ANNAMALAI UNIBERSITY

Faculty of Marine Sciences

DEPARTMENT OF CAS IN MARINE BIOLOGY								
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S. N	**Course Name**	**Course Code**	**Name of the Group**	**Date of creation of the Group**	**No of Members in the Group**	**Name of the Course Teacher**	**Reading Materials Provided (Yes/No)**	**Assignment Topics given to students (Yes/No)**
1	FISH IMMUNOLOGY AND HEALTH MANAGEMENT	MBTC 204	Fish immunology First year	20.12.2019	7	**Dr. B. Deivasigamani Associate Professor**	Yes	Yes

Fish Immunology: Important Topic

Theory

- 1 - General concepts in immunology
- 2 - Evolution of immune system
- 3 - Nonspecific immunity
- 4 - Fish Leucocytes
- 5- Specific defence mechanism in Fish
- 6- Ontogeny of Fish Immune System
- 7 - Lymphocytes
- 8 - Antibody (Immunoglobulin)
- 9- Immune response
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- 12 - Stress and immune response
- 13 - Defence mechanisms in crustaceans
- 14 - Vaccine
- 15 - Adjuvants
- 16 - Immuno-stimulants

Concept of Fish Immunology

Immune systems protect animals from threats by parasites, bacteria and viruses. Most of what we know today about the composition, function and regulation of the two fundamental branches of the immune system — innate immunity and adaptive immunity — comes from studies on mice and humans. However, recently there has been an increased interest in fish immunology for several reasons. For evolutionary biologists, fish immune systems provide important comparative outgroups for understanding the evolution of the immune system. Such comparisons should eventually lead to an increased understanding of general principles of immune system design. At the same time, fish immune systems are also interesting in their own right, as in fish very different mechanisms may have evolved as solutions to immunological problems. Additionally, there are practical reasons, as the cost of infections to aquaculture can be great, making failure of immunity a major risk for commercial fish farming. In biological research, several small fish species have increased in popularity as model organisms for developmental, physiological, and biomedical research. Particularly prominent among these has been the zebrafish (Danio rerio), a small cyprinid teleost, which offers researchers the attractive combination of genetic tractability, rapid ex vivo development, optical transparency, a genome sequencing and annotation project nearing completion, and a rapidly expanding resource of genetic and biochemical reagents including numerous mutant and transgenic lines. The study of immunology in aquatic organisms is as old as the immunology itself, as Metchnikoff's observations in the wounded larvae of starfish initiated his interest in the phagocyte and stimulated his exploration of the cellular basis of the interaction between an organism and invasions from its environment.

Evolution of immune system

Innate immune mechanisms can be found in species at almost every level of the evolutionary tree of life.

When life evolved the primitive organisms like of bacteria, archaea and eukaryotes brought change in environment, also increased the concentration of atmospheric oxygen, this facilitated the evolution of multicellular organisms (metazoans) around 600 million years ago. Evolution in multicellular organisms provided new host opportunities for microbial pathogens so these multicellular organisms developed new mechanisms of defence to protect themselves form pathogens, thus the origin of defence mechanisms begin and evolved along with the life.

The most primitive metazoans like sponges and coelenterates possess an epithelial layer of cells within an intermediate mesogleal layer which performs the role of digestive cells and defensive cells that engulf foreign organisms. These phagocytic cells lead the way for the development of vertebrate macrophage. This innate immunity uses germline encoded pattern recognition receptors for pathogens to distinguish between self and foreign.

Second layer of complex immune defences called adaptive immunity evolved in vertebrates around 500 million years ago. The adaptive immunity have not evolved overnight but it took several years. The unique feature of an adaptive immune system is the development of lymphocytes, where each lymphocyte possess an antigen recognition receptor that can be used to trigger specific defence mechanism. This lymphocytes are the key cells in evolution of specific immune system. Lymphoid cells found first in pre-vertebrate named Deuterostomes. Later these lymphocytes evolved along with the life and the development of the diverse lymphocyte receptor allows vertebrates to recognize almost any potential pathogen or toxin and can generate antigen-specific responses to it. Antigen-activated lymphocytes differentiate into mature lymphocytes with cytotoxic and pro-inflammatory functions or into plasma cells that secrete antibodies and also provide protective memory of the antigen to fight the pathogen in future invasion.

Two types of adaptive immune system have evolved in vertebrates:

Adaptive immune system in jawless vertebrates (hagfish and lamprey)

Adaptive immune system of jawed vertebrates

Adaptive immune system in jawless vertebrates (hagfish and lamprey)

Lymphocytes present in jawless fish are indistinguishable from mammalian cells. But these Fish lymphocytes lack MHC (major histocompatibility complex) molecules, T-cell receptors and B-cell receptors. So jawless fishes use different types of antigen recognition receptor but use similar lymphocyte differentiation process to elicit specific immune response.

Adaptive immune system of jawed vertebrates

MHC (major histocompatibility complex) molecules, T-cell receptors and B-cell receptors based adaptive immune system is detected in cartilaginous fish but absent in lower chordates. For the evolution of adaptive immune system in jawed vertebrates it is believed that two macroevolutionary events that had provided the plat form for evolution of specific immune system are:-

The invasion of recombination-activating gene transposon (RAG transposon)

Whole-genome duplication (WGDs)

Immunoglobulins

The development of adaptive immune system lead to the production of immunoglobulins where, among living animals immunoglobulins are first found in cartilaginous fish like sharks, skates and rays. These are generated by a somatic recombination mechanism. Among immunoglobulins, IgM is the most ancient antibody class and conserved the same function in all

gnathostomes. In cartilaginous fish, IgM is abundantly secreted as monomer, but in bony fish it is secreted as tetramer. In addition to IgM and IgD other immunoglobulins are also found in higher vertebrates. IgG, which is involved in memory responses, Ig E, involved in inflammatory (and allergic) responses at epithelial surfaces. IgA involved in mucosal antibody. IgG and IgE are evolved from IgY, this IgY was found in amphibians and perform the function similar to IgG.

General concepts in immunology

Immunology is the study of the immune system and its responses to invading pathogens. The immune system includes the molecules, cells, tissues, and organs that are associated with immunity in the host defense mechanisms. The coordinated reaction of these cells and molecules to invading pathogen is called immune response. The organs of the immune system are positioned throughout the body. They are called lymphoid organs. The generation of an immune response of either the innate or acquired variety requires the interaction of specific molecules, cells, and tissues.

The immune system of fish is almost similar to that of higher vertebrates. Immunity is referred as the state of acquired or innate resistance or protection from a pathogenic microorganism or its products or from the effect of toxic substances. The cells of the immune system consist of lymphocytes, specialized cells that capture and display microbial antigens, and efffector cells that eliminate pathogens.

