[2] Fish and Shellfish Bio-Defense

1.6. Conclusion

Specific biodefense (immunity) is necessary in order to prevent the disease since the non-specific biodefense is not always effective against obligate pathogens. On the other hand, the conditional pathogens intrude into the host when their non-specific defense activity is weak. A better understanding of non-specific defense mechanisms of fish and the conditions (such as immune modulators and stress) makes the damage or loss caused by pathogens improves in sustainable aquaculture. For this purpose, it is necessary to reveal the remaining questions of non-specific defense mechanisms in fish in the future.

Glossary

APC: Antigen presenting cells,

MAC: Membrane-attack complex,

MBL: Mannose-binding lectin,

MASP: MBL-associated serine protease,

PRRs: Pattern recognition receptors,

TLR: Toll-like receptor,

RIG-I: Retinoic acid-inducible gene 1,

RLR: RIG-I-like receptor,

NLR: NOD-like receptor,

CLR: c-type lectin-like receptor,

IFN: Interferon,

NK cells: Natural killer cells,

JAK: Janus kinase,

STAT: Signal transducer and activator of transcription,

ISG: Interferon stimulated gene,

DC: Dendritic cells,

NCC: Nonspecific cytotoxic cells

2. ADAPTIVE IMMUNITY IN FISH

Teruyuki Nakanishi

2.1. Synopsis

There are three major classes of living fish, i.e. agnathans (jawless vertebrates), elasmobranchs and teleosts. Agnathans have different immune system from other class of fish and does not have immunoglobulin (Ig) but variable lymphocyte receptors (VLRs). Teleosts and elasmobranchs are the lowest vertebrates which possess adaptive immunity akin to mammalian one having Igs, the major histocompatibility complex (MHC)/T cell receptor (TCR) system and lymphocyte populations analogous to B cells,

T cells, NK cells. Fish evoke specific immune responses against a variety of antigens with memory. However, fish immune system is different from that of mammals in terms of differentiation of lymphoid tissues, *i.e.* lack of bone marrow and lymph node, and limited number of Ig subclasses, *i.e.* IgM, IgD and IgT for teleosts and IgM, IgW, IgNAR (new antigen receptor) for elasmobranchs and temperature dependence. On the other hand, they have multiple isoforms in immune-related molecules, e.g. cytokines: TNFα, IL-1β; lymphocyte cell surface markers: CD4, CD8; compliment components: C2, C3, etc. The additional number of genes resulting from genome duplication may have creative roles in evolution such as speciation, adaptation, diversification, and promotion of new functions, although differential roles of the isoforms have yet to be clarified in most cases.

2.2. Cells Involved in Adaptive Immunity

Adaptive immunity is mediated by two lymphocyte populations classified as B cells and T cells. Conventional T cells all possess a TCR and CD3 together with co-stimulatory and co-inhibitory surface molecules and are divided into two functional groups of cytotoxic and helper T cells. In teleosts, three major B cell lineages have been described, those expressing either IgT or IgD, and the most common lineage which co-expresses IgD and IgM. Recently, B cell subsets with phagocytic and intracellular bactericidal activities have been reported (Li et al, 2006). This finding led to the existence B cells with phagocytic and microbicidal abilities even in mammals (Sunyer, 2012).

Toda et al. (2011) demonstrated *in vitro* proliferation of $CD4^+$ T cells by allogeneic combination of mixed leukocyte culture (MLC) and antigen-specific proliferation of $CD4^+$ T cells after *in vitro* sensitization with OVA suggesting the primordial functions of helper T cells in fish. Recently, a culture system of $CD4^+$ αβ T cells has been established in carp and $CD4^+$ αβ T cell clones sharing some features with mammalian Th2 cells were obtained by picking single cells from the bulk culture of helper T cells (Yamaguchi et al, 2013). In channel catfish five groups of clones including alloantigen specific TCR $\alpha\beta^+$ cytotoxic clones (presumably CTLs), NK-like cells were identified employing MLC followed by limiting dilution (Stuge et al, 2000). Effector cells in CMC against allogeneic cells and/or virus-infected syngeneic cells were first characterized as surface Ig (sIg) negative cells and, later on, as cells expressing CD8α and/or TCR α or β mRNA. Only $CD8\alpha^+$ CTLs among $CD8\alpha^+$, $CD4^+$, $sIgM^+$ and $CD8\alpha^-CD4^-sIgM^-$ cells showed specific cytotoxicity against allogeneic cells, while $sIgM^+$ cells including NK-like cells exhibited non-specific killing (Toda et al, 2009). This is the first demonstration of the presence of CTLs in a defined T cell subset in fish.

