane having considerable homology with â$_2$ micro-globulin and the characteristic Ig domain. It is seen that I-J region code for more number proteins that it can hold. This includes the suppressor and helper T lymphocyte receptors, immunoglobulins and other peptides. Probably the gene alleles mediate the selection of structurally elated molecules indirectly, perhaps through idiopathic interactions involving T cell receptors. In the immunoglobulin system, variability is achieved in each individual with highly polymorphic system based on multiple alleles. It is very high in class I molecules. Multiple allelic forms generated by variety of mechanisms, point mutation, recombination and homologous but unequal crossing over and gene conversion. The mechanism has been detected in mice. Most of the mutations contain clusters of multiple amino acid substitution and seen to arise by transfer of up to 95 nucleotides from class I Qa genes to á$_1$and á$_2$ domains of H 2 K. these findings have indicated that the large number of functionless Qa genes may represent a stockpile of genetic information for the generation of polymorphic diversity in the working class I molecules. Evidence for gene conversion has also been obtained for the class II genes.



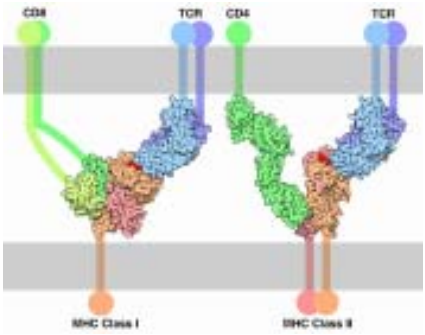
MHC Class II Molecule

All nucleated cells express class I molecules. These are abundant on lymphoid cells, less so on liver, kidney and only sparsely on brain and striated muscles. Class II molecules are restricted to B lymphocytes, macrophages and antigen presenting cells; however, when stimulated by cytokines, capillary endothelium and many epithelial cells express class II molecules. MHC molecules have several physiological functions in addition to immunological functions. It is a known fact that detection non-self MHC activates the T lymphocytes. The T cells can recognize an antigen in association with MHC. The Class I molecule synthesized in rough endoplasmic reticulum and transported to cell surface in a transport vehicle. The MHC molecules have a groove which carries a native peptide (â$_2$). The cells with CD8 receptors search the MHC I molecules. In viral infected cells the peptide is replaced by viral encoded peptides or in cancer cells and old cells the peptide has a different constitution. The detection of changed configuration in MHC leads to the multiplication and secretion of cytotoxic molecules by CD8 t cells. Hence, destruction of the cell occurs. The MHC class II also has a groove. The molecule is synthesized in RER but held in cytosol. In B cell the antigen is captured by surface antibody receptor. It is delivered inside the cell. The antigen is broken down into small peptides; the class II molecule grab the peptide and moves to surface (macrophage also degrades the antigens and fragments are exhibited on MHC II). The CD4 T lymphocytes combine with the antigen presented on MHC II molecule. In nan infection the B cells and macrophages

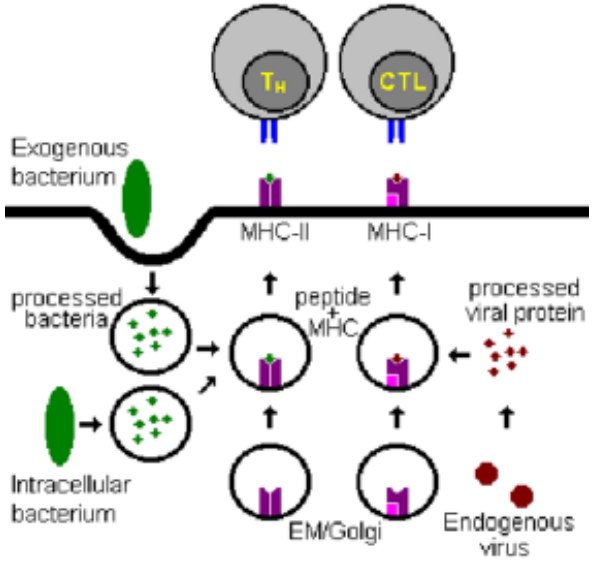
express another molecule B-7. This combines with CD 28 on the T cells. These bindings trigger T cells to secrete cytokines, which initiate B lymphocyte proliferation and antibody secretions. The production of B-7 is essential for activating the T cell secretion of lymphokines and proliferation of B lymphocytes..



MHC Class I Molecule Processing in a Cell



Association of CD 8 and CD4 Molcules with Mhc I and MHCII



The Processing of Antigen in Relation to MHC Classes (I &II)

Clonal Selection Theory of Immune Response

This theory proposes that the cells of the antibody forming system have developed from random mutations resulting in the emergence of small numbers of cells which differentiate so as to be capable of producing one or very small number of specific antibodies. Contact by such differentiated cells with self or foreign antigens during foetal life before cells have reached maturity, would lead to suppression because cells are annihilated by apoptosis.

After intial contact with antigen the cells of immune system retain memory, subsequent contact with antigen lead to quicker as well as amplified immune response. The immune system becomes more skilled with continued experience with the same antigen. The antibodies increase its combining capacity-avidity. This is due to the expansion of the clone. Memory involves generation of long lived T and B lymphocytes and changes in receptor involving generation of high affinity receptors bearing cells.

The antibody has a variable region, which is situated in Fab end and comprises both heavy chain and light chain peptides. The genes for these variable regions are inherited as fragments and these fragments are joined together to form complete genes in individual lymphocytes as they develop. The enzymes that combine the gene fragments add random DNA bases to the ends and as a result new genes are formed. Further diversity results from the assembly of protein chain into a complete receptor. Antibodies are made from two pairs of protein chains a heavy chain and light chain. Each B cell produces one kind of light chain and heavy chain so that B cell makes unique receptor. The genes for receptors of T and B cells mutate extremely rapid, when antigens activate them

Each heavy chain and light chain has variable (V) and constant (C) domain. The V domain is in the N terminal of the both chains. V domain chain is formed by the recombination of four gene fragment- J (joint), D (Diversity) and V (Hyper variable segment). J segment forms the joint with the constant region C. The V region forms hyper variable region and has 100 alleles in mouse, D12 and J 4. Thus in mouse heavy chain itself 4800 varieties can be produced. Considering the light chain variability the antibody types are innumerable.

Suggested Reading

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