Microorganisms in the environment are blocked by physical barriers like skin, mucus etc from being enter into the host. If the pathogens overcome this barrier and invade the host the innate immune mechanism is activated and destroys the pathogen. If the pathogen survives the innate immunity the adaptive immunity is activated and combats the pathogen, added to it adaptive immunity also keeps the memory of the pathogen which helps the immune system during the secondary invasion of the same pathogen.

Uni -I. Ontogeny of Fish Immune System

In fish, as in all vertebrates, the thymus is the first lymphoid organ to develop and to become lymphoid. Later kidney and spleen develops as lymphoid organ and remaining throughout life. In the early life of the carp, the thymus contribute majorly to lymphoid cell pool (70% or about 3 X l05 cells at day 28). Later as the organism grows say at 2 months of age, the total lymphocyte cell pool (5 x l06 cells) was distributed among the lymphoid organs with 38% in the mesonephros, 32% in the thymus, 15% in the pronephros, 12% in the peripheral blood, and 3% in the spleen.

The development of the lymphoid system in fish is explained by two hypotheses.

Natural selection will favor individuals in which development of the immune system coincides with their first exposure to potential pathogens either from their environment or in their food (i.e., hatching in oviparous species, parturition is viviparous species, or at the time of first feeding).

Development of the lymphoid organs occurs at a preset time during embryogenesis. Once a certain "physiological age" has been reached lymphoid organs develop and is independent of first exposure to antigenic challenge.

The state of immunological maturity of the fish may be correlated with the age, weight, and water temperature. The weight of fish is approximately 0.24 g when the first antibody is detected.

2. Evidence for Ig in the Eggs and Passive Transfer of Immunity from Mother to Young

Nonspecific humoral factors, such as C-reactive protein and lectin-like agglutinins are found in fish egg, in addition to that Immunoglobulin has aslo been detected in both fertilized and unfertilized eggs of carp. The levels in the whole fish increased slowly to reach a peak, in terms of milligrams per gram of tissue, at the time of hatching, and slowly decline. IgM-like protein was maternally derived and that it was depleted by the time of first feeding, after which the larvae start to produce their own.

3. Ontogeny of T and B Cells in Lymphoid Organ Development

The sIg+ cells (cells bearing Ig on their surface) could be detected in the thymus and pronephros from day 14 after fertilization, and in the mesonephros and spleen from day 28. Cytoplasmic Ig+ cells (cells bearing Ig in their cytoplasm) were first seen in the pronephros at day 21. The percentage of sIg' cells increased gradually to the age of 16 months, with 15.8-48.1% for blood, pronephros 9.7-21.6%, mesonephros 7.2-16.8% , spleen 15.9-21.6% , and thymus 1.5-3.7%. but in the case of cIg+ cells, the percentage was very low or even absent in spleen, thymus, and blood. Plasma cells were not found in the kidney of the fishes of the age below 1 month old and 0.17% can be seen in the fishes of 1 month old followed by increase in plasma cells, and increased to 1% at the age of 8 months.

4. Ontogeny of nonspecific immunity

Along with maternally derived immune protection in newly hatched fry a variety of nonspecific defence mechanisms develops prior to specific immunity. Nonspecific immunity is the major defence mechanism in earlier life before the specific mechanism is fully developed. Where the nonspecific lectins and hemagglutinins found in fish eggs and fry, in addition to this presence phagocytic cells can be seen in newly hatched fry. This nonspecific immunity helps in defending

the fish throughout the life. Non specific defence system also plays a significant role when the specific immune response is suppressed.

5. Ontogeny of specific immunity

Matured T lymphocytes that can function in defence system can be seen soon after their morphological appearance in about 14-18 days after hatching. The presence circulating antibodies in the blood indicates activation of specific immunity in tern the presence of matured plasma cell can be seen in carp at the age of 4 month. Also the development of these immune system depends on temperature.

Specific defence mechanism in Fish

Specific immune system can be seen in vertebrates which is also referred as advance defence system in organisms. Specific immune system are made up of two cellular systems:

- Humoral antibody system (B cells)
- cell-mediated immunity (T cells)

2. Humoral Antibody System (B cells)

Humoral antibody uses B cells where the B cells differentiate into antibody producing Plasma cells and Memory cells. Series that involved in stimulating B cells to produce antibody are:-
Antigen presentation
The antigen-presenting macrophages recognize the pathogen (e.g., virus) engulf the pathogen, digests it and displays antigen on their surface. The processing involves proteolysis, which presumably occurs within acidic subcellular compartments. These macrophages display residual foreign antigen on the cell membrane and this is l0 times more effective than unbound antigen in promoting immune response.

- On the other hand, other viral particles infect nearby host cells. The macrophages also secrete a protein called interleukin- 1 (IL- 1) which activates the helper-T cells. Besides macrophage, the B cell also acts as antigen presenting cell due to its antigen-binding receptor.

3. Helper T cells

These T cells are activated when macrophages-bound antigen linked to class II Major Histocompatibility Complex (MHC) in the presence of interleukin-l and they secrete two factors like B cell growth factor (IL-4) and B cell differentiating factor (IL-5). The IL- l, IL-4 and IL-5 act together to provoke B cell to respond quickly.

4. B cell activation

If B cell immunoglobulin receptor molecules are crosslinked by foreign antigen and its IL-1 and IL-4 receptors are stimulated, the B cells are activated and express new class II MHC antigen. The membrane receptors for antigen are concentrated at a particular position on the surface of the B cells. The B cells divide repeatedly to form antibody-secreting plasma cells and memory cells. These plasma cells are widely distributed throughout the body but concentrated in the head kidney and spleen of fish. The plasma cells usually die after 3-6 days of secretion and the immunoglobulin levels in the serum decline gradually. The second populations of cells derived from antigen- sensitive B cells and morphologically indistinguishable from parent cells are known as memory cells.

5. Cell-Mediated Immunity (T cells)

Antigenic fragments present on the macrophage alert a specific type of T lymphocyte ("helper" T) about the attack of intruder. These helper T cells recognizes antigen particles and binds to the macrophage via an antigen receptor. Helper T cells are unique to a specific antigen.
This binding stimulates production of chemical substances such as interleukin-1 (IL-1), tumor necrosis factor (TNF) by macrophage
Helper T cells generates interleukin-2 and gamma interferon (IFN-y)
All substances facilitate intercellular communication

Fish Leucocytes

Lymphocytes, macrophages/monocytes, granulocytes (neutrophilic, eosinophilic, and basophilic), and nonspecific cytotoxic cells (NCCs) can be seen in teleost, but there is a difference in occurrence, morphology and function in leukocytes of fish depending on the species of fish and this differences make difficult in generalizations about fish leukocytes.

The cell involved in non-specific cellular response are macrophages/monocytes, granulocytes (neutrophilic, eosinophilic, and basophilic), and nonspecific cytotoxic cells (NCCs). These cells play a major role in combating the pathogen once it enters into the fish.

