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REVIEW

Adaptive Immune System in Fish

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Abstract

The immune system of all jawed vertebrates is composed of two major subsystems, the innate (non-specific) and adaptive (specific) immune system. The innate immune system is the first to respond to infectious agents; however, it does not provide long-lasting protection. The adaptive immune system is activated later and responds to pathogens with specificity and memory. The main components of the adaptive immune system, including T cell receptors (TCRs), major histocompatibility complex (MHC), immunoglobulins (Igs), and recombination-activating gene (RAG) arose in the first jawed fish (cartilaginous and teleost fish). This review explores and discusses components of the adaptive immune system in teleost fish and recent developments in comparative immunology. Similar to mammals, the adaptive immune system in teleost fish is divided into two components: cellular-mediated responses and humoral-mediated responses. T cells, the principal elements of cellular-mediated adaptive immune responses, differentiate into effector helper T (Th) cells or effector cytotoxic T cells (CTLs). The central elements involved in the differentiation of Th subsets in mammals, cytokines and master transcription factors, have also been identified in teleost fish. In addition, each subset of Th cells, defined with a particular cytokine to control the immune responses, has been described in teleost fish. Similar to mammals, CTLs contribute to cellular cytotoxicity in teleost fish. B cells are central players in humoral-mediated adaptive immunity by producing opsonizing, neutralizing and complement-binding antibodies and inducing antibody-dependent cellular cytotoxicity (ADCC). Three classes of antibodies named IgM, IgD, and IgT/Z have been characterized in teleost fish. The presence of an adaptive immune system and consequent immune memory in teleost fish allows vaccination, the most appropriate method for disease control in aquaculture. Immunological studies in fish provide a comprehensive assessment of the fish immune system, which is crucial for understanding the evolution of the mammalian immune system.

Introduction

All jawed vertebrates, including cartilaginous and teleost (bony) fish, share essential fundamentals of the immune system, consisting of two main parts: innate (non-specific) and adaptive (specific) immune responses. Innate immunity, the first line of the defense system, recognizes pathogens in a non-specific manner and creates a fast reaction to eliminate the microbes, but does not provide long-term protection (Turvey & Broide, 2010). If the innate immune system is insufficient to clear the infectious agents, adaptive immunity is activated by non-specific immunity and interferes with pathogens by reacting with specificity

and long-lasting protection (memory) (Rauta et al., 2012). The adaptive immune system is composed of highly specialized cells, T- and B-lymphocytes, and proteins that destroy and inhibit the growth of invaders (Spiering, 2015). In contrast to innate immunity, adaptive immune responses rely on the highly diverse antigen-specific receptors expressed on T- and B-cells by a few hundred germ-line-encoded gene elements that are assembled by somatic hypermutation and recombination mechanisms of the variable (V), diversity (D), and joining (J) gene segments (Chaplin, 2010; Litman et al., 2010; Tonegawa, 1983). Therefore, the adaptive immune system possesses exquisite specificity for many different pathogens. Another key property of adaptive immunity is memory, as the adaptive immune system generates memory cells that provide long-lived specific immunity, thus playing a crucial role in protection against recurrent infections with a quick and efficient response to the same pathogens (Tangye & Tarlinton, 2009).

Cartilaginous and teleost (bony) fish are the earliest vertebrate group containing basic principles of immunity similar to mammals, despite certain differences between them. Although teleost fish include approximately 50% of all vertebrate species, most of the studies in fish immunology have been defined over the last decades (Volff, 2005). Previously, we highlighted recent knowledge on the innate immune responses and antigen-presenting cells (APCs) in teleost fish (Kordon et al., 2018). In this review, we describe the major components of the adaptive immune system in teleost fish.

Adaptive Immunity in Fish

The major specific components of the adaptive immune system, such as T cell receptors (TCRs), the major histocompatibility complex (MHC), immunoglobulins (Igs), and the recombination-activating gene (RAG), appeared roughly 450-500 million years ago in the first jawed fish (cartilaginous and teleost fish) (Table 1) (Brazeau & Friedman, 2015; Flajnik & Kasahara, 2010). Like in mammals, adaptive immunity in teleost fish is divided into two main components: cellular and humoral responses (Figure 1) (Biller-Takahashi J. D. & C., 2014). T cells, key elements of cellular adaptive immunity, mature in the thymus and differentiate into effector cells, including cytotoxic T cells (CTLs) or helper T (Th) cells (Nakanishi et al., 2015). Although B cells, key elements of humoral adaptive immunity, are derived from bone marrow in mammals, they are produced in the anterior kidney of teleost fish and differentiate into plasma cells that produce antibodies (Carsetti, 2000; Zwollo et al., 2005). Antibodies can be present in two different forms: a soluble form, known as immunoglobulins (Igs), and a membrane-bound form, known as B cell receptor (BCR) (Smith et al., 2019).

The BCR and TCR, expressed on B and T cells, are antigen-specific receptors formed by V(D)J recombination and somatic hypermutation (Nemazee, 2000). Rearrangement of V(D)J genes in different combinations generates a wide-range diversity of antigen-receptor specificities (Carmona et al., 2016). Proteins encoded by recombination-activating genes (RAG1 and RAG2) mediate V(D)J gene rearrangement by recognition of recombination signal sequences (RSSs) and cleavage of target DNA (Carmona & Schatz, 2017; Fugmann, 2001; Schatz et al., 1989). V(D)J gene segments are present in teleost fish, such as zebrafish, rainbow trout, and fugu (Mashoof & Criscitiello, 2016). In addition, the expression of RAG1 and RAG2 genes have been identified in teleost fish, and the V(D)J gene rearrangement in teleost fish is dependent on RAG proteins (Dickerson & Findly, 2017; Greenhalgh & Steiner, 1995; Lee et al., 2014; Willett et al., 1997). Moreover, somatic hypermutation in mammals is initiated by an enzyme, activation-induced cytidine deaminase (AID), that is also expressed in teleost fish (Barreto et al., 2005). In addition to the formation of TCRs and BCRs, these mechanisms induce an irreversible change in the DNA of each cell, and the progenies of these cells inherit the genes that encode the same receptor specificity, including memory T and B cells that provide long-lived specific immunity (Firdaus-Nawi & Zamri-Saad, 2016).

In addition to antigen specificity, immunological memory is defined as the fundamental feature of the adaptive immune system. The majority of specific immune cells die after clearance of the invading pathogens, but a small percentage of cells give rise to long-lived memory cells that mediate a rapid and protective immune response against previously encountered pathogens (Pennock et al., 2013; Youngblood et al., 2017). Effective immunization relies on the combination of antigen specificity and memory in adaptive immunity. Like mammals, memory T and B cells have been identified in teleost fish (Scapigliati et al., 2018). The proliferation of memory T cells has been described in immunized carp by IL-10 modulation (Piazzon et al., 2015).

Cell-Mediated Responses in Adaptive Immunity

T cells play a fundamental role in cell-mediated responses of adaptive immunity by either involving the regulation of other leukocytes functions or directly killing infected host cells. T cells are developed in the thymus; therefore, they are also called thymocytes. The transcription factors for T cell development in teleost fish, such as ikaros (expressed by early T-cells) and lck (expressed later in development) seem to be similar in mammals (Willett et al., 2001). In addition to molecular mechanisms, cellular mechanisms of T cell development are conserved in jawed vertebrates (Boehm et al., 2012; Boehm & Swann, 2014; Hirano et al., 2011; Langenau &

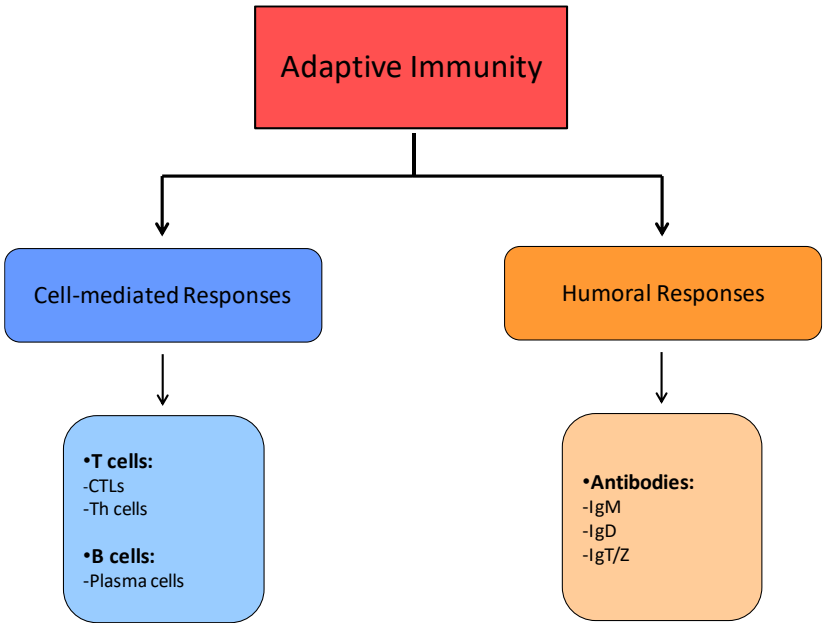

Figure 1. The components of adaptive immunity in teleost (bony) fish. The adaptive immune system of teleost fish consists of two major components: 1) Cell-mediated responses, and 2) Humoral responses.

Table 1. Comparative assessment of general adaptive immune components between teleost fish and mammals

	Teleost (Bony) Fish	Mammals
T and B cells	+	+
BCR	+	+
TCR	+	+
Mechanism of Diversity	V(D)J recombination	V(D)J recombination
RAG genes	+	+
AID	+	+
SHM	+	+
Germinal Center	-	+
CSR	-	+
Ig Isotypes	IgM, IgD, and IgT/Z	IgM, IgD, IgG, IgE, and IgA

Zon, 2005). Recently, the development of T cells in teleost fish was comprehensively reviewed elsewhere (Bajoghli et al., 2019). Mature T-cells are present in lymphoid tissues of teleost fish, such as thymus, kidney, spleen, and mucosa-associated lymphoid tissues, including the intestine, skin, and gills (Nakanishi et al., 2015).

All T cells possess a TCR, formed by RAG-mediated V(D)J gene rearrangement and recognize specific antigens. In mammals, TCR is a type I transmembrane glycoprotein with a short cytoplasmic tail and is associated with the CD3 complex, a transmembrane protein complex with an intracellular signaling domain (immunoreceptor tyrosine-based activation motif (ITAM)), which forms the TCR:CD3 complex (Birnbaum et al., 2014; Love & Hayes, 2010). The structure of the TCR:CD3 complex is conserved between mammals and teleost fish (Castro et al., 2011; Øvergård et al., 2009; Shang et al., 2008). Moreover, T cells in mammals are present mainly in two sublineages based on the nature of heterodimeric receptor chains with TCR: αβ-TCR or γδ-TCR (Smith et al., 2019). Most of the T cells express αβ-TCR found in blood and lymphoid organs, whereas γδ-T cells represent a small fraction of total T cell population in mammals (~5 %) (Carding & Egan, 2002; Janeway et al., 1988). Similar to mammals, TCR genes encode the TCR α, β, γ, and δ chains in teleost fish, such as channel catfish, zebrafish, and Atlantic salmon (Bilal et al., 2018; Castro et al., 2011; Fischer et al., 2002; Haire et al., 2000; Hordvik et al., 2004; Nam et al., 2003; Wilson et al., 1998). Also, the γδ-T cells constitute 7-20% of total lymphocytes in blood and lymphoid tissues of zebrafish (Wan et al., 2017).

Although γδ-T cells recognize antigens directly, αβ-T cells recognize peptides of antigens bound to major histocompatibility complex (MHC) molecules (Konigshofer & Chien, 2006). The αβ-T cells in mammals are further divided into two major populations based on

their function, cytotoxic CD8+ T cells (CTLs) and helper CD4+ T (Th) cells. CD8+ T cells are activated by peptides derived from intracellular antigens and presented by MHC class I molecules, and their function is to kill infected host cells. Helper CD4+ T cells are stimulated by peptides derived from extracellular antigens and presented by MHC class II molecules, and they regulate the responses of other leukocytes (Andersen et al., 2006; Banchereau & Steinman, 1998; Kordon et al., 2018). The functional roles of both T cells and T cell-related molecules, CD4, CD8, MHC class I and II, have been described in teleost fish (Fischer et al., 2013; Koppang et al., 2010; Nakanishi et al., 2015; Hideaki Toda, Yasutaka Saito, et al., 2011).

Major Histocompatibility Complex and Activation of Naïve T Cells

Major histocompatibility complex (MHC) class I and II molecules play a pivotal role in adaptive immunity. MHC class I molecules are composed of two domains (α1 and α2), forming antigen binding platform, and one membrane-spanning domain (α3). The microglobulin domain (β2) is bound non-covalently to the membrane-spanning domain (Chen et al., 2010; Chen et al., 2017). On the other hand, the binding region of MHC class II molecules are formed by α1 and β1 domains, and two membrane-spanning domains (α2 and β2) are present in MHC class II molecule (Chen et al., 2010; Chen et al., 2017; Yamaguchi & Dijkstra, 2019). Although both MHC class I and II genes were described in most teleost fish and are conserved in jawed vertebrates, some teleost fish, such as Atlantic cod lack MHC class II genes (Smith et al., 2019; Star et al., 2011). However, more genes-related to MHC class I molecules were identified in Atlantic cod to compensate for the absence of MHC class II counterparts compared to other teleost fish (Smith et al., 2019; Star & Jentoft, 2012; Star et al., 2011). Also, the heterodimer complexes and the conserved structure for peptide-ligand binding of MHC molecules in teleost fish are similar to those found in mammals (Smith et al., 2019; Yamaguchi & Dijkstra, 2019).

