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Cytokine Regulation of Teleost Inflammatory Responses

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1. Introduction

The inflammatory response is a highly regulated process initiated by tissue damage, infiltrating pathogens or both. The primary role of inflammation is the resolution of tissue damage, including the elimination of damaged or dead cells and any infiltrating pathogens, and restoration of homeostasis. The initial recognition of tissue damage and/or pathogens is mediated by tissue resident macrophages primarily through various sentinel pattern recognition receptors such as toll-like receptors. In response to and in accordance with distinct stimuli, macrophages become activated to produce a wide range of bioactive molecules, some of which attract other cells to the site of inflammation, and others that dictate the course of an inflammatory response and eventual tissue repair. Recent evidence suggests that there are at least two activation states of monocytes/ macrophages [4, 130, 211, 214]. The classically activated monocytes/macrophages possess significant antimicrobial armamentarium, secrete a plethora of factors that propagate and enhance the microbicidal activities and in general mediate pathogen clearance. The non-classically or alternatively activated monocytes/macrophages secrete factors that ablate the destructive components of the inflammatory response and promote tissue healing, repair and angiogenesis and will not be addressed further here.

The processes involved in the onset, progression and resolution of inflammation are complex and remain to be fully elucidated in vertebrates. However, it is widely believed that myeloid lineage cells are intimately involved in inflammatory reactions and their function is controlled by cytokines. This review focuses on the recent advancements in the understanding of the biology of hallmark fish pro-inflammatory cytokines, tumor necrosis factor alpha (TNF α), interferon gamma (IFN γ) and interleukin-1 beta (IL-1 β) and their receptors.

2. Antimicrobial responses of fish phagocytes

It is well established that fish phagocytes possess oxidative burst responses, comparable to those of mammals. Absence of readily available fish cytokines limited the early research of fish phagocytes to employing pathogen products and/or crude activated cell supernatants, presumed to contain hallmark “activating” agents. This fundamental work of fish phagocyte-mediated inflammatory processes has been comprehensively reviewed [138]. Since then the specific genes encoding the components of the fish NADPH oxidase complex (Fig. 1) have been cloned in various fish species [13, 84, 119] and their expression correlated with reactive oxygen radical production [13, 63, 141]. The priming of the fish phagocyte ROI responses by recombinant fish cytokines such as TNF α [67, 133, 217], IFN γ [62, 63, 215] and IL-1 β [54, 97, 144] has also been reported. Similar to the mammalian monocyte/macrophage paradigm [22], fish monocytes have greater ability to generate reactive oxygen intermediates (ROI) following short stimulation [67, 136, 152], whereas mature fish macrophages require relatively prolonged immune stimulations to achieve comparable magnitudes of this response [58, 59, 136, 171].

Phagocytes (primarily mature macrophages) also produce microbicidal/tumoricidal reactive nitrogen intermediates in a stimulus-specific manner. This response, catalyzed by the inducible nitric oxide synthase enzyme (iNOS, Fig. 2), involves the conversion of arginine to citrulline and results in the production of nitric oxide (NO) and other products including nitrite, nitrate, and nitrosamines [86, 134, 184]. The NADPH oxidase produced superoxide anion may also react with NO to form the peroxynitrite intermediate [ONOO $^-$] that also has potent microbiocidal activity [35, 156, 191, 213]. The biology of the iNOS enzyme has been reviewed in references [2, 111, 126].

The ability of fish phagocytes to produce microbicidal NOs has been well established (reviewed in reference [138]). The iNOS gene transcript has been identified in several fish species [101, 102, 163, 200] and fish macrophages have been demonstrated to up-regulate iNOS expression and produce copious amounts of NO in response to a plethora of immune stimuli [62-64, 66, 67, 85, 88, 140, 158, 180, 210]. The inflammatory cytokine regulation of fish iNOS and NO production is described below.

3. Cytokine regulation of inflammatory responses

The onset, progression and resolution of the inflammatory responses are tightly regulated through soluble mediators (cytokines, monokines, chemokines) that orchestrate inflammation. Pro-inflammatory cytokines such as TNF α , IFN γ , and IL-1 β enhance antimicrobial functions of immune cells and facilitate the pathogen clearance, while anti-inflammatory cytokines such as TGF β and IL-10 down-regulate inflammatory processes and skew cell functions towards tissue repair mechanisms. It is important to note that in addition to the hallmark cytokines discussed in this review, other cytokines such as chemokines and growth factors also participate in the regulation of an inflammatory response.

The last decade has yielded significant advances in the understanding of inflammatory responses of lower vertebrates, such as bony fish. The genes encoding hallmark cytokines have been identified and characterized in a number of fish species. Interestingly, many of these exhibit structural similarities and gene synteny organization comparable to their higher vertebrate counterparts. Conversely, multiple isoforms of certain cytokines are present in distinct fish species.

3.1. Tumor necrosis factor alpha (TNF α)

Tumor necrosis factor alpha is a central inflammatory mediator, initially identified as a serum component capable of eliciting “hemorrhagic necrosis” of certain tumors [20]. Since discovery, TNF α has been found to be produced by many cell-types and confer an incredible range of immune processes [44, 203, 209]. In the context of inflammatory responses, TNF α promotes the chemotaxis of neutrophils and monocytes/macrophages [123, 207], enhances their phagocytic capacity [95, 105, 194], primes ROI and NO responses [42, 135], chemoattracts fibroblasts [168] and elicits platelet activating factor production [19, 73, 104].

The mammalian TNF α functions as a 26 kDa type II trans-membrane protein as well as a 17 kDa soluble moiety, released by the TNF α cleaving metalloproteinase enzyme (TACE)-mediated cleavage [98, 128, 148]. A homotrimerized TNF α (soluble or membrane bound) engages one of two cognate receptors, TNF-R1 or TNF-R2, which in turn trimerize around the ligand [7, 45]. Currently, there is no consensus as to the respective contribution of these receptors to the biological effects caused by TNF α . Some evidence suggests that TNF-R1 propagates the signal from the soluble TNF α , while the membrane-bound TNF α acts exclusively through TNF-R2 [70]. Other evidence suggest that TNF-R1 is primarily involved in induction of apoptosis while the TNF-R2 functions in proliferation and cell survival [129], while other contributions suggest cooperation between the two receptors [204]. The prevailing theory proposes that TNF-R1 confers signal propagation, while TNF-R2 binds and redistributes TNF α to TNF-R1 in a process coined “ligand passing” [28, 43, 197]. Despite this, more recent literature suggests that TNF-R2 is directly involved in many inflammatory processes including the activation of T lymphocytes [93, 94], stimulation of myofibroblasts [190], as well as tumor suppression [212]. The TNF-R1 and TNF-R2 utilize largely non-overlapping signaling mechanisms, relying on recruitment of numerous downstream signaling molecules, the relative abundance of which ultimately dictates signaling outcomes (for a current review of TNF α signal transduction see recent review [143]). It is likely that the roles of respective TNF receptors in the biological outcomes of TNF α stimulation are cell type and cell activation state dependent.

3.1.1. Identification of TNF α in fish

Presence of an endogenous bony fish TNF system was first suggested in the early 1990s where the human recombinant TNF α elicited ROI production in trout leukocytes while administration of a monoclonal anti-TNF-R1 antibody blocked this response [75, 87]. Hirono *et al.* [76] identified and characterized the first cDNA transcript encoding a Japanese flounder TNF α , which had only 20-30% amino acid identity with the mammalian TNFs, but had very

similar intron/exon organization. The expression of this flounder TNF α gene increased in PBLs following LPS, ConA or PMA stimulation, suggesting a conserved role for this cytokine in fish inflammatory responses. Shortly thereafter, the rainbow trout TNF α was identified, shown to be constitutively expressed in the gill and kidney tissues, and upregulated in LPS-stimulated or IL-1 β -treated kidney leukocytes and in the trout macrophage cell line, RTS11 [103]. The catfish TNF α was also constitutively expressed in healthy fish and several catfish immune cell lines including macrophage and T cell lines, but not in B cell or fibroblast cell lines [218]. Notably, all fish TNF α proteins possess the TNF family signature, [LV]-x-[LIVM]-x₃-G-[LIVMF]-Y-[LIMVMFY]₂-x₂-[QEKHL] [103], underlining the evolutionary conservation of this cytokine.

3.1.2. Isoforms of TNF α in fish

Trout were reported to possess an additional TNF α isoform [219] and the presence of multiple bony fish TNFs was confirmed in common carp, in which first two [162], and soon thereafter a third carp TNF α isoform [167] were identified. Interestingly, polymorphisms in the gene encoding the carp TNF α 2 isoform were linked to carp resistance to the protozoan parasite, *T. borreli* [162].

3.1.3. TNF α receptors of fish

Tumor necrosis factor alpha has now been identified in a number of fish species including flounder [76], trout [103, 114], catfish [218], carp [162, 167], sea bream [18, 56], tilapia [151], turbot [142], ayu [193], fugu [165], zebrafish [165], sea bass [133], goldfish [67] and tuna [89]. Additionally, novel TNF-like molecules with unique intron/exon organization have been identified in zebrafish and flounder [165]. Despite this, knowledge regarding the cognate receptors for the teleost TNF proteins has been relatively limited.

A death domain containing TNF receptor was identified in zebrafish ovarian tissues and coined the ovarian TNF receptor (OTR) [12]. Predicted zebrafish TNF-R1 and TNF-R2 sequences are in the NCBI database, with zebrafish TNF-R1 showing high sequence homology to the OTR. The goldfish TNF-R1 and TNF-R2 cDNAs were identified based on the zebrafish sequences [61] and display conserved regions included cysteine residues and predicted docking sites for downstream signaling. Also, the goldfish TNF-R1 possesses a conserved death domain including the highly conserved motif (W/E)-X₃₁-L-X₂-W-X₁₂-L-X₃-L (with R in the W/E position) and six conserved or semi-conserved residues, crucial for the mammalian TNF-R1-mediated cytotoxicity [189]. Interestingly, while the mammalian TNF-R1 and TNF-R2 and the fish TNF-R2 contain four TNF homology domains (THD, defined by specific cysteine residues), the fish TNF-R1 proteins exhibit only 3 full THDs [61].

Recombinant extracellular domains of goldfish TNF-R1 and TNF-R2 bound to both goldfish recombinant TNF α 1 or TNF α 2 in *in vitro* binding assays [61]. As with the sea bream TNF α [56], the goldfish recombinant TNF α ligands and receptors all adopted dimeric conformations and interacted as dimers rather than trimers *in vitro*. Interestingly, there have also been several reports indicating dimerization of the mammalian TNF-R1 [131, 132, 147]. Further-

more, the mammalian p75/NTR neurotrophin receptor, a member of the TNF superfamily of proteins with many structural similarities to teleost and mammalian TNF-R1, binds to its cognate NTR ligand as a dimer [26, 57].

By examining the TNF system in lower vertebrates such as teleost fish, we can gain insight into the evolutionary origins of our own immune systems and the selective pressures that shaped them. As in mammals, TNF α appears to be central to the regulation of inflammatory responses of bony fish. Further examination of biological effects of this molecule using different lineages of fish immune cells will yield a more concrete understanding of this system in teleosts.

3.1.4. Inflammatory roles of the fish TNF α

The first functional characterization of a fish TNF α was reported in 2003 when Zou et al. [217] demonstrated that recombinant trout TNF α 1 and TNF α 2 (isoforms) both induced the IL1 β , TNF α 1, TNF α 2, IL-8 and COX-2 gene expression in primary kidney leukocytes and in the RTS11 trout macrophage cell line [217]. These recombinant TNF α isoforms also elicited dose-dependent chemotaxis of trout kidney leukocytes and phagocytosis of yeast particles. SDS-PAGE analysis of the recombinant TNF α 1 and TNF α 2 suggested that both of the recombinant molecules existed in monomeric, dimeric and trimeric states. Together, these findings suggested conservation in the pro-inflammatory roles for teleost TNF α .

Intraperitoneal administration of a mature recombinant (cleaved) form of gilthead sea bream TNF α to fish resulted in rapid recruitment of phagocytic granulocytes to the sites of injection, granulopoiesis and the priming for enhanced ROI of peritoneal and primary kidney leukocytes [56]. Intriguingly, size exclusion chromatography of this recombinant sea bream TNF α suggested that this protein existed primarily in a dimeric state [56], in contrast to the trimeric state of the mammalian TNF α .

Subsequent reports indicated that the pro-inflammatory effects of sea bream and zebrafish TNF α s were not direct but resulted from the stimulation of endothelial cells [155]. When sea bream peritoneal and head kidney leukocytes were primed with recombinant TNF α (100 ng/mL) or bacterial DNA (*Vibrio anguillarum*, 50 μ g/mL) for 16 hours, TNF α elicited significant ROI responses, albeit modest when compared to those induced by *V. anguillarum* DNA. Intraperitoneal injections of the recombinant TNF α resulted in increased expression of several pro-inflammatory genes of sea bream peritoneal leukocytes, while TNF α treatments of sea bream endocardium endothelial cells (EECs) *in vitro* also increased their immune gene expression. Notably, while the *in vitro* TNF α stimulation of EECs and macrophages resulted in substantially elevated pro-inflammatory gene expression in both cell types, the stimulated macrophages exhibited significantly more robust transcriptional responses. Although this TNF α failed to chemoattract leukocytes, TNF α -stimulated EEC-conditioned medium and supernatants from TNF α -injected peritoneal exudate cells elicited leukocyte chemoattraction. Furthermore, the zebrafish TNF α conferred neutrophil recruitment but also increased fish susceptibility to bacterial and viral infections. Accordingly, the authors of these studies proposed that unlike the mammalian cytokine, the fish TNF α elicits inflammatory functions indirectly, through non-immune cells.

In similar studies, supernatants from recombinant carp TNF α -stimulated cardiac endothelial cells primed kidney phagocyte ROI responses while recombinant carp TNF α 1 and 2 did not enhance phagocyte antimicrobial functions [155]. Notably, the recombinant forms of the zebrafish, trout, sea bream and carp TNF α proteins exhibited lytic activity towards *Trypanoplasma borreli*, akin to the trypanolytic capacity of the mammalian TNFs [52], where the membrane form of the fish TNF α was thought to be responsible for these effects. In summation of the above observations, Florenza *et al.* (2009) suggested that while the trypanolytic roles of TNF α are evolutionarily conserved, the pro-inflammatory mechanisms elicited by this molecule were not [155].

