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Insights into teleost interferon-gamma biology: An update

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ABSTRACT

Interferon-gamma (IFN-ϒ) is probably one of the most relevant cytokines orchestrating the immune response in vertebrates. Although the activities mediated by this molecule are well known in mammals, several aspects of the IFN-ϒ system in teleosts remain a riddle to scientists. Numerous studies support a potentially similar role of the fish IFN-ϒ signalling pathway in some well-described immunological processes induced by this cytokine in mammals. Nevertheless, the existence in some teleost species of duplicated *ifng* genes and an additional gene derived from *ifng* known as interferon-γ-related (*ifngrel*), among other things, raises new interesting questions about the mode of action of these various molecules in fish. Moreover, certain IFN-ϒ-mediated activities recently observed in mammals are still fully unknown in fish. Another attractive but mainly unexplored curious property of IFN-ϒ in vertebrates is its potential dual role depending on the type of pathogen. In addition, some aspects mediated by this molecule could favour the resolution of a bacterial infection but be harmful in the context of a viral disease, and vice versa. This review collects old and new aspects of IFN-ϒ research in teleosts and discusses new questions and pathways of investigation based on recent discoveries in mammals.

1. Introduction: IFN-ϒ in mammals

Type II IFN, also known as IFN-gamma (IFN-ϒ), is a cytokine mainly produced by T lymphocytes (specifically the Th1 subset) and natural killer (NK) cells, although other immune cells are also producers of this cytokine [1]. IFN-ϒ is implicated in several aspects of immunity, including activation of macrophages and T helper (Th) lymphocytes, stimulation of antigen presentation, control of cell proliferation and apoptosis, induction of antiviral state, immunomodulation, inflammation and leukocyte trafficking, among other functions [2].

The activity of IFN-ϒ is mediated by the recognition of IFN-ϒ homodimers through specific receptors. This recognition triggers different immune activities through the JAK/STAT signalling pathway [3]. The IFN-ϒ receptor consists of a tetrameric receptor complex composed of two subunits: interferon-gamma receptor 1 (IFNGR1) and 2 (IFNGR2) [1]. Two ligand-binding chains (IFNGR1) interact with two signal-transducing chains (IFNGR2), and whereas IFNGR1 is highly expressed, IFNGR2 is usually the limiting factor in the IFN-ϒ signalling pathway since its expression is tightly regulated based on the cellular status [4]. After ligand binding, the Janus tyrosine kinases JAK1 and JAK2, which are constitutively associated with IFNGR1 and IFNGR2, respectively, become phosphorylated and subsequently phosphorylate signal transducer and activator of transcription 1 (STAT1) [4]. STAT1 phosphorylation induces its homodimerization and translocation into the nucleus to interact with the gamma-activated sequence (GAS) in the promoter region of genes induced by IFN-ϒ [3]. The nuclear localization signal (NLS) at the C-terminal tail of mammalian IFN-ϒ is essential for the bioactivity of this molecule and the correct translocation of STAT1 into the nucleus [5].

After IFN-ϒ signalling activation, the complex IFN-ϒ-IFNGR is internalized through endocytosis [6]. Although the internalization of the type I IFN receptors (IFNAR1 and IFNAR2) is essential for JAK/STAT signalling, this process does not seem to be necessary for IFN-ϒ activity [6]. Clathrin- and caveolin-dependent endocytosis is involved in the degradation or recycling of IFNGR to the cell surface [7,8]. Thus, the cells can regulate the presence of IFNGR on the cell surface and therefore their responsiveness to IFN-ϒ [8]. Some pathogens can downregulate the expression of IFNG receptors in immune cells by increasing their endocytosis rates as a strategy to limit the host immune response [9,10]. Another paradigmatic endocytic process of the IFN-ϒ-IFNGR complex is the translocation of this structure to the nucleus and the potential regulatory effects of this importation, although the mechanisms mediated by this phenomenon remain poorly understood [8].