In mammals, MHC class I molecule is expressed by all nucleated cells and presents the peptides from intracellular antigens to CD8+ T cells (Cytotoxic T cells) (Hewitt, 2003). However, MHC class II molecule presents the peptides processed from endocytosed/phagocytosed antigens (extracellular antigens) on only professional APCs, dendritic cells, macrophages, and B cells to CD4+ T cells (helper T cells) (Cho & Roche, 2013). Similar to mammals, MHC class II molecules are expressed by all professional APCs of teleost fish (Yamaguchi & Dijkstra, 2019)(ref). However, MHC class I molecules are ubiquitously expressed and detected in multiple tissues, including spleen and anterior kidney (Grimholt et al., 2002; Smith et al., 2019). Also, the roles of teleost MHC class I and II molecules are similar to those in mammals. For example, upon antigen stimulation, the expression of both MHC class I and II genes were upregulated in teleost fish similarly to mammals (Scharsack et al., 2007). Also, the elevated expression of MHC class I and II genes increased the survival and resistance of Atlantic salmon in *Aeromonas salmonicida* infection (Kjøglum et al., 2008; Smith et al., 2019). Furthermore, increases in expression of MHC class II and CD4 genes correlated with enhanced adaptive immune responses to *Edwardsiella ictaluri* (*E. ictaluri*) live attenuated vaccine strains in channel catfish lymphoid organs (Kordon, Abdelhamed, et al., 2019; Kordon et al., 2021).

In addition, the interaction between TCR and the MHC: peptide complex on APCs lack enough signal to activate fully naïve T cells in mammals. Therefore the priming of T cells requires the combination of three different signals delivered in sequence: 1) antigen recognition (TCR:MHC), 2) costimulation, and 3) cytokines (Figure 2) (Sckisel et al., 2015). The co-stimulatory signal is generated by the interaction of a co-stimulatory factor, CD28, expressed on T cells and B7.1 (CD80) and B7.2 (CD86) ligands found on APC. As a third signal, cytokines secreted by APCs determine the differentiation of activated T cell into a particular effector T cell subset (Muñoz-Wolf & Lavelle, 2018; Nace et al., 2012). Similar to mammals, co-stimulatory molecules (CD28, B7.1, and B7.2) have been identified in several teleost fish, such as rainbow trout and zebrafish (Bernard et al., 2006; Hansen et al., 2009; Sugamata et al., 2009). Cytokines for T cell differentiation have been determined in teleost fish, similar to mammals (Ashfaq et al., 2019).

Effector CD4+ T helper Cells

CD4+ T cells accomplish numerous crucial functions in mammals by stimulating cell-mediated immunity of macrophages, granulocytes (neutrophils, eosinophils, and basophils), CD8+ T cells, and B cells to produce antibodies through the production of several cytokines (Reiner, 2007). Similar functions of CD4+ T cells have been described in teleost fish: in particular, the effective bacterial killing triggered by CD4+ T cells in ginbuna carp (Nayak & Nakanishi, 2013). Moreover, the upregulation of cytokine, IL-12, related to CD4+ T cells was documented in fugu during extracellular parasitic infection (Yoshiura et al., 2003). Although the mammalian CD4 molecule contains four Ig-like domains (D1-D4), teleost fish possess two CD4 genes: CD4-1 containing four Ig-like domains and CD4-2 containing two or three Ig-like domains (Ashfaq et al., 2019; Maddon et al., 1987). Recently, we showed the increased expression of both CD4-1 and CD4-2 genes in the lymphoid tissues of channel catfish infected with *E. ictaluri* strains (Kordon, Abdelhamed, et al., 2019; Kordon et al., 2021).

Upon activation, the naïve CD4+ T cells differentiate into specific subsets named Th1, Th2, Th9, Th17, Tfh cells, and Tregs, with each subset defined by a

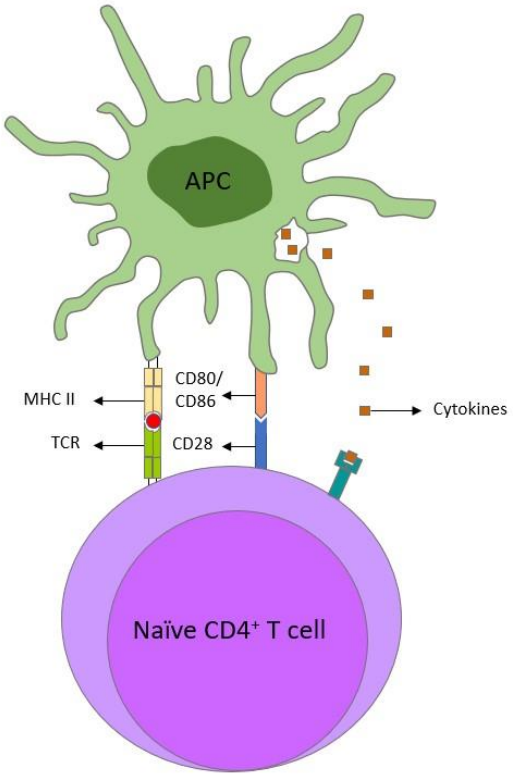

Figure 2. Three signals delivered by APCs to prime naïve T cells. **Signal 1** is generated from the interaction between MHC: peptide complex and TCR and required for activation of naïve T cells. **Signal 2** is a co-stimulatory signal (CD80/CD96:CD28) delivered by the same APC for survival and proliferation of the T cell. **Signal 3** is a cytokine signal. Cytokines are secreted by APC and other innate lymphocytes for differentiation of the T cells into a particular subtype.

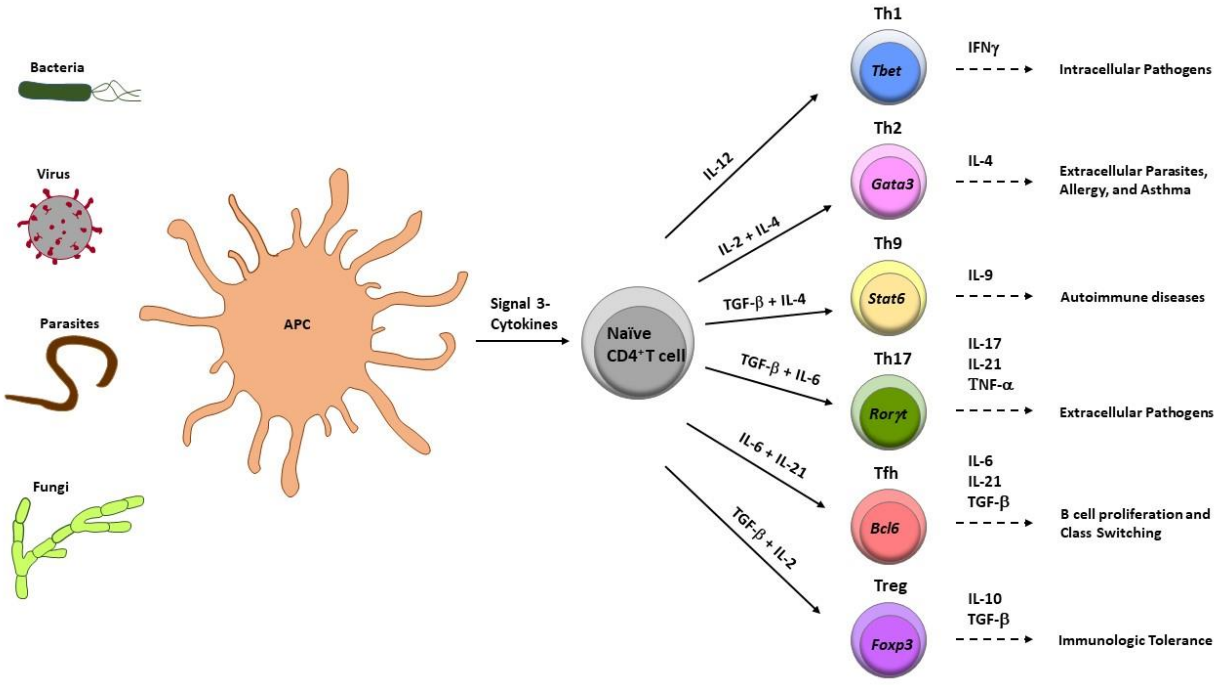

Figure 3. Pathogen-induced differentiation of naïve CD4+ T cell into different subsets. Followed by interaction with an APC infected with different pathogens (bacteria, viruses, parasites, or fungi), Th cells may differentiate into several effector cell subsets depending on the cytokine signal (solid arrows). Each Th type secrets a unique set of cytokines (dashed arrows) and promotes the pathogen-specific immune responses.

particular cytokine profile control over the immune responses (Figure 3) (Annunziato & Romagnani, 2009; Dardalhon et al., 2008; Saito et al., 2010; Wan & Flavell, 2009). In mammals, APCs activated through the pattern recognition receptors produce large amounts of interleukin 12 (IL-12) that induce natural killer cells (NK) to secrete interferon γ (IFN-γ), thus initiating Th1 differentiation (Luckheeram et al., 2012; Trinchieri et al., 2003). Also, several transcription factors, including the T-box transcription factor (T-bet), signal the transducer and activator of transcription-1 (STAT-1), and STAT-4 are involved in full differentiation of the Th1 cells (Ashfaq et al., 2019). In teleost fish, multiple isoforms of IL-12 and IFN-γ are key cytokines for Th1 differentiation (Nascimento et al., 2007; Yoshiura et al., 2003). In addition, the T-bet gene has been characterized in teleost fish, such as zebrafish, rainbow trout, and grass carp, and T-bet is considered to be a master transcription factor for both fish and mammals (S. Mitra et al., 2010; Wang et al., 2013; Wang, Holland, et al., 2010). The Th1 cells mount an immune response to intracellular pathogens by the release of IFN-γ that activates macrophages and enhances their phagocytic activity (Luckheeram et al., 2012). The Th1 cells in teleost fish generate one or two forms of IFN-γ, IFN-γ and IFN- γ rel (Smith et al., 2019). In *E. ictaluri* infection, we reported elevated expression levels of the IFN-γ gene, correlated with CD4-1 and CD4-2 genes' elevated expression in the lymphoid organs of channel catfish (Kordon, Abdelhamed, et al., 2019; Kordon et al., 2021).

The IL-2 and IL-4 are critical cytokines responsible for Th2 differentiation through the regulation of transcription factors containing STAT-5, STAT-6, and GATA-3 (Ashfaq et al., 2019; Zhu et al., 2001). Similarly, two genes of both IL-2 and IL-4 have been identified in teleost fish (Díaz-Rosales et al., 2009; Li et al., 2007). In addition to key cytokines, the master transcription factor GATA-3 has been described in distinct fish species, including zebrafish, salmonids, and grass carp (Kumari et al., 2009; Neave et al., 1995; Wang et al., 2013). The Th2 cells are effective against extracellular parasites, inducing the degranulation of cells and mast cells, and the production of antibodies by producing key cytokines including IL-4, IL-5, IL-9, IL-10, IL-13 (Walker & McKenzie, 2018). Similar to mammals, Th2-related immune responses were detected in fish gills and skin with a higher expression of IL-4/13A and GATA-3 against parasites (Takizawa, Koppang, et al., 2011).

Th17 cells, another Th cell lineage, are involved in the elimination of extracellular pathogens, including bacteria and fungi. The main cytokines IL-6, IL-21, IL-23, and transforming growth factor-beta (TGF-β) drive the differentiation of Th17 cells, along with regulators including retinoic acid receptor-related orphan receptors gamma (RORγ) and alpha (RORα) and the signal transducer and activator of transcription 3 (STAT-3) (Zhu et al., 2010). The major elements required for mammalian Th17 cell segregation pathway are also found in teleost fish, containing signature cytokines (IL-6, IL-21, IL-23, and TGF-β) and the master transcription factor RORγ (Ashfaq et al., 2019). Th17 cells in mammals produce IL-17 (IL-17A, IL-17F), IL-21, and IL-22, which mediate inflammatory responses, inducing the recruitment of inflammatory cells, such as neutrophils to site of infection, and the production of pro-inflammatory cytokines, such as IL-1, IL-6, and TNFα (Luckheeram et al., 2012). Multiple isoforms of IL-17A/F genes (IL-17A/F1, IL-17A/F2, and IL-17A/F3) have been characterized in teleost fish, such as zebrafish, rainbow trout, and fugu (Gunimaladevi et al., 2006; Kono et al., 2008; Monte et al., 2013). Additionally, IL-17A/F2 stimulated antibacterial defenses by inducing the expression of antibacterial peptides and pro-inflammatory cytokines, IL-6 and IL-8, in rainbow trout (Secombes et al., 2011). Moreover, Th17-like immune response has been identified in zebrafish mucosal tissues after live attenuated *Vibrio anguillarum* infection (Zhang et al., 2014).