Contrary to the sea bream and carp studies, other literature suggests that akin to the mammalian cytokine, teleost TNF α also directly elicits pro-inflammatory functions. The tilapia non-specific cytotoxic cells (NCCs) constitutively express membrane bound as well as soluble forms of TNF α (in addition to granzymes and Fas ligand) to confer cytolytic activity towards target cells, and when stimulated with recombinant tilapia TNF α , become protected from activation-induced apoptosis [151]. While the turbot recombinant TNF α did not enhance macrophage ROI, it elicited *in vitro* NO production and *in vivo* inflammatory cell recruitment and activation [142]. The Ayu fish, recombinant TNF α induced ROI production by kidney cells [193] and the blue fin tuna recombinant TNF α 1 and 2 enhanced the phagocytic responses of tuna PBLs [89].

Zebrafish TNF α elicited cell signaling and conferred increased resistance to *Mycobacterium marinum* [30] while knockdown of the TNF-R1 led to enhanced mycobacterial disease progression, increased fish mortality, accelerated bacterial growth, granuloma breakdown and necrotic macrophage cell death [30]. Thus, it appears that the zebrafish TNF α is pivotal in the maintenance of encapsulated *M. marinum* granulomas and the restriction of the growth of this pathogen. Our recent work supports these findings, where the pre-treatment of goldfish macrophages with recombinant goldfish TNF α 2 ablated the *M. marinum*-mediated down-regulation of NO production by these cells and reduced the survival of intracellular bacteria [65].

We recently identified two isoforms of the goldfish TNF α and functionally characterized a recombinant goldfish TNF α 2 (rgTNF α 2) in the context of primary kidney-derived goldfish macrophage cultures (PKMs) [67]. This rgTNF α 2 induced dose-dependent chemotaxis of goldfish macrophages, enhanced their phagocytic abilities, NO production and primed the ROI responses of PKMs.

The extent of the conservation in the biology of teleost TNF α will become more evident with increased availability of tools, reagents and cell culture systems. While some literature proposes a lack of conservation of the inflammatory roles of teleost TNF α , others strongly implicate this molecule in the regulation of fish antimicrobial functions. It is also possible that the discrepancies in the above findings stem from the culture systems employed. For example, both the sea bream and carp studies that failed to observe direct effects of fish TNF α utilized freshly isolated adherent kidney phagocyte populations [52, 155]. Notably, freshly isolated mammalian myeloid populations are highly variable in their antimicrobial capabilities [116]. Similarly, goldfish PKMs exhibit temporal gain and loss of antimicrobial capabili-

ties after prolonged cultivation [67, 136]. Specifically, ROI is primarily mediated by PKM-monocytes while NO is predominantly produced by mature macrophages [67, 136].

3.2. Interferon-gamma (IFN γ)

Interferon gamma is a pleiotropic, pro-inflammatory and anti-viral cytokine, identified in the supernatants of PHA-activated lymphocytes for its unique anti-viral properties [205]. IFN γ is primarily produced by activated Th1 phenotype CD4⁺ cells [127], CD8⁺ cells [161] and natural killer (NK) cells [149] and is of central importance in host defense against intracellular and extracellular pathogens [8, 91, 99, 179, 182]. For example, IFN γ gene knock-out mice are incapable of controlling infections with *Leishmania major* [202], *Listeria monocytogenes* [81], and *Mycobacterium* [32], underlining the importance of this cytokine in the regulation of antimicrobial responses [8, 10, 21, 51, 115].

The mammalian IFN γ dimer ligates the interferon gamma receptor 1 (IFNGR1), which then associates with IFNGR2, forming a signaling complex and activating the Janus kinases (Jak) 1 and 2, associated with the receptor chains 1 and 2, respectively [83]. Jak1 and Jak2 in turn activate the IFNGR1-associated signal transducer of activation-1 (Stat1) transcription factor [33]. The IFNGR ligation may also activate and utilize Stat2 [187], albeit to lesser extent than Stat1. Subsequent transcriptional regulation of several other genes then ensues through homodimeric Stat1, heterodimeric Stat1: Stat2, through the transcription factor complexes ISGF3 and Stat1-p48, composed of Stat1: Stat2:IRF-9 and Stat1: Stat1:IRF-9, respectively [11, 118, 187, 188]. These confer transcriptional changes through recognition of IFN γ -activated sequences (GAS) in the promoter regions of target genes [188]. Within 30 minutes of IFN γ receptor ligation, there are increased transcript levels for several interferon regulatory factors (IRFs), which then modulate subsequent waves of gene expression in the IFN γ signaling cascade [208].

3.2.1. Identification of IFN γ in bony fish

Trout mitogen-simulated leukocyte supernatants possess macrophage activating capabilities (MAFs) akin to the mammalian IFN γ [58, 59], suggesting the existence of an IFN γ counterpart(s) in fish. It was also established that downstream signaling factors employed by the mammalian IFN γ (Stats), were present in fish [160] where the antibody-purified fish Stat-like factor was shown to bind the mammalian IFN γ promoter [160].

The initial fish IFN γ homolog discovery came from examination of fugu gene scaffolds [220]. Fugu homologs of mammalian genes syntenic to IFN γ were also present on the same fugu gene scaffold. This fugu IFN γ had 4 exon / 3 intron organization similar to its mammalian counterpart and shared 32.3-32.7 % and 34.9-43.3% identities with bird and mammalian IFN γ sequences, respectively. An identified trout IFN γ sequence exhibited low sequence identity with other vertebrate IFN γ proteins, but possessed the conserved signature motif ([IV]-Q-X-[KQ]-A-X₂-E-[LF]-X₂-[IV]) and C-terminal nuclear localization signal (NLS), characteristic of the mammalian IFN γ proteins [215].

3.2.2. Inflammatory roles of the fish IFN γ

The recombinant trout IFN γ (rtIFN γ) elicited increased immune gene expression in the RTS-11 monocyte-macrophage cell line [199], while this response was pharmacologically abrogated with ERK (transcription factor) or protein kinase C (PKC) inhibitors as well as by deleting the C-terminal NLS on the rtIFN γ [215]. Also, rtIFN γ stimulation of trout kidney leukocytes primed their ROI production, suggesting functional similarities between the trout and mammalian type II IFNs [215].

Adult zebrafish microinjected with recombinant IFN γ did not exhibit increased immune gene expression or enhanced protection against *Streptococcus iniae* and the spring viremia carp virus (SVCV) [110]. However, this lack of response could stem from IFN γ being bound up by cells expressing only the IFNGR1 without eliciting a detectable level of response from the relatively few cells expressing both IFNGR1 and IFNGR2. If this is the case, these results underline the localized nature of the zebrafish IFN γ function rather than the efficacy of regulation of antimicrobial functions by this cytokine.

We [62] and others [3] have functionally characterized the cyprinid IFN γ . A recombinant goldfish IFN γ (rgIFN γ) primed goldfish monocytes ROI response in a concentration dependent manner [62] and, as in mammals [72, 157], at lower concentrations rgIFN γ conferred additive ROI priming effects. The rgIFN γ also enhanced the expression of the ROI enzyme, NADPH oxidase, catalytic subunits, p67^{phox} and gp91^{phox}. Similarly, the recombinant carp IFN γ (rcIFN γ) also primed carp kidney phagocytes for enhanced ROI production [3].

The goldfish rIFN γ elicited modest but significant enhancement of phagocytosis and NO production by goldfish monocytes and macrophages, respectively [62]. This was paralleled with increased iNOS gene expression in rgIFN γ -stimulated macrophages. In contrast, carp kidney phagocytes only displayed significant iNOS gene expression and NO production when treated with a combination of carp recombinant IFN γ and 30 μ g/mL LPS, but not following rcIFN γ treatments alone [3].

Since carp and goldfish are closely related species, the above discrepancies presumably stem from differences in experimental systems. Akin to bone-marrow-derived macrophages, which acquire antimicrobial capabilities with culture time, we have observed that the antimicrobial capabilities of goldfish kidney-derived phagocytes are dynamic [136, 137]. For this reason, we used culture-derived cells, while the carp studies used freshly isolated phagocytes. Additionally, it is well established that in mammals the production of ROIs is seen as early as 1 hour after immune stimulation, whereas the production RNIs is first detected approximately 24 hours post stimulation [169]. This is very similar to what we have observed with rgIFN γ -elicited iNOS expression and NO responses.

The treatment of mature goldfish macrophages with rgIFN γ resulted in increased expression of TNF α and IL-1 β isoforms; IL-12 p35 and p40; IFN γ ; IL-8 (CXCL-8); CCL-1; and viperin (an anti-viral molecule) [62]. The treatment of carp phagocytes with a combination of carp IFN γ and LPS resulted in increased expression of TNF α ; IL-1 β ; IL-12 subunits p35 and isoforms of IL-12 p40 subunit [3]. The carp IFN γ also induced the expression of the CXCL-10 like chemokine, CXCLb, while inhibiting the LPS-induced expression of CXCL-8 isoforms,

CXCL-8_L1 and CXCL-8_L2 [3]. The latter is reminiscent of the mammalian IFN γ and CXCL-8 relationship, where shorter treatments of mammalian granulocytes with IFN γ causes decreased CXCL-8 production [23, 90, 120], while prolonged treatments increase the CXCL-8 mRNA and protein levels [90]. Additionally, mammalian blood monocytes and macrophage cell lines stimulated with IFN γ up-regulate CXCL-8 mRNA transcripts and protein levels [14, 36] due to post-transcriptional stabilization of the CXCL-8 mRNAs rather than gene expression [14]. Together, these findings suggest that the pro-inflammatory roles of IFN γ , including synergism with LPS, are conserved in cyprinid fish, while it is probable that the discrepancies in gene expression profiles may be due to distinct immune cell model systems used (primary kidney phagocytes versus cultured mature macrophages).

3.2.3. Interferon-gamma-related (IFN γ rel) cytokine

Using gene synteny analysis, Igawa *et al.* (2006) discovered two tandem IFN γ isoforms next to the fish IL-22 and IL-26 genes [82]. The corresponding IFN γ sequences, coined IFN γ 1 and IFN γ 2, were later renamed IFN γ -related (rel) and IFN γ respectively [166]. These proteins share only 17 % amino acid identity, but exhibit exon/intron organization similar to that of mammalian and fugu IFN γ , and have the IFN γ signature motif ([IV]-Q-X-[KQ]-A-X₂-E-[LF]-X₂-[IV]). Interestingly, only IFN γ but not IFN γ rel has a C-terminal nuclear localization signal (NLS).

The existence of two IFN γ isoforms, sharing all of the above characteristics was soon confirmed in siluriformes and other cypriniformes when IFN γ and IFN γ rel were identified in the catfish [122] and common carp [183], respectively. Catfish and carp were both reported to possess two distinct, alternatively spliced IFN γ transcripts, as well as a single transcript of IFN γ rel [122, 183]. The pattern of tissue gene expression of catfish IFN γ and IFN γ rel differed while both cytokines were expressed in various cell lines and immune cells, with highest expression observed in macrophages, T cells and NK cells [122]. Interestingly, low transcript levels of IFN γ rel but not IFN γ were also reported in a catfish B-cell line. Expression of the carp IFN γ increased in T cells after stimulation with PHA, while the mRNA levels of carp IFN γ rel increased in IgM⁺, B lymphocyte-enriched immune cell fractions, stimulated with high doses of LPS [183]. Furthermore, increased transcript levels of the carp IFN γ , but not IFN γ rel, were observed in the head kidneys of fish infected with *T. borreli*. The goldfish IFN γ and IFN γ rel exhibited similar mRNA levels across tissues and immune cell types [63].

3.2.4. Inflammatory roles of fish IFN γ rel

While the siluriforme IFN γ rel has yet to be functionally characterized, there has been some insight into the functions of cyprinid IFN γ rel. Zebrafish IFN γ rel mRNA is detectable in freshly laid eggs, suggesting maternal supply of this transcript [174]. The IFN γ rel mRNA levels persist throughout embryonic development, while the expression of IFN γ is not detected until later stages of development. Embryos injected with *in vitro* transcribed IFN γ or

IFN γ rel mRNAs individually, exhibited similar immune gene expression changes, while combined injections further increased certain expression profiles, suggesting non-overlapping roles for the respective IFN γ proteins.

Morpholino knock-downs of either IFN γ or IFN γ rel alone had negligible effects on zebrafish embryo survival following *Escherichia coli* challenge, while knock-down of both IFN γ s resulted in substantially diminished survival following infection [174]. Interestingly, individual morpholino knockdowns of IFN γ or IFN γ rel caused decreased survival rates of embryos infected with *Yersinia ruckeri*, while double knockdowns had a further deleterious effect on embryo survival. Presumably IFN γ and IFN γ rel elicit some overlapping and some distinct antimicrobial mechanisms such that the presence of one cytokine may be sufficient for dealing with certain pathogens but not others. It is noteworthy that while the *E. coli* (strain DH5 α) is not a natural fish pathogen, the *Y. ruckeri* is [159].

A comprehensive functional investigation of recombinant goldfish (rg) IFN γ rel and rgIFN γ revealed that these molecules differed in respective capacities to modulate pro-inflammatory responses of goldfish PKMs [63]. While rgIFN γ conferred long-lasting ROI priming effects, rgIFN γ rel induced short-lived ROI priming, causing subsequent unresponsiveness to ROI priming by other recombinant cytokines (rgIFN γ or rgTNF α 2). While rgIFN γ elicited modest phagocytosis and nitric oxide responses in goldfish monocytes and macrophages, respectively [62, 63], rgIFN γ rel was a highly potent inducer of both responses. Interestingly, rgIFN γ and rgIFN γ rel induced different gene expression profiles in goldfish monocytes, where rgIFN γ rel elicited significantly greater expression of key inflammatory genes. Notably, while both cytokines induced the phosphorylation of Stat1, its nuclear translocation was only observed following rgIFN γ treatment. Together, these findings suggest a functional segregation of the goldfish type II interferons in the regulation macrophage antimicrobial functions.