2. Monocytes/Macrophages

Macrophages differentiated from mononuclear cells derived from circulating blood monocytes. In fish macrophages are mainly involved in phagocytosis which is one of the most ancient immune mechanism, and macrophages were observed in almost all lymphoid tissues. Several subpopulations of macrophages are also present in fish. Distribution of macrophages in the fish is species dependent but these macrophages are commonly observed in both lymphoid organ

(spleen, kidney, and thymus) and non lymphoid organ (liver, gonads, and heart) tissue. The environmental change influence on the size, pigmentation, and number of macrophages.

3. Granulocytes

Fish granulocytes are involved in non-specific defence mechanisms and activate in the presence of foreign material in the body but do not stimulated by specific antigens. These cell are involved in phagocytosis, respiratory burst activity, and chemotaxis. Granulocytes are generated in the haemopoietic tissues of the kidney, have wide morphological variations in granulocyte subpopulations and immature (blast) cells are circulated throughout the fish.

In fish, we can find three types of granulocytes they are neutrophils and eosinophils are the most common while basophils are much rare in fish.

Neutrophil: This is the most abundant among leucocyte. Neutrophils has no affinity for acidic or basic dyes, but stainable with neutral dye. They can migrate into the tissues from blood to engulf bacteria.

Eosinophil: This is a polymorphonuclear leucocyte that can be stained with eosin which is an acidic dye. Eosinophils can neutralize internal parasites and also can modulate allergic inflammatory reactions.

Basophil: These basophils are present only in few fish species, even though they are present their numbers are normally very low in blood. Basophils shows an affinity for basic dyes. Basophils also participate in neutralizing microorganisms.

4. Nonspecific Cytotoxic Cells

These cells are involve in nonspecific cytotoxic activity against the invading foreign materials. These nonspecific cytotoxic cells can be seen in head kidney, spleen, and blood. Circulating nonspecific cytotoxic cells are morphologically and functionally different from the nonspecific cytotoxic cells present in the spleen.

5. Lymphocytes

Lymphocytes are produced in both thymus and kidney and are of 2 types namely B lymphocytes and T lymphocytes. These lymphocytes function in developing specific immune mechanisms in fish. Lymphocytes contain prominent nucleus and basophilic cytoplasm with few mitochondria and ribosomes present in it. The size and number of lymphocytes vary among species and also

with season. Specific antigen responses of lymphocytes differ between cells produced by each organ. There is also a belief that fish lymphocytes may involve in phagocytic activity.

Lymphoid organs in fish

There are two types of lymphoid organs can be seen in fish. Primary lymphoid organs include thymus and head kidney that produce and mature stem cells' The secondary lymphoid organs include kidney, spleen, and Mucosa lymphoid tissue. Besides, liver, skin, intestine and heart are also important organs that take part in the defence. The endothelial cells and macrophages present in these organs are highly endocytic towards 'self' or 'non-self' substances. The development lymphoid organs does not necessarily correspond to the maturation of immune functions' Even though the organs develop simultaneously in trout and salmon, the surface IgM positive cells appear 8 days pre-hatching in frout and 45 days post-hatch in salmon.

2. Primary lymphoid organs

Primary lymphoid organs mainly contains thymus and anterior kidney.

Thymus

It is situated in the dorsolateral region of the gill chamber close to the opercular cavity. Thymus is as the primary lymphoid organ and mostly contains T cells and few populations of B cells. The thymus starts developing from 24 hours after fertilization and it is the first lymphoid tissue to become populated by lymphocytes during development. Once developed the thymocytes migrate from thymus to spleen and kidney. Along with sIg– and sIg+ lymphocytes thymus also possess epithelial cells monocytes, macrophages, neuroendocrine cells etc.

Anterior kidney

The anatomy of the kidney varies from species to species. The kidney of teleost fish not only function as the excretory organ, it also contains the medullary and cortical adrenal homologs and hematopoietic tissue. Kidney is situated along the dorsal wall of the body cavity. Based on location and function kidney of teleost fish has been classified into two types they are:- 1) anterior or head kidney, and 2) posterior or trunk kidney.

Anterior kidney losses its excretory role after the fish matures and Mature anterior kidney is the primary hematopoietic organ in adult teleosts. It is the first lymphoid organ to possess sIg+ cells. Based on the species the anterior kidney may be bifurcated or single extension. B cells will generate and mature in head kidney. Anterior kidney can even act as secondary lymphoid organ.

3. Secondary lymphoid organs

Secondary lymphoid organs contains kidney, spleen, mucosa.

Kidney

The head kidney which also works as secondary lymphoid organ is important in mounting immune response. The head kidney is a major organ where antibody producing cells are formed. The presence of monocytes, macrophages and neutrophils and the lack of lymphocytes in the early life of many fish species indicate the importance of innate immune system during early stage of life. The mature kidney in Indian major carp contains mostly lymphocytes besides other blood cells.

Spleen

Spleen is the secondary lymphoid organ and it is the last organ to form during the development of the lymphoid organs in teleosts. In most teleosts, the spleen is present as an encapsulated organ with abundant red pulp and poorly developed white pulp but in majority of the teleosts these pulp are not clearly distinct. The red pulp occupies most of the space in spleen and contains lymphocytes and macrophages. The white pulp occupies less space and contains poorly developed ellipsoids and numerous melano-macrophage aggregations. Ellipsoids purifying the blood by trapping immunocomplexes and later digested by the macrophages.

Mucosa

Mucosa is highly diffused and unorganized lymphoid tissue that consists of granulocytes, macrophages in the gut, gills and skin. These lymphoid tissue occur mainly under the epithelial cells of the gut and in lamina propria. Macrophages and sIg+ lymphocytes also present in the mucosal lymphoid tissues

Unit I1- Fish Immunology

1. Introduction

All antibodies are proteins known as immunoglobulins. An antibody is defined as “an immunoglobulin capable of specific combination with the antigen that caused its production in a susceptible animal.” Antibodies are produced by plasma cells which are differentiated by B cells, in response to the foreign proteins, called antigens. The part of the antigen to which an antibody binds is called the epitope. The epitope is a short amino acid sequence that the antibody is able to recognize. Antibodies contain four polypeptide chains and are arranged in Y-shape.

2. Basic Structure and Isotypes

Antibody molecule contain 4 polypeptide chains, among them two are identical heavy chains and two identical light chains. These chains are held together by disulphide bonds, some are inter-chain and some are intra-chain. Each part of the molecule has different functions:

The fab fragment: Fab is for "fragment antigen binding" this Fab part of the antibody that binds to antigens. Variable amino acid sequences can be seen in this fragment.

The Fc fragment: Fc is for "fragment crystallizable". The Fc fragment is where complement binds and also the anti-antibodies (anti-IgG) will bind. The amino acid sequences present here are constant.

There are five different isotypes of antibody depending on their difference in heavy chain. These includes:
IgG, IgM, IgA, IgD and IgE.
Heavy chains of IgG, IgM, IgA, IgD, and IgE, are known as gamma, mu, alpha, delta, and epsilon, respectively.