Regulatory T cells (Treg) regulate the maintenance of immunologic tolerance to self and foreign antigens. The key cytokines, TGF-β and IL-10, play a vital role in the differentiation of Treg cells through the main transcription factor, forkhead box P3 (FoxP3) (Yuan & Malek, 2012). Similar to mammals, distinct isoforms of TGF-β and IL-10-type genes are also present in fish (Ashfaq et al., 2019; Zhan & Jimmy, 2000). In addition to key cytokines, the master regulator FoxP3 has been identified in teleost fish (Suman Mitra et al., 2010; Wang, Monte, et al., 2010). Mammalian Tregs produce the same effector cytokines, TGF-β and IL-10, that suppress pro-inflammatory responses after the clearance of pathogens to prevent tissue damage, and maintain peripheral tolerance (Ouyang et al., 2011). The IL-10 produced by Tregs has been identified in most teleost fish species, and its expression was highly elevated at 2-5 weeks post-infection (Piazzon et al., 2017). Also, Tregs in teleost fish preserve peripheral tolerance in the skin of rainbow trout (Leal et al., 2016).

In mammals, follicular helper T cells (Tfh) regulate the proliferation of B cells and Ig class switching, especially in the germinal centers of lymphoid tissues. The major cytokines, IL-6 and IL-21, and transcription factor Bcl6 are involved in differentiation process of Tfh cells (Nurieva & Chung, 2010). The effector Tfh cells secrete IL-6, IL-21, and TGF-β to perform their function (Srivastava et al., 2018). In contrast to mammals, teleost fish lack germinal centers and Ig isotype switching. Recently, Th9 cells were defined as a subgroup of CD4+ T cells contributing to immune responses against intestinal worms and autoimmune diseases, including multiple sclerosis (MS), inflammatory bowel disease (IBD), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), psoriasis, and cancer, and they exhibit potent anti-tumor properties (Chen et al., 2020; Srivastava et al., 2018). The cytokines TGF-β and IL-4 and

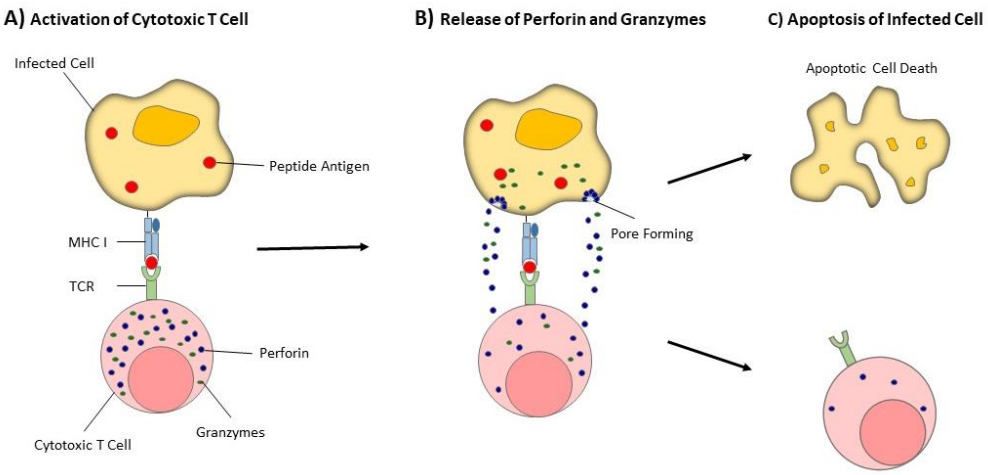

Figure 4. Clearance of infected host cells by effector CD8+ T (Cytotoxic T) cells in teleost fish. Cytotoxic T cells bind by their TCRs that recognize peptide: MHC class I complex on the infected host cells (A). Cytotoxic T cells secrete perforin and granzymes, that induce the formation of pores and release of cytotoxins in the cytoplasm of infected host cell (B). The infected host cells undergo apoptotic cell death, and cytotoxic T cells are detached from infected cells (C).

master transcriptional factor STAT-6 are responsible for Th9 cell differentiation (Chen et al., 2020). The significant cytokine IL-9 is secreted by Th9 cells (Srivastava et al., 2018). On the other hand, two subpopulations of CD4+ T cells, Tfh and Th9 cells, have not yet been detected in teleost fish species.

Effector CD8+ Cytotoxic T Cells

CD8+ T cells play a crucial role in immune defense against intracellular pathogens, especially virus infection. IL-12 released from the APC is the key cytokine driving the differentiation of mammalian CD8+ T cells through the master transcription regulators, including T-bet and eomesodermin (Eomes) (Ahuja et al., 2007; Intlekofer et al., 2005). In teleosts, homologues of IL-12 subunits have been reported (Yoshiura et al., 2003). Similarly, *T-bet* has been characterized in many teleost fish species, and gene expression analysis showed that T-bet triggers the activation of CD8+ T cells. Also, Eomes has been identified in teleost fish, such as zebrafish, Atlantic salmon, and rainbow trout (Yamaguchi et al., 2019). Furthermore, the highest level of Eomes expression was observed in rainbow trout CD8+ T cells (Takizawa et al., 2014). The CD8 molecule in mammals is present in two forms: a homodimer of two α chains or a heterodimer of α and β chains (Cole & Gao, 2004). Both α and β chains of the CD8 molecule have also been described in most species of teleost fish (Forlenza et al., 2008; Takizawa, Dijkstra, et al., 2011). Furthermore, we demonstrated that an intracellular pathogen, *E. ictaluri* strains, induced the upregulation of both CD8α and CD8β gene expressions in the lymphoid tissues of channel catfish (Kordon, Abdelhamed, et al., 2019; Kordon et al., 2021).

Mammalian CD8+ T cells induce the apoptosis of infected host cells by two pathways: the secretory and non-secretory mechanisms. The secretory pathway is characterized by the release of granular toxins, including perforin and serine proteases known as granzyme, while the non-secretory pathway requires the engagement of target-cell death receptors, such as Fas, located on the CD8+ T cell surface (Jaime-Sanchez et al., 2020). The secretory pathway in teleost fish is similar to that of mammals (Figure 4). A perforin-like molecule has been identified in multiple teleost species (Hwang et al., 2004; Praveen et al., 2004). CD8α+ cells in ginbuna crucian carp kill virus-infected cells by using perforin, and perforin inhibitor, concanamycin A, inhibited the killing function of CD8α+ lymphocytes in channel catfish and ginbuna crucian carp (H. Toda et al., 2011; Zhou et al., 2001). These studies showed that perforin induces a similar pathway of killing in teleost fish (Figure 4). In addition to perforin, granzyme with a similar structure to that of mammals has been identified in teleost fish (Figure 4) (Huang et al., 2010; Praveen et al., 2004). The granzyme B-lime molecule was involved in the cytotoxicity of CD8+ T cells in fish (Hideaki Toda, Takeshi Yabu, et al., 2011). Moreover, highly elevated levels of granzyme mRNA was detected in CD8+ T cells of ginbuna crucian carp in *Edwardsiella tarda* (*E. tarda*) infection (Matsuura et al., 2014). Granzyme A-like serine protease in ginbuna crucian carp contributed cell-mediated immunity through cytotoxic activity (Matsuura et al., 2016). Furthermore, the non-secretory pathway has been studied in teleost fish. For instance, the FasL-like molecule, apoptosis ligand, has been characterized in several teleost species, such as channel catfish, tilapia, and gilthead sea bream (Cuesta et al., 2003; Long et al., 2004; Ma et al., 2014). The FasL-like protein in tilapia

Table 2. General structure of Ig isotypes in teleost fish

Ig Isotypes	Teleost (Bony) Fish	Mammals
IgM	Tetramer No J chain Disulfide bonds-associated Serum Mucosal in the absence of IgT/Z	Pentamer J chain-associated Serum
IgD	Transmembrane Secreted: No V domain C domain ranging from 2 to 16	Monomer
IgT/Z	Unique to teleost fish Mucosal Tetramer in mucosal tissue Monomer in serum	N**ot** present

resulted in cytotoxicity to the Hela cells through apoptosis (Ma et al., 2014).

Teleost CD8+ T cells are involved in cell-mediated cytotoxicity that control intracellular pathogens. The population of CD8α+ cells killed virus-infected target cells in fish (Somamoto et al., 2013; Hideaki Toda, Takeshi Yabu, et al., 2011; Yamaguchi et al., 2019). Increased cytotoxic activity of CD8+ T cells with elevated numbers of CD8α+ cells contribute to the elimination of *E. tarda*-infected cells and bacteria in the spleen and kidney of fish (Yamasaki et al., 2013). Furthermore, adoptive transfer of CD8α+ lymphocytes from carp hematopoietic necrosis virus-infected fish to naïve recipients provided effective protection in ginbuna crucian carp at the latter virus challenge (Somamoto et al., 2013).

Humoral-Mediated Responses in Adaptive Immunity

B cells mediate the humoral immunity branch of the adaptive immune system by secreting antibodies. Antibodies are divided into two groups: secreted soluble form, immunoglobulins (Igs) or antibodies, or B cell receptors (BCR), membrane-bound form. Mammalian Igs are composed of two heavy chains (IgH) and two light chains (IgL) that are engaged by disulfide bonds resulting in a 'Y' shape molecule (Chiu et al., 2019). Both IgH and IgL chains are comprised of one variable region, called the Fab region (fragment, antigen-binding) that give specificity to the antibody for antigen binding, and one or more constant domains, called Fc region (fragment, crystallizable) that determine the effector functions of the antibody (Smith et al., 2019). Antibodies induce the neutralization, internalization, and elimination of pathogens, as well as antibody-dependent cellular cytotoxicity (ADCC) through Fc receptor-bearing effector cells (Forthal, 2014; Teillaud, 2001). Antibodies also activate a complement cascade that mediates internalization of complement-coated pathogens by phagocytic cells and lysis of pathogens by membrane attack complex formation (Forthal, 2014). The structure and function of Igs in teleost fish are similar to those in mammals (Mashoof & Criscitiello, 2016). Three classes of Igs have been identified in teleost fish: IgM, IgD, and IgZ/T (Table 2) (Mashoof & Criscitiello, 2016).

The BCR in mammals consists of the membrane-bound antibody associated with the Ig-α/Ig-β heterodimer, known as CD79a/b, that is crucial for signal transduction because of the presence of ITAM domain (Treanor, 2012). Like TCR, antigen specificity of BCR and Igs are formed by V(D)J gene rearrangement mediated by RAG genes (Nguyen et al., 2016). Similar to higher vertebrates, the IgH gene in teleost fish is arranged by the configuration of multiple V(D)J gene segments, followed by constant (C) segments (V-D-J-C). However, the IgL chain is arranged in a repeating set of V-J-C segments (Bao et al., 2010; N. Danilova et al., 2005; J. Hikima et al., 2011).

In mammals, bone marrow is the main hematopoiesis site that produces B cells. However, teleost fish lack bone marrow, and B cells are developed in the anterior kidney, the main site of hematopoiesis in teleosts. Mammalian mature B cells are found in secondary lymphoid tissues, such as spleen and lymph nodes. Although lymph nodes are not present in teleost fish, mature B cells are found in the spleen and posterior kidney of teleost fish (Zwollo et al., 2005). Also, B cells are found in distinct tissues, such as the lamina propria of intestine and in the epithelium of skin and gill (Salinas, 2015; Salinas et al., 2011). Upon recognition of an antigen, B cells differentiate into short-lived plasma cells (SLPC) formed in extra-follicular sites of secondary lymphoid tissues, with a life span of 3-5 days, or long-lived plasma cells (LLPC) formed in germinal centers of secondary lymphoid organs, with a life span of several months to a lifetime (Nutt et al., 2015). A similar differentiation pattern of B cells to plasma cells has been described in teleost fish (Ye et al., 2011; Zwollo et al., 2005). The plasma cells of teleost fish are divided into SLPCs formed in the spleen and LLPCs located only in the anterior kidney of fish (Kaattari et al., 2005; Ye et al., 2011).

The germinal center (GC) is a microenvironment where somatic hypermutation and class-switch recombination reaction for antibody diversification and affinity maturation occur (De Silva & Klein, 2015).

Activation-induced cytidine deaminase (AID) mediates somatic hypermutation to increase the affinity of antibodies and mediates class-switch recombination to generate antibodies with specialized effector functions (Leeman-Neill et al., 2018). Hence, long-lived plasma cells produce high-affinity class-switched antibodies, whereas short-lived cells express low-affinity IgM antibodies (Khodadadi et al., 2019). While teleost fish lack GCs in immune tissues, melano-macrophage centers are formed by numerous macrophages in lymphoid tissues, such as the spleen, and have a similar function as GCs (Agius & Roberts, 2003). Furthermore, the AID enzyme of teleost fish that mediates somatic hypermutation and catalyzes class-switch recombination in mammalian B cells *in vitro* was first identified in channel catfish (Barreto et al., 2005; Magor, 2015; Saunders & Magor, 2004). Despite the presence of AID in teleost fish, they still lack class-switch recombination due to the IgH gene structure, thus resulting in low efficient affinity maturation response compared to mammals (Wakae et al., 2006).