The production and activity of IFN-ϒ is regulated by different molecules, which are critical for the effects mediated by this cytokine. After infection, antigen-presenting cells (APCs) secrete interleukin-12

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(IL-12), IL-18 and IL-1β, which are the main promoters of IFN-ϒ synthesis [2]. Nevertheless, the synthesis of this cytokine is modulated by a fine-tuned regulation, and it is repressed by its negative regulators, including IL-4, IL-10, transforming growth factor-beta (TGF-β) and glucocorticoids (GCs) [2]. The induction of IFN-ϒ by IL-12 is mediated by the phosphorylation of the transcription factor STAT4, but IL-18 and IL-1β mediate its induction via activator protein 1 (AP-1) activation [11]. The inhibitory effects of IL-4 are mediated by the activation of the STAT6 signalling pathway [12], and IL-10, via activation of STAT3, generates the expression of suppressor of cytokine signalling (SOCS) proteins, which are inhibitors of IFN-ϒ-signalling [13]. TGF-β pathway activation has been shown to inhibit the expression of T box expressed in T cells (T-bet) and STAT4, both mediating the overexpression of IFN-ϒ [14]. Finally, GCs inhibit IFN-ϒ signalling through the down-regulation of STAT1 expression, reducing IFN-ϒ-inducible gene expression [15]. At the same time, IFN-ϒ is able to modulate the expression of these molecules.

The activation of macrophages by IFN-ϒ has been well described in mammals. The broad activities of this molecule in the activation of innate responses in macrophages include the production of pro-inflammatory cytokines and chemokines, potentiation of microbial killing, and enhancement of antigen presentation [3]. Macrophages can be divided into two polarized subsets referred to as classically activated (M1) and alternatively activated (M2). Macrophage differentiation into M1 or M2 macrophages relies on microenvironmental signals [16], and these include the level of IFN-ϒ. IFN-ϒ promotes the polarization of macrophages towards an M1 profile [17]. M1 macrophages are characterized by the production of pro-inflammatory cytokines, are efficient producers of reactive oxygen species (ROS) and nitric oxide (NO) and promote the differentiation of Th cells in Th1 lymphocytes, but M2 macrophages present an anti-inflammatory and immunoregulatory profile and promote the generation of Th2 lymphocytes [16].

IFN-ϒ, in addition to the modulation of immune factors, induces metabolic reprogramming of immune cells [18–20], which has been especially studied in macrophages and Th cells. Upregulated glycolysis and oxidative phosphorylation were found to be required to produce IFN-ϒ in NK cells [21]. The effects of IFN-ϒ on the activation of macrophages have been mainly attributed to the interaction of STAT1 with the GAS sequence in the promoter region of genes induced by IFN-ϒ (pro-inflammatory cytokines, chemokines, nitric oxide synthase, antiviral genes, etc.). Nevertheless, recent investigations concluded that these activities are a consequence of the interplay between metabolic and immune factors due to the ability of IFN-ϒ to alter macrophage metabolism via suppression of mechanistic target of rapamycin complex 1 (mTORC1), which is a central metabolic regulator [19]. Su et al. [19] found that IFN-ϒ alters the macrophage cell state to potentiate Toll-like receptor-mediated responses through the suppression of mitogen-activated protein kinase (MAPK)-interacting kinases (MNKs) and mTORC1, which directly impact the activity of 5' cap-binding eukaryotic initiation factor 4E (eIF4E) and modulate mRNA translation. Another consequence of mTORC1 inhibition mediated by IFN-ϒ is increased autophagy [19], a mechanism promoting microbial killing and antigen presentation. Translational regulation of IFN-ϒ selectively affects pathways involved in cytokine expression, protein synthesis and cell metabolism [19]. When the authors investigated the mechanism by which IFN-ϒ inhibited mTORC1, they found that this effect is mediated by the induction of indoleamine-2-3-dioxygenase (IDO) [19]. Some amino acids are potent activators of the recruitment of mTORC1 to lysosomes, including tryptophan, and IDO catalyses tryptophan degradation, suppressing mTORC1 activity. Therefore, the higher autophagy induced by IFN-ϒ is directly related to its inhibitory effect on mTOR activity. Modulation of oxidative phosphorylation and mitochondrial pathways was also observed [19]. Then, it was found that the macrophage activation by lipopolysaccharide (LPS) includes a rapid activation of aerobic glycolysis and a reduction of oxidative phosphorylation ("Warburg effect"), which is mediated by IFN-ϒ [20]. M1 and M2 macrophages show different metabolic responses to activation. Whereas M1 macrophages obtain energy from glycolysis, M2 macrophages are dependent on oxidative phosphorylation [20].