Further confirmation of this functional dichotomy between the fish type II IFNs is warranted using *in vivo* and other *in vitro* fish models. Notably, the zebrafish IFN γ rel has recently also been demonstrated to elicit more robust pro-inflammatory gene expression than IFN γ in larvae microinjected with respective IFN expression constructs [110]. Furthermore, these zebrafish IFN γ rel-mediated effects were dependent on the myeloid transcription factor SP1, underlying the specificity of this cytokine for macrophage-lineage cells.

3.2.5. IFN γ receptors in fish

Despite the growing knowledge regarding the teleost type II IFNs, the receptor systems employed by these fish molecules remain poorly understood. The IFNGR1 and IFNGR2 chains were recently identified in the rainbow trout [55]. The expression of the IFNGR1 was generally greater than that of IFNGR2 and was subject to decrease following rIFN γ or rIL1 β stimulation. Furthermore RTG-2 trout fibroblast cells transfected with an IFNGR1 construct, or CHO cells transfected with constructs expressing IFNGR1 and IFNGR2, both bound rIFN γ , where, as in mammals, the expression of the IFNGR2 chain was essential for the IFN γ -induced activity. It should be noted that a reliable trout IFN γ reporter cell line has been estab-

lished and was effectively demonstrated to specifically increase luciferase reporter expression following IFN γ stimulation but not in response to a range of other stimuli [24, 25]. Hence, a system is now in place to elucidate the distinct binding and signaling mechanisms involved in the salmonid IFN γ biological processes.

In light of the functional differences between IFN γ and IFN γ rel, we postulated that these cytokines might function through distinct receptors. When we performed gene synteny analysis of IFNGR1, we observed that while some genes were localized to the chromosome bearing the known zebrafish IFNGR1 gene, other syntenic neighbours of the mammalian IFNGR1 were present on a distinct zebrafish chromosome. Further analysis of the chromosomal region flanked by these genes revealed a second gene, encoding a distinct IFNGR1 protein [60], (these genes were denoted IFNGR1-1 and IFNGR1-2). The corresponding goldfish receptor cDNA transcripts were identified. The fish IFNGR1 sequences displayed putative Jak1 and Stat1 binding sites, pivotal for the biological functions of the mammalian IFN γ [49, 68, 69]. While the zebrafish receptors displayed comparable tissue expression, the goldfish IFNGR1-1 exhibited substantially greater mRNA levels than the IFNGR1-2 in all tissues and immune cell types examined. In order to elucidate possible binding partners for the goldfish IFNGR1-1 and IFNGR1-2, recombinant forms of their extracellular domains were produced and *in vitro* binding assays were performed. While IFNGR1-1 bound exclusively to IFN γ rel (IFN γ 1), IFNGR1-2 bound strictly to IFN γ (IFN γ 2, receptors were named after the fact). It has recently been reported that morpholino knockdowns of IFNGR1-1, IFNGR1-2 or a putative IFNGR2 abolished zebrafish IFN γ -induced gene expression [1]. In contrast, only the knockdown of IFNGR1-1, but not the knockdown of IFNGR1-2 or IFNGR2, abrogated gene expression elicited by IFN γ rel. It was suggested that IFN γ might signal through a heterodimer of IFNGR1-1 and IFNGR1-2 and a homodimer of IFNGR2 while the IFN γ rel would ligate with a homodimeric IFNGR1-1 and an as of yet unidentified receptor 2 chains. Alternatively, since IFN γ rel is present in cyprinids early in development, the knockdown of its putative receptor, IFNGR1-1 in embryos might effect development of the components required for IFN γ function. A biological relationship of such nature would be phenotypically manifest as a loss of IFN γ function (as seen in the above zebrafish study).

It would appear that certain teleost species possess receptor signaling systems to facilitate a dichotomy of type II IFN functions. Presumably, the cyprinid IFNGR1 genes arose from duplications of an ancestral IFNGR1 and subsequently diverged in respective signaling mechanisms used, where the IFN γ rel-induced Stat1-phosphorylation might be an artifact, remnant of the ancestral gene. Indeed, the importance of the C-terminal NLS of fish (and mammalian) IFN γ has been demonstrated [217] while the lack of this NLS on IFN γ rel proteins is the key distinguishing feature of the latter cytokine. The leading model for mammalian IFN γ signaling [185] suggests that after IFN γ receptor ligation, Stat1 is delivered into the nucleus via the IFN γ NLS in a complex consisting of Stat1:IFNGR1:IFN γ . Therefore, due to a lack of an NLS, IFN γ rel may have evolved to utilize distinct signaling mechanism.

3.3. Interleukin-1 beta (IL-1 β)

3.3.1. Interleukin-1 cytokine family

The interleukin-1 cytokine family is becoming increasingly diverse, with new family members being discovered and/or assigned to this family. In addition to the well characterized IL-1 α , IL-1 β , IL-1 receptor antagonist (IL-1Ra) and IL-18 the new members of the family now include the IL-1F5-10, and IL-1F11 [34, 192]. Accordingly, it has been proposed that IL-1 α , IL-1 β , IL-1 receptor antagonist (IL-1Ra) should be renamed IL-1F1, IL-1F2, IL-1F3 and IL-1F4, respectively [177]. The exact biological roles of these new IL-1 cytokines (IL-1F5-11) have not been fully elucidated, and it was reported that concentrations of 100-1000 fold greater than those of IL-1 β are required to them to induce biological effects [177, 178]. These new family members will not be discussed further, instead the remainder of this section will deal with the classical IL-1 cytokines and primarily IL-1 β .

The IL-1 α [106] and IL-1 β [6] were initially identified as monocyte transcripts with only 23% amino acid identity but with structural similarities. Both of these cytokines are produced as leaderless 31 kDa pro-peptides that are cleaved to generate mature 17 kDa molecules, which mediated their respective effects by binding to the IL-1RI [37, 38, 40]. The synthesis of IL-1 has been reported in several cell types, including keratinocytes, Langerhan's cells, synovial fibroblasts, mesangial cells, astrocytes, microglia, corneal cells, gingival cells, thymic epithelial cells, in addition to myeloid and some lymphoid cell types (reviewed in references [38, 40]). Although IL-1 α and IL-1 β signal through the same receptor, they induce different biological functions. It has been suggested that IL-1 α is produced primarily by epithelial cells and keratinocytes [125] and is involved in mediating local inflammatory processes. In contrast, IL-1 β is synthesized by cells such as monocytes, macrophages, Langerhan's cells and dendritic cells [38], and mediates systemic inflammatory responses [37]. This is corroborated by the fact that while IL-1 α may act as a membrane-bound pro-IL-1 α through myristoylation of the protein [100, 181], IL-1 β requires intracellular processing for activation.

As mentioned, IL-1 α and IL-1 β are both produced as pro-peptides and while IL-1 α can mediate biological effects as pro-IL-1 α or as a mature IL-1 α following enzymatic cleavage (e.g. with calpain) [96], only the proteolytically cleaved mature IL-1 β , but not the pro-IL-1 β can elicit immune functions. In fact, the release of IL-1 β appears to be a two-step process, whereby the first stimulus such myeloid cell encounter of a pathogen results in increased synthesis and cytosolic accumulation of pro-IL-1 β , while a second (as of yet poorly defined) stimulus induces the proteolytic processing of pro-IL-1 β by caspase-1/IL-1 β -converting enzyme (ICE), and the subsequent release of the biologically active mature IL-1 β [39]. This processing event is further enhanced by the presence of extracellular ATP, which is recognized by the ATP receptor P2X₇R, causing an efflux of K⁺ and a concomitant activation of ICE [50]. A number of mechanisms have been proposed for the release of the mature IL-1 β into the extracellular milieu. These include: exocytosis of IL-1 β containing lysosomes; through microvesicular budding of the plasma membrane; release in exosomes by fusion of multivesicular bodies with the plasma membrane; export through specific membrane transporters; and cell-lysis-mediated release (reviewed in [46]). Possibly, these mechanisms are mutually ex-

clusive and/or cell type dependent. Further research in multiple model organism and cell types is warranted before specific mechanism(s) can be defined.

Both IL-1 α and IL-1 β bind to IL-1RI, resulting in recruitment of the IL-1R associated protein (IL-1RAcP), which amplifies the signal transduction [176]. The IL-1RI is structurally related to the toll-like pattern recognition receptors where the signal propagation through the IL-1RI involves many of the same downstream signaling components (MyD88, IL-1R associated kinase (IRAK), and NF κ B) as those employed in TLR signaling [139]. IL-1 α and IL-1 β also bind to the IL-1RII, but because this receptor lacks the intracellular signaling components of IL-1RI, it functionally serves as a “decoy” receptor by dampening the IL-1 α/β signal transduction [31]. Additionally, the IL-1 receptor antagonist (IL-1Ra) exhibits competitive inhibition of the IL-1 α/β signaling by interacting with the IL-1RI without eliciting the downstream signaling events [31].

Unlike most other cytokines and growth factors IL-1 α and IL-1 β (primarily IL-1 β) target nearly every cell type and induce a range of biological processes (reviewed in references [37, 38, 41]). Some of the numerous pro-inflammatory roles of IL-1 β include increases in collagen and pro-collagenase synthesis; increase of chondrocyte protease and proteoglycan release; increase in osteoclast-activating factor release and hence bone resorption; induction of the synthesis of lipid mediators such as PGE₂; and enhancement of the proliferation of fibroblasts, keratinocytes, mesangial, glial cells and smooth muscle cells. IL-1 β also induces chemotaxis of T and B cells, the synthesis of thromboxane by neutrophils and monocytes, basophil histamine release and eosinophil degranulation. Additionally, IL-1 β induces synthesis of type I IFNs, endothelial plasminogen activator inhibitors, and expression of leukocyte adherence receptors on endothelial cell surfaces. Based on the above, it is not surprising that IL-1 β has been implicated in numerous disorders including cardiac disease [15], rheumatoid arthritis [92], and neurodegenerative diseases [71].

3.3.2. Identification of IL-1 in fish

Fish were first suspected of possessing an IL-1 homolog when the mammalian PBL-derived IL-1 was demonstrated to enhance the proliferation of catfish T-lymphocytes in response to ConA [74], while carp epithelial cells [175], carp macrophages and granulocytes [195] and catfish monocytes [47], were shown to produce factor(s) with properties similar to those of the mammalian IL-1. Gel filtration analysis of catfish monocyte revealed two distinct bands of approximately 70 kDa and 15 kDa, recognized by polyclonal antibodies raised against mammalian IL-1 α and IL-1 β . Paradoxically, the catfish 70 kDa molecule activated catfish (but not mouse) T cells, while the 15 kDa molecule activated mouse (but not catfish) T cells [47].

The first fish IL-1 β cDNA sequence was identified in trout and exhibited 49-56% amino acid identity to the mammalian IL-1 β [170]. Notably, the trout IL-1 β did not possess a putative ICE cleavage site required for the maturation-cleavage of the mammalian IL-1 β , while the gene expression of this trout cytokine could be induced in tissues and head kidney leukocytes following LPS stimulation [170, 216], suggesting its pro-inflammatory nature.

Although there have been no reports of a fish IL-1 α , recently a novel IL-1 family member, nIL-1F has been identified in trout and *Tetraodon* spp. and was reported to have IL-1 family signature motif as well as a putative ICE cleavage site [198]. The expression of nIL-1F increased after activation of macrophages with LPS or recombinant IL-1 β . Furthermore, a recombinant form of the C-terminal nIL-1F abrogated the rIL-1 β -induced immune gene expression in RTS-11 trout macrophage-like cells, suggesting a possible competitive inhibition through the trout IL-1RI. This mechanism would be analogous to the mammalian IL-1Ra, which also competes with IL-1 β for IL-1RI binding, without inducing downstream events.

3.3.3. Isoforms of IL-1 β in fish

Because many fish species are tetraploid and have undergone genome duplication events, it is not surprising that additional isoforms of IL-1 β exist in certain fish species. An identified second trout IL-1 β , denoted IL-1 β 2, also has the 6 exon/5 intron organization, 82% amino acid identity to the trout IL-1 β 1 and no putative ICE cleavage site [150]. In catfish, two IL-1 β genes have been described and shown to undergo distinct expression patterns following challenge with *Edwardsiella ictaluri* [201]. Additionally identified cDNA of another carp IL-1 β , IL-1 β 2, exhibited 74% identity with the carp IL-1 β 1 and 95-99% identity across individual IL-1 β 2 transcripts [48]. Since several distinct IL-1 β 2 sequences were identified in a homozygous individual, it was suggested that there might be multiple IL-1 β 2 genes. The expression of the carp IL-1 β 1 and IL-1 β 2 differed following immune stimuli where the expression of IL-1 β 1 gene was on average at least ten fold greater than that of IL-1 β 2. It was also reported that transcripts of IL-1 β 2 had high substitution numbers in the coding regions, including key areas predicted to be involved in receptor binding. In light of the above, it was suggested that the IL-1 β 2 may be a pseudogene [48]. Our observations in the goldfish, a close carp relative, support this theory. Notably, the predicted goldfish IL-1 β 2 protein is truncated compared to the IL-1 β 1, and while the expression of both IL-1 β isoforms is subject to change following immune stimuli, specific treatments elicit more robust changes in the expression of IL-1 β 1 compared to IL-1 β 2 (unpublished observations).