Similar to T cells, B cells differentiate into memory B cells in GCs of lymphoid tissues after activation by antigenic interaction (as discussed in detail in ref. (Akkaya et al., 2020). Also, teleost fish have developed immunological memory that provides a rapid and effective response to a pathogen previously encountered. For example, the formation of memory B cells in rainbow trout resulted in a faster and larger magnitude of secondary response to trinitrophenylated-keyhole limpet hemocyanin (Arkoosh & Kaattari, 1991). In addition, antibody affinity was higher in salmonids during the secondary response to the same antigen because of memory B cells (Ma et al., 2013). Moreover, vaccination with attenuated Viral Hemorrhagic Septicemia rhabdovirus (VHSV) induced the generation of memory B cells that elevated titers of serum IgM in rainbow trout in response to the same antigen (Magadan et al., 2018). Therefore, vaccination is a prophylactic method to protect teleost fish against economically devastating pathogens for several years post-immunization due to immunological memory (Findly R. C. et al., 2013; Firdaus-Nawi & Zamri-Saad, 2016).

In addition to the crucial role of B cells in adaptive immunity, two subsets of mammalian B cells, B-1 cells and marginal zone (MZ) B cells, contribute to innate immunity, including cytokine production, phagocytic capability, antigen presentation, and intracellular killing (Sunyer, 2013; Zouali & Richard, 2011). Similar to B-1 and MZ B cells of mammals, B cells in teleost fish are capable of phagocytosis and microbial killing of ingested bacteria. The phagocytic ability of B cells has been described in rainbow trout, and the formation of phagolysosome was observed in these fish (Li J et al., 2006). Moreover, zebrafish B cells showed strong phagocytic ability for particulate and soluble antigens and could present antigens to T cells like mammalian B cells (Zhu et al., 2013). Furthermore, the phagocytic capacity of B cells isolated from the anterior kidney and peripheral blood was higher than neutrophils in Atlantic cod (Øverland et al., 2010). Previously, it was reported that large amounts of B cells in catfish blood were phagocytic (Esteban et al., 2015). Recently, our research group demonstrated both active uptake of *E. ictaluri* strains by anterior kidney B cells in catfish at 30^{o} and 4^{o} C and phagosome and/or phagolysosome formation in the cytoplasm of B cells (Kordon, Kalindamar, et al., 2019; Kordon et al., 2020). Also, internalized *E. ictaluri* strains were destroyed by catfish B cells at 30^{o} C, but not at 4^{o} C (Kordon et al., 2020).

Immunoglobulin M

IgM is known as the most ancient class of antibody found in all jawed vertebrates with unique features (Flajnik, 2002). In mammals, secreted IgM is present as a pentameric form associated with the joining (J) chain (Keyt et al., 2020). Immunoglobulin M, the first identified in plasma and the most prevalent in teleost fish, is described in two different forms: secreted and transmembrane form (Mashoof & Criscitiello, 2016). The secreted form of IgM is multimerized into a tetrameric form polymerized by interchain disulfide bonds due to the absence of J chain (Table 2) (Castro & Flajnik, 2014). However, some teleost fish species, such as rainbow trout, have monomer IgM in their serum (Elcombe et al., 1985; Wilson & Warr, 1992). In rainbow trout, the binding affinity of both monomeric and tetrameric IgM is similar, but the tetrameric form of IgM is more efficient to activate the complement system (Elcombe et al., 1985). In addition, the transmembrane form of IgM in B cells is one domain shorter than the secreted form, due to alternative splicing (Sahoo et al., 2008). Moreover, IgM, the only isotype in teleost fish, has two sub-types identified in Atlantic salmon and brown trout (Hordvik et al., 2002). Similar to mammals, teleost IgM contributes to both innate and adaptive immune responses, including complement activation, induced opsonization, lysis of pathogens and mediating cellular cytotoxicity through ADCC (Boshra et al., 2004; Smith et al., 2019). Also, IgM regulates agglutination for phagocytosis that results in the clearance of pathogens (Ye et al., 2013). In addition to plasma, IgM was detected in mucosal tissues, such as the skin and intestine of fish (Peleteiro & Richards, 1988; Zhang Y et al., 2010). Immunization induces an elevated titer of IgM in fish serum, but results in a weak improvement in affinity maturation compared to mammals (Kaattari et al., 2002; Ye et al., 2013). Recently, our research team detected significantly increased IgM levels in channel catfish serum following vaccination with *E. ictaluri* strains (Kordon et al, unpublished observation).

Immunoglobulin D

Immunoglobulin D is the second class of Igs identified in fish that shares sequence similarity with IgD

in mammals (Wilson M et al., 1997). However, teleost fish contain unique features of IgD due to the many forms (from 2 to 16) of IgD constant domains (Table 2) (Edholm et al., 2011; Parra et al., 2016; F. Ramirez-Gomez et al., 2012). In addition, the only transmembrane form of IgD has been found in teleost fish, with the exception of channel catfish, Japanese puffer, and rainbow trout, which have two forms, transmembrane and secretory (J.-i. Hikima et al., 2011; Francisco Ramirez-Gomez et al., 2012). Secreted IgD (which lacks the V domain) binds directly to Fc receptor of basophils functioning as a pattern recognition receptor. Thus, it can induce inflammatory responses, including the production of pro-inflammatory cytokines, antimicrobial, opsonizing, and B cell-activating factors (Chen et al., 2009; Edholm et al., 2010). Immunoglobulin D in teleost fish is mainly found in serum and also expressed in the anterior and posterior kidneys, spleen, and gill (Bengtén & Wilson, 2015). Furthermore, the level of IgD was higher in the gill of rainbow trout compared to IgM level (Smith et al., 2019).

Immunoglobulin T/Z

IgT/Z, the only teleost fish-specific Ig, was first identified in rainbow trout (IgT) and in zebrafish (IgZ) (Table 2) (Nadia Danilova et al., 2005; Hansen et al., 2005). IgT/Z functions similar to mammalian IgA (Mashoof et al., 2014). The IgT/Z level in the gut of rainbow trout was much higher (63 times) than that in serum, whereas the concentration of IgM in the serum was much higher compared to IgT/Z (Zhang Y et al., 2010). The IgT/Z in the gut of teleost fish contributes to immune responses against intestinal parasites and bacteria (Zhang Y et al., 2010). For example, the number of IgT+ B cells elevated in the gut of rainbow trout after parasitic intestinal infection, although the number of IgM+ B cells did not change in the same tissue (Zhang Y et al., 2010). In addition to IgT+ B cells, the IgT concentration was increased in the gut of surviving rainbow trout. However, the titer of parasite-specific IgM was higher in the sera of surviving animals (Zhang Y et al., 2010). Moreover, IgT+ B cells in teleost fish were found in skin-associated lymphoid tissue and secrete IgT into skin mucus (Xu et al., 2013). IgT is present in fish serum as a monomer but has a tetramer form in the gut mucus (Zhang Y et al., 2010). Some teleost fish species, such as channel catfish, lack IgT; therefore, IgM is the main Ig class in both serum and mucosal immunity (Findly R. C et al., 2013; Maki & Dickerson, 2003; Zhao et al., 2008).

Conclusion

Recent findings in fish immunology show that significant progress has been made in the mechanistic understanding of fish immune responses. Two branches of the fish immune system, innate and adaptive immunity, provide important information for understanding the evolution of the immune system. Studies of fish with new reagents and powerful sequencing and knockout procedures will provide a valuable framework for assessment of the highly complex mammalian adaptive immune responses. In addition, the costs of fish infections to aquaculture can be overwhelming, making the failure of immunity a major risk for commercial fish farming. The presence of an adaptive immune system and, consequently, immune memory in teleost fish allows vaccination, the most appropriate method for disease control in aquaculture. In addition to vaccination, different approaches have been developed to control infectious diseases in aquaculture, such as high-quality diets including probiotics, prebiotics, and medicinal plants and treatment with antibiotics (Chinabut & Puttinaowarat, 2005). Furthermore, farm-level biosecurity precautions, including egg disinfection, water treatments, clean food, traffic control, intense quarantine measures, removal and disposal of mortalities, are preventive approaches to keep the facility safe before any disease outbreaks (Assefa & Abunna, 2018).

References

Agius, C., & Roberts, R. J. (2003). Melano-macrophage centres and their role in fish pathology. *Journal of Fish Diseases*, *26*(9), 499-509. https://doi.org/10.1046/j.1365-2761.2003.00485.x

Ahuja, S. S., Estrada, C. A., & Lindsey, M. L. (2007). Crosstalk Between Cytotoxic T-Lymphocyte-Associated Antigen-4 and Interleukin-12 in Cytotoxic T-Lymphocyte-; Mediated Myocarditis. *Circulation Research*, *101*(3), 218-220. https://doi.org/doi:10.1161/CIRCRESAHA.107.158238

Akkaya, M., Kwak, K., & Pierce, S. K. (2020). B cell memory: building two walls of protection against pathogens. *Nature Reviews Immunology*, *20*(4), 229-238. https://doi.org/10.1038/s41577-019-0244-2

Andersen, M. H., Schrama, D., thor Straten, P., & Becker, J. C. (2006). Cytotoxic T Cells. *Journal of Investigative Dermatology*, *126*(1), 32-41. https://doi.org/http://dx.doi.org/10.1038/sj.jid.5700001

Annunziato, F., & Romagnani, S. (2009). Heterogeneity of human effector CD4(+) T cells. *Arthritis Research & Therapy*, *11*(6), 257-257. https://doi.org/10.1186/ar2843

Arkoosh, M. R., & Kaattari, S. L. (1991). Development of immunological memory in rainbow trout (Oncorhynchus mykiss). I. An immunochemical and cellular analysis of the B cell response. *Developmental and comparative immunology*, *15*(4), 279-293. https://doi.org/10.1016/0145-305x(91)90021-p

Ashfaq, H., Soliman, H., Saleh, M., & El-Matbouli, M. (2019). CD4: a vital player in the teleost fish immune system. *Veterinary Research*, *50*(1), 1. https://doi.org/10.1186/s13567-018-0620-0

Bajoghli, B., Dick, A. M., Claasen, A., Doll, L., & Aghaallaei, N. (2019). Zebrafish and Medaka: Two Teleost Models of T-Cell and Thymic Development. *Int J Mol Sci*, *20*(17), 4179. https://doi.org/10.3390/ijms20174179

Banchereau, J., & Steinman, R. M. (1998). Dendritic cells and the control of immunity. *Nature*, *392*(6673), 245-252. https://doi.org/10.1038/32588

Bao, Y., Wang, T., Guo, Y., Zhao, Z., Li, N., & Zhao, Y. (2010). The immunoglobulin gene loci in the teleost Gasterosteus aculeatus. *Fish Shellfish Immunol*, *28*(1), 40-48. https://doi.org/10.1016/j.fsi.2009.09.014

Barreto, V. M., Pan-Hammarstrom, Q., Zhao, Y., Hammarstrom, L., Misulovin, Z., & Nussenzweig, M. C. (2005). AID from bony fish catalyzes class switch recombination. *The Journal of Experimental Medicine*, *202*(6), 733-738. https://doi.org/10.1084/jem.20051378

Bengtén, E., & Wilson, M. (2015). Antibody Repertoires in Fish. *Results Problems in Cell Differentiation*, *57*, 193-234. https://doi.org/10.1007/978-3-319-20819-0_9

Bernard, D., Riteau, B., Hansen, J. D., Phillips, R. B., Michel, F., Boudinot, P., & Benmansour, A. (2006). Costimulatory Receptors in a Teleost Fish: Typical CD28, Elusive CTLA4. *The Journal of Immunology*, *176*(7), 4191-4200. https://doi.org/10.4049/jimmunol.176.7.4191

Bilal, S., Lie, K. K., Sæle, Ø., & Hordvik, I. (2018). T Cell Receptor Alpha Chain Genes in the Teleost Ballan Wrasse (Labrus bergylta) Are Subjected to Somatic Hypermutation [Original Research]. *Frontiers in Immunology*, *9*(1101). https://doi.org/10.3389/fimmu.2018.01101

Biller-Takahashi J. D., & C., U. E. (2014). Fish Immunology. The modification and manipulation of the innate immune system: Brazilian studies. *Anais da Academia Brasileira de Ciências*, *86*, 1484-1506. http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0001-37652014000301484&nrm=iso

Birnbaum, M. E., Berry, R., Hsiao, Y.-S., Chen, Z., Shingu-Vazquez, M. A., Yu, X., Waghray, D., Fischer, S., McCluskey, J., Rossjohn, J., Walz, T., & Garcia, K. C. (2014). Molecular architecture of the αβ T cell receptor–CD3 complex. *Proceedings of the National Academy of Sciences*, *111*(49), 17576-17581. https://doi.org/10.1073/pnas.1420936111

