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Cytokine network regulating inflammatory response in fish

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Abstract

Cytokines are family of low molecular weight proteins that coordinate the whole body's immune response. Cytokines are important immune system regulators and finding these genes in fish has potential applications for the development of aquaculture vaccines and/or immunostimulants. Many CC family cytokines which includes a number of ILs, including IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 play vital roles in mammalian adaptive immunity have been identified in teleost fish. The expression of cytokine gene in fish organs can be regulated by Pathogen-associated molecular patterns (PAMPs), Toll like receptors (TLRs), bacterial subcellular components, Lipopolysaccharide (LPS) and probiotic cellular components. This review will focus on the role of the main cytokine and cell signalling molecules, and how cytokine network regulates the inflammatory cells (Neutrophil, Dendritic cells and Macrophage) in fish.

Keywords: Cytokine, pamps, immunostimulants, immune cells

1. Introduction

Cytokines are peptides that play a basic role in immune system communication, generally less than 80 kDa in size (a family of low molecular weight proteins) that control a broad variety of biological functions, including innate and acquired immunity, hematopoiesis, inflammation and repair and proliferation, mostly by extracellular signals^[1]. Cytokines are often glycosylated and secreted by activated immune cells when they are induced by different pathogens such as parasites, bacterial or viral elements^[2]. Cytokines regulate endothelial lymphatic cells that guide the entry of antigens / dendritic cells (DCs) into the lymph node in order to effectively initiate adaptive immune responses^[3]. In addition to antigen and co-stimulation signals, particular cytokine-dependent signals play a key role in the management of naive CD4 + T (Th) cells, which are vital regulators for adaptive immune responses to many infectious agents^[4, 5]. When naive CD4+T cells acknowledge a foreign antigen-derived peptide presented on antigen-presenting cells in the framework of a significant Class II histocompatibility complex, they undergo significant proliferation and differentiation in at least four distinct mammalian Th-cell subsets (Th1, Th2, Th17 and induced regulatory cells)^[6]. Macrophages, highlighting their role as "first responders" can secrete TNF- α , IL-1, IL-6 and IL-12 in innate immunity, as well as chemokines such as MCP-1(CCL2) and IL-8 (CXCL8) that regulates neutrophil migration by signaling through the CXCR2 receptor, all of which are crucial for the recruitment of macrophages and lymphocytes into infected tissues and their instigation as pathogen eliminators^[7, 8].

2. CC cytokine family

Many cytokines that play critical roles in mammalian adaptive immunity have been identified in teleost fish including the cc cytokine family, a number of ILs, and transforming growth factor-b that contributes significantly to adaptive immune responses^[9]. One of the major type I cytokines is the cc family; these contain four α -helical bundles and share the same CC receptor chains. Various ILs, including IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 belong to this family^[10]. Schematic representation of the cytokine network that regulates inflammatory cells in fish is presented in Figure 1.

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3. Pathogen-associated molecular patterns (PAMPS)

Toll-like receptors (TLRs) are single, non-catalytic, membrane-spanning receptors of pattern recognition in invertebrates and vertebrates. They act by specifically recognizing pathogen-associated molecular patterns (PAMPs) of a variety of microbes and activate signalling cascades to induce innate immunity [11]. PAMPs include polysaccharides, LPS, (Peptidoglycan) PGNs, bacterial DNA, double-stranded viral RNA and other molecules [11]. Suppressors of cytokine signaling (SOCS) family members are emerging as one of the most important regulators of Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathways [12]. So

far eight members of the SOCS family, including cytokine-inducible SRC homology 2- (SH2-) domain-containing protein (CISH) and SOCS1–7, have been identified in mammals [13]. Most SOCS proteins are induced by cytokines and thus act to inhibit cytokine signal transduction in a classic negative feedback loop. However, various other stimuli such as pathogen-associated molecular patterns (PAMPs) and bacterial, viral, and parasitic infections also induce them [14]. SOCS gene expression inhibition may be a potential target in future studies aimed at modulating the development and function of T-helper cells with a view to improving vaccine efficacy and enhancing dietary resistance in fish.