Regulatory T cell (T_{reg})-like cells with the phenotype CD4-2^+, CD25-$like^+$, Foxp3-$like^+$ have been reported from a pufferfish which showed suppressive effect on MLR and nonspecific cytotoxic cell (NCC) activity in vitro (Wen et al, 2011).Recently, antigen presenting cell (APC) resembling mammalian dendritic cells (DCs) have been identified in zebrafish. Zebrafish DCs possess the classical morphological features of DCs and exhibit expressions of genes associated with DC function and activate T lymphocytes in an antigen-dependent manner (Lugo-Villarino et al, 2010).

2.3. Molecules Involved in Adaptive Immunity

2.3.1. Immunoglobulins

Teleost B cells share many similarities with mammalian B cells, including immunoglobulin (Ig) gene rearrangements, allelic exclusion, production of membrane Ig and secreted Ig forms (reviewed in (Edholm et al, 2011)). As opposed to other vertebrate taxa, IgM is the primary antibody present in teleost serum and cutaneous mucus, although the capabilities of IgD as a cytophilic effector molecule and predominant role of IgT in gut mucosal infections have been recently reported. In most teleost, serum IgM is expressed as a tetramer, although IgM monomers have been described in some fishes. In contrast, serum IgT is expressed as a monomer in rainbow trout serum, and a tetramer in gut mucous (Zhang et al, 2010). Teleost IgM possess varying levels of inter-monomeric disulfide polymerization, yielding tetramers, trimmers, dimers, and monomers. A direct association of affinity with disulfide polymerization has been reported in IgM. Polymerization of IgM is suggested to contribute the affinity maturation in teleost which lack class-switching (Ye et al, 2011). Teleost IgT and IgM have comparable genomic structures with mammalian TCRδ and TCRα.

Three Ig isotypes, sIgM, IgW, IgNAR are present in elasmobranch and IgNAR is only found in this group. IgNAR binds antigen by means of a single V domain and IgNARV gene undergoes extensive hypermutaion resulting in affinity maturation (Criscitiello et al, 2006). Shark Ig loci are found in many “clusters” as opposed to the single translocon organization common to mammals. Each of the hundreds of Ig loci in the shark genome contains V, D, J and C genes.

2.3.2. T-Cell Receptors

TCR is divided into two forms, αβ-T cells expressing a heterodimer of α and β chains and γδ-T cells expressing a heterodimer of γ and δ chains. In mammals αβ-T cells are the more abundant in lymphoid organs and blood, whereas γδ-T cells are distributed in mucosal tissues. The initial description of teleost TCR (TCRβ) was reported in rainbow trout (Partula et al, 1995) and in shark (Rast et al, 1994). Orthologs for all four TCR chains have been reported in teleosts and elasmobranchs (see review (Laing et al, 2011)). Basic structure of TCR is well conserved in both teleosts and elasmobranchs. Only the conventional α, β, γ, and δ TCR chains with single C and V domains have been described from shark, although shark Ig loci shows cluster organization and horned shark TRB was multi-cluster as an exception. However, fish TCR display novel characteristics not observed for mammals. For instance, teleost TCRβ chain locus contains two highly divergent constant domain regions and salmonids express 5 distinct constant region genes for TCRγ. Sharks possess a novel TCR-δ variant with which a variable domain of IgNAR is recombined.

2.3.3. MHC Class I/II

MHC genes including class IA, B2m, class IIA and class IIB have been reported from a number of fish species including elasmobranchs. In teleost, MHC class I and II genes are separately located on different chromosomes, although the MHC I and II linkage is

observed in sharks as in mammals (Stet et al, 2003). Extensive polymorphism of classic MHC class I (Ia) genes has been observed in rainbow trout and shark. Trans-species polymorphism is a common feature throughout vertebrates, e.g. the amino acid sequence of the α2 domain of MHC class I a gene is more closely related to that of the carp and zebrafish than that of other salmonids. Ubiquitous expression of MHC Ia genes has been reported in many species of fish. Enhanced expression of MHC class II has been noted in lymphoid tissues of Atlantic salmon following vaccination (Fischer et al, 2013). Important role of the MHC class II linkage group in tissue rejection has been reported in Gila topminnow. MHC class I linkage group was found to be the major determinant for *in vivo* allograft rejection. Correlation between polymorphism in MHC class Ia genes with behavioral traits such as aggression has been reported in rainbow trout (see review (Nakanishi et al, 2011)).