IFN-ϒ has the same effect on the polarization of Th cell populations. IFN-ϒ, together with IL-12, favours the differentiation of naïve T lymphocytes into Th1 cells [22], which tend to produce pro-inflammatory responses and are the highest producers of IFN-ϒ among the immune cell types [23]. On the other hand, Th1-derived IFN-ϒ exerts an anti-proliferative effect on Th cells, especially affecting the Th2 subset due to the higher expression of the IFNGR2 subunit in these cells [24–26]. Although IFN-ϒ downregulates the expression of its own receptor in both Th cell subsets [26], the lower level of IFNGR2 in Th1 lymphocytes favours the Th1 rather than Th2 immunity during IFN-ϒ signalling [24–26]. Because Th1 cells promote cell-mediated immunity and Th2 humoral responses, the Th1−Th2 phenotype switch mediated by IFN-ϒ is a pivotal mechanism in the immune response. In addition to this, key metabolic pathways, such as glycolysis, fatty acid synthesis and mitochondrial metabolism, impact T cell activation and proliferation [27]. As occurs with the macrophage populations, Th1 cells possess a high glycolytic rate based on aerobic glycolysis, whereas Th2 cells depend on oxidative phosphorylation [28].

Therefore, it seems clear that IFN-ϒ mediates metabolic and immune functions that condition the response against pathogens. Although the activation of immune cells by IFN-ϒ, especially macrophages, has been documented in fish, the metabolic changes induced by this cytokine and their consequences in fighting infections remain unknown.

In this review, the objective was to explore the current knowledge of the IFN-ϒ system in teleosts, the similarities and differences with mammals, and future perspectives that would help to better understand this mechanism. The signalling pathway mediated by IFN-ϒ seems to also be highly conserved due to the identification of numerous homologue genes and equivalent mechanisms [29], and therefore, a very similar role of teleost IFN-ϒ to that observed in mammals could be assumed. Nevertheless, we are still far away from fully understanding the complete repertoire of functional activities regulated by IFN-ϒ in fish.

2. Teleost IFN-ϒ

IFN-ϒ, among other immune-relevant functions, is a powerful activator of macrophages. For that reason, it was initially coined as a macrophage-activating factor (MAF) [30]. Its activity was described in fish a long time ago [31,32], although it was difficult in that moment to fully characterize this molecule and to identify if it corresponded to the homologue molecule of the mammalian IFN-ϒ. As in mammals, fish *ifng* genes were located on the same cluster containing two class II cytokine genes belonging to the interleukin-10 family (*il22* and *il26*) [33]. Therefore, it is not strange that the first identification of a teleost *ifng* gene, reported in 2004 for fugu (*Takifugu rubripes*), was conducted by using synteny comparative analysis between human and fugu genomes [34]. Since then, numerous *ifng* genes have been reported for teleosts, including rainbow trout (*Oncorhynchus mykiss*) [35], zebrafish (*Danio rerio*) [36], spotted green pufferfish (*Tetraodon nigroviridis*) [36], channel catfish (*Ictalurus punctatus*) [37], Atlantic salmon (*Salmo salar*) [38], common carp (*Cyprinus carpio*) [39], goldfish (*Carassius auratus*) [40] and turbot (*Scophthalmus maximus*) [41], among others (Table 1; Fig. 1). Two slightly different *ifng* copies were found in Atlantic salmon [29], rainbow trout [42], or ginbuna crucian carp (*Carassius auratus langsdorfii*) [43] (Fig. 1). In addition to the three common teleost genome duplications, salmonids have suffered a 4th round of genome duplication [44], which facilitates the existence of several copies of different genes. This was also observed in common carp [45], although genome duplications in other carp species cannot be discounted.

A high degree of synteny conservation, genomic structure (4 exons/3 introns) and sequence similarity of mammalian and teleost IFN-ϒ

Table 1
Summary of *ifng* and *ifngrel* genes characterized in teleosts.