IgG

This is the principle antibody found in blood and body fluids. Nearly 75% of the antibody circulating in the blood is IgG. IgG is a monomeric immunoglobulin, built of two heavy chains and two light chains. Each molecule has two antigen binding sites. This is the only isotype that can pass through the placenta, thereby providing protection to the fetus in its first weeks of life before its own immune system has developed.

IgA

IgA represents about 15% to 20% of immunoglobulins in the blood. IgA is involved in mucosal immunity and prevent the colonization of bacteria in the digestive and respiratory tracts. It does not activate complement.

IgM

The IgM isotype is expressed on the surface of B cells and it is also secreted by plasma cells. IgM present in the form of polymers where multiple immunoglobulins are covalently linked together with disulfide bonds, normally as a pentamer or occasionally as a hexamer. Each monomer has two antigen binding sites, so an pentameric IgM has 10 antigen binding sites, however it cannot bind 10 antigens at the same time because they hinder each other. It is also a "natural antibody" where it is found in the serum without any prior contact with antigen. Due to its polymeric nature, a single IgM-antigen complex can trigger the complement cascade, whereas multiple IgG-antigen complexes are required.

IgD

The function of IgD is not well known but it makes up about 1% of proteins in the plasma membranes of immature B-lymphocytes. It is also in serum in very small level. It is monomeric in nature. IgD's function is currently unknown but it may function as a regulatory antigen receptor.

IgE

IgE is a monomeric immunoglobulin which is heat labile and plays an important role in defending against parasitic worms. IgE is mainly responsible for allergies and this is through their ability to trigger the release of chemicals from the granulocytes when the antibody reacts with specific antigen. The IgE antibodies do not activate complement.

Antibody functions

The antibodies have two primary functions: they bind to antigens they combine with different immunoglobulin receptors specific for them and exert effector functions.

Antigen binding

Immunoglobulins bind specifically to the antigens. Antigen binding by antibodies is the primary function of antibodies and result in protection of the host. If an antibody binds to an antigen on the virus surface then the antibody hinders the virus from binding to the receptor so that the infection cannot be established. Thus, the antibodies neutralize the virus infectivity. Similarly in

the case with microbial toxins where antibodies to toxins bind and prevent the induction of biological effects of toxins.

Effector functions

In addition to binding to specific antigen, the antibodies participate in a number of other biological activities known as effector functions. These functions are initiated once the antibody binds to an antigen. The effector functions are mediated by the heavy chain constant regions (Fc regions) of the antibody. Different antibody classes possess different heavy chain constant regions and hence are specialized to perform different effector functions. The various effector functions carried out by antibodies are described below.

Complement activation

Complement refers to a group of serum proteins where the product of one reaction catalyze a second reaction, the product form the second reaction catalyze a third reaction and so on. The complement activation pathway is triggered by antigen-antibody reactions. The Fc region of antibody is involved in the activation of the first component of complement. Then a series of reactions takes place and form a 'membrane attack complex' (MAC). This MAC is able to attack the membrane of the pathogen and cause the lysis of pathogen by forming a hole on membrane that releases the cell components.

Opsonization

Opsonization is a process where macrophages and neutrophiis are involved. These phagocytes carry Fc receptors on their surfaces and once the pathogen is coated with antibody molecules, it encances the opsonizing activity because of the Fc region of antibody, the phagocytes bind the antibodies through Fc receptors, which help them to phagocytose the target organism. Once the phathgen is engulfed it is killed inside the macrophage.

Antibody-dependent cell-mediated cytotoxicity (ADCC)

Natural Killer (NK) cells present in fish also possess Fc receptors. Antibodies after binding to the target cell (e.g., virus infected cell) activate the NK cells to kill the target cell by extracellular mechanism. This process is known as antibody-dependent cell-mediated cytotoxicity.

Transcytosis

Transcytosis is a process by which some antibodies can cross the epithelial layer to reach the mucosal surfaces of the respiratory, gastro–intestinal tract and can bind antigens. The process of transcytosis depends on the properties of constant region of antibody molecules. IgA is the major antibody that can undergo transcytosis.

IgE-mediated function

Mast cells and Basophils (which are rarely seen in fish) contain Fc receptors for IgE antibodies. When IgE binds with antigen the Fc region induces these cells to degranulate. These granular contents initiates an inflammatory response by attracting various molecular and cellular immune effectors which intern destroy the pathogen. This type of defence is effective particularly against parasitic infection.

4.Monoclonal anitbody

Each B lymphocyte in an organism synthesizes only one kind of antibody and a huge population of different types of B cells can be seen in an organism capable of producing specific antibodies to the various antigens that the organism had been exposed to. Specific population of B cells would produce a specific type of antibody so that we can get a single kind of antibody. Monoclonal antibodies are single B lymphocyte generating antibodies to one specific epitope.

In 1975 Kohler and Milstein developed a technology to fuse immortal Myleoma cells with B cells, using poly ethylglycol (PEG). The resulting cell type is called a hybridoma. This hybridoma takes on the characteristics of both the B cell (that produce antibody) and Myeloma cell (which is immortal), creating an immortal cell with the ability to produce antibody. This hybridoma cells are selectively grown using HAT media. As the hybridoma cells grow they produce a specific antibody which is called a Monoclonal antibody.

Advantages

Once hybridomas are made it is a constant and renewable source and all batches will be identical

They are less likely to cross-react with other proteins

Disadvantage

High technology required.

Training is required for the technology used.

Time scale is long for hybridomas.

5. Polyclonal antibody

Bacterial cell possess different epitopes, upon injection each epitope will stimulate the proliferation and differentiation of B-cell to produce antibody, each particular antibody derived from a B cell that recognizes a particular epitope. Hence large numbers of antibodies are produced with different specificities and epitope affinities, The resulting antibodies in the serum are heterogeneous in nature, each specific for one epitope.
Thus polyclonal antibodies are antibodies that are obtained from different B cell resources and are combination of immunoglobulin molecules secreted against a specific antigen, each identifying a different epitope.
So these antibodies are purified from the serum of immunised animals were the antigen of

interest stimulates the B-lymphocytes to produce a diverse range of immunoglobulin's specific to that antigen.

Advantages

- Inexpensive to produce
- Technology and skills required for production low
- Production time scale is short

Disadvantages:

- Prone to batch to batch variability.
- Multiple epitopes may cross-reactivity and give false result.

Immunity

1.2. Innate immunity

Innate immunity also called natural or native immunity, takes part in the initial protection against Pathogens. Innate immunity in healthy host is prepared to block the entry of microbes and to rapidly destroy pathogens that enter into host tissues.

Innate immunity acts non specifically where the nature or quality of the reaction to a foreign substance does not change when the organism encounters repeatedly.