2.4. Cell-Mediated Immunity

CTL-mediated virus-specific cytotoxicity in fish was first described by Somamoto et al. (2000), although a few earlier papers had described the lysis of virus-infected cells by NK-like cells in fish (See review (Nakanishi et al, 2002)). Convincing data showing the essential roles of CTLs against viral infection were reported by Somamoto et al. (2002). Recently, Utke et al. (2007) reported that PBL from low dose viral haemorrhagic septicaemia virus (VHSV)-infected rainbow trout killed MHC class I-matched VHSV-infected cells. More recently, presentation of viral antigen derived peptides by MHC Ia and its regulation by IFN has been reported in grass carp (Chen et al, 2010).

CTLs kill their cellular targets via either of the two mechanisms that each require direct contact between the effector and target cells, i.e. the secretory and non-secretory pathways mediated by perforin/granzymes and Fas/FasL, respectively. In fish, the presence of FasL has been reported at both protein and gene levels in several fishes (Toda et al, 2011). Recombinant FasL protein induced apoptosis in a Japanese flounder cell line indicating that fish possess a Fas ligand system (Kurobe et al, 2007). A major role for the perforin/granzyme pathway in the killing mechanism of alloantigen specific CTLs has been reported in channel catfish, carp and ginbuna (Toda et al, 2011; Zhou et al, 2001). These studies strongly suggest that pathways of killing similar to those of mammals are operative in fish.

2.5. Transplantation Immunity

Skin and/or scale allograft rejection is a representative phenomenon of specific cell-mediated immunity. Cellular reactions, that occur at the grafting site are essentially the same as those in mammals, as characterized by specificity and memory (reviewed in (Manning et al, 1996)). Agnathans and elasmobranchs reject first-set grafts in a chronic manner, while teleosts can evoke allograft rejection in an acute fashion. Accelerated response on second-set grafts is commonly observed in all groups of fish. However, the precise mechanism of allograft rejection has yet to be investigated, although the involvement of T cells in allograft rejection has been suggested in sea bass (Abelli, 1999).

The Graft-Versus-Host Reaction (GVHR) is a phenomenon of cell-mediated immunity in which CTLs play the major role. The presence of GVHR in a teleost fish has been demonstrated in ginbuna and amago salmon (see review (Nakanishi et al, 2011). Most features of acute GVHD in fish are quite similar to those reported for mammals, suggesting the existence of similar mechanisms. More recently, essential roles of donor-derived $CD8\alpha^+$ T cells together with $CD4^+$ T cells in the induction of acute GVHR/D in teleost have been reported (Shibasaki, 2010).

Glossary

Ig: Immunoglobulin,

MHC: The major histocompatibility complex,

TCR: T cell receptor,

MLC: Mixed leukocyte culture,

CTLs: Cytotoxic T lymphocytes,

NCC: Nonspecific cytotoxic cell,

APC: Antigen presenting cell ,

DCs: Dendritic cells,

GVHR: Graft-Versus-Host Reaction,

GVHD: Graft-Versus-Host Disease

3. SHRIMP BIO-DEFENSE

Ikuo Hirono and Sheryll G. Hipolito

3.1. Synopsis

Because of the importance of penaeid shrimps in world aquaculture, there is much interest in understanding their immune system to improve their resistance to pathogenic microorganisms. Basic knowledge of shrimp immunity is needed to develop strategies for prophylaxis and control of diseases in shrimp aquaculture. Shrimps possess an innate immunity that is composed of both humoral and cellular responses. However, little is known about these systems particularly the mechanisms involved at the molecular level. Here, some recent researches of shrimp immune responses against microbial pathogens are presented.

3.2. Introduction

Shrimps are one of the most important aquaculture species not only for commercial products but also for animal protein source for human consumption. Annual shrimp production is growing year by year after the 1980's. However, the growing shrimp aquaculture was accompanied by the outbreak of infectious diseases.

Although devoid of an adaptive immune system, shrimp have an innate immune system that combats invading pathogens. This includes phagocytic activity of hemocytes,