Species	*ifng* form	Acc. number	Publications
Takifugu rubripes	*ifng*	AJ616216	Zou et al. (2004) [34]
Oncorhynchus mykiss	*ifng1*	AJ616215	Zou et al. (2005) [35]; Purcell et al. (2009) [42]
Danio rerio	*ifng*	NM_212,864	Igawa et al. (2006) [36]
Tetraodon nigroviridis	*ifng*	KJ524455	Igawa et al. (2006) [36]; Yi et al. (2014) [148]
Ictalurus punctatus	*Ifng1* (*ifng* transcript variant)	DQ124250	Milev-Milovanovic et al. (2006) [37]
Ictalurus punctatus	*ifng2* (*ifng* transcript variant)	DQ124251	Milev-Milovanovic et al. (2006) [37]
Salmo salar	*ifng1*	FJ263446	Unpublished
Salmo salar	*ifng2*	AY795563	Robertsen (2006) [38]
Cyprinus carpio	*ifng*	AM168523	Stolte et al. (2008) [39]
Oncorhynchus mykiss	*ifng2*	FJ184375	Purcell et al. (2009) [42]
Carassius auratus	*ifng*	EU909368	Grayfer & Belosevic (2009) [40]
Gadus morhua	*ifng*	FJ356235	Furnes et al. (2009) [151]
Paralichthys olivaceus	*ifng*	AB435093	Matsuyama et al. (2009) [152]
Carassius auratus langsdorfii	*ifng1*	AB570431	Yabu et al. (2011) [43]
Carassius auratus langsdorfii	*ifng2*	AB570432	Yabu et al. (2011) [43]
Ctenopharyngodon idella	*ifng*	JX196701	Yang et al. (2013) [63]
Larimichthys crocea	*ifng*	XM_010751697	Chen et al. (2015) [153]
Labeo rohita	*ifng*	HQ667144	Parhi et al. (2015) [53]
Scophthalmus maximus	*ifng*	KX360748	Pereiro et al. (2016) [41]
Acanthopagrus schlegelii	*ifng*	KY921614	Xiang et al. (2017) [112]
Oreochromis niloticus	*ifng*	XM_003448130	Velázquez et al. (2017) [109]
Epinephelus coioides	*ifng*	Not provided	Peng et al. (2018) [51]
	***ifngrel* sequences**		
Ictalurus punctatus	*ifngrel*	DQ124249	Milev-Milovanovic et al. (2006) [37]
Danio rerio	*ifngrel*	NM_001020793	Igawa et al.(2006) [36]
Tetraodon nigroviridis	*ifngrel*	KJ524454	Igawa et al. (2006) [36]; Yi et al. (2014) [148]
Cyprinus carpio	*ifngrel*	AM261214	Stolte et al. (2008) [39]
Carassius auratus	*ifngrel*	GQ149696	Grayfer & Belosevic (2009) [47]
Ctenopharyngodon idella	*ifngrel*	FJ695519	Chen et al. (2010) [48]
Carassius auratus langsdorfii	*ifngrel1*	AB570433	Shibasaki et al. (2014) [49]
Carassius auratus langsdorfii	*ifngrel2*	AB614642	Shibasaki et al. (2014) [49]
Labeo rohita	*ifngrel*	KJ874352	Swain et al. (2015) [50]
Epinephelus coioides	*ifngrel*	Not provided	Peng et al. (2018) [51]

genes is observed [34,36,46]. As in mammals, teleost *ifng* genes possess the conserved IFN-ϒ signature motif ([IV]-Q-X-[KQ]-A-X_2-E – [LF]-X_2-[IV]). Indeed, deletion of the C-terminal region of rainbow trout Ifng resulted in impaired activity that abolished the expression of the *IFN-gamma-inducible protein 10* (*ip10*; *cxcl10*) gene [35].