3.3.4. Maturation cleavage of the fish IL-1 β

As alluded to above, despite the functional similarities of the fish IL-1 β s and their mammalian counterpart, all fish IL-1 β proteins identified to date lack the typical ICE cleavage site necessary for the functional maturation of the mammalian IL-1 β 1. Despite this, evidence suggests that indeed the fish IL-1 β proteins undergo cleavage. Early fish IL-1 studies using anti-mammalian IL-1 α/β serum to surveys supernatants of activated fish cells demonstrated the recognition of distinct, multiple IL-1 protein species in these supernatants. While the catfish monocyte supernatants contained 70 kDa and 15 kDa molecules with IL-1-like activity [47], carp macrophages secreted 22 kDa and 15 kDa IL-1-like factors that were recognized by mammalian anti-IL-1 antibodies [195]. The immunoprecipitation experiments using the anti-mammalian IL-1 polyclonal antibodies recognized the 15kDa factor from the macrophage supernatants, suggesting possible maturation events of the carp IL-1 [195]. In an independent survey of PHA-stimulated carp leukocytes using a monoclonal anti-carp IL-1 β antibody,

the 15 kDa protein was confirmed to be a mature carp IL-1 β , with the putative carp IL-1 β cleavage site situated approximately 15 amino acids downstream of the mammalian ICE site [117]. Analysis of the supernatants of the trout macrophage cell line, RTS-11, using an anti-trout IL-1 β polyclonal antibody detected putative native, as well as potentially cleaved trout IL-1 β proteins of 29 kDa and 24 kDa, respectively [79]. When the RTS-11 cells were transfected with a plasmid encoding a C-terminally His⁶ tagged trout IL-1 β , and the RTS-11 supernatants assayed using an Ni-NTA-column specific for the His⁶ tagged IL-1 β , the authors detected primarily a mature, 24 kDa IL-1 β protein, confirming that the trout IL-1 β underwent maturation cleavage.

In an elegant set of studies it was demonstrated that combined immune stimuli of sea bream head kidney leukocytes resulted in an accumulation of a 30 kDa, pro-IL-1 β protein, which, unlike the mammalian counterpart [113], did not exhibit a maturation cleavage or secretion following stimulation of the cells with extra-cellular ATP [146]. Furthermore, sea bream peritoneal acidophilic granulocytes and peripheral blood leukocytes accumulated the 30 kDa form of IL-1 β following challenge with *Vibrio anguillarum* [27]. In contrast, the sea bream SAF-1 fibroblast cell line shed a mature, 22 kDa IL-1 β protein through microvesicular plasma membrane budding within 30 minutes of treatment with extracellular ATP [113]. Interestingly this IL-1 β maturation/shedding process could be ablated with a pharmacological inhibitor of the mammalian ICE, suggesting a presence of an orthologous sea bream enzyme responsible for this process.

The P2X₇R receptor is the primary receptor responsible for the recognition of extracellular ATP and the concomitant release of mature IL-1 β [50]. Upon ATP treatment and activation of the HEK 293 mammalian cell line expressing the rat P2X₇R, human ICE and sea bream IL-1 β , a non-cleaved (30 kDa) sea bream IL-1 β was secreted by these cells [109]. Interestingly, neither sea bream nor zebrafish P2X₇R expression in ATP-stimulated HEK 293 cells resulted in sea bream or mammalian IL-1 β secretion. In contrast, the expression of a chimeric P2X₇R bearing the sea bream ATP-binding and rat intracellular domains led to maturation/secretion of the mammalian IL-1 β , while the outcomes of this combination on the sea bream IL-1 β were not addressed [109]. The authors of this work suggest that the mechanisms involved in IL-1 β secretion are conserved across vertebrates while the distinct stimuli that elicit the maturation events are not. We propose that alternatively, functional specificity may stem from the P2X₇R intracellular signaling, activation of species specific ICE (or alternative maturation mechanisms), and the substrate specificity of the fish Caspase1/ICE. Notably, the above group also identified the sea bream Caspase 1 and demonstrated that the recombinant form of this fish enzyme cleaved a commercially available substrate, for which the mammalian counterpart holds specificity [107]. Additional studies are needed to establish the association between the sea bream Caspase 1/ICE, P2X₇R and IL-1 β .

3.3.5. IL-1 receptors of fish

Majority of the IL-1 receptor signaling components have been described in fish. A fish IL-1RI was first described in Atlantic salmon, with 43-44% similarity to chicken and 31% similarity to the human IL-1RI, respectively [186]. The expression of this gene increased in fish tissues

following LPS injection. The identification of a carp IL-1R1 was also described and the receptor characterized in the context of acute stress conditions [121].

The trout IL-1RII was identified through a selective subtractive hybridization of genes up-regulated following immune stimulation [164]. This receptor displayed low sequence identity with the mammalian IL-1RII, but exhibited surprisingly similar overall gene organization including a very short intracellular domain. The gene expression of the identified sea bream IL-1RII increased in stimulated macrophages to levels 15 times greater than IL-1 β expression [108], suggesting a conservation in the roles of the sea bream IL-1RII as a “decoy” receptor. Furthermore, sea bream IL-1RII expressed on HEK 293 cells bound the recombinant IL-1 β , confirming the specificity of this receptor-ligand pair.

Enhanced understanding of the evolutionary mechanisms responsible for shaping the vertebrate IL-1 biological pathways will be achieved through studies that examine the IL-1 receptor-ligand relationships and biological outcomes of these interactions in teleosts.

3.3.6. Inflammatory roles of the fish IL-1 β

Using the selective subtractive hybridization technique, a carp IL-1 β was identified [53] and the C-terminus of the cytokine produced as a recombinant protein [206]. This recombinant carp IL-1 β dimerized and enhanced carp antibody responses to *Aeromonas hydrophila*, where sera from carp co-injected with the IL-1 β and *A. hydrophila* had a greater agglutinating capacity than the respective controls.

A number of studies have since utilized recombinant technology to investigate the functions of the fish IL-1 β . A recombinant form of the mature trout IL-1 β (rtIL-1 β) was produced by Hong and co-workers [80] and shown to enhance the expression of the MHCII β chain, IL-1 β and COX-2 genes in trout head kidney leukocytes and the macrophage cell line, RTS-11. Functionally, rtIL-1 β elicited the proliferation of trout head kidney cells as well as the proliferation of a murine cell line, D10.G4.1, known for its dependence on the mammalian IL-1 β [80]. Also, rtIL-1 β enhanced the phagocytosis of yeast particles by trout head kidney cells [80] while peritoneal administrations of rtIL-1 β induced migration of trout leukocytes to the site of injection, enhanced phagocytosis of peritoneal cells and increased the systemic expression of IL-1 β , COX-2 and lysozyme II [77]. The injection of fish with rtIL-1 β also enhanced trout resistance to infection with the fish pathogen *A. salmonicida*. Additionally, the rtIL-1 β and the recombinant sea bass IL-1 β were demonstrated to induce Ca²⁺ mediated downstream signaling events, abrogated by leukocyte trypsin-treatments and indicating a requirement for receptor engagement [9]. Interestingly, these authors reported that IL-1 β of trout, sea bass and humans were highly species specific, which is in contradiction of the early fish IL-1 β carp and catfish work, where cross-reactivity was observed [9, 47, 74, 175, 195].

The distinct biological roles of individual sub-domains of the trout IL-1 β were also examined by generating appropriate peptides [78, 144, 145]. While a control scrambled peptide (P2) had no effect and the peptide corresponding to the putative trout IL-1 β receptor binding region (P1) had little effect on its own, a peptide (P3) corresponding to an alternative receptor binding area of the mammalian IL-1 β [5, 196], had chemotactic properties towards

head kidney cells alone or in combination with the P1 peptide (at low concentrations) [145]. Intraperitoneal injections of the rtIL-1 β P3 peptide induced peritoneal leukocyte migration and enhanced phagocytosis and reactive oxygen production by peritoneal cells. Additionally, the rtIL-1 β P3 conferred fish resistance to viral hemorrhagic septicemia virus (VHCV) early after injection. Surprisingly, injection of rtIL-1 β P1 caused an increase in *in vivo* expression of the antiviral gene Mx and inhibition of trout TNF α expression, while having no effects on the expression of a panel of other pro-inflammatory genes [78]. The injection of the rtIL-1 β P3 peptide resulted in a more robust, widespread pro-inflammatory response with increased expression in trout rtIL-1 β , IL-8 and lysozyme. This work suggests that the fish IL-1 β utilizes a highly complex receptor-ligand system, possibly unique to that of mammals.

The functional roles of the trout IL-1 β have been corroborated in others fish species. For example, injection of carp with a plasmid encoding the carp IL-1 β gene caused enhanced PHA-induced proliferation of carp lymphocytes, increased carp macrophage reactive oxygen production, enhanced phagocytosis and improved protection against *A. hydrophila* challenge [97]. A sea bass recombinant IL-1 β exhibited immuno-adjuvant properties when combined with rsIL-1 β in immunization trials against the pathogen, *V. anguillarum*. The sea bass IL-1 β also induced the proliferation of the murine IL-1 β reporter cell line (D10.G4.1), sea bass thymocytes, enhanced kidney leukocyte phagocytosis and activated peritoneal macrophages when administered i.p. [16, 17, 29]. The orange spotted grouper rIL-1 β stimulated the proliferation of grouper head kidney leukocytes and increased the gene expression of IL-1 β and COX-2 through p38 MAPK and Jnk signaling pathways [112]. Together, the above findings suggest that the functions of IL-1 β have been evolutionarily conserved in vertebrates.

4. Concluding remarks

A successful inflammatory response is defined by the presence and proficient coordination of cytokine networks consisting of hallmark mediators such as TNF α , IFN γ and IL-1 β . The synchronized involvement of these pleiotropic yet functionally distinct agents in the recruitment, regulation and functional polarization of inflammatory cells dictates the outcome of the mounted response. Thus, it can be argued that the inflammatory processes are largely defined by the efficacy of the individual and interdependent cytokine pathways.

The regulation of the vertebrate inflammatory response is complex, involving numerous mechanisms, some of which are poorly understood while others remain to be identified. This is particularly true for the teleost model systems, where lack of specific reagents for different fish species hampers our ability to examine different aspects of the regulation of inflammation at a mechanistic level. However, there is growing evidence that the key immune components required for effective inflammatory responses are present in teleosts. Notably, certain fish species possess additional pathways that regulate inflammatory processes (for example IFN γ rel and its receptor IFNGR1-1, novel chemokines and PRRs) that are distinct from those reported in mammals. The elucidation of the coordination of inflammatory responses by these factors may shed new light on the evolution of innate host defense mechanisms in lower vertebrates.

Figure 1. Schematic representation of the NADPH oxidase complex mobilization and reactive oxygen production following phagocyte activation. In a resting state, the gp91^{phox} and p22^{phox} components are membrane bound while the p40^{phox}, p47^{phox} and p67^{phox} components are located in the cytosol and the small G-protein Rac is GDP bound. Upon cell activation, Rac is rapidly converted from a GDP- to a GTP-bound state and facilitates the translocation and assembly of the cytosolic NADPH oxidase components at the cell membrane. Following PKC activation, Rap1 is thought to serve as the final switch in the activation of the NADPH complex. The activated NADPH oxidase complex accepts the electrons from the reduced NADPH and transfers these to molecular oxygen, forming the superoxide anion (O₂⁻). The generated O₂⁻ can subsequently be converted to other reactive oxygen species. Reviewed in references [124, 153, 154, 173].

Figure 2. Schematic representation of iNOS gene expression, iNOS protein synthesis and enzymatic production of nitric oxide by the iNOS dimer complex. Upon phagocyte activation, there is a substantial increase in the expression from the iNOS gene and de novo synthesis of the iNOS enzyme. The iNOS enzyme forms a dimer and associates with a Ca²⁺ bound calmodulin, stabilizing the structure and facilitating the function of the enzymatic complex. The iNOS dimer catalyzes the transfer of electrons from a reduced NADPH, through a series of cofactors (FAD, FMN, Fe, BH₄) in the oxidation of L-Arginine to L-Citruilline with a concomitant production of nitric oxide (NO[•]). The generated NO[•] can subsequently be converted to other reactive nitrogen species. Reviewed in references [2, 111, 126, 172].

Abbreviations

ConA: concanavalin A; **GAS:** γ -IFN-activated sequence; **Jak:** janus activated kinase; **ICE:** IL-1 cleaving enzyme; **IFN:** interferon; **IFNGR:** interferon gamma receptor; **IL:** interleukin; **IL-1R:** interleukin-1 receptor; **IL-1RAcP:** IL-1R associated protein; **iNOS:** inducible nitric oxide synthase; **IRAK:** IL-1R associated kinase; **IRF:** interferon regulatory factor; **MAF:** macrophage activating factor(s); **NK:** natural killer; **NLS:** nuclear localization signal; **NO:** nitric oxide; **NTR:** neurotrophin receptor; **PBL:** peripheral blood leukocyte; **PHA:** phytohemagglutinin; **PKC:** protein kinase C; **PKM:** primary kidney macrophage; **PMA:** phorbol myristate acetate; **PRR:** pattern recognition receptor; **rg:** recombinant goldfish; **RNI:** reactive nitrogen intermediates; **ROI:** reactive oxygen intermediates; **Stat:** signal transducer of activation transcription factor; **TACE:** TNF α cleaving enzyme; **TLR:** toll-like receptor; **TNF:** tumor necrosis factor; **TNFR:** tumor necrosis factor receptor.

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References

- [1] Aggad, D., C. Stein, D. Sieger, M. Mazel, P. Boudinot, P. Herbomel, J. P. Levrard, G. Lutfalla, and M. Leptin. 2010. In vivo analysis of Ifn-gamma1 and Ifn-gamma2 signaling in zebrafish. *J Immunol* 185:6774-6782.
- [2] Andrew, P. J., and B. Mayer. 1999. Enzymatic function of nitric oxide synthases. *Cardiovasc Res* 43:521-531.
- [3] Arts, J. A., E. J. Tijhaar, M. Chadzinska, H. F. Savelkoul, and B. M. Verburg-van Kemenade. 2010. Functional analysis of carp interferon-gamma: evolutionary conservation of classical phagocyte activation. *Fish Shellfish Immunol* 29:793-802.
- [4] Auffray, C., D. Fogg, M. Garfa, G. Elain, O. Join-Lambert, S. Kayal, S. Sarnacki, A. Cumano, G. Lauvau, and F. Geissmann. 2007. Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. *Science* 317:666-670.
- [5] Auron, P. E. 1998. The interleukin 1 receptor: ligand interactions and signal transduction. *Cytokine Growth Factor Rev* 9:221-237.

