

Unit 3 - Nonspecific immunity

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 containing potentially harmful agents. This type of response requires a series of mechanisms that involve humoral factors, cell and tissue, antimicrobial peptides and complement factors. Humoral factors may be cellular receptors or molecules that are soluble in plasma and other body fluids.

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.

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 °C and 25 °C and can remain active at temperatures as low as 0 – 4 °C which is in contrast to mammalian complement with an optimal temperature of 37 °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 directly 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- α (TNF α) and TNF β , interleukin-1 β (IL-1 β), IL-2, IL-4, IL-6, IL-10, IL-11, IL-12, IL-15, IL-18, IL-21, IL-22, IL-26, IFN- γ and chemokines IL-8 or CXCL8, gIP-10, CK-1 and CK-2.

Tumor necrosis factor (TNF)

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

Interleukins (IL)

IL present in teleost 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 anterior kidney, spleen, liver and gills after stimulation with LPS and TNF- α , suggesting a role for the IL-1 receptor in regulating IL-1 β 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 anterior kidney, spleen, liver and gills after stimulation with LPS and TNF- α , suggesting a role for the IL-1 receptor in regulating IL-1 β 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)

INF α and β are cytokines with a nonspecific anti-viral function that is based on the inhibition of nucleic acid replication within infected cells. INF plays an important role in the defence against viral infection in vertebrate host cells, which secrete INF α / 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 inhibitors is to maintain body fluid homeostasis. These molecules are involved in acute phase reactions and defence against pathogens that secrete proteolytic 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 serum, 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 action 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 stimulation of cells that are equivalent to B1 cells. These natural antibodies are found in high levels in the serum of fish, where they provide immediate and broad protection against bacterial and viral pathogens, making these factors key components 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 conditions.

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 pentraxins 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 glycoprotein 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.

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 antibacterial 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 temperature. 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 granzymes 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.