Some bony fishes possess an additional *ifng* gene (Table 1; Fig. 1), and whereas the classical *ifng* is considered the orthologue to mammalian IFN-ϒ, the other one, named IFN-ϒ-related (*ifngrel*), is exclusive to teleosts. In this sense, the nomenclature adopted in the first identifications of these two *ifng* forms results in confusion. The "true" *ifng* gene was first named IFN-ϒ-2, and *ifngrel* was referred to as IFN-ϒ-1 [36,37,39]. Currently, the terms *ifng* and *ifngrel* are the most accepted. Due to the tandem location of both genes on the genome and to their existent, although low, similarities, it is supposed that this additional gene originated from a teleost-specific tandem duplication of *ifng* [36]. The presence of this complementary form of type II IFN was observed in

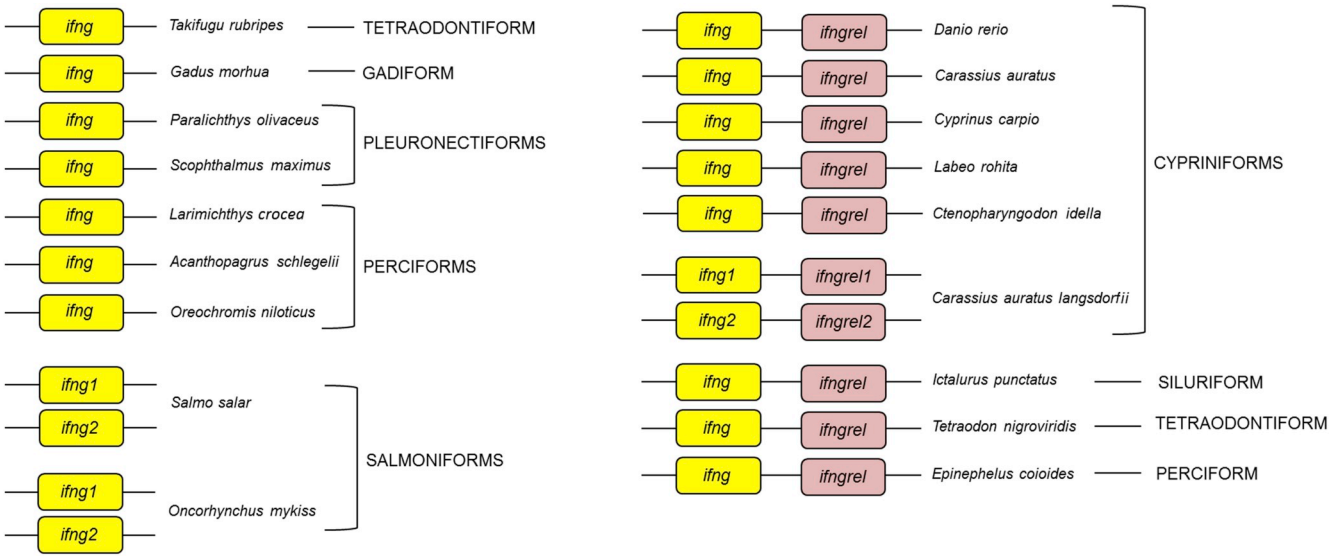

Fig. 1. Representation of the *ifng* and *ifngrel* genes described in different species of teleost fish. In addition to the orthologue gene to mammalian IFN-ϒ, some teleost species, especially cyprinids, possess an additional type II IFN named *ifngrel*. Moreover, additional whole-genome duplication occurred in salmonids and some cyprinids extended the repertoire of *ifng* and/or *ifngrel* genes in these species.

cyprinids [36,39,47–50] (Fig. 1), but also in some non-cyprinid species, such as channel catfish [37], spotted green pufferfish [36], and orange-spotted grouper (*Epinephelus coioides*) [51] (Fig. 1). In ginbuna crucian carp, even two *ifngrel* genes were identified [49]. This additional form possesses the IFN-ϒ signature motif but, in general, lacks the NLS characteristic of IFN-ϒ [37,39,48,51], although an NLS-like motif was found in some Ifngrel proteins [49]. Indeed, for the two Ifngrel proteins described for ginbuna crucian carp (Ifngrel1 and Ifngrel2), one of them have a functional NLS-like motif (Ifngrel1), whereas Ifngrel2 lacks the NLS-like sequence [49]. In goldfish, both Ifng and Ifngrel seems to dimerize to form homodimers [47], as occurs with mammalian Ifng. Nevertheless, in ginbuna crucian carp Ifng forms a homodimer, whereas Ifngrel seems to be monomeric [43,49], which could also occur in other species possessing Ifngrel.