- [6] Auron, P. E., A. C. Webb, L. J. Rosenwasser, S. F. Mucci, A. Rich, S. M. Wolff, and C. A. Dinarello. 1984. Nucleotide sequence of human monocyte interleukin 1 precursor cDNA. *Proc Natl Acad Sci U S A* 81:7907-7911.
- [7] Banner, D. W., A. D'Arcy, W. Janes, R. Gentz, H. J. Schoenfeld, C. Broger, H. Loetscher, and W. Lesslauer. 1993. Crystal structure of the soluble human 55 kd TNF receptor-human TNF beta complex: implications for TNF receptor activation. *Cell* 73:431-445.
- [8] Belosevic, M., C. E. Davis, M. S. Meltzer, and C. A. Nacy. 1988. Regulation of activated macrophage antimicrobial activities. Identification of lymphokines that cooperate with IFN-gamma for induction of resistance to infection. *J Immunol* 141:890-896.
- [9] Benedetti, S., E. Randelli, F. Buonocore, J. Zou, C. J. Secombes, and G. Scapigliati. 2006. Evolution of cytokine responses: IL-1beta directly affects intracellular Ca²⁺ concentration of teleost fish leukocytes through a receptor-mediated mechanism. *Cytokine* 34:9-16.
- [10] Berton, G., L. Zeni, M. A. Cassatella, and F. Rossi. 1986. Gamma interferon is able to enhance the oxidative metabolism of human neutrophils. *Biochem Biophys Res Commun* 138:1276-1282.
- [11] Bluysen, H. A., R. Muzaffar, R. J. Vliestra, A. C. van der Made, S. Leung, G. R. Stark, I. M. Kerr, J. Trapman, and D. E. Levy. 1995. Combinatorial association and abundance of components of interferon-stimulated gene factor 3 dictate the selectivity of interferon responses. *Proc Natl Acad Sci U S A* 92:5645-5649.
- [12] Bobe, J., and F. W. Goetz. 2001. Molecular cloning and expression of a TNF receptor and two TNF ligands in the fish ovary. *Comp Biochem Physiol B Biochem Mol Biol* 129:475-481.
- [13] Boltana, S., C. Donate, F. W. Goetz, S. MacKenzie, and J. C. Balasch. 2009. Characterization and expression of NADPH oxidase in LPS-, poly(I:C)- and zymosan-stimulated trout (*Oncorhynchus mykiss* W.) macrophages. *Fish Shellfish Immunol* 26:651-661.
- [14] Bosco, M. C., G. L. Gusella, I. Espinoza-Delgado, D. L. Longo, and L. Varesio. 1994. Interferon-gamma upregulates interleukin-8 gene expression in human monocytic cells by a posttranscriptional mechanism. *Blood* 83:537-542.
- [15] Bujak, M., and N. G. Frangogiannis. 2009. The role of IL-1 in the pathogenesis of heart disease. *Arch Immunol Ther Exp (Warsz)* 57:165-176.
- [16] Buonocore, F., M. Forlenza, E. Randelli, S. Benedetti, P. Bossu, S. Meloni, C. J. Secombes, M. Mazzini, and G. Scapigliati. 2005. Biological activity of sea bass (*Dicentrarchus labrax* L.) recombinant interleukin-1beta. *Mar Biotechnol (NY)* 7:609-617.
- [17] Buonocore, F., M. Mazzini, M. Forlenza, E. Randelli, C. J. Secombes, J. Zou, and G. Scapigliati. 2004. Expression in *Escherichia coli* and purification of sea bass (*Dicentrarchus labrax* L.) recombinant interleukin-1beta. *Mar Biotechnol (NY)* 6:609-617.

- trarchus labrax) interleukin 1beta, a possible immunoadjuvant in aquaculture. *Mar Biotechnol* (NY) 6:53-59.
- [18] Cai, Z. H., L. S. Song, C. P. Gao, L. T. Wu, L. H. Qiu, and J. H. Xiang. 2003. [Cloning and expression of tumor necrosis factor (TNFalpha) cDNA from red seabream *pagrus major*]. *Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao* (Shanghai) 35:1111-1116.
 - [19] Camussi, G., F. Bussolino, G. Salvidio, and C. Baglioni. 1987. Tumor necrosis factor/cachectin stimulates peritoneal macrophages, polymorphonuclear neutrophils, and vascular endothelial cells to synthesize and release platelet-activating factor. *J Exp Med* 166:1390-1404.
 - [20] Carswell, E. A., L. J. Old, R. L. Kassel, S. Green, N. Fiore, and B. Williamson. 1975. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci U S A* 72:3666-3670.
 - [21] Cassatella, M. A., F. Bazzoni, R. M. Flynn, S. Dusi, G. Trinchieri, and F. Rossi. 1990. Molecular basis of interferon-gamma and lipopolysaccharide enhancement of phagocyte respiratory burst capability. Studies on the gene expression of several NADPH oxidase components. *The Journal of biological chemistry* 265:20241-20246.
 - [22] Cassatella, M. A., V. Della Bianca, G. Berton, and F. Rossi. 1985. Activation by gamma interferon of human macrophage capability to produce toxic oxygen molecules is accompanied by decreased Km of the superoxide-generating NADPH oxidase. *Biochem Biophys Res Commun* 132:908-914.
 - [23] Cassatella, M. A., I. Guasparri, M. Ceska, F. Bazzoni, and F. Rossi. 1993. Interferon-gamma inhibits interleukin-8 production by human polymorphonuclear leucocytes. *Immunology* 78:177-184.
 - [24] Castro, R., S. A. Martin, S. Bird, J. Lamas, and C. J. Secombes. 2008. Characterisation of gamma-interferon responsive promoters in fish. *Molecular immunology* 45:3454-3462.
 - [25] Castro, R., S. A. Martin, J. Zou, and C. J. Secombes. 2010. Establishment of an IFN-gamma specific reporter cell line in fish. *Fish Shellfish Immunol* 28:312-319.
 - [26] Chapman, B. S., and I. D. Kuntz. 1995. Modeled structure of the 75-kDa neurotrophin receptor. *Protein Sci* 4:1696-1707.
 - [27] Chaves-Pozo, E., P. Pelegrin, J. Garcia-Castillo, A. Garcia-Ayala, V. Mulero, and J. Meseguer. 2004. Acidophilic granulocytes of the marine fish gilthead seabream (*Sparus aurata* L.) produce interleukin-1beta following infection with *Vibrio anguillarum*. *Cell Tissue Res* 316:189-195.
 - [28] Chen, G., and D. V. Goeddel. 2002. TNF-R1 signaling: a beautiful pathway. *Science* 296:1634-1635.
 - [29] Chistiakov, D. A., F. V. Kabanov, O. D. Troepolskaya, and M. M. Tischenko. 2010. A variant of the interleukin-1beta gene in European sea bass, *Dicentrarchus labrax* L., is

associated with increased resistance against *Vibrio anguillarum*. *J Fish Dis* 33:759-767.

- [30] Clay, H., H. E. Volkman, and L. Ramakrishnan. 2008. Tumor necrosis factor signaling mediates resistance to mycobacteria by inhibiting bacterial growth and macrophage death. *Immunity* 29:283-294.
- [31] Colotta, F., F. Re, M. Muzio, R. Bertini, N. Polentarutti, M. Sironi, J. G. Giri, S. K. Dower, J. E. Sims, and A. Mantovani. 1993. Interleukin-1 type II receptor: a decoy target for IL-1 that is regulated by IL-4. *Science* 261:472-475.
- [32] Cooper, A. M., D. K. Dalton, T. A. Stewart, J. P. Griffin, D. G. Russell, and I. M. Orme. 1993. Disseminated tuberculosis in interferon gamma gene-disrupted mice. *The Journal of experimental medicine* 178:2243-2247.
- [33] Darnell, J. E., Jr., I. M. Kerr, and G. R. Stark. 1994. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* 264:1415-1421.
- [34] Debets, R., J. C. Timans, B. Homey, S. Zurawski, T. R. Sana, S. Lo, J. Wagner, G. Edwards, T. Clifford, S. Menon, J. F. Bazan, and R. A. Kastelein. 2001. Two novel IL-1 family members, IL-1 delta and IL-1 epsilon, function as an antagonist and agonist of NF-kappa B activation through the orphan IL-1 receptor-related protein 2. *J Immunol* 167:1440-1446.
- [35] Denicola, A., H. Rubbo, D. Rodriguez, and R. Radi. 1993. Peroxynitrite-mediated cytotoxicity to *Trypanosoma cruzi*. *Arch Biochem Biophys* 304:279-286.
- [36] Dhawan, S., A. Heredia, R. B. Lal, L. M. Wahl, J. S. Epstein, and I. K. Hewlett. 1994. Interferon-gamma induces resistance in primary monocytes against human immunodeficiency virus type-1 infection. *Biochem Biophys Res Commun* 201:756-761.
- [37] Dinarello, C. A. 1996. Biologic basis for interleukin-1 in disease. *Blood* 87:2095-2147.
- [38] Dinarello, C. A. 1988. Biology of interleukin 1. *Faseb J* 2:108-115.
- [39] Dinarello, C. A. 1998. Interleukin-1 beta, interleukin-18, and the interleukin-1 beta converting enzyme. *Ann N Y Acad Sci* 856:1-11.
- [40] Dinarello, C. A. 1998. Interleukin-1, interleukin-1 receptors and interleukin-1 receptor antagonist. *Int Rev Immunol* 16:457-499.
- [41] Dinarello, C. A. 1987. The biology of interleukin 1 and comparison to tumor necrosis factor. *Immunol Lett* 16:227-231.
- [42] Ding, A. H., C. F. Nathan, and D. J. Stuehr. 1988. Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages. Comparison of activating cytokines and evidence for independent production. *J Immunol* 141:2407-2412.

- [43] Dranoff, G. 2004. Cytokines in cancer pathogenesis and cancer therapy. *Nat Rev Cancer* 4:11-22.
- [44] Dubravec, D. B., D. R. Spriggs, J. A. Mannick, and M. L. Rodrick. 1990. Circulating human peripheral blood granulocytes synthesize and secrete tumor necrosis factor alpha. *Proc Natl Acad Sci U S A* 87:6758-6761.
- [45] Eck, M. J., B. Beutler, G. Kuo, J. P. Merryweather, and S. R. Sprang. 1988. Crystallization of trimeric recombinant human tumor necrosis factor (cachectin). *J Biol Chem* 263:12816-12819.
- [46] Eder, C. 2009. Mechanisms of interleukin-1beta release. *Immunobiology* 214:543-553.
- [47] Ellsaesser, C. F., and L. W. Clem. 1994. Functionally distinct high and low molecular weight species of channel catfish and mouse IL-1. *Cytokine* 6:10-20.
- [48] Engelsma, M. Y., R. J. Stet, J. P. Saeij, and B. M. Verburg-van Kemenade. 2003. Differential expression and haplotypic variation of two interleukin-1beta genes in the common carp (*Cyprinus carpio* L.). *Cytokine* 22:21-32.
- [49] Farrar, M. A., J. Fernandez-Luna, and R. D. Schreiber. 1991. Identification of two regions within the cytoplasmic domain of the human interferon-gamma receptor required for function. *The Journal of biological chemistry* 266:19626-19635.
- [50] Ferrari, D., C. Pizzirani, E. Adinolfi, R. M. Lemoli, A. Curti, M. Idzko, E. Panther, and F. Di Virgilio. 2006. The P2X7 receptor: a key player in IL-1 processing and release. *J Immunol* 176:3877-3883.
- [51] Fertsch, D., and S. N. Vogel. 1984. Recombinant interferons increase macrophage Fc receptor capacity. *J Immunol* 132:2436-2439.
- [52] Forlenza, M., S. Magez, J. P. Scharsack, A. Westphal, H. F. Savelkoul, and G. F. Wiegertjes. 2009. Receptor-mediated and lectin-like activities of carp (*Cyprinus carpio*) TNF-alpha. *J Immunol* 183:5319-5332.
- [53] Fujiki, K., D. H. Shin, M. Nakao, and T. Yano. 2000. Molecular cloning and expression analysis of carp (*Cyprinus carpio*) interleukin-1 beta, high affinity immunoglobulin E Fc receptor gamma subunit and serum amyloid A. *Fish Shellfish Immunol* 10:229-242.
- [54] Gabbiani, G. 1979. The role of contractile proteins in wound healing and fibrocontractive diseases. *Methods Achiev Exp Pathol* 9:187-206.
- [55] Gao, Q., P. Nie, K. D. Thompson, A. Adams, T. Wang, C. J. Secombes, and J. Zou. 2009. The search for the IFN-gamma receptor in fish: Functional and expression analysis of putative binding and signalling chains in rainbow trout *Oncorhynchus mykiss*. *Developmental and comparative immunology* 33:920-931.
- [56] Garcia-Castillo, J., E. Chaves-Pozo, P. Olivares, P. Pelegrin, J. Meseguer, and V. Mulero. 2004. The tumor necrosis factor alpha of the bony fish seabream exhibits the in

vivo proinflammatory and proliferative activities of its mammalian counterparts, yet it functions in a species-specific manner. *Cell Mol Life Sci* 61:1331-1340.