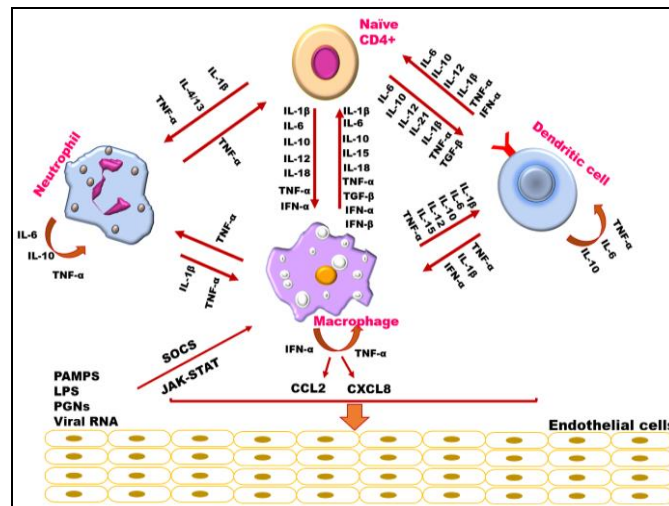


Fig 1: Schematic representation of the cytokine network that regulates inflammatory cells in fish

4. Activation of pathway by toll like receptors (TLRs)

To understand the comparative immunology between warm-blooded and aquatic animals, functional analysis of novel TLRs in aquatic animals is very important. Binding of agonists to the ectodomain of the receptor initiates TLR activation through a variety of Toll / Interleukin-1 receptor homology (TIR)—domain which comprised of canonical adaptor MyD88, leading to the assembly of signaling cascade of protein kinases that ultimately trigger the activation of transcription factors (NFκBs, IRFs) to promote proinflammatory response and expression of genes involved in the immune response [15]. Therefore, the use of LPS, a commonly used PAMP in fish immunostimulant diets, seems contradictory as fish are highly resistant to LPS toxic effects [16], negatively regulates NFκB activation [17] and the vast majority of fish do not express TLR4 [11]. In contrast, (peptidoglycan) PGN-stimulated rainbow trout macrophages induced the expression of MyD88, IRAK and a TNF-activated factor (TRAF6), suggesting TLR's involvement in PGN-mediated inflammatory trout responses [18]. However, while knowledge of the innate immune response of TLR-mediated fish is increasing, further investigation is needed to determine the underlying effects of PAMP-associated immunostimulants on the recognition and activation of the immune response of fish.

5. Regulation by bacterial subcellular components

Recent research has shown that the expression of cytokine gene in fish organs can be regulated by bacterial subcellular components. For example, elevation of TNF-α and IL-1β in the blood of *Cyprinus carpio* was noted in dietary supplementation of (extracellular products) ECPs from the

intestinal autochthonous bacteria *Flavobacterium sasangense* BA-3 and *Aeromonas veronii* BA-1 [19]. Upregulation of mRNA expression of TNF-α, IL-1, IL-6, IL-10, COX-2, and Mx was observed by the incubation with the ECPs of the probiotic *Vagococcus fluvialis* L21 in the head kidney leucocytes of sea bass [20]. In addition, transcriptome analysis was performed by using heat-killed lactic acid bacteria *Lactobacillus paracasei* spp. *paracasei* and *Lactobacillus plantarum* (isolated from mongolian dairy products) to confirm the cytokine responses in *Takifugu rubripes* head kidney cells [21]. Significant elevation of all the cytokine genes was studied by both probiotic bacterial stimulants compared with the unstimulated control [22]. Pro-inflammatory cytokine such as IL-1β, TNF-α, TNF-N, IL-6 and IL-17A/F-3, anti-inflammatory cytokine like IL-10, intra-cellular pathogen killing cytokine named I-IFN-1 and IFNγ, cytokine which induce cell-mediated immunity such as IL-12p35, IL-12p40 and IL-18 and the cytokine which regulate peripheral T-cell expansion and survival named TGF-b1, IL-2, IL-7, IL-15 and IL-21 were investigated in treated fish [22].