Boehm, T., Iwanami, N., & Hess, I. (2012). Evolution of the immune system in the lower vertebrates. *Annu Rev Genomics Hum Genet*, *13*, 127-149. https://doi.org/10.1146/annurev-genom-090711-163747

Boehm, T., & Swann, J. B. (2014). Origin and evolution of adaptive immunity. *Annu Review of Animal Biosciences*, *2*, 259-283. https://doi.org/10.1146/annurev-animal-022513-114201

Boshra, H., Gelman, A. E., & Sunyer, J. O. (2004). Structural and Functional Characterization of Complement C4 and C1s-Like Molecules in Teleost Fish: Insights into the Evolution of Classical and Alternative Pathways. *The Journal of Immunology*, *173*(1), 349-359. https://doi.org/10.4049/jimmunol.173.1.349

Brazeau, M. D., & Friedman, M. (2015). The origin and early phylogenetic history of jawed vertebrates. *Nature*, *520*(7548), 490-497. https://doi.org/10.1038/nature14438

Carding, S. R., & Egan, P. J. (2002). γδ T cells: functional plasticity and heterogeneity. *Nature Reviews Immunology*, *2*(5), 336-345. https://doi.org/10.1038/nri797

Carmona, L. M., Fugmann, S. D., & Schatz, D. G. (2016). Collaboration of RAG2 with RAG1-like proteins during the evolution of V(D)J recombination. *Genes & development*, *30*(8), 909-917. https://doi.org/10.1101/gad.278432.116

Carmona, L. M., & Schatz, D. G. (2017). New insights into the evolutionary origins of the recombination-activating gene proteins and V(D)J recombination. *The FEBS Journal*, *284*(11), 1590-1605. https://doi.org/10.1111/febs.13990

Carsetti, R. (2000). The development of B cells in the bone marrow is controlled by the balance between cell-autonomous mechanisms and signals from the microenvironment. *The Journal of Experimental Medicine*, *191*(1), 5-8. https://doi.org/10.1084/jem.191.1.5

Castro, C. D., & Flajnik, M. F. (2014). Putting J chain back on the map: how might its expression define plasma cell development? *Journal of Immunology 193*(7), 3248-3255. https://doi.org/10.4049/jimmunol.1400531

Castro, R., Bernard, D., Lefranc, M. P., Six, A., Benmansour, A., & Boudinot, P. (2011). T cell diversity and TcR repertoires in teleost fish. *Fish & Shellfish Immunology*, *31*(5), 644-654.https://doi.org/http://dx.doi.org/10.1016/j.fsi.2010.08.016

Chaplin, D. D. (2010). Overview of the immune response. *The Journal of allergy and clinical immunology*, *125*(2 Suppl 2), S3-S23. https://doi.org/10.1016/j.jaci.2009.12.980

Chen, K., Xu, W., Wilson, M., He, B., Miller, N. W., Bengtén, E., Edholm, E.-S., Santini, P. A., Rath, P., Chiu, A., Cattalini, M., Litzman, J., B Bussel, J., Huang, B., Meini, A., Riesbeck, K., Cunningham-Rundles, C., Plebani, A., & Cerutti, A. (2009). Immunoglobulin D enhances immune surveillance by activating antimicrobial, proinflammatory and B cell–stimulating programs in basophils. *Nature immunology*, *10*(8), 889-898. https://doi.org/10.1038/ni.1748

Chen, T., Guo, J., Cai, Z., Li, B., Sun, L., Shen, Y., Wang, S., Wang, Z., Wang, Z., Wang, Y., Zhou, H., Cai, Z., & Ye, Z. (2020). Th9 Cell Differentiation and Its Dual Effects in Tumor Development [Review]. *Frontiers in Immunology*, *11*(1026). https://doi.org/10.3389/fimmu.2020.01026

Chiu, M. L., Goulet, D. R., Teplyakov, A., & Gilliland, G. L. (2019). Antibody Structure and Function: The Basis for Engineering Therapeutics. *Antibodies (Basel, Switzerland)*, *8*(4), 55. https://doi.org/10.3390/antib8040055

Cole, D. K., & Gao, G. F. (2004). CD8: adhesion molecule, co-receptor and immuno-modulator. *Cellular & Molecular Immunology*, *1*(2), 81-88.

Cuesta, A., Esteban, M. A., & Meseguer, J. (2003). Identification of a FasL-like molecule in leucocytes of the teleost fish gilthead seabream (Sparus aurata L.). *Developmental and comparative immunology*, *27*(1), 21-27. https://doi.org/10.1016/s0145-305x(02)00041-1

Danilova, N., Bussmann, J., Jekosch, K., & Steiner, L. A. (2005). The immunoglobulin heavy-chain locus in zebrafish: identification and expression of a previously unknown isotype, immunoglobulin Z [10.1038/ni1166]. *Natute Immunology*, *6*(3), 295-302. https://doi.org/http://www.nature.com/ni/journal/v6/n3/suppinfo/ni1166_S1.html

Danilova, N., Bussmann, J., Jekosch, K., & Steiner, L. A. (2005). The immunoglobulin heavy-chain locus in zebrafish: identification and expression of a previously unknown isotype, immunoglobulin Z. *Nat Immunol*, *6*(3), 295-302. https://doi.org/10.1038/ni1166

Dardalhon, V., Awasthi, A., Kwon, H., Galileos, G., Gao, W., Sobel, R. A., Mitsdoerffer, M., Strom, T. B., Elyaman, W., Ho, I. C., Khoury, S., Oukka, M., & Kuchroo, V. K. (2008).

Interleukin 4 inhibits TGF-β-induced-Foxp3(+) T cells and generates, in combination with TGF-β, Foxp3(−) effector T cells that produce interleukins 9 and 10. *Nature immunology*, *9*(12), 1347-1355. https://doi.org/10.1038/ni.1677

De Silva, N. S., & Klein, U. (2015). Dynamics of B cells in germinal centres. *Nature Reviews Immunology*, *15*(3), 137-148. https://doi.org/10.1038/nri3804

Díaz-Rosales, P., Bird, S., Wang, T. H., Fujiki, K., Davidson, W. S., Zou, J., & Secombes, C. J. (2009). Rainbow trout interleukin-2: cloning, expression and bioactivity analysis. *Fish Shellfish Immunol*, *27*(3), 414-422. https://doi.org/10.1016/j.fsi.2009.06.008

Dickerson, H. W., & Findly, R. C. (2017). Vertebrate Adaptive Immunity—Comparative Insights from a Teleost Model [Mini Review]. *Frontiers in Immunology*, *8*(1379). https://doi.org/10.3389/fimmu.2017.01379

Edholm, E. S., Bengtén, E., Stafford, J. L., Sahoo, M., Taylor, E. B., Miller, N. W., & Wilson, M. (2010). Identification of two IgD+ B cell populations in channel catfish, Ictalurus punctatus. *J Immunol*, *185*(7), 4082-4094. https://doi.org/10.4049/jimmunol.1000631

Edholm, E. S., Bengten, E., & Wilson, M. (2011). Insights into the function of IgD. *Developmental and comparative immunology*, *35*(12), 1309-1316. https://doi.org/10.1016/j.dci.2011.03.002

Elcombe, B. M., Chang, R. J., Taves, C. J., & Winkelhake, J. L. (1985). Evolution of antibody structure and effector functions: comparative hemolytic activities of monomeric and tetrameric IgM from rainbow trout, Salmo gairdnerii. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *80*(4), 697-706. https://doi.org/http://dx.doi.org/10.1016/0305-0491(85)90448-1

Esteban, M., Cuesta, A., Chaves-Pozo, E., & Meseguer, J. (2015). Phagocytosis in Teleosts. Implications of the New Cells Involved. *Biology*, *4*(4), 907. http://www.mdpi.com/2079-7737/4/4/907

Findly R. C, Zhao X, Noe, J., Camus, A. C., & Dickerson, H. W. (2013). B cell memory following infection and challenge of channel catfish with Ichthyophthirius multifiliis. *Developmental & Comparative Immunology*, *39*(3), 302-311.https://doi.org/http://dx.doi.org/10.1016/j.dci.2012.08.007

Findly R. C., Zhao X, Noe, J., Camus, A. C., & Dickerson, H. W. (2013). B cell memory following infection and challenge of channel catfish with Ichthyophthirius multifiliis. *Developmental & Comparative Immunology*, *39*(3), 302-311.https://doi.org/http://dx.doi.org/10.1016/j.dci.2012.08.007

Firdaus-Nawi, M., & Zamri-Saad, M. (2016). Major Components of Fish Immunity: A Review [Article]. *Pertanika Journal of Tropical Agricultural Science*, *39*(4), 393-420. https://login.proxy.library.msstate.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=121809219&site=eds-live

Fischer, C., Bouneau, L., Ozouf-Costaz, C., Crnogorac-Jurcevic, T., Weissenbach, J., & Bernot, A. (2002). Conservation of the T-Cell Receptor α/δ Linkage in the Teleost Fish Tetraodon nigroviridis. *Genomics*, *79*(2), 241-248. https://doi.org/https://doi.org/10.1006/geno.2002.6688

Fischer, U., Koppang, E. O., & Nakanishi, T. (2013). Teleost T and NK cell immunity. *Fish & Shellfish Immunology*, *35*(2), 197-206. https://doi.org/http://dx.doi.org/10.1016/j.fsi.2013.04.018

Flajnik, M. F. (2002). Comparative analyses of immunoglobulin genes: surprises and portents. *Nature Reviews Immunology*, *2*(9), 688-698.

Flajnik, M. F., & Kasahara, M. (2010). Origin and evolution of the adaptive immune system: genetic events and selective pressures. *Nature Reviews Genetics*, *11*(1), 47-59. https://doi.org/10.1038/nrg2703

Forlenza, M., de Carvalho Dias, J. D., Veselý, T., Pokorová, D., Savelkoul, H. F., & Wiegertjes, G. F. (2008). Transcription of signal-3 cytokines, IL-12 and IFN alpha beta, coincides with the timing of CD8 alpha beta up-regulation during viral infection of common carp (Cyprinus carpio L). *Mol Immunol*, *45*(6), 1531-1547. https://doi.org/10.1016/j.molimm.2007.10.010

Forthal, D. N. (2014). Functions of Antibodies. *Microbiology spectrum*, *2*(4), 1-17. https://pubmed.ncbi.nlm.nih.gov/25215264 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159104/

Fugmann, S. D. (2001). RAG1 and RAG2 in V(D)J recombination and transposition. *Immunologic Research*, *23*(1), 23-39. https://doi.org/10.1385/IR:23:1:23

Greenhalgh, P., & Steiner, L. A. (1995). Recombination activating gene 1 (Rag1) in zebrafish and shark [journal article]. *Immunogenetics*, *41*(1), 54-55. https://doi.org/10.1007/bf00188438

Gunimaladevi, I., Savan, R., & Sakai, M. (2006). Identification, cloning and characterization of interleukin-17 and its family from zebrafish. *Fish & Shellfish Immunology*, *21*(4), 393-403. https://doi.org/http://dx.doi.org/10.1016/j.fsi.2006.01.004

Haire, R. N., Rast, J. P., Litman, R. T., & Litman, G. W. (2000). Characterization of three isotypes of immunoglobulin light chains and T-cell antigen receptor alpha in zebrafish. *Immunogenetics*, *51*(11), 915-923. https://doi.org/10.1007/s002510000229

Hansen, J. D., Du Pasquier, L., Lefranc, M. P., Lopez, V., Benmansour, A., & Boudinot, P. (2009). The B7 family of immunoregulatory receptors: a comparative and evolutionary perspective. *Mol Immunol*, *46*(3), 457-472. https://doi.org/10.1016/j.molimm.2008.10.007

Hansen, J. D., Landis, E. D., & Phillips, R. B. (2005). Discovery of a unique Ig heavy-chain isotype (IgT) in rainbow trout: Implications for a distinctive B cell developmental pathway in teleost fish. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(19), 6919-6924. https://doi.org/10.1073/pnas.0500027102

Hikima, J.-i., Jung, T.-S., & Aoki, T. (2011). Immunoglobulin genes and their transcriptional control in teleosts. *Developmental & Comparative Immunology*, *35*(9), 924-936.https://doi.org/https://doi.org/10.1016/j.dci.2010.10.011

Hikima, J., Jung, T. S., & Aoki, T. (2011). Immunoglobulin genes and their transcriptional control in teleosts. *Developmental and comparative immunology*, *35*(9), 924-936. https://doi.org/10.1016/j.dci.2010.10.011

Hirano, M., Das, S., Guo, P., & Cooper, M. D. (2011). The evolution of adaptive immunity in vertebrates. *Advances in Immunology*, *109*, 125-157. https://doi.org/10.1016/b978-0-12-387664-5.00004-2

Hordvik, I., Berven, F. S., Solem, S. T., Hatten, F., & Endresen, C. (2002). Analysis of two IgM isotypes in Atlantic salmon and brown trout. *Mol Immunol*, *39*(5-6), 313-321. https://doi.org/10.1016/s0161-5890(02)00114-1