- [57] Gong, Y., P. Cao, H. J. Yu, and T. Jiang. 2008. Crystal structure of the neurotrophin-3 and p75NTR symmetrical complex. *Nature* 454:789-793.
- [58] Graham, S., and C. J. Secombes. 1990. Do fish lymphocytes secrete interferon- γ ? *J. Fish Biol.* 36:563-573.
- [59] Graham, S., and C. J. Secombes. 1988. The production of a macrophage-activating factor from rainbow trout *Salmo gairdneri* leucocytes. *Immunology* 65:293-297.
- [60] Grayfer, L., and M. Belosevic. 2009. Molecular characterization of novel interferon gamma receptor 1 isoforms in zebrafish (*Danio rerio*) and goldfish (*Carassius auratus* L.). *Molecular immunology* 46:3050-3059.
- [61] Grayfer, L., and M. Belosevic. 2009. Molecular characterization of tumor necrosis factor receptors 1 and 2 of the goldfish (*Carassius auratus* L.). *Molecular immunology* 46:2190-2199.
- [62] Grayfer, L., and M. Belosevic. 2009. Molecular characterization, expression and functional analysis of goldfish (*Carassius auratus* L.) interferon gamma. *Developmental and comparative immunology* 33:235-246.
- [63] Grayfer, L., E. G. Garcia, and M. Belosevic. 2010. Comparison of macrophage antimicrobial responses induced by type II interferons of the goldfish (*Carassius auratus* L.). *J Biol Chem* 285:23537-23547.
- [64] Grayfer, L., P. C. Hanington, and M. Belosevic. 2009. Macrophage colony-stimulating factor (CSF-1) induces pro-inflammatory gene expression and enhances antimicrobial responses of goldfish (*Carassius auratus* L.) macrophages. *Fish Shellfish Immunol* 26:406-413.
- [65] Grayfer, L., J. W. Hodgkinson, and M. Belosevic. 2011. Analysis of the antimicrobial responses of primary phagocytes of the goldfish (*Carassius auratus* L.) against *Mycobacterium marinum*. *Developmental and comparative immunology* 35:1146-1158.
- [66] Grayfer, L., J. W. Hodgkinson, S. J. Hitchen, and M. Belosevic. 2010. Characterization and functional analysis of goldfish (*Carassius auratus* L.) interleukin-10. *Molecular immunology* doi:10.1016/j.molimm.2010.10.013.
- [67] Grayfer, L., J. G. Walsh, and M. Belosevic. 2008. Characterization and functional analysis of goldfish (*Carassius auratus* L.) tumor necrosis factor- α . *Developmental and comparative immunology* 32:532-543.
- [68] Greenlund, A. C., M. A. Farrar, B. L. Viviano, and R. D. Schreiber. 1994. Ligand-induced IFN gamma receptor tyrosine phosphorylation couples the receptor to its signal transduction system (p91). *The EMBO journal* 13:1591-1600.

- [69] Greenlund, A. C., M. O. Morales, B. L. Viviano, H. Yan, J. Krolewski, and R. D. Schreiber. 1995. Stat recruitment by tyrosine-phosphorylated cytokine receptors: an ordered reversible affinity-driven process. *Immunity* 2:677-687.
- [70] Grell, M., E. Douni, H. Wajant, M. Lohden, M. Clauss, B. Maxeiner, S. Georgopoulos, W. Lesslauer, G. Kollias, K. Pfizenmaier, and P. Scheurich. 1995. The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor. *Cell* 83:793-802.
- [71] Griffin, W. S., J. G. Sheng, M. C. Royston, S. M. Gentleman, J. E. McKenzie, D. I. Graham, G. W. Roberts, and R. E. Mrak. 1998. Glial-neuronal interactions in Alzheimer's disease: the potential role of a 'cytokine cycle' in disease progression. *Brain Pathol* 8:65-72.
- [72] Gupta, J. W., M. Kubin, L. Hartman, M. Cassatella, and G. Trinchieri. 1992. Induction of expression of genes encoding components of the respiratory burst oxidase during differentiation of human myeloid cell lines induced by tumor necrosis factor and gamma-interferon. *Cancer Res* 52:2530-2537.
- [73] Hajjar, K. A., D. P. Hajjar, R. L. Silverstein, and R. L. Nachman. 1987. Tumor necrosis factor-mediated release of platelet-derived growth factor from cultured endothelial cells. *J Exp Med* 166:235-245.
- [74] Hamby, B. A., E. M. Huggins, Jr., L. B. Lachman, C. A. Dinarello, and M. M. Sigel. 1986. Fish lymphocytes respond to human IL-1. *Lymphokine Res* 5:157-162.
- [75] Hardie, L. J., L. H. Chappell, and C. J. Secombes. 1994. Human tumor necrosis factor alpha influences rainbow trout *Oncorhynchus mykiss* leucocyte responses. *Vet Immunol Immunopathol* 40:73-84.
- [76] Hirono, I., B. H. Nam, T. Kurobe, and T. Aoki. 2000. Molecular cloning, characterization, and expression of TNF cDNA and gene from Japanese flounder *Paralichthys olivaceus*. *J Immunol* 165:4423-4427.
- [77] Hong, S., S. Peddie, J. J. Campos-Perez, J. Zou, and C. J. Secombes. 2003. The effect of intraperitoneally administered recombinant IL-1beta on immune parameters and resistance to *Aeromonas salmonicida* in the rainbow trout (*Oncorhynchus mykiss*). *Developmental and comparative immunology* 27:801-812.
- [78] Hong, S., and C. J. Secombes. 2009. Two peptides derived from trout IL-1beta have different stimulatory effects on immune gene expression after intraperitoneal administration. *Comp Biochem Physiol B Biochem Mol Biol* 153:275-280.
- [79] Hong, S., J. Zou, B. Collet, N. C. Bols, and C. J. Secombes. 2004. Analysis and characterisation of IL-1beta processing in rainbow trout, *Oncorhynchus mykiss*. *Fish Shellfish Immunol* 16:453-459.
- [80] Hong, S., J. Zou, M. Crampe, S. Peddie, G. Scapigliati, N. Bols, C. Cunningham, and C. J. Secombes. 2001. The production and bioactivity of rainbow trout (*Oncorhynchus mykiss*) recombinant IL-1 beta. *Vet Immunol Immunopathol* 81:1-14.

- [81] Huang, S. H., W., Althage, A., Hemmi, S., Bluethmann, H., Kamijo, R., Vilcek, J., Zinkernagel, R. M., Aguet, M. 1993. Immune response in mice that lack the interferon- γ receptor. *Science* (New York, N.Y) 259.
- [82] Igawa, D., M. Sakai, and R. Savan. 2006. An unexpected discovery of two interferon gamma-like genes along with interleukin (IL)-22 and -26 from teleost: IL-22 and -26 genes have been described for the first time outside mammals. *Molecular immunology* 43:999-1009.
- [83] Ihle, J. N., and I. M. Kerr. 1995. Jaks and Stats in signaling by the cytokine receptor superfamily. *Trends Genet* 11:69-74.
- [84] Inoue, Y., S. Kamota, K. Ito, Y. Yoshiura, M. Ototake, T. Moritomo, and T. Nakanishi. 2005. Molecular cloning and expression analysis of rainbow trout (*Oncorhynchus mykiss*) interleukin-10 cDNAs. *Fish Shellfish Immunol* 18:335-344.
- [85] Ishibe, K., T. Yamanishi, Y. Wang, K. Osatomi, K. Hara, K. Kanai, K. Yamaguchi, and T. Oda. 2009. Comparative analysis of the production of nitric oxide (NO) and tumor necrosis factor-alpha (TNF-alpha) from macrophages exposed to high virulent and low virulent strains of *Edwardsiella tarda*. *Fish Shellfish Immunol* 27:386-389.
- [86] Iyengar, R., D. J. Stuehr, and M. A. Marletta. 1987. Macrophage synthesis of nitrite, nitrate, and N-nitrosamines: precursors and role of the respiratory burst. *Proc Natl Acad Sci U S A* 84:6369-6373.
- [87] Jang, S. I., L. J. Hardie, and C. J. Secombes. 1995. Elevation of rainbow trout *Oncorhynchus mykiss* macrophage respiratory burst activity with macrophage-derived supernatants. *Journal of leukocyte biology* 57:943-947.
- [88] Jurecka, P., I. Irnazarow, J. L. Stafford, A. Ruszczuk, N. Taverne, M. Belosevic, H. F. Savelkoul, and G. F. Wiegertjes. 2009. The induction of nitric oxide response of carp macrophages by transferrin is influenced by the allelic diversity of the molecule. *Fish Shellfish Immunol* 26:632-638.
- [89] Kadowaki, T., H. Harada, Y. Sawada, C. Kohchi, G. Soma, Y. Takahashi, and H. Inagawa. 2009. Two types of tumor necrosis factor-alpha in bluefin tuna (*Thunnus orientalis*) genes: Molecular cloning and expression profile in response to several immunological stimulants. *Fish Shellfish Immunol* 27:585-594.
- [90] Kasama, T., R. M. Strieter, N. W. Lukacs, P. M. Lincoln, M. D. Burdick, and S. L. Kunkel. 1995. Interferon gamma modulates the expression of neutrophil-derived chemokines. *J Investig Med* 43:58-67.
- [91] Kerr, I. M., and G. R. Stark. 1992. The antiviral effects of the interferons and their inhibition. *Journal of interferon research* 12:237-240.
- [92] Khan, S., J. D. Greenberg, and N. Bhardwaj. 2009. Dendritic cells as targets for therapy in rheumatoid arthritis. *Nat Rev Rheumatol* 5:566-571.

- [93] Kim, E. Y., J. J. Priatel, S. J. Teh, and H. S. Teh. 2006. TNF receptor type 2 (p75) functions as a costimulator for antigen-driven T cell responses in vivo. *J Immunol* 176:1026-1035.
- [94] Kim, E. Y., and H. S. Teh. 2004. Critical role of TNF receptor type-2 (p75) as a costimulator for IL-2 induction and T cell survival: a functional link to CD28. *J Immunol* 173:4500-4509.
- [95] Klebanoff, S. J., M. A. Vadas, J. M. Harlan, L. H. Sparks, J. R. Gamble, J. M. Agosti, and A. M. Waltersdorff. 1986. Stimulation of neutrophils by tumor necrosis factor. *J Immunol* 136:4220-4225.
- [96] Kobayashi, Y., K. Yamamoto, T. Saido, H. Kawasaki, J. J. Oppenheim, and K. Matsushima. 1990. Identification of calcium-activated neutral protease as a processing enzyme of human interleukin 1 alpha. *Proc Natl Acad Sci U S A* 87:5548-5552.
- [97] Kono, T., K. Fujiki, M. Nakao, T. Yano, M. Endo, and M. Sakai. 2002. The immune responses of common carp, *Cyprinus carpio* L., injected with carp interleukin-1beta gene. *J Interferon Cytokine Res* 22:413-419.
- [98] Kriegler, M., C. Perez, K. DeFay, I. Albert, and S. D. Lu. 1988. A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. *Cell* 53:45-53.
- [99] Krishnan, L., L. J. Guilbert, T. G. Wegmann, M. Belosevic, and T. R. Mosmann. 1996. T helper 1 response against *Leishmania major* in pregnant C57BL/6 mice increases implantation failure and fetal resorptions. Correlation with increased IFN-gamma and TNF and reduced IL-10 production by placental cells. *J Immunol* 156:653-662.
- [100] Kurt-Jones, E. A., D. I. Beller, S. B. Mizel, and E. R. Unanue. 1985. Identification of a membrane-associated interleukin 1 in macrophages. *Proc Natl Acad Sci U S A* 82:1204-1208.
- [101] Laing, K. J., P. S. Grabowski, M. Belosevic, and C. J. Secombes. 1996. A partial sequence for nitric oxide synthase from a goldfish (*Carassius auratus*) macrophage cell line. *Immunol Cell Biol* 74:374-379.
- [102] Laing, K. J., L. J. Hardie, W. Aartsen, P. S. Grabowski, and C. J. Secombes. 1999. Expression of an inducible nitric oxide synthase gene in rainbow trout *Oncorhynchus mykiss*. *Developmental and comparative immunology* 23:71-85.
- [103] Laing, K. J., T. Wang, J. Zou, J. Holland, S. Hong, N. Bols, I. Hirono, T. Aoki, and C. J. Secombes. 2001. Cloning and expression analysis of rainbow trout *Oncorhynchus mykiss* tumour necrosis factor-alpha. *Eur J Biochem* 268:1315-1322.
- [104] Li, J., M. Zhang, and Y. C. Rui. 1997. Tumor necrosis factor mediated release of platelet-derived growth factor from bovine cerebral microvascular endothelial cells. *Zhongguo Yao Li Xue Bao* 18:133-136.

- [105] Lohmann-Matthes, M. L., B. Luttig, and S. Hockertz. 1991. Involvement of membrane-associated TNF in the killing of *Leishmania donovani* parasites by macrophages. *Behring Inst Mitt*:125-132.
- [106] Lomedico, P. T., U. Gubler, C. P. Hellmann, M. Dukovich, J. G. Giri, Y. C. Pan, K. Collier, R. Semionow, A. O. Chua, and S. B. Mizel. 1984. Cloning and expression of murine interleukin-1 cDNA in *Escherichia coli*. *Nature* 312:458-462.
- [107] Lopez-Castejon, G., M. P. Sepulcre, I. Mulero, P. Pelegrin, J. Meseguer, and V. Mulero. 2008. Molecular and functional characterization of gilthead seabream *Sparus aurata* caspase-1: the first identification of an inflammatory caspase in fish. *Molecular immunology* 45:49-57.
- [108] Lopez-Castejon, G., M. P. Sepulcre, F. J. Roca, B. Castellana, J. V. Planas, J. Meseguer, and V. Mulero. 2007. The type II interleukin-1 receptor (IL-1RII) of the bony fish gilthead seabream *Sparus aurata* is strongly induced after infection and tightly regulated at transcriptional and post-transcriptional levels. *Molecular immunology* 44:2772-2780.
- [109] Lopez-Castejon, G., M. T. Young, J. Meseguer, A. Surprenant, and V. Mulero. 2007. Characterization of ATP-gated P2X7 receptors in fish provides new insights into the mechanism of release of the leaderless cytokine interleukin-1 beta. *Molecular immunology* 44:1286-1299.
- [110] Lopez-Munoz, A., F. J. Roca, M. P. Sepulcre, J. Meseguer, and V. Mulero. 2011. Zebrafish larvae are unable to mount a protective antiviral response against waterborne infection by spring viremia of carp virus. *Developmental and comparative immunology* 34:546-552.
- [111] Lowenstein, C. J., and E. Padalko. 2004. iNOS (NOS2) at a glance. *J Cell Sci* 117:2865-2867.
- [112] Lu, D. Q., J. X. Bei, L. N. Feng, Y. Zhang, X. C. Liu, L. Wang, J. L. Chen, and H. R. Lin. 2008. Interleukin-1beta gene in orange-spotted grouper, *Epinephelus coioides*: molecular cloning, expression, biological activities and signal transduction. *Molecular immunology* 45:857-867.
- [113] MacKenzie, A., H. L. Wilson, E. Kiss-Toth, S. K. Dower, R. A. North, and A. Surprenant. 2001. Rapid secretion of interleukin-1beta by microvesicle shedding. *Immunity* 15:825-835.
- [114] MacKenzie, S., J. V. Planas, and F. W. Goetz. 2003. LPS-stimulated expression of a tumor necrosis factor-alpha mRNA in primary trout monocytes and in vitro differentiated macrophages. *Developmental and comparative immunology* 27:393-400.
- [115] Martin, E., C. Nathan, and Q. W. Xie. 1994. Role of interferon regulatory factor 1 in induction of nitric oxide synthase. *J Exp Med* 180:977-984.
- [116] Martin, J. H., and S. W. Edwards. 1993. Changes in mechanisms of monocyte/macrophage-mediated cytotoxicity during culture. Reactive oxygen intermediates are in-

volved in monocyte-mediated cytotoxicity, whereas reactive nitrogen intermediates are employed by macrophages in tumor cell killing. *J Immunol* 150:3478-3486.