Innate immunity does not discriminate between the pathogens. The mechanisms of innate immunity provide the initial defense against infections. Majority of the microbes are blocked by physical barrier. If the pathogens succeed in entering the host then these pathogens are eliminated by phagocytes, NK cells, complement system etc.

There are two types of innate immunity

Humoral immunity and

Cell-mediated immunity

Humoral immunity

Humoral immunity is mediated by the antimicrobial peptides, cytokines etc where the cellular substance are involved.

Cell-mediated immunity

Cell-mediated immunity is mediated by the non specific immune cells like macrophages, NK cells, granular cells etc.

1.3. Adaptive immunity

Adaptive immunity also called specific or acquired immunity develops more slowly and take part after innate immunity, adaptive immunity is even more effective against pathogens. Adaptive immunity is stimulated by the presence of pathogens.

If the pathogen survives the innate immunity, adaptive immunity develops later and mediated by lymphocytes and their products. Where antibodies block infections and eliminate pathogens, T lymphocytes also helps in eradicating intracellular microbes.

There are two types of adaptive immunity

Humoral immunity and

Cell-mediated immunity

Humoral immunity

Humoral immunity is mediated by the antibodies which are immunoglobulins and these immunoglobulins are produced by plasma cells differentiated from B cells.

Antibodies are formed against antigens, secreted into the blood and mucosal fluids so these antibodies can neutralize and eliminate pathogens and microbial toxins that are harmful to host.

Cell-mediated immunity

Cell-mediated immunity is mediated by different immune cells that are formed to provide protection against pathogen.

Antibodies cannot reach the pathogen that divide inside infected cells. Defense against such intracellular pathogens is mediated by cell mediated immunity. Cell mediated immunity is mainly through T lymphocytes. Here a type of T lymphocyte, stimulates the phagocytes to quickly recognize the pathogen and destroy them. Other T lymphocytes kill any type of host cells that are harboring infectious microbes in their cytoplasm.

Adaptive immunity may be sub-divided into two major types depending on how the immunity was introduced.

Naturally acquired immunity occurs through contact with a pathogen in environment, whereas artificially acquired immunity develops with vaccination.

Both naturally and artificially acquired immunity can be further subdivided depending on whether immunity is induced in the host or passively transferred from an immune host. Passive immunity is acquired through transfer of antibodies or activated T-cells from an actively immunized host, passive immunity may last only a few months, whereas active immunity is induced in the host by vaccination, and lasts longer duration may be life-long.

An individual exposed to an antigen of a microbe develops an active immune response to eradicate the infection and develops resistance to later infection by that microbe. Such and individual is said to be immune to that microbe.

The most important properties of adaptive immunity is the fine specificity for antigens and memory to the prior exposed antigen.

The tissue of the immune system consist of primary lymphoid organs, in which T and B lymphocytes mature and become competent to respond to antigens, and the peripheral or secondary lymphoid organs, in which adaptive immune response to microbes are initiated.

Nonspecific immunity

3.1. Nonspecific immunity

The innate immune system is believed to be the first line of host defense against invading pathogenic organisms and other foreign material. These components of the innate response are evolutionarily conserved in organisms lacking the typical adaptive immunity of vertebrates.

In fish, the innate immune response has been considered as an essential and primary component in combating pathogens due to limitations of the specific immune response and also their poikilothermic nature. The innate immune system is commonly divided into three compartments: the Physical (epithelial/mucosal) barrier, the humoral parameters and the cellular components.

The epithelial and mucosal barrier of the skin, gills and alimentary tract is an extremely important disease barrier in fish, being constantly immersed in media contain¬ing potentially harmful agents. This type of response requires a series of mecha¬nisms that involve humoral factors, cell and tissue, antimicrobial peptides and complement factors. Humoral factors may be cellular receptors or mol¬ecules that are soluble in plasma and other body fluids.

3.2. Physical barriers

The epithelial and mucosal barrier of the skin, scales, gills and alimentary tract act as the first barrier to infection. This physical barrier is much important in fish because the fish being aquatic in nature and constantly immersed in water that contain potentially harmful agents. The mucus of fish contains lectins, pentraxins, lysozymes, complement proteins, antibacterial peptides and immunoglobulin M (IgM), which have an important role in inhibiting the entry of pathogens. In addition, the epidermis is able to react by thickening and cellular hyperplasia to different attacks and its integrity is essential for osmotic balance and to prevent the entry of foreign agents. On the other hand, defending cells such as lymphocytes, macrophages and eosinophilic granular cells are also present.

3.3. Nonspecific Humoral defence

Teleost fish have been shown to possess non-specific humoral defense substances which are physicochemical and functionally similar to mammals, but still different features.

Antimicrobial polypeptides

Antimicrobial polypeptides are small molecular peptides which are active against microorganisms and have been identified as a component of the innate immune response and been found in the tissues of teleost fishes, These peptides have been found in the mucus, liver and gill tissue of teleost fish. These low molecular weight polypeptides have the ability to break down cell wall of both Gram-positive and Gram-negative bacteria and also some of the evolutionarily conserved cationic, bactericidal polypeptides have been found.

Complement

Fish complement, in general, exhibits highest activity between 15 ^{0}C and 25 ^{0}C and can remain active at temperatures as low as 0 ¬- 4 ^{0}C which is in contrast to mammalian complement with an optimal temperature of 37 ^{0}C.

The complement system in teleosts, as well as that in higher vertebrates, can be activated in three ways:

The classical pathway which is triggered by antibody binding to the cell surface, but can also be activated by proteins such as ligand-bound C-reactive protein or directly by viruses, bacteria and virus-infected cells

The alternative pathway, which is independent of antibodies and is activated di¬rectly by foreign microorganisms, and

The lectin pathway, which is activated by the binding of a protein complex consisting of mannose/mannan-binding lectin in bacterial cells.

Cytokines

A cascade of pro-inflammatory cytokines is released as part of the non-specific innate immune response. The major drawback in identifying fish cytokines is the low sequence identity compared to their mammalian counterparts. The low sequence identities also limit the detection of proteins of fish cytokines by using the antibodies of human cytokines. In general, however, fish appear to possess a repertoire of cytokines similar to that of mammals.

Several cytokine homologues that are observed in fish species are

tumour necrosis factor-a (TNFa) and TNFb,

interleukin-1b (IL-1b), IL-2, IL-4, IL-6, IL-10, IL-11, IL-12, IL-15, IL-18, IL-21, IL-22, IL-26, IFN-g and

chemokines IL-8 or CXCL8, gIP-10, CK-1 and CK-2.

Tumor necrosis factor (TNF)

TNF-α and -β are impor¬tant activators of macrophages leading to increased respiratory activity, phagocytosis and nitric oxide production.