Hordvik, I., Torvund, J., Moore, L., & Endresen, C. (2004). Structure and organization of the T cell receptor alpha chain genes in Atlantic salmon. *Mol Immunol*, *41*(5), 553-559. https://doi.org/10.1016/j.molimm.2004.03.006

Huang, R., Zhong, S., Liu, H., Kong, R., Wang, Y., Hu, W., & Guo, Q. (2010). Identification and characterization of common carp (Cyprinus carpio L.) granzyme A/K, a cytotoxic cell granule-associated serine protease. *Fish & Shellfish Immunology*, *29*(3), 388-398. https://doi.org/10.1016/j.fsi.2010.04.002

Hwang, J. Y., Ohira, T., Hirono, I., & Aoki, T. (2004). A pore-forming protein, perforin, from a non-mammalian organism, Japanese flounder, Paralichthys olivaceus. *Immunogenetics*, *56*(5), 360-367. https://doi.org/10.1007/s00251-004-0688-8

Intlekofer, A. M., Takemoto, N., Wherry, E. J., Longworth, S. A., Northrup, J. T., Palanivel, V. R., Mullen, A. C., Gasink, C. R., Kaech, S. M., Miller, J. D., Gapin, L., Ryan, K., Russ, A. P., Lindsten, T., Orange, J. S., Goldrath, A. W., Ahmed, R., & Reiner, S. L. (2005). Effector and memory CD8+ T cell fate coupled by T-bet and eomesodermin. *Nature immunology*, *6*(12), 1236-1244. https://doi.org/10.1038/ni1268

Jaime-Sanchez, P., Uranga-Murillo, I., Aguilo, N., Khouili, S. C., Arias, M. A., Sancho, D., & Pardo, J. (2020). Cell death induced by cytotoxic CD8(+) T cells is immunogenic and primes caspase-3-dependent spread immunity against endogenous tumor antigens. *Journal for ImmunoTherapy of Cancer*, *8*(1). https://doi.org/10.1136/jitc-2020-000528

Janeway, C. A., Jones, B., & Hayday, A. (1988). Specificity and function of T cells bearing γδ receptors. *Immunology Today*, *9*(3), 73-76. https://doi.org/https://doi.org/10.1016/0167-5699(88)91267-4

Kaattari, S., Bromage, E., & Kaattari, I. (2005). Analysis of long-lived plasma cell production and regulation: Implications for vaccine design for aquaculture. *Aquaculture*, *246*(1), 1-9. https://doi.org/http://dx.doi.org/10.1016/j.aquaculture.2004.12.024

Kaattari, S. L., Zhang, H. L., Khor, I. W., Kaattari, I. M., & Shapiro, D. A. (2002). Affinity maturation in trout: clonal dominance of high affinity antibodies late in the immune response. *Developmental & Comparative Immunology*, *26*(2), 191-200. https://doi.org/https://doi.org/10.1016/S0145-305X(01)00064-7

Keyt, B. A., Baliga, R., Sinclair, A. M., Carroll, S. F., & Peterson, M. S. (2020). Structure, Function, and Therapeutic Use of IgM Antibodies. *Antibodies (Basel, Switzerland)*, *9*(4). https://doi.org/10.3390/antib9040053

Khodadadi, L., Cheng, Q., Radbruch, A., & Hiepe, F. (2019). The Maintenance of Memory Plasma Cells [Review]. *Frontiers in Immunology*, *10*(721). https://doi.org/10.3389/fimmu.2019.00721

Konigshofer, Y., & Chien, Y.-h. (2006). γδ T cells — innate immune lymphocytes? *Current Opinion in Immunology*, *18*(5), 527-533. https://doi.org/https://doi.org/10.1016/j.coi.2006.07.008

Kono, T., Bird, S., Sonoda, K., Savan, R., Secombes, C. J., & Sakai, M. (2008). Characterization and expression analysis of an interleukin-7 homologue in the Japanese pufferfish, Takifugu rubripes. *The FEBS Journal*, *275*(6), 1213-1226. https://doi.org/10.1111/j.1742-4658.2008.06281.x

Koppang, E. O., Fischer, U., Moore, L., Tranulis, M. A., Dijkstra, J. M., Köllner, B., Aune, L., Jirillo, E., & Hordvik, I. (2010). Salmonid T cells assemble in the thymus, spleen and in novel interbranchial lymphoid tissue. *Journal of Anatomy*, *217*(6), 728-739. https://doi.org/10.1111/j.1469-7580.2010.01305.x

Kordon, A. O., Abdelhamed, H., Ahmed, H., Baumgartner, W., Karsi, A., & Pinchuk, L. M. (2019). Assessment of the Live Attenuated and Wild-Type Edwardsiella ictaluri-Induced Immune Gene Expression and Langerhans-Like Cell Profiles in the Immune-Related Organs of Catfish [Original Research]. *Frontiers in Immunology*, *10*(392). https://doi.org/10.3389/fimmu.2019.00392

Kordon, A. O., Abdelhamed, H., Karsi, A., & Pinchuk, L. M. (2021). Adaptive immune responses in channel catfish exposed to Edwardsiella ictaluri live attenuated vaccine and wild type strains through the specific gene expression profiles. *Developmental and comparative immunology*, *116*, 103950. https://doi.org/10.1016/j.dci.2020.103950

Kordon, A. O., Kalindamar, S., Majors, K., Abdelhamed, H., Tan, W., Karsi, A., & Pinchuk, L. M. (2019). Effects of Live Attenuated Vaccine and Wild Type Strains of Edwardsiella ictaluri on Phagocytosis, Bacterial Killing, and Survival of Catfish B Cells [Original Research]. *Frontiers in Immunology*, *10*(2383). https://doi.org/10.3389/fimmu.2019.02383

Kordon, A. O., Kalindamar, S., Majors, K., Abdelhamed, H., Tan, W., Karsi, A., & Pinchuk, L. M. (2020). Live attenuated *Edwardsiella ictaluri* vaccines enhance the protective innate immune responses of channel catfish B cells. *Developmental & Comparative Immunology*, *109*, 103711.https://doi.org/https://doi.org/10.1016/j.dci.2020.103711

Kordon, A. O., Karsi, A., & Lesya, P. (2018). Innate Immune Responses in Fish: Antigen Presenting Cells and Professional Phagocytes. *Turkish Journal of Fisheries and Aquatic Sciences*, *18*, 1123-1139. https://doi.org/10.4194/1303-2712-v18_9_11

Kumari, J., Bogwald, J., & Dalmo, R. A. (2009). Transcription factor GATA-3 in Atlantic salmon (Salmo salar): Molecular characterization, promoter activity and expression analysis. *Mol Immunol*, *46*(15), 3099-3107. https://doi.org/https://doi.org/10.1016/j.molimm.2009.06.008

Langenau, D. M., & Zon, L. I. (2005). The zebrafish: a new model of T-cell and thymic development. *Nat Rev Immunol*, *5*(4), 307-317. https://doi.org/10.1038/nri1590

Leal, E., Granja, A. G., Zarza, C., & Tafalla, C. (2016). Distribution of T Cells in Rainbow Trout (Oncorhynchus mykiss) Skin and Responsiveness to Viral Infection. *PLoS ONE*, *11*(1), e0147477. https://doi.org/10.1371/journal.pone.0147477

Lee, J.-W., Yang, H., Noh, J. K., Kim, H. C., Park, C.-J., Park, J.-W., Hwang, I. J., Kim, S. Y., & Lee, J.-H. (2014). RAG-1 and IgM Genes, Markers for Early Development of the Immune System in Olive Flounder, Paralichthys olivaceus. *Development & reproduction*, *18*(2), 99-106. https://doi.org/10.12717/DR.2014.18.2.099

Leeman-Neill, R. J., Lim, J., & Basu, U. (2018). The Common Key to Class-Switch Recombination and Somatic Hypermutation: Discovery of AID and Its Role in Antibody Gene Diversification. *The Journal of Immunology*, *201*(9), 2527-2529. https://doi.org/10.4049/jimmunol.1801246

Li J, DR., B., Yong-An, Z., Hani, B., E., G. A., Scott, L., Lluis, T., & Oriol, S. J. (2006). B lymphocytes from early vertebrates have potent phagocytic and microbicidal abilities [10.1038/ni1389]. *Nature immunology*, *7*(10), 1116-1124.https://doi.org/http://www.nature.com/ni/journal/v7/n10/suppinfo/ni1389_S1.html

Li, J. H., Shao, J. Z., Xiang, L. X., & Wen, Y. (2007). Cloning, characterization and expression analysis of pufferfish interleukin-4 cDNA: the first evidence of Th2-type cytokine in fish. *Mol Immunol*, *44*(8), 2078-2086. https://doi.org/10.1016/j.molimm.2006.09.010

Litman, G. W., Rast, J. P., & Fugmann, S. D. (2010). The origins of vertebrate adaptive immunity. *Nature reviews. Immunology*, *10*(8), 543-553. https://doi.org/10.1038/nri2807

Long, S., Wilson, M., Bengtén, E., Clem, L. W., Miller, N. W., & Chinchar, V. G. (2004). Identification and characterization of a FasL-like protein and cDNAs encoding the channel catfish death-inducing signaling complex. *Immunogenetics*, *56*(7), 518-530. https://doi.org/10.1007/s00251-004-0701-2

Love, P. E., & Hayes, S. M. (2010). ITAM-mediated signaling by the T-cell antigen receptor. *Cold Spring Harbor perspectives in biology*, *2*(6), a002485-a002485. https://doi.org/10.1101/cshperspect.a002485

Luckheeram, R. V., Zhou, R., Verma, A. D., & Xia, B. (2012). $CD4^+$T cells: differentiation and functions. *Clinical & developmental immunology*, *2012*, 925135-925135. https://doi.org/10.1155/2012/925135

Ma, C., Ye, J., & Kaattari, S. L. (2013). Differential compartmentalization of memory B cells versus plasma cells in salmonid fish. *European Journal of Immunology*, *43*(2), 360-370. https://doi.org/https://doi.org/10.1002/eji.201242570

Ma, T. Y., Wu, J. Y., Gao, X. K., Wang, J. Y., Zhan, X. L., & Li, W. S. (2014). Molecular cloning, functional identification and expressional analyses of FasL in Tilapia, Oreochromis niloticus. *Developmental and comparative immunology*, *46*(2), 448-460. https://doi.org/10.1016/j.dci.2014.06.003

Maddon, P. J., Molineaux, S. M., Maddon, D. E., Zimmerman, K. A., Godfrey, M., Alt, F. W., Chess, L., & Axel, R. (1987). Structure and expression of the human and mouse T4 genes. *Proceedings of the National Academy of Sciences of the United States of America*, *84*(24), 9155-9159. https://doi.org/10.1073/pnas.84.24.9155

Magadan, S., Jouneau, L., Puelma Touzel, M., Marillet, S., Chara, W., Six, A., Quillet, E., Mora, T., Walczak, A. M., Cazals, F., Sunyer, O., Fillatreau, S., & Boudinot, P. (2018). Origin of Public Memory B Cell Clones in Fish After Antiviral Vaccination [Original Research]. *Frontiers in Immunology*, *9*(2115). https://doi.org/10.3389/fimmu.2018.02115

Magor, B. G. (2015). Antibody Affinity Maturation in Fishes-Our Current Understanding. *Biology*, *4*(3), 512-524. https://doi.org/10.3390/biology4030512

Maki, J. L., & Dickerson, H. W. (2003). Systemic and Cutaneous Mucus Antibody Responses of Channel Catfish Immunized against the Protozoan Parasite Ichthyophthirius multifiliis. *Clinical and Diagnostic Laboratory Immunology*, *10*(5), 876-881. https://doi.org/10.1128/cdli.10.5.876-881.2003

Mashoof, S., & Criscitiello, M. F. (2016). Fish Immunoglobulins. *Biology*, *5*(4), 45. https://doi.org/10.3390/biology5040045

Mashoof, S., Pohlenz, C., Chen, P. L., Deiss, T. C., Gatlin, D., Buentello, A., & Criscitiello, M. F. (2014). Expressed IgH μ and τ transcripts share diversity segment in ranched Thunnus orientalis. *Developmental & Comparative Immunology*, *43*(1), 76-86. https://doi.org/http://dx.doi.org/10.1016/j.dci.2013.10.015

Matsuura, Y., Yabu, T., Shiba, H., Moritomo, T., & Nakanishi, T. (2014). Identification of a novel fish granzyme involved in cell-mediated immunity. *Developmental & Comparative Immunology*, *46*(2), 499-507. https://doi.org/https://doi.org/10.1016/j.dci.2014.06.006

Matsuura, Y., Yabu, T., Shiba, H., Moritomo, T., & Nakanishi, T. (2016). Purification and characterization of a fish granzymeA involved in cell-mediated immunity. *Developmental & Comparative Immunology*, *60*, 33-40. https://doi.org/https://doi.org/10.1016/j.dci.2016.02.011

Mitra, S., Alnabulsi, A., Secombes, C. J., & Bird, S. (2010). Identification and characterization of the transcription factors involved in T-cell development, t-bet, stat6 and foxp3, within the zebrafish, Danio rerio. *Febs j*, *277*(1), 128-147. https://doi.org/10.1111/j.1742-4658.2009.07460.x