- [117] Mathew, J. A., Y. X. Guo, K. P. Goh, J. Chan, B. M. Verburg-van Kemenade, and J. Kwang. 2002. Characterisation of a monoclonal antibody to carp IL-1 β and the development of a sensitive capture ELISA. *Fish Shellfish Immunol* 13:85-95.
- [118] Matsumoto, M., N. Tanaka, H. Harada, T. Kimura, T. Yokochi, M. Kitagawa, C. Schindler, and T. Taniguchi. 1999. Activation of the transcription factor ISGF3 by interferon-gamma. *Biol Chem* 380:699-703.
- [119] Mayumi, M., Y. Takeda, M. Hoshiko, K. Serada, M. Murata, T. Moritomo, F. Takizawa, I. Kobayashi, K. Araki, T. Nakanishi, and H. Sumimoto. 2008. Characterization of teleost phagocyte NADPH oxidase: molecular cloning and expression analysis of carp (*Cyprinus carpio*) phagocyte NADPH oxidase. *Molecular immunology* 45:1720-1731.
- [120] Meda, L., S. Gasperini, M. Ceska, and M. A. Cassatella. 1994. Modulation of proinflammatory cytokine release from human polymorphonuclear leukocytes by gamma interferon. *Cell Immunol* 157:448-461.
- [121] Metz, J. R., M. O. Huising, K. Leon, B. M. Verburg-van Kemenade, and G. Flik. 2006. Central and peripheral interleukin-1 β and interleukin-1 receptor I expression and their role in the acute stress response of common carp, *Cyprinus carpio* L. *J Endocrinol* 191:25-35.
- [122] Milev-Milovanovic, I., S. Long, M. Wilson, E. Bengten, N. W. Miller, and V. G. Chinchar. 2006. Identification and expression analysis of interferon gamma genes in channel catfish. *Immunogenetics* 58:70-80.
- [123] Ming, W. J., L. Bersani, and A. Mantovani. 1987. Tumor necrosis factor is chemotactic for monocytes and polymorphonuclear leukocytes. *J Immunol* 138:1469-1474.
- [124] Mizrahi, A., Y. Berdichevsky, Y. Ugolev, S. Molshanski-Mor, Y. Nakash, I. Dahan, N. Alloul, Y. Gorzalczy, R. Sarfstein, M. Hirshberg, and E. Pick. 2006. Assembly of the phagocyte NADPH oxidase complex: chimeric constructs derived from the cytosolic components as tools for exploring structure-function relationships. *J Leukoc Biol* 79:881-895.
- [125] Mizutani, H., R. Black, and T. S. Kupper. 1991. Human keratinocytes produce but do not process pro-interleukin-1 (IL-1) β . Different strategies of IL-1 production and processing in monocytes and keratinocytes. *J Clin Invest* 87:1066-1071.
- [126] Mori, M. 2007. Regulation of nitric oxide synthesis and apoptosis by arginase and arginine recycling. *J Nutr* 137:1616S-1620S.
- [127] Mosmann, T. R., and R. L. Coffman. 1989. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 7:145-173.

- [128] Moss, M. L., S. L. Jin, J. D. Becherer, D. M. Bickett, W. Burkhart, W. J. Chen, D. Hassler, M. T. Leesnitzer, G. McGeehan, M. Milla, M. Moyer, W. Rocque, T. Seaton, F. Schoenen, J. Warner, and D. Willard. 1997. Structural features and biochemical properties of TNF-alpha converting enzyme (TACE). *J Neuroimmunol* 72:127-129.
- [129] Mukhopadhyay, A., J. Suttles, R. D. Stout, and B. B. Aggarwal. 2001. Genetic deletion of the tumor necrosis factor receptor p60 or p80 abrogates ligand-mediated activation of nuclear factor-kappa B and of mitogen-activated protein kinases in macrophages. *J Biol Chem* 276:31906-31912.
- [130] Nahrendorf, M., F. K. Swirski, E. Aikawa, L. Stangenberg, T. Wurdinger, J. L. Figueiredo, P. Libby, R. Weissleder, and M. J. Pittet. 2007. The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. *J Exp Med* 204:3037-3047.
- [131] Naismith, J. H., B. J. Brandhuber, T. Q. Devine, and S. R. Sprang. 1996. Seeing double: crystal structures of the type I TNF receptor. *J Mol Recognit* 9:113-117.
- [132] Naismith, J. H., T. Q. Devine, T. Kohno, and S. R. Sprang. 1996. Structures of the extracellular domain of the type I tumor necrosis factor receptor. *Structure* 4:1251-1262.
- [133] Nascimento, D. S., P. J. Pereira, M. I. Reis, A. do Vale, J. Zou, M. T. Silva, C. J. Secombes, and N. M. dos Santos. 2007. Molecular cloning and expression analysis of sea bass (*Dicentrarchus labrax* L.) tumor necrosis factor-alpha (TNF-alpha). *Fish Shellfish Immunol* 23:701-710.
- [134] Nathan, C., and Q. W. Xie. 1994. Nitric oxide synthases: roles, tolls, and controls. *Cell* 78:915-918.
- [135] Neumann, M., and E. Kownatzki. 1989. The effect of adherence on the generation of reactive oxygen species by human neutrophilic granulocytes. *Agents Actions* 26:183-185.
- [136] Neumann, N. F., D. R. Barreda, and M. Belosevic. 2000. Generation and functional analysis of distinct macrophage sub-populations from goldfish (*Carassius auratus* L.) kidney leukocyte cultures. *Fish Shellfish Immunol* 10:1-20.
- [137] Neumann, N. F., D. Fagan, and M. Belosevic. 1995. Macrophage activating factor(s) secreted by mitogen stimulated goldfish kidney leukocytes synergize with bacterial lipopolysaccharide to induce nitric oxide production in teleost macrophages. *Developmental and comparative immunology* 19:473-482.
- [138] Neumann, N. F., J. L. Stafford, D. Barreda, A. J. Ainsworth, and M. Belosevic. 2001. Antimicrobial mechanisms of fish phagocytes and their role in host defense. *Developmental and comparative immunology* 25:807-825.
- [139] O'Neill, L. A. 2000. The interleukin-1 receptor/Toll-like receptor superfamily: signal transduction during inflammation and host defense. *Sci STKE* 2000:re1.

- [140] Oladiran, A., and M. Belosevic. 2009. Trypanosoma carassii hsp70 increases expression of inflammatory cytokines and chemokines in macrophages of the goldfish (*Carassius auratus* L.). *Developmental and comparative immunology* 33:1128-1136.
- [141] Olavarria, V. H., L. Gallardo, J. E. Figueroa, and V. Mulero. Lipopolysaccharide primes the respiratory burst of Atlantic salmon SHK-1 cells through protein kinase C-mediated phosphorylation of p47phox. *Developmental and comparative immunology* 34:1242-1253.
- [142] Ordas, M. C., M. M. Costa, F. J. Roca, G. Lopez-Castejon, V. Mulero, J. Meseguer, A. Figueras, and B. Novoa. 2007. Turbot TNFalpha gene: molecular characterization and biological activity of the recombinant protein. *Molecular immunology* 44:389-400.
- [143] Parameswaran, N., and S. Patial. 2010. Tumor necrosis factor-alpha signaling in macrophages. *Crit Rev Eukaryot Gene Expr* 20:87-103.
- [144] Peddie, S., P. E. McLauchlan, A. E. Ellis, and C. J. Secombes. 2003. Effect of intraperitoneally administered IL-1beta-derived peptides on resistance to viral haemorrhagic septicaemia in rainbow trout *Oncorhynchus mykiss*. *Dis Aquat Organ* 56:195-200.
- [145] Peddie, S., J. Zou, C. Cunningham, and C. J. Secombes. 2001. Rainbow trout (*Oncorhynchus mykiss*) recombinant IL-1beta and derived peptides induce migration of head-kidney leucocytes in vitro. *Fish Shellfish Immunol* 11:697-709.
- [146] Pelegrin, P., E. Chaves-Pozo, V. Mulero, and J. Meseguer. 2004. Production and mechanism of secretion of interleukin-1beta from the marine fish gilthead seabream. *Developmental and comparative immunology* 28:229-237.
- [147] Pennica, D., W. J. Kohr, B. M. Fendly, S. J. Shire, H. E. Raab, P. E. Borchardt, M. Lewis, and D. V. Goeddel. 1992. Characterization of a recombinant extracellular domain of the type 1 tumor necrosis factor receptor: evidence for tumor necrosis factor-alpha induced receptor aggregation. *Biochemistry* 31:1134-1141.
- [148] Perez, C., I. Albert, K. DeFay, N. Zachariades, L. Gooding, and M. Kriegler. 1990. A nonsecretable cell surface mutant of tumor necrosis factor (TNF) kills by cell-to-cell contact. *Cell* 63:251-258.
- [149] Perussia, B. 1991. Lymphokine-activated killer cells, natural killer cells and cytokines. *Curr Opin Immunol* 3:49-55.
- [150] Pleguezuelos, O., J. Zou, C. Cunningham, and C. J. Secombes. 2000. Cloning, sequencing, and analysis of expression of a second IL-1beta gene in rainbow trout (*Oncorhynchus mykiss*). *Immunogenetics* 51:1002-1011.
- [151] Praveen, K., D. L. Evans, and L. Jaso-Friedmann. 2006. Constitutive expression of tumor necrosis factor-alpha in cytotoxic cells of teleosts and its role in regulation of cell-mediated cytotoxicity. *Molecular immunology* 43:279-291.

- [152] Rieger, A. M., B. E. Hall, and D. R. Barreda. 2010. Macrophage activation differentially modulates particle binding, phagocytosis and downstream antimicrobial mechanisms. *Developmental and comparative immunology* 34:1144-1159.
- [153] Robinson, J. M. 2009. Phagocytic leukocytes and reactive oxygen species. *Histochem Cell Biol* 131:465-469.
- [154] Robinson, J. M. 2008. Reactive oxygen species in phagocytic leukocytes. *Histochem Cell Biol* 130:281-297.
- [155] Roca, F. J., I. Mulero, A. Lopez-Munoz, M. P. Sepulcre, S. A. Renshaw, J. Meseguer, and V. Mulero. 2008. Evolution of the inflammatory response in vertebrates: fish TNF-alpha is a powerful activator of endothelial cells but hardly activates phagocytes. *J Immunol* 181:5071-5081.
- [156] Rubbo, H., A. Denicola, and R. Radi. 1994. Peroxynitrite inactivates thiol-containing enzymes of *Trypanosoma cruzi* energetic metabolism and inhibits cell respiration. *Arch Biochem Biophys* 308:96-102.
- [157] Ruddle, N. H. 1986. Activation of human polymorphonuclear neutrophil functions by interferon-gamma and tumor necrosis factors. *J Immunol* 136:2335-2336.
- [158] Russo, R., C. A. Shoemaker, V. S. Panangala, and P. H. Klesius. 2009. In vitro and in vivo interaction of macrophages from vaccinated and non-vaccinated channel catfish (*Ictalurus punctatus*) to *Edwardsiella ictaluri*. *Fish Shellfish Immunol* 26:543-552.
- [159] Ryckaert, J., P. Bossier, K. D'Herde, A. Diez-Fraile, P. Sorgeloos, F. Haesebrouck, and F. Pasmans. 2010. Persistence of *Yersinia ruckeri* in trout macrophages. *Fish Shellfish Immunol* 29:648-655.
- [160] Ryczczyn, M. A., M. R. Wilson, E. Bengten, G. W. Warr, L. W. Clem, and N. W. Miller. 1998. Mitogen and growth factor-induced activation of a STAT-like molecule in channel catfish lymphoid cells. *Molecular immunology* 35:127-136.
- [161] Sad, S., R. Marcotte, and T. R. Mosmann. 1995. Cytokine-induced differentiation of precursor mouse CD8⁺ T cells into cytotoxic CD8⁺ T cells secreting Th1 or Th2 cytokines. *Immunity* 2:271-279.
- [162] Saeij, J. P., R. J. Stet, B. J. de Vries, W. B. van Muiswinkel, and G. F. Wiegertjes. 2003. Molecular and functional characterization of carp TNF: a link between TNF polymorphism and trypanotolerance? *Developmental and comparative immunology* 27:29-41.
- [163] Saeij, J. P., R. J. Stet, A. Groeneveld, L. B. Verburg-van Kemenade, W. B. van Muiswinkel, and G. F. Wiegertjes. 2000. Molecular and functional characterization of a fish inducible-type nitric oxide synthase. *Immunogenetics* 51:339-346.
- [164] Sangrador-Vegas, A., S. A. Martin, P. G. O'Dea, and T. J. Smith. 2000. Cloning and characterization of the rainbow trout (*Oncorhynchus mykiss*) type II interleukin-1 receptor cDNA. *Eur J Biochem* 267:7031-7037.