Interleukins (IL)

IL present in tel¬eost fish species are involved in the regulation of immunity through the stimulation of T cells. The expression of the IL-1 receptor in fish appears to be in the ante¬rior kidney, spleen, liver and gills after stimula¬tion with LPS and TNF-α, suggesting a role for the IL-1 receptor in regulating IL-1β during the inflammatory response. The expression of the IL-1 receptor in fish appears to be found in all tissues and is regulated in the ante¬rior kidney, spleen, liver and gills after stimula¬tion with LPS and TNF-α, suggesting a role for the IL-1 receptor in regulating IL-1β during the inflammatory response.

Chemokines

Chemokines are a superfamily of cytokines, produced by different cell types that have, among other functions, chemoattractant properties stimulating the recruitment, activation, and adhesion of cells to sites of infection or injury.

Chemokines play a key role in the movement of immune effector cells to sites of infection and it is becoming increasingly clear that their function is also necessary to translate an innate immune response into an acquired adaptive response.

Innate immune stimuli activate toll receptors and set in motion the expression of chemokines from resident tissue macrophages and dendritic cells and, modulate the expression of chemokine receptors on dendritic cells. These changes in chemokine/chemokine-receptor expression direct the movement of antigen-loaded dendritic cells from the tissue into lymphoid tissue to activate native T and B cells and initiate the adaptive response.

Interferon (INF)

INFα and β are cytokines with a nonspecific anti¬viral function that is based on the inhibition of nu¬cleic acid replication within infected cells. INF plays an important role in the defence against viral infec¬tion in vertebrate host cells, which secrete INFα/β upon recognition of viral nucleic acid. These INFs protect other cells from viral infection by binding to different receptors, which results in the induction of several hundred genes that are stimulated by INF (ISGs).

Protease inhibitors

Several protease inhibitors are present in the serum and other body fluids of fish. The main function of protease inhibi¬tors is to maintain body fluid homeostasis. These molecules are involved in acute phase reactions and defence against pathogens that secrete pro¬teolytic enzymes. The most widely studied of the protease inhibitors is the α-2 macroglobulin, which has a high specificity for inhibiting the physical encapsulation of protease.

Lysozyme

Lysozyme is a bacteriolytic enzyme that is widely distributed throughout the body and is part of the nonspecific defence mechanisms in most animals. lysozyme has been detected in se¬rum, secretions, mucous membranes and tissues rich in leucocytes, mainly the kidney and intestine. Apparently, the main sources of lysozyme are monocytes/macrophages and neutrophils. The bactericidal ac¬tion of this enzyme involves the hydrolyzation of the peptidoglycan of bacterial cell walls resulting in cell lysis. Furthermore, this enzyme is known to trigger an opsonin of the complement system and phagocytic cells.

Natural antibodies

Natural antibodies are produced in fish at a level that is regulated in the absence of antigenic stimu¬lation of cells that are equivalent to B1 cells. These natural antibodies are found in high levels in the serum of fish, where they provide im¬mediate and broad protection against bacterial and viral pathogens, making these factors key compo¬nents of nonspecific immunity. Natural antibodies are also linked to adaptive immunity. Teleost fish are capable of generating specific IgM-type natural antibodies against various antigens. The intensity of this response, however, has been shown to vary between different species and environmental con¬ditions.

Pentraxins

C-reactive protein (CRP) and serum amyloid protein (SAA) are present in the body fluids of vertebrates and invertebrates and are commonly associated with the acute phase response of inflammation. Different stimuli, such as tissue damage, trauma or infection, generate various patterns of CRP production in teleost fish, in which either the level of CRP is decreased in serum (negative acute phase protein) or the level of CRP is increased in serum (positive acute phase protein). Although the pentraxinas of teleosts have a recognized role in defence mechanisms.

Transferrin

Iron is an essential element in the establishment of infection by many pathogens, but the availability of iron in the tissue fluids of vertebrates is extremely low due to its high affinity for the blood protein transferrin. Transferrin is a globular glycopro¬tein with a high iron chelator activity. This protein is the major iron ion transport protein in animals and plants. Only bacteria with high affinity systems for iron absorption are able to maintain sufficient iron levels to grow in the host.

3.4. Nonspecific Cellular Defence

Cell types that are involved in the non-specific cellular defense responses of teleost fish are the phagocytic cells, monocytes/macrophages, the non-specific cytotoxic cells (NCC) and granulocytes (neutrophils). Some teleosts have both acidophilic and basophilic granulocytes in peripheral blood in addition to the neutrophils, but in others only the neutrophils has been found.

Phagocytes

Fish phagocytic cells are formed mainly in the head-kidney from stem cells, as they mature, they spread throughout the body. They are most frequent in tissues underlying epithelial barriers, their principal locations being the head kidney, blood, spleen, gut-associated lymphoid tissue, liver, atrium of heart and gills.

Phagocytes are divided into two main types,

neutrophils and

macrophages

These phagocytes are involved in ingestion of microbes.

Neutrophils

These cells also called polymorphonuclear leukocytes (PMNs) play a key role in the development of acute inflammation. In addition to the phagocytic nature, neutrophils also contain granules that contain acidic and alkaline phosphatases, defensins and peroxidase which necessary for successful elimination of the pathogens.

Macrophages

Macrophages termed as monocytes when present in the blood stream, these are large cells. The function of macrophages include phagocytosis and antigen presentation to T cells. Macrophages are long-lived cells. Macrophages can produce chemicals that can act as antibacte¬rial agents, peroxynitrites and hydroxyl groups

Phagocytosis

Phagocytosis is one of the most important processes in poikilothermic animals because it is the process that is least influenced by tempera¬ture. Phagocytosis is the process by which cells engulf microorganisms and particles. During phagocytosis the phagocytes are attracted towards the microbe by the chemical signals. Phagocyte attaches to the microbe with the help of either microbial sugar residues present on its surface or with the help of complement/antibody which is bound to the pathogen. Once attached the phagocytic cell engulf the microbe and form

phagosome. This phagosome fuses with lysosomes to form a phagolysosome. Which leads to the destruction of the pathogen.

Natural killer (NK) cells

NK cells are large granular lymphocytes that are mainly found in the blood. These NK cells of fish are morphologically distinct from the large granular lymphocytes of mammals but they are functionally similar. They contain two unique cell surface receptors known as killer activation receptor and killer inhibition receptor.

The activation of NK cells, killer activation receptor initiates the release of cytokine molecules, while the activation of killer inhibition receptor inhibits the release of cytokine molecules. NK cells attack virally-infected cells and certain tumour cells and destroy these cells by releasing perforins and granyzymes from its granules. NK cells also secrete interferon-γ (IFN-γ), where this interferon molecule to prevent healthy host cells from becoming infected by a virus and it also increase the T cell response to other virally infected cells.

Unit III

Immune response

Primary immune response

When a new pathogen enters in to the body, specific immune system is activated and mounts an immune response against that pathogen, this immune response to the new antigens is known as primary response. Primary immune response results in a proliferation of B-cells that were stimulated by pathogens. If the antigen is given as vaccine then the the first injection of the antigen producing primary immune response is called priming dose.