Mitra, S., Alnabulsi, A., Secombes, C. J., & Bird, S. (2010). Identification and characterization of the transcription factors involved in T-cell development, t-bet, stat6 and foxp3, within the zebrafish, Danio rerio. *The FEBS Journal*, *277*(1), 128-147. https://doi.org/doi:10.1111/j.1742-4658.2009.07460.x

Monte, M. M., Wang, T., Holland, J. W., Zou, J., & Secombes, C. J. (2013). Cloning and characterization of rainbow trout interleukin-17A/F2 (IL-17A/F2) and IL-17 receptor A: expression during infection and bioactivity of recombinant IL-17A/F2. *Infection and Immunity*, *81*(1), 340-353. https://doi.org/10.1128/iai.00599-12

Muñoz-Wolf, N., & Lavelle, E. C. (2018). A Guide to IL-1 family cytokines in adjuvanticity. *The FEBS Journal*, *285*(13), 2377-2401. https://doi.org/https://doi.org/10.1111/febs.14467

Nace, G., Evankovich, J., Eid, R., & Tsung, A. (2012). Dendritic cells and damage-associated molecular patterns: endogenous danger signals linking innate and adaptive immunity. *Journal of Innate Immunity*, *4*(1), 6-15. https://doi.org/10.1159/000334245

Nakanishi, T., Shibasaki, Y., & Matsuura, Y. (2015). T Cells in Fish. *Biology*, *4*(4), 640-663. https://doi.org/10.3390/biology4040640

Nam, B.-H., Hirono, I., & Aoki, T. (2003). The Four TCR Genes of Teleost Fish: The cDNA and Genomic DNA Analysis of Japanese Flounder (Paralichthys olivaceus)

TCR α-, β-, γ-, and δ-Chains. *The Journal of Immunology*, *170*(6), 3081-3090. https://doi.org/10.4049/jimmunol.170.6.3081

Nascimento, D. S., do Vale, A., Tomás, A. M., Zou, J., Secombes, C. J., & dos Santos, N. M. (2007). Cloning, promoter analysis and expression in response to bacterial exposure of sea bass (Dicentrarchus labrax L.) interleukin-12 p40 and p35 subunits. *Mol Immunol*, *44*(9), 2277-2291. https://doi.org/10.1016/j.molimm.2006.11.006

Nayak, S. K., & Nakanishi, T. (2013). Direct antibacterial activity of CD8+/CD4+ T-cells in ginbuna crucian carp, Carassius auratus langsdorfii. *Fish & Shellfish Immunology*, *34*(1), 136-141. https://doi.org/https://doi.org/10.1016/j.fsi.2012.10.016

Neave, B., Rodaway, A., Wilson, S. W., Patient, R., & Holder, N. (1995). Expression of zebrafish GATA 3 (gta3) during gastrulation and neurulation suggests a role in the specification of cell fate. *Mech Dev*, *51*(2-3), 169-182. https://doi.org/10.1016/0925-4773(95)00351-7

Nemazee, D. (2000). Receptor selection in B and T lymphocytes. *Annual Review of Immunology*, *18*, 19-51. https://doi.org/10.1146/annurev.immunol.18.1.19

Nguyen, T. V., Pawlikowska, P., Firlej, V., Rosselli, F., & Aoufouchi, S. (2016). V(D)J recombination process and the Pre-B to immature B-cells transition are altered in Fanca−/− mice. *Scientific Reports*, *6*(1), 36906. https://doi.org/10.1038/srep36906

Nurieva, R. I., & Chung, Y. (2010). Understanding the development and function of T follicular helper cells. *Cellular & Molecular Immunology*, *7*(3), 190-197. https://doi.org/10.1038/cmi.2010.24

Nutt, S. L., Hodgkin, P. D., Tarlinton, D. M., & Corcoran, L. M. (2015). The generation of antibody-secreting plasma cells. *Nature Reviews Immunology*, *15*(3), 160-171. https://doi.org/10.1038/nri3795

Ouyang, W., Rutz, S., Crellin, N. K., Valdez, P. A., & Hymowitz, S. G. (2011). Regulation and functions of the IL-10 family of cytokines in inflammation and disease. *Annual Review of Immunology*, *29*, 71-109. https://doi.org/10.1146/annurev-immunol-031210-101312

Øvergård, A.-C., Hordvik, I., Nerland, A. H., Eikeland, G., & Patel, S. (2009). Cloning and expression analysis of Atlantic halibut (Hippoglossus hippoglossus) CD3 genes. *Fish & Shellfish Immunology*, *27*(6), 707-713. https://doi.org/https://doi.org/10.1016/j.fsi.2009.08.011

Øverland, H. S., Pettersen, E. F., Rønneseth, A., & Wergeland, H. I. (2010). Phagocytosis by B-cells and neutrophils in Atlantic salmon (Salmo salar L.) and Atlantic cod (Gadus morhua L.). *Fish & Shellfish Immunology*, *28*(1), 193-204. https://doi.org/https://doi.org/10.1016/j.fsi.2009.10.021

Parra, D., Korytář, T., Takizawa, F., & Sunyer, J. O. (2016). B cells and their role in the teleost gut. *Developmental and comparative immunology*, *64*, 150-166. https://doi.org/10.1016/j.dci.2016.03.013

Peleteiro, M. C., & Richards, R. H. (1988). Immunocytochemical studies on immunoglobulin-containing cells in the epidermis of rainbow trout Salmo gairdneri Richardson: influence of bath vaccination*. *Journal of Fish Biology*, *32*(6), 845-858. https://doi.org/10.1111/j.1095-8649.1988.tb05428.x

Pennock, N. D., White, J. T., Cross, E. W., Cheney, E. E., Tamburini, B. A., & Kedl, R. M. (2013). T cell responses: naive to memory and everything in between. *Advances in Physiology Education*, *37*(4), 273-283. https://doi.org/10.1152/advan.00066.2013

Piazzon, M. C., Wentzel, A. S., Tijhaar, E. J., Rakus, K. Ł., Vanderplasschen, A., Wiegertjes, G. F., & Forlenza, M. (2015). Cyprinid Herpesvirus 3 Il10 Inhibits Inflammatory Activities of Carp Macrophages and Promotes Proliferation of Igm⁺ B Cells and Memory T Cells in a Manner Similar to Carp Il10. *The Journal of Immunology*, *195*(8), 3694-3704. https://doi.org/10.4049/jimmunol.1500926

Piazzon, M. C., Wentzel, A. S., Wiegertjes, G. F., & Forlenza, M. (2017). Carp Il10a and Il10b exert identical biological activities in vitro, but are differentially regulated in vivo. *Developmental & Comparative Immunology*, *67*, 350-360.https://doi.org/https://doi.org/10.1016/j.dci.2016.08.016

Praveen, K., Evans, D. L., & Jaso-Friedmann, L. (2004). Evidence for the existence of granzyme-like serine proteases in teleost cytotoxic cells. *J Mol Evol*, *58*(4), 449-459. https://doi.org/10.1007/s00239-003-2566-7

Ramirez-Gomez, F., Greene, W., Rego, K., Hansen, J. D., Costa, G., Kataria, P., & Bromage, E. S. (2012). Discovery and characterization of secretory IgD in rainbow trout: secretory IgD is produced through a novel splicing mechanism. *J Immunol*, *188*(3), 1341-1349. https://doi.org/10.4049/jimmunol.1101938

Ramirez-Gomez, F., Greene, W., Rego, K., Hansen, J. D., Costa, G., Kataria, P., & Bromage, E. S. (2012). Discovery and Characterization of Secretory IgD in Rainbow Trout: Secretory IgD Is Produced through a Novel Splicing Mechanism. *The Journal of Immunology*, *188*(3), 1341-1349. https://doi.org/10.4049/jimmunol.1101938

Rauta, P. R., Nayak, B., & Das, S. (2012). Immune system and immune responses in fish and their role in comparative immunity study: A model for higher organisms. *Immunology Letters*, *148*(1), 23-33. https://doi.org/https://doi.org/10.1016/j.imlet.2012.08.003

Reiner, S. L. (2007). Development in Motion: Helper T Cells at Work. *Cell*, *129*(1), 33-36. https://doi.org/https://doi.org/10.1016/j.cell.2007.03.019

Sahoo, M., Edholm, E.-S., Stafford, J. L., Bengtén, E., Miller, N. W., & Wilson, M. (2008). B cell receptor accessory molecules in the channel catfish, Ictalurus punctatus. *Developmental & Comparative Immunology*, *32*(11), 1385-1397. https://doi.org/https://doi.org/10.1016/j.dci.2008.05.008

Saito, S., Nakashima, A., Shima, T., & Ito, M. (2010). Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. *American journal of reproductive immunology 63*(6), 601-610. https://doi.org/10.1111/j.1600-0897.2010.00852.x

Salinas, I. (2015). The Mucosal Immune System of Teleost Fish. *Biology*, *4*(3), 525-539. https://doi.org/10.3390/biology4030525

Salinas, I., Zhang, Y.-A., & Sunyer, J. O. (2011). Mucosal immunoglobulins and B cells of Teleost fish. *Developmental and comparative immunology*, *35*(12), 1346-1365. https://doi.org/10.1016/j.dci.2011.11.009

Saunders, H. L., & Magor, B. G. (2004). Cloning and expression of the AID gene in the channel catfish. *Developmental and comparative immunology*, *28*(7-8), 657-663. https://doi.org/10.1016/j.dci.2004.01.002

Scapigliati, G., Fausto, A. M., & Picchietti, S. (2018). Fish Lymphocytes: An Evolutionary Equivalent of Mammalian Innate-Like Lymphocytes? [Review]. *Frontiers in Immunology*, *9*(971). https://doi.org/10.3389/fimmu.2018.00971

Schatz, D. G., Oettinger, M. A., & Baltimore, D. (1989). The V(D)J recombination activating gene, RAG-1. *Cell*, *59*(6), 1035-1048. https://doi.org/https://doi.org/10.1016/0092-8674(89)90760-5

Sckisel, G. D., Bouchlaka, M. N., Monjazeb, A. M., Crittenden, M., Curti, B. D., Wilkins, D. E., Alderson, K. A., Sungur, C. M., Ames, E., Mirsoian, A., Reddy, A., Alexander, W., Soulika, A., Blazar, B. R., Longo, D. L., Wiltrout, R. H., & Murphy, W. J. (2015). Out-of-Sequence Signal 3 Paralyzes Primary CD4(+) T-Cell-Dependent Immunity. *Immunity*, *43*(2), 240-250. https://doi.org/10.1016/j.immuni.2015.06.023

Secombes, C. J., Wang, T., & Bird, S. (2011). The interleukins of fish. *Developmental & Comparative Immunology*, *35*(12), 1336-1345. https://doi.org/https://doi.org/10.1016/j.dci.2011.05.001

Shang, N., Sun, X.-F., Hu, W., Wang, Y.-P., & Guo, Q.-L. (2008). Molecular cloning and characterization of common carp (Cyprinus carpio L.) TCRγ and CD3γ/δ chains. *Fish & Shellfish Immunology*, *24*(4), 412-425. https://doi.org/https://doi.org/10.1016/j.fsi.2007.12.007

Smith, N. C., Rise, M. L., & Christian, S. L. (2019). A Comparison of the Innate and Adaptive Immune Systems in Cartilaginous Fish, Ray-Finned Fish, and Lobe-Finned Fish [Review]. *Frontiers in Immunology*, *10*(2292). https://doi.org/10.3389/fimmu.2019.02292

Somamoto, T., Nakanishi, T., & Nakao, M. (2013). Identification of anti-viral cytotoxic effector cells in the ginbuna crucian carp, Carassius auratus langsdorfii. *Developmental & Comparative Immunology*, *39*(4), 370-377.https://doi.org/https://doi.org/10.1016/j.dci.2012.11.001

Spiering, M. J. (2015). Primer on the Immune System. *Alcohol research: current reviews*, *37*(2), 171-175. https://pubmed.ncbi.nlm.nih.gov/26695756 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4590614/

Srivastava, R. K., Dar, H. Y., & Mishra, P. K. (2018). Immunoporosis: Immunology of Osteoporosis—Role of T Cells [Review]. *Frontiers in Immunology*, *9*(657). https://doi.org/10.3389/fimmu.2018.00657

Sugamata, R., Suetake, H., Kikuchi, K., & Suzuki, Y. (2009). Teleost B7 Expressed on Monocytes Regulates T Cell Responses. *The Journal of Immunology*, *182*(11), 6799-6806. https://doi.org/10.4049/jimmunol.0803371

Sunyer, J. O. (2013). Fishing for mammalian paradigms in the teleost immune system. *Nature immunology*, *14*(4), 320-326. https://doi.org/10.1038/ni.2549

Takizawa, F., Araki, K., Ohtani, M., Toda, H., Saito, Y., Lampe, V. S., Dijkstra, J. M., Ototake, M., Moritomo, T., Nakanishi, T., & Fischer, U. (2014). Transcription analysis of two Eomesodermin genes in lymphocyte subsets of two teleost species. *Fish & Shellfish Immunology*, *36*(1), 215-222. https://doi.org/https://doi.org/10.1016/j.fsi.2013.11.004