- [165] Savan, R., T. Kono, D. Igawa, and M. Sakai. 2005. A novel tumor necrosis factor (TNF) gene present in tandem with the TNF- α gene on the same chromosome in teleosts. *Immunogenetics* 57:140-150.
- [166] Savan, R., S. Ravichandran, J. R. Collins, M. Sakai, and H. A. Young. 2009. Structural conservation of interferon gamma among vertebrates. *Cytokine Growth Factor Rev* 20:115-124.
- [167] Savan, R., and M. Sakai. 2004. Presence of multiple isoforms of TNF α in carp (*Cyprinus carpio* L.): genomic and expression analysis. *Fish Shellfish Immunol* 17:87-94.
- [168] Schirren, C. G., K. Scharffetter, R. Hein, O. Braun-Falco, and T. Krieg. 1990. Tumor necrosis factor α induces invasiveness of human skin fibroblasts in vitro. *J Invest Dermatol* 94:706-710.
- [169] Schroder, K., P. J. Hertzog, T. Ravasi, and D. A. Hume. 2004. Interferon- γ : an overview of signals, mechanisms and functions. *J Leukoc Biol* 75:163-189.
- [170] Secombes, C., J. Zou, G. Daniels, C. Cunningham, A. Koussounadis, and G. Kemp. 1998. Rainbow trout cytokine and cytokine receptor genes. *Immunol Rev* 166:333-340.
- [171] Secombes, C. J. 1987. Lymphokine-release from rainbow trout leucocytes stimulated with concanavalin A. Effects upon macrophage spreading and adherence. *Developmental and comparative immunology* 11:513-520.
- [172] Shen, Y., M. Naujokas, M. Park, and K. Ireton. 2000. InIB-dependent internalization of *Listeria* is mediated by the Met receptor tyrosine kinase. *Cell* 103:501-510.
- [173] Sheppard, F. R., M. R. Kelher, E. E. Moore, N. J. McLaughlin, A. Banerjee, and C. C. Silliman. 2005. Structural organization of the neutrophil NADPH oxidase: phosphorylation and translocation during priming and activation. *J Leukoc Biol* 78:1025-1042.
- [174] Sieger, D., C. Stein, D. Neifer, A. M. van der Sar, and M. Leptin. 2009. The role of gamma interferon in innate immunity in the zebrafish embryo. *Dis Model Mech* 2:571-581.
- [175] Sigel, M. M., B. A. Hamby, and E. M. Huggins, Jr. 1986. Phylogenetic studies on lymphokines. Fish lymphocytes respond to human IL-1 and epithelial cells produce an IL-1 like factor. *Vet Immunol Immunopathol* 12:47-58.
- [176] Sims, J. E. 2002. IL-1 and IL-18 receptors, and their extended family. *Curr Opin Immunol* 14:117-122.
- [177] Sims, J. E., M. J. Nicklin, J. F. Bazan, J. L. Barton, S. J. Busfield, J. E. Ford, R. A. Kastelein, S. Kumar, H. Lin, J. J. Mulero, J. Pan, Y. Pan, D. E. Smith, and P. R. Young. 2001. A new nomenclature for IL-1-family genes. *Trends Immunol* 22:536-537.

- [178] Smith, D. E., B. R. Renshaw, R. R. Ketchum, M. Kubin, K. E. Garka, and J. E. Sims. 2000. Four new members expand the interleukin-1 superfamily. *J Biol Chem* 275:1169-1175.
- [179] Staeheli, P. 1990. Interferon-induced proteins and the antiviral state. *Adv Virus Res* 38:147-200.
- [180] Stafford, J. L., E. C. Wilson, and M. Belosevic. 2004. Recombinant transferrin induces nitric oxide response in goldfish and murine macrophages. *Fish Shellfish Immunol* 17:171-185.
- [181] Stevenson, F. T., S. L. Bursten, C. Fanton, R. M. Locksley, and D. H. Lovett. 1993. The 31-kDa precursor of interleukin 1 alpha is myristoylated on specific lysines within the 16-kDa N-terminal propiece. *Proc Natl Acad Sci U S A* 90:7245-7249.
- [182] Stevenson, M. M., M. F. Tam, M. Belosevic, P. H. van der Meide, and J. E. Podoba. 1990. Role of endogenous gamma interferon in host response to infection with blood-stage *Plasmodium chabaudi* AS. *Infection and immunity* 58:3225-3232.
- [183] Stolte, E. H., H. F. Savelkoul, G. Wiegertjes, G. Flik, and B. M. Lidy Verburg-van Kemenade. 2008. Differential expression of two interferon-gamma genes in common carp (*Cyprinus carpio* L.). *Developmental and comparative immunology* 32:1467-1481.
- [184] Stuehr, D. J., and C. F. Nathan. 1989. Nitric oxide. A macrophage product responsible for cytostasis and respiratory inhibition in tumor target cells. *J Exp Med* 169:1543-1555.
- [185] Subramaniam, P. S., B. A. Torres, and H. M. Johnson. 2001. So many ligands, so few transcription factors: a new paradigm for signaling through the STAT transcription factors. *Cytokine* 15:175-187.
- [186] Subramaniam, S., C. Stansberg, L. Olsen, J. Zou, C. J. Secombes, and C. Cunningham. 2002. Cloning of a *Salmo salar* interleukin-1 receptor-like cDNA. *Developmental and comparative immunology* 26:415-431.
- [187] Takaoka, A., Y. Mitani, H. Suemori, M. Sato, T. Yokochi, S. Noguchi, N. Tanaka, and T. Taniguchi. 2000. Cross talk between interferon-gamma and -alpha/beta signaling components in caveolar membrane domains. *Science* 288:2357-2360.
- [188] Takaoka, A., and H. Yanai. 2006. Interferon signalling network in innate defence. *Cell Microbiol* 8:907-922.
- [189] Tartaglia, L. A., T. M. Ayres, G. H. Wong, and D. V. Goeddel. 1993. A novel domain within the 55 kd TNF receptor signals cell death. *Cell* 74:845-853.
- [190] Theiss, A. L., J. G. Simmons, C. Jobin, and P. K. Lund. 2005. Tumor necrosis factor (TNF) alpha increases collagen accumulation and proliferation in intestinal myofibroblasts via TNF receptor 2. *J Biol Chem* 280:36099-36109.

- [191] Thomson, L., A. Denicola, and R. Radi. 2003. The trypanothione-thiol system in *Trypanosoma cruzi* as a key antioxidant mechanism against peroxynitrite-mediated cytotoxicity. *Arch Biochem Biophys* 412:55-64.
- [192] Towne, J. E., K. E. Garka, B. R. Renshaw, G. D. Virca, and J. E. Sims. 2004. Interleukin (IL)-1F6, IL-1F8, and IL-1F9 signal through IL-1Rrp2 and IL-1RAcP to activate the pathway leading to NF-kappaB and MAPKs. *J Biol Chem* 279:13677-13688.
- [193] Uenobe, M., C. Kohchi, N. Yoshioka, A. Yuasa, H. Inagawa, K. Morii, T. Nishizawa, Y. Takahashi, and G. Soma. 2007. Cloning and characterization of a TNF-like protein of *Plecoglossus altivelis* (ayu fish). *Molecular immunology* 44:1115-1122.
- [194] van Strijp, J. A., M. E. van der Tol, L. A. Miltenburg, K. P. van Kessel, and J. Verhoef. 1991. Tumour necrosis factor triggers granulocytes to internalize complement-coated virus particles. *Immunology* 73:77-82.
- [195] Verburg-van Kemenade, B. M., F. A. Weyts, R. Debets, and G. Flik. 1995. Carp macrophages and neutrophilic granulocytes secrete an interleukin-1-like factor. *Developmental and comparative immunology* 19:59-70.
- [196] Vigers, G. P., L. J. Anderson, P. Caffes, and B. J. Brandhuber. 1997. Crystal structure of the type-I interleukin-1 receptor complexed with interleukin-1beta. *Nature* 386:190-194.
- [197] Wallach, D., E. E. Varfolomeev, N. L. Malinin, Y. V. Goltsev, A. V. Kovalenko, and M. P. Boldin. 1999. Tumor necrosis factor receptor and Fas signaling mechanisms. *Annu Rev Immunol* 17:331-367.
- [198] Wang, T., S. Bird, A. Koussounadis, J. W. Holland, A. Carrington, J. Zou, and C. J. Secombes. 2009. Identification of a novel IL-1 cytokine family member in teleost fish. *J Immunol* 183:962-974.
- [199] Wang, T., J. W. Holland, A. Carrington, J. Zou, and C. J. Secombes. 2007. Molecular and functional characterization of IL-15 in rainbow trout *Oncorhynchus mykiss*: a potent inducer of IFN-gamma expression in spleen leukocytes. *J Immunol* 179:1475-1488.
- [200] Wang, T., M. Ward, P. Grabowski, and C. J. Secombes. 2001. Molecular cloning, gene organization and expression of rainbow trout (*Oncorhynchus mykiss*) inducible nitric oxide synthase (iNOS) gene. *Biochem J* 358:747-755.
- [201] Wang, Y., Q. Wang, P. Baoprasertkul, E. Peatman, and Z. Liu. 2006. Genomic organization, gene duplication, and expression analysis of interleukin-1beta in channel catfish (*Ictalurus punctatus*). *Molecular immunology* 43:1653-1664.
- [202] Wang, Z. E., S. L. Reiner, S. Zheng, D. K. Dalton, and R. M. Locksley. 1994. CD4+ effector cells default to the Th2 pathway in interferon gamma-deficient mice infected with *Leishmania major*. *J Exp Med* 179:1367-1371.

- [203] Warner, S. J., and P. Libby. 1989. Human vascular smooth muscle cells. Target for and source of tumor necrosis factor. *J Immunol* 142:100-109.
- [204] Weiss, T., M. Grell, K. Sieminski, F. Muhlenbeck, H. Durkop, K. Pfizenmaier, P. Scheurich, and H. Wajant. 1998. TNFR80-dependent enhancement of TNFR60-induced cell death is mediated by TNFR-associated factor 2 and is specific for TNFR60. *J Immunol* 161:3136-3142.
- [205] Wheelock, E. F. 1965. Interferon-Like Virus-Inhibitor Induced in Human Leukocytes by Phytohemagglutinin. *Science* 149:310-311.
- [206] Yin, Z., and J. Kwang. 2000. Carp interleukin-1 beta in the role of an immuno-adjuvant. *Fish Shellfish Immunol* 10:375-378.
- [207] Yonemaru, M., K. E. Stephens, A. Ishizaka, H. Zheng, R. S. Hogue, J. J. Crowley, J. R. Hatherill, and T. A. Raffin. 1989. Effects of tumor necrosis factor on PMN chemotaxis, chemiluminescence, and elastase activity. *J Lab Clin Med* 114:674-681.
- [208] Young, H. A., and K. J. Hardy. 1995. Role of interferon-gamma in immune cell regulation. *J Leukoc Biol* 58:373-381.
- [209] Young, J. D., C. C. Liu, G. Butler, Z. A. Cohn, and S. J. Galli. 1987. Identification, purification, and characterization of a mast cell-associated cytolytic factor related to tumor necrosis factor. *Proc Natl Acad Sci U S A* 84:9175-9179.
- [210] Zhang, M., Z. Z. Xiao, and L. Sun. 2011. Suppressor of cytokine signaling 3 inhibits head kidney macrophage activation and cytokine expression in *Scophthalmus maximus*. *Developmental and comparative immunology* 35:174-181.
- [211] Zhao, C., H. Zhang, W. C. Wong, X. Sem, H. Han, S. M. Ong, Y. C. Tan, W. H. Yeap, C. S. Gan, K. Q. Ng, M. B. Koh, P. Kourilsky, S. K. Sze, and S. C. Wong. 2009. Identification of novel functional differences in monocyte subsets using proteomic and transcriptomic methods. *J Proteome Res* 8:4028-4038.
- [212] Zhao, X., M. Mohaupt, J. Jiang, S. Liu, B. Li, and Z. Qin. 2007. Tumor necrosis factor receptor 2-mediated tumor suppression is nitric oxide dependent and involves angiostasis. *Cancer Res* 67:4443-4450.
- [213] Zhu, L., C. Gunn, and J. S. Beckman. 1992. Bactericidal activity of peroxynitrite. *Arch Biochem Biophys* 298:452-457.
- [214] Ziegler-Heitbrock, L. 2007. The CD14⁺ CD16⁺ blood monocytes: their role in infection and inflammation. *J Leukoc Biol* 81:584-592.
- [215] Zou, J., A. Carrington, B. Collet, J. M. Dijkstra, Y. Yoshiura, N. Bols, and C. Secombes. 2005. Identification and bioactivities of IFN-gamma in rainbow trout *Oncorhynchus mykiss*: the first Th1-type cytokine characterized functionally in fish. *J Immunol* 175:2484-2494.

- [216] Zou, J., P. S. Grabowski, C. Cunningham, and C. J. Secombes. 1999. Molecular cloning of interleukin 1beta from rainbow trout *Oncorhynchus mykiss* reveals no evidence of an ice cut site. *Cytokine* 11:552-560.
- [217] Zou, J., S. Peddie, G. Scapigliati, Y. Zhang, N. C. Bols, A. E. Ellis, and C. J. Secombes. 2003. Functional characterisation of the recombinant tumor necrosis factors in rainbow trout, *Oncorhynchus mykiss*. *Developmental and comparative immunology* 27:813-822.
- [218] Zou, J., C. J. Secombes, S. Long, N. Miller, L. W. Clem, and V. G. Chinchar. 2003. Molecular identification and expression analysis of tumor necrosis factor in channel catfish (*Ictalurus punctatus*). *Developmental and comparative immunology* 27:845-858.
- [219] Zou, J., T. Wang, I. Hirono, T. Aoki, H. Inagawa, T. Honda, G. I. Soma, M. Ototake, T. Nakanishi, A. E. Ellis, and C. J. Secombes. 2002. Differential expression of two tumor necrosis factor genes in rainbow trout, *Oncorhynchus mykiss*. *Developmental and comparative immunology* 26:161-172.
- [220] Zou, J., Y. Yoshiura, J. M. Dijkstra, M. Sakai, M. Ototake, and C. Secombes. 2004. Identification of an interferon gamma homologue in Fugu, *Takifugu rubripes*. *Fish Shellfish Immunol* 17:403-409.