The period between the invasion of pathogen and expression of immune response is known as "latent period" which can vary from several hours to days. This latent period depends on the factors like type of antigen, amount of antigen entered, rout of entry, health status of the individual etc. In this latent period the immune system prepares to activate and proliferate the lymphocytes which produce plasma cells and memory cells.

Plasma cells secrete antibodies and memory cells store memory of the pathogen. During primary response IgM type of antibodies are produced with a half life of 5 days. Secreted antibodies are different at different stages of latent period.

The amount of antibodies produced by the immune response to antigen is called antibody titre. The antibody titre plotted against time gives a sigmoid curve called immune response curve. The curve obtained for primary immune response is

called primary immune response curve.

In the initial phase of infection non specific immune response activates followed by specific immune response and that's the reason in initial stage antibodies against the antigens are almost absent, and this period is known as "Lag phase". Later the concentration of antibodies rises and this raising period is referred as "Log phase". Once the antibody level attains maximum it remains constant for some period called as "plateau phase". If the level of antibody remains constant for several days indicates that the antibodies are produced and replaced the antibodies that were lost. After plateau phase the level of antibodies starts to decrease called decline phase.

The antigen is necessary to stimulate immune response which intern produce antibodies from plasma cells. After the removal of antigens by the antibodies, B-cells are not stimulated which intern stops producing plasma cells. Some of the B-cells are stored as memory cells.

Secondary immune response

During vaccine, the injection of the same antigen for the second time is called secondary dose or booster dose.

When the animal is invaded by the same pathogen that caused primary response, the immune system responds very quickly because of the presence of memory cells. Secondary immune response differ both qualitatively and quantitatively from primary response. In the case of secondary response the log phase is very short because of the presence of memory B cells specific for antigen induce the production of antibody with the level of antibody peaking up in a short time. The rise of antibody is around 100 to 1000 times higher than the primary response.

The antibodies formed in response to the secondary antibody is IgG type. IgG type of antibodies have a life span of around 3 weeks so the duration of plateau phase is much extended in the secondary response, and the antibodies decline very steadily even if the plasma cells does not produce antibody.

What Is Fish Health Management?

Fish health management is a term used in aquaculture to describe management practices which are designed to prevent fish disease. Once fish get sick it can be difficult to salvage them.

Successful fish health management begins with prevention of disease rather than treatment. Prevention of fish disease is accomplished through good water quality management, nutrition, and sanitation. Without this foundation it is impossible to prevent outbreaks of opportunistic diseases. The fish is constantly bathed in potential pathogens, including bacteria, fungi, and parasites. Even use of sterilization technology (i.e., ultraviolet sterilizers, ozonation) does not eliminate all potential pathogens from the environment. Suboptimal water quality, poor nutrition, or immune system suppression generally associated with stressful conditions allow these potential pathogens to cause disease. Medications used to treat these diseases provide a means of

buying time for fish and enabling them to overcome opportunistic infections, but are no substitute for proper animal husbandry.

Daily observation of fish behavior and feeding activity allows early detection of problems when they do occur so that a diagnosis can be made before the majority of the population becomes sick. If treatment is indicated, it will be most successful if it is implemented early in the course of the disease while the fish are still in good shape.

The Significance of Fish Disease to Aquaculture

Fish disease is a substantial source of monetary loss to aquaculturists. Production costs are increased by fish disease outbreaks because of the investment lost in dead fish, cost of treatment, and decreased growth during convalescence. In nature we are less aware of fish disease problems because sick animals are quickly removed from the population by predators. In addition, fish are much less crowded in natural systems than in captivity. Parasites and bacteria may be of minimal significance under natural conditions, but can cause substantial problems when animals are crowded and stressed under culture conditions.

Disease is rarely a simple association between a pathogen and a host fish. Usually other circumstances must be present for active disease to develop in a population. These circumstances are generally grouped under the umbrella term "Stress" (Figure 1). Stress is discussed in greater detail in the UF/IFAS Extension Circular 919 Stress - Its Role in Fish Disease. Management practices directed at limiting stress are likely to be most effective in preventing disease outbreaks.

Disease rarely results from simple contact between the fish and a potential pathogen. Environmental problems, such as poor water quality, or other stressors often contribute to the outbreak of disease.

Determining if Your Fish are Sick

The most obvious sign of sick fish is the presence of dead or dying animals. However, the careful observer can usually tell that fish are sick before they start dying because sick fish often stop feeding and may appear lethargic. Healthy fish should eat aggressively if fed at regularly scheduled times. Pond fish should not be visible except at feeding time. Fish that are observed hanging listlessly in shallow water, gasping at the surface, or rubbing against objects indicate something may be wrong. These behavioral abnormalities indicate that the fish are not feeling well or that something is irritating them.

In addition to behavioral changes, there are physical signs that should alert producers to potential disease problems in their fish. These include the presence of sores (ulcers or hemorrhages), ragged fins, or abnormal body confirmation (i.e., a distended abdomen or "dropsy" and exopthalmia or "popeye"). When these abnormalities are observed, the fish should be evaluated for parasitic or bacterial infections.

What to Do if Your Fish are Sick

If you suspect that fish are getting sick, the first thing to do is check the water quality. If you do not have a water quality test kit, contact your county extension office; some counties have been issued these kits, and your extension agent may be able to help you. If your county is not equipped with a water quality test kit, call the aquaculture extension specialist nearest to you (see the list at the end of this publication). Anyone contemplating commercial production of fish should invest in a water quality test kit and learn how to use it. An entry level kit for freshwater aquaculture can be purchased for about $200, and can save thousands of dollars worth of fish with its first use.

Low oxygen is a frequent cause of fish mortality in ponds, especially in the summer. High levels of ammonia are also commonly associated with disease outbreaks when fish are crowded in vats or tanks. Separate extension fact sheets are available that explain oxygen cycles, ammonia cycles, and management of these water quality problems. In general, check dissolved oxygen, ammonia, nitrite, and pH, during a minimum water quality screen associated with a fish disease outbreak. The parameters of significance include total alkalinity, total hardness, nitrate (saltwater systems) and chlorine (if using city water).

Ideally, daily records should be available for immediate reference when a fish disease outbreak occurs. These should include the dates fish were stocked, size of fish at stocking, source of fish, feeding rate, growth rate, daily mortality and water quality. This information is needed by the aquaculture specialist working with you to solve your fish disease problem. Good records, a description of behavioral and physical signs exhibited by sick fish, and results of water quality tests provide a complete case history for the diagnostician working on your case.

Professional assistance is available to Florida residents through the Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences (IFAS) at the University of Florida; the Department of Agriculture and Consumer Services, Division of Animal Industries and Division of Aquaculture, as well as several private laboratories and veterinary practices. A list of public resources is included at the end of this publication.