Takizawa, F., Dijkstra, J. M., Kotterba, P., Korytář, T., Kock, H., Köllner, B., Jaureguiberry, B., Nakanishi, T., & Fischer, U. (2011). The expression of CD8α discriminates distinct T cell subsets in teleost fish. *Developmental & Comparative Immunology*, *35*(7), 752-763. https://doi.org/https://doi.org/10.1016/j.dci.2011.02.008

Takizawa, F., Koppang, E. O., Ohtani, M., Nakanishi, T., Hashimoto, K., Fischer, U., & Dijkstra, J. M. (2011). Constitutive high expression of interleukin-4/13A and GATA-3 in gill and skin of salmonid fishes suggests that these tissues form Th2-skewed immune environments. *Mol Immunol*, *48*(12), 1360-1368. https://doi.org/http://dx.doi.org/10.1016/j.molimm.2011.02.014

Tangye, S. G., & Tarlinton, D. M. (2009). Memory B cells: effectors of long-lived immune responses. *European Journal of Immunology*, *39*(8), 2065-2075. https://doi.org/10.1002/eji.200939531

Teillaud, J. L. (2001). Antibody-dependent Cellular Cytotoxicity (ADCC). *In eLS, (Ed.).* https://doi.org/https://doi.org/10.1002/9780470015902.a0000498.pub2

Toda, H., Araki, K., Moritomo, T., & Nakanishi, T. (2011). Perforin-dependent cytotoxic mechanism in killing by CD8 positive T cells in ginbuna crucian carp, Carassius auratus langsdorfii. *Developmental and comparative immunology*, *35*(1), 88-93. https://doi.org/10.1016/j.dci.2010.08.010

Toda, H., Saito, Y., Koike, T., Takizawa, F., Araki, K., Yabu, T., Somamoto, T., Suetake, H., Suzuki, Y., Ototake, M., Moritomo, T., & Nakanishi, T. (2011). Conservation of characteristics and functions of CD4 positive lymphocytes in a teleost fish. *Developmental & Comparative Immunology*, *35*(6), 650-660. https://doi.org/http://dx.doi.org/10.1016/j.dci.2011.01.013

Toda, H., Yabu, T., Shiba, H., Moritomo, T., & Nakanishi, T. (2011). Evaluating antigen-specific cytotoxicity of CD8+ T cells in fish by granzyme B-like activity. *Veterinary Immunology and Immunopathology*, *141*(1), 168-172. https://doi.org/https://doi.org/10.1016/j.vetimm.2011.02.020

Tonegawa, S. (1983). Somatic generation of antibody diversity. *Nature*, *302*(5909), 575-581. https://doi.org/10.1038/302575a0

Treanor, B. (2012). B-cell receptor: from resting state to activate. *Immunology*, *136*(1), 21-27. https://doi.org/10.1111/j.1365-2567.2012.03564.x

Trinchieri, G., Pflanz, S., & Kastelein, R. A. (2003). The IL-12 family of heterodimeric cytokines: new players in the regulation of T cell responses. *Immunity*, *19*(5), 641-644. https://doi.org/10.1016/s1074-7613(03)00296-6

Turvey, S. E., & Broide, D. H. (2010). Chapter 2: Innate Immunity. *The Journal of allergy and clinical immunology*, *125*(2 Suppl 2), S24-S32. https://doi.org/10.1016/j.jaci.2009.07.016

Volff, J. N. (2005). Genome evolution and biodiversity in teleost fish. *Heredity*, *94*(3), 280-294. https://doi.org/10.1038/sj.hdy.6800635

Wakae, K., Magor, B. G., Saunders, H., Nagaoka, H., Kawamura, A., Kinoshita, K., Honjo, T., & Muramatsu, M. (2006).

Evolution of class switch recombination function in fish activation-induced cytidine deaminase, AID. *International Immunology*, *18*(1), 41-47. https://doi.org/10.1093/intimm/dxh347

Walker, J. A., & McKenzie, A. N. J. (2018). TH2 cell development and function. *Nature Reviews Immunology*, *18*(2), 121-133. https://doi.org/10.1038/nri.2017.118

Wan, F., Hu, C.-B., Ma, J.-X., Gao, K., Xiang, L.-X., & Shao, J.-Z. (2017). Characterization of γδ T Cells from Zebrafish Provides Insights into Their Important Role in Adaptive Humoral Immunity. *Frontiers in Immunology*, *7*, 675-675. https://doi.org/10.3389/fimmu.2016.00675

Wan, Y. Y., & Flavell, R. A. (2009). How Diverse—CD4 Effector T Cells and their Functions. *Journal of molecular cell biology*, *1*(1), 20-36. https://doi.org/10.1093/jmcb/mjp001

Wang, L., Shang, N., Feng, H., Guo, Q., & Dai, H. (2013). Molecular cloning of grass carp (Ctenopharyngodon idellus) T-bet and GATA-3, and their expression profiles with IFN-γ in response to grass carp reovirus (GCRV) infection. *Fish Physiology and Biochemistry*, *39*(4), 793-805. https://doi.org/10.1007/s10695-012-9741-y

Wang, T., Holland, J. W., Martin, S. A., & Secombes, C. J. (2010). Sequence and expression analysis of two T helper master transcription factors, T-bet and GATA3, in rainbow trout Oncorhynchus mykiss and analysis of their expression during bacterial and parasitic infection. *Fish & Shellfish Immunology*, *29*(5), 705-715. https://doi.org/10.1016/j.fsi.2010.06.016

Wang, T., Monte, M. M., Huang, W., Boudinot, P., Martin, S. A., & Secombes, C. J. (2010). Identification of two FoxP3 genes in rainbow trout (Oncorhynchus mykiss) with differential induction patterns. *Mol Immunol*, *47*(16), 2563-2574. https://doi.org/10.1016/j.molimm.2010.06.015

Willett, C. E., Cherry, J. J., & Steiner, L. A. (1997). Characterization and expression of the recombination activating genes (rag1 and rag2) of zebrafish. *Immunogenetics*, *45*(6), 394-404. https://doi.org/10.1007/s002510050221

Willett, C. E., Kawasaki, H., Amemiya, C. T., Lin, S., & Steiner, L. A. (2001). Ikaros expression as a marker for lymphoid progenitors during zebrafish development. *Developmental Dynamics*, *222*(4), 694-698. https://doi.org/10.1002/dvdy.1223

Wilson M, Bengtén E, Miller, N. W., Clem, L. W., Du Pasquier, L., & Warr, G. W. (1997). A novel chimeric Ig heavy chain from a teleost fish shares similarities to IgD. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(9), 4593-4597. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC20768/

Wilson, M. R., & Warr, G. W. (1992). Fish immunoglobulins and the genes that encode them. *Annual Review of Fish Diseases*, *2*, 201-221. https://doi.org/http://dx.doi.org/10.1016/0959-8030(92)90064-5

Wilson, M. R., Zhou, H., Bengtén, E., Clem, L. W., Stuge, T. B., Warr, G. W., & Miller, N. W. (1998). T-cell receptors in channel catfish: structure and expression of TCR alpha and beta genes. *Mol Immunol*, *35*(9), 545-557. https://doi.org/10.1016/s0161-5890(98)00037-6

Xu, Z., Parra, D., Gómez, D., Salinas, I., Zhang, Y.-A., von Gersdorff Jørgensen, L., Heinecke, R. D., Buchmann, K., LaPatra, S., & Sunyer, J. O. (2013). Teleost skin, an ancient mucosal surface that elicits gut-like immune responses. *Proceedings of the National Academy of Sciences*, *110*(32), 13097-13102. https://doi.org/10.1073/pnas.1304319110

Yamaguchi, T., Takizawa, F., Furihata, M., Soto-Lampe, V., Dijkstra, J. M., & Fischer, U. (2019). Teleost cytotoxic T cells. *Fish & Shellfish Immunology*, *95*, 422-439. https://doi.org/https://doi.org/10.1016/j.fsi.2019.10.041

Yamasaki, M., Araki, K., Nakanishi, T., Nakayasu, C., Yoshiura, Y., Iida, T., & Yamamoto, A. (2013). Adaptive immune response to Edwardsiella tarda infection in ginbuna crucian carp, Carassius auratus langsdorfii. *Veterinary Immunology and Immunopathology*, *153*(1-2), 83-90. https://doi.org/10.1016/j.vetimm.2013.02.004

Ye, J., Kaattari, I., & Kaattari, S. (2011). Plasmablasts and plasma cells: Reconsidering teleost immune system organization. *Developmental & Comparative Immunology*, *35*(12), 1273-1281. https://doi.org/http://dx.doi.org/10.1016/j.dci.2011.03.005

Ye, J., Kaattari, I. M., Ma, C., & Kaattari, S. (2013). The teleost humoral immune response. *Fish & Shellfish Immunology*, *35*(6), 1719-1728. https://doi.org/http://dx.doi.org/10.1016/j.fsi.2013.10.015

Yoshiura, Y., Kiryu, I., Fujiwara, A., Suetake, H., Suzuki, Y., Nakanishi, T., & Ototake, M. (2003). Identification and characterization of Fugu orthologues of mammalian interleukin-12 subunits. *Immunogenetics*, *55*(5), 296-306. https://doi.org/10.1007/s00251-003-0582-9

Youngblood, B., Hale, J. S., Kissick, H. T., Ahn, E., Xu, X., Wieland, A., Araki, K., West, E. E., Ghoneim, H. E., Fan, Y., Dogra, P., Davis, C. W., Konieczny, B. T., Antia, R., Cheng, X., & Ahmed, R. (2017). Effector CD8 T cells dedifferentiate into long-lived memory cells. *Nature*, *552*(7685), 404-409. https://doi.org/10.1038/nature25144

Yuan, X., & Malek, T. R. (2012). Cellular and molecular determinants for the development of natural and induced regulatory T cells. *Hum Immunol*, *73*(8), 773-782. https://doi.org/10.1016/j.humimm.2012.05.010

Zhan, Y., & Jimmy, K. (2000). Molecular isolation and characterisation of carp transforming growth factor beta 1 from activated leucocytes. *Fish & Shellfish Immunology*, *10*(4), 309-318. https://doi.org/10.1006/fsim.1999.0239

Zhang, H., Shen, B., Wu, H., Gao, L., Liu, Q., Wang, Q., Xiao, J., & Zhang, Y. (2014). Th17-like immune response in fish mucosal tissues after administration of live attenuated Vibrio anguillarum via different vaccination routes. *Fish & Shellfish Immunology*, *37*(2), 229-238. https://doi.org/http://dx.doi.org/10.1016/j.fsi.2014.02.007

Zhang Y, Salinas I, Li, J., Parra, D., Bjork, S., Xu, Z., LaPatra, S. E., Bartholomew, J., & Sunyer, J. O. (2010). IgT, a primitive immunoglobulin class specialized in mucosal immunity. *Nature immunology*, *11*(9), 827-835. https://doi.org/10.1038/ni.1913

Zhao, X., Findly, R. C., & Dickerson, H. W. (2008). Cutaneous antibody-secreting cells and B cells in a teleost fish. *Developmental & Comparative Immunology*, *32*(5), 500-508. https://doi.org/http://dx.doi.org/10.1016/j.dci.2007.08.009

Zhou, H., Stuge, T. B., Miller, N. W., Bengten, E., Naftel, J. P., Bernanke, J. M., Chinchar, V. G., Clem, L. W., & Wilson, M. (2001). Heterogeneity of Channel Catfish CTL with Respect to Target Recognition and Cytotoxic Mechanisms Employed. *The Journal of Immunology*, *167*(3), 1325-1332., https://doi.org/10.4049/jimmunol.167.3.1325

Zhu, J., Guo, L., Watson, C. J., Hu-Li, J., & Paul, W. E. (2001). Stat6 is necessary and sufficient for IL-4's role in Th2 differentiation and cell expansion. *Journal of Immunology*, *166*(12), 7276-7281. https://doi.org/10.4049/jimmunol.166.12.7276

Zhu, J., Yamane, H., & Paul, W. E. (2010). Differentiation of effector CD4 T cell populations (*). *Annu Rev Immunol*, *28*, 445-489. https://doi.org/10.1146/annurev-immunol-030409-101212

Zhu, L.-y., Nie, L., Shao, T., Dong, W.-r., Xiang, L.-x., & Shao, J.-z. (2013). B cells in primitive vertebrate act as pivotal antigen presenting cells in priming adaptive immunity (P5035). *The Journal of Immunology*, *190*(1 Supplement), 110.118-110.118.

Zouali, M., & Richard, Y. (2011). Marginal Zone B-Cells, a Gatekeeper of Innate Immunity. *Frontiers in Immunology*, *2*, 63. https://doi.org/10.3389/fimmu.2011.00063

Zwollo, P., Cole, S., Bromage, E., & Kaattari, S. (2005). B Cell Heterogeneity in the Teleost Kidney: Evidence for a Maturation Gradient from Anterior to Posterior Kidney. *The Journal of Immunology*, *174*(11), 6608-6616. https://doi.org/10.4049/jimmunol.174.11.6608