6. Role of lipopolysaccharide (LPS)

Expression of immune-related genes in zebra fish (*Danio rerio*) embryos were investigated with the exposure to *Escherichia coli* - derived LPS [23]. Upregulation of TNF-α and IL-1β and concomitant deregulation of IL-6 in head kidney cells of *Cyprinus carpio* was observed by supplementing LPS for 30–60 days as oral dose [24]. Further, in rainbow trout, peptidoglycan (PGN) as dietary supplementation showed beneficial role on antimicrobial protein gene expression in the mucosal tissues [25]. Novel beta-defensin antimicrobial peptides (omDB-3 and omDB-4) was up regulated in rainbow trout by supplementing the PGN

[26]. 2015). From the aforementioned studies, it is confirmed that fish innate immunity was boosted with the administration of PGN or LPS [27], however, strong immune response in fish was not observed by advocating ultra-pure LPS when compared to PGN, which up regulates IL6 and IL-1 β and release prostaglandin E2. Above evidences highlight the differences in the fish immune response between purified LPS and PGN.

7. Probiotic cellular components as immunostimulants

Expression of cytokine genes, that is IL-10, IL-1 β , IL-12p35, IL-12p40, TNF α , NF κ B, COX-2, IFN- α and IFN- γ in the head kidney macrophages of *Labeo rohita* were stimulated by (Heat Killed Whole Cell Products) HKWCs of the probiotic *Pseudomonas aeruginosa* VSG2 [28]. Further, *in-vitro* results were validated by an *in-vivo* study [29]. In addition, immune response in *Labeo rohita* fingerlings was confirmed with intraperitoneally injection of 0.1 mL PBS supplemented with 0.1 mg of any of the following cellular components: ICPs of VSG1, HKWCs of VSG2 or ICPs of VSG3. The above results further confirmed the augmentation of cytokines, IL-1 β , COX-2, iNOS and IL-10 by supplementing probiotic cellular components versus control. Upregulation of TNF- α and NF κ B were noted in immunized fish up to 14 days post immunization. Recent investigation showed that dietary supplementation of heat-inactivated *Bacillus pumilus* SE5 increased the cytokines IL-8, IL-1 β and TLR2 in the head kidney of grouper (*Epinephelus coioides*) [30].

Another recent investigation by Giri *et al.* (2015b) [31] confirmed the efficiency of (Intracellular products) ICPs of the probiotics *Bacillus subtilis* Variant Surface Glycoprotein (VSG1) and *Lactobacillus plantarum* VSG3 in stimulating cytokine genes in the head kidney macrophages of *Labeo rohita*. Significant elevation of all the cytokine genes IL-10, IL-1 β , IL-12p35, IL-12p40, IL-18, TNF- α , NF κ B, COX-2, IFN-1 and IFN- γ were observed when compared to positive control (LPS) or negative control (phosphate buffer solution - PBS).

All the above studies, suggested that probiotic cellular components may be useful as immunostimulants in fish culture and perhaps an adjuvant for vaccines in aquaculture.

8. Conclusion

In teleost fish, many CC family cytokines including IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 play essential roles in mammalian adaptive immunity have been recognized. Cytokines IL-3, IL-4, IL-5 IL-7, IL-9 and IL-13 are yet to be discovered from fish. Most cytokine signaling suppressors (SOCS) proteins are induced by cytokines and thus behave in a classic adverse feedback loop to inhibit cytokine signal transduction. In future research directed at modulating the growth and function of T-helper cells with a perspective to improving vaccine effectiveness and enhancing nutritional resistance in fish, SOCS gene expression inhibition may be a potential target. So, further investigation is needed to determine the underlying effects of PAMP-associated immunostimulants on the recognition and activation of the immune response of fish.

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