If you decide to submit fish to a diagnostic laboratory you should collect live, sick fish, place them in a freezer bag (without water), and ship them on ice to the nearest facility. Small fish can be shipped alive by placing them in plastic bags which are partially filled (30%) with water. Oxygen gas can be injected into the bag prior to sealing it. An insulated container is recommended for shipping live, bagged fish as temperature fluctuations during transit are

minimized. In addition to fish samples, a water sample collected in a clean jar should also be submitted. Detailed information on submitting samples is available in UF/IFAS Fact Sheet FA-55, Submission of Fish for Diagnostic Evaluation.

Types of Fish Diseases

There are two broad categories of disease that affect fish, infectious and non-infectious diseases. Infectious diseases are caused by pathogenic organisms present in the environment or carried by other fish. They are contagious diseases, and some type of treatment may be necessary to control the disease outbreak. In contrast, non-infectious diseases are caused by environmental problems, nutritional deficiencies, or genetic anomalies; they are not contagious and usually cannot be cured by medications.

Infectious diseases. Infectious diseases are broadly categorized as parasitic, bacterial, viral, or fungal diseases.

Parasitic diseases of fish are most frequently caused by small microscopic organisms called protozoa which live in the aquatic environment. There are a variety of protozoans which infest the gills and skin of fish causing irritation, weight loss, and eventually death. Most protozoan infections are relatively easy to control using standard fishery chemicals such as copper sulfate, formalin, or potassium permanganate. Information on specific diseases and proper use of fishery chemicals is available from your aquaculture extension specialist.

Bacterial diseases are often internal infections and require treatment with medicated feeds containing antibiotics which are approved for use in fish by the Food and Drug Administration. Typically fish infected with a bacterial disease will have hemorrhagic spots or ulcers along the body wall and around the eyes and mouth. They may also have an enlarged, fluid-filled abdomen, and protruding eyes. Bacterial diseases can also be external, resulting in erosion of skin and ulceration. Columnaris is an example of an external bacterial infection which may be caused by rough handling.

Viral diseases are impossible to distinguish from bacterial diseases without special laboratory tests. They are difficult to diagnose and there are no specific medications available to cure viral infections of fish. The most important viral infection which affects fish production in the southeastern United States is Channel Catfish Virus Disease, caused by a herpes virus. Consultation with an aquaculture or fish health specialist is recommended if you suspect a bacterial or viral disease is killing your fish.

Fungal diseases are the fourth type of infectious disease. Fungal spores are common in the aquatic environment, but do not usually cause disease in healthy fish. When fish are infected with an external parasite, bacterial infection, or injured by handling, the fungi can colonize damaged tissue on the exterior of the fish. These areas appear to have a cottony growth or may appear as brown matted areas when the fish are removed from the water. Formalin or potassium

permanganate are effective against most fungal infections. Since fungi are usually a secondary problem it is important to diagnose the original problem and correct it as well.

Non-infectious diseases. Non-infectious diseases can be broadly categorized as environmental, nutritional, or genetic.

Environmental diseases are the most important in commercial aquaculture. Environmental diseases include low dissolved oxygen, high ammonia, high nitrite or natural or man-made toxins in the aquatic environment. Proper techniques of managing water quality will enable producers to prevent most environmental diseases. There are separate IFAS publications which address water quality management in greater detail.

Nutritional diseases can be very difficult to diagnose. A classic example of a nutritional disease of catfish is "broken back disease," caused by vitamin C deficiency. The lack of dietary vitamin C contributes to improper bone development, resulting in deformation of the spinal column. Another important nutritional disease of catfish is "no blood disease" which may be related to a folic acid deficiency. Affected fish become anemic and may die. The condition seems to disappear when the deficient feed is discarded and a new feed provided. Additional information on nutrition of fish is available through your aquaculture veterinary extension specialist.

Genetic abnormalities include conformational oddities such as lack of a tail or presence of an extra tail. Most of these are of minimal significance; however, it is important to bring in unrelated fish for use as broodstock every few years to minimize inbreeding.

Fish disease outbreaks are often complex, involving both infectious and non-infectious processes. Appropriate therapy often involves medication and changes in husbandry practices. Assistance from UF/IFAS aquaculture extension specialists is available to help you manage disease outbreaks and develop management programs to prevent them. A list of public laboratories available to assist with diagnoses of fish disease is provided for your convenience at the end of this publication. There are many private veterinarians willing to see fish or aquaculture species in their practice. Your aquaculture veterinary extension specialist may be able to refer you to a veterinarian in your area.

Immunological Memory

1. Introduction

The immune system to work successfully it must recognize a large number of microorganisms and molecules that it has never seen before, and it must decide how to respond to them. It should also differentiate between self and non self antigens such as viruses, bacteria, parasites and toxins. The body to defend itself against non self specific invading pathogen it has developed a resistance referred as immunity. Immunity have a memory for most invading pathogens invaded

before, and for this invading pathogen they form memory cells that can last for decades. During the second invasion the immune system remembers pathogen it has seen before leading the rapid proliferation of memory cells and stimulates the release of antibodies. These antibodies are capable of eliminating the pathogens before disease occurs reffered as immunological memory. It is also proved that in vertebrates exposed to antigens in early life has resistance for the same antigen causing illness later. The concept of vaccination is based on immunological memory.

2. Recognition

The immune system uses a large number of highly specific B and T cells to recognize antigen. The number of possible distinct antigens is to be in the range 1012 to 1016. B and T cell receptors are stimulated by antigen. Typically around105 B cells of an individual are stimulated by antigens. B-cells that are activated but not differentiate into plasma cells are transformed into memory B-cells. These memory cells can provide memory for longer period. This was explained by theories like:-

- Memory cells live for a long time
- Memory cells are restimulated at some low level
- Small amounts of the antigen are retained in lymph nodes
- Related environmental antigens provide cross-stimulation

3. Response

B and T cells that are stimulated by antigen divide, during division B cell receptor sometimes mutates leading to increase in the affinity of its daughter cells for the antigen. This B cells change into plasma cells and secrete antibodies which eliminate the antigen. Once the antigen is cleared the B cells with increased affinity for the antigen are selected for keeping memory for an antigen.

Important Topic

Theory

- 1 - General concepts in immunology
- 2 - Evolution of immune system
- 3 - Nonspecific immunity
- 4 - Fish Leucocytes
- 5- Specific defence mechanism in Fish
- 6- Ontogeny of Fish Immune System
- 7 - Lymphocytes
- 8 - Antibody (Immunoglobulin)

- 9- Immune response
- 10 - Immunological Memory
- 11 - Immunologic tolerance
- 12 - Stress and immune response
- 13 - Defence mechanisms in crustaceans
- 14 - Vaccine
- 15 - Adjuvants
- 16 - Immuno-stimulants

Reference

1. 5th Edition Kuby General Immunology book
2. Fish immunology, K.M.Shankar and Jaculine Periera