Both Ifng and Ifngrel proteins present different numbers of N-glycosylation sites. Although the glycosylation of these molecules does not seem to be essential for their activities, it was shown that the glycan residues of IFN-ϒ enhance its resistance to protease degradation, providing a longer half-life [52]. In contrast to avian and mammalian IFN-ϒ, which contain at least two N-glycosylation sites, teleost Ifng possess only one [35,37,51,53], and Ifngrel possess one or two potential glycosylation sites [37,51].

3. Teleost IFN-ϒ receptors

Whereas the discovery of teleost *ifng* genes had raised expectations, identification of orthologue receptor sequences was also conducted in some teleosts but in a more modest way (Table 2). The first *in silico* analysis for identification of the teleost IFN-ϒ receptors was based on synteny and phylogenetic analyses of pufferfish, spotted green pufferfish [33] and zebrafish [54]. Indeed, a high degree of synteny conservation was also found for both IFN-ϒ receptors from fish to mammals [33,54,55].

The full-length rainbow trout Ifngr1 and Ifngr2 were cloned, and *in silico* analysis revealed significant homology and common characteristics with mammalian IFNGR1 and IFNGR2, respectively [55]. Transfection of the Chinese hamster ovary (CHO) cell line with expression plasmids encoding both Ifngr genes and treatment with recombinant Ifng showed, using immunofluorescence assays, the physical interaction of the Ifngr1/Ifngr2 complex with Ifng [55]. As in mammals, Ifngr2 seems to be the limiting chain [55].

Evidence about the binding of Ifng and Ifngrel to different receptor complexes was first reported for goldfish [47] and zebrafish [56]. In goldfish, two Ifngr1 were identified, Ifngr1-1 and Ifngr1-2, and whereas Ifngrel was preferentially recognized by Ifngr1-1, Ifng showed higher affinity for Ifngr1-2 than for Ifngr1-1 [47]. The existence of these isoforms was also observed in zebrafish. Aggad et al. [56] found, by using morpholinos, that zebrafish Ifng and Ifngrel bind to different receptor complexes, and whereas the Ifng receptor complex includes cytokine receptor family B (Crfb6) (Ifngr2), Crfb13 (Ifngr1-2) and Crfb17 (Ifngr1-1), Ifngrel only bound to Crfb17. This result suggested that zebrafish Ifng binds to a heterodimer composed of Ifngr1-1 and Ifngr1-2 and an Ifngr2 homodimer, but Ifngrel uses a homodimer of Ifngr1-1 and probably a different unknown Ifngr2 [56].

In ginbuna crucian carp, which possesses two *ifng* and two *ifngrel* genes [43,49], two sequences with homology to *ifngr1* were identified and were designated *ifngr1-1* and *ifngr1-2* [43]. Transfection of these receptors in HeLa cells and incubation with both recombinant Ifng proteins (Ifng1 and Ifng2) or one of the Ifngrel proteins showed that the Ifngr1-2 receptor is activated by Ifng2 and Ifng1-1 by Ifng1, but any of them was affected by Ifngrel [43]. Due to the homology of the ginbuna crucian carp Ifngr1-1 with the goldfish Ifngr1-1, the authors speculated that this receptor was probably specific for Ifngrel [43], as occurred in goldfish [47]. However, both ginbuna crucian carp Ifngr1 proteins are specific for two different forms of Ifng, and the specific receptor of Ifngrel proteins remains to be identified [43]. In spotted green pufferfish, the transfection of COS-7 cells with expression plasmids encoding Ifngr1-1 (Crfb17) or Ifngr1-2 (Crfb13) revealed that Ifng and Ifngrel bind to both Ifngr1-1 and Ifngr1-2; although in the case of Ifngrel, the binding was weaker to Ifngr1-2 than to Ifngr1-1 [57]. Because these results were obtained in a chimeric model, which implies the transfection of expression plasmids encoding fish Ifng receptors in mammalian cells, they should be considered cautiously.

According to the variety of *ifng* and *ifngrel* genes found in some teleosts, it is not surprising to observe the existence of more than one Ifngr1 and even the potential presence of more than one Ifngr2 on the genome, especially in those species with *ifngrel* (Fig. 2). In Atlantic salmon, although it does not possess *ifngrel*, two paralog *ifngr2* genes were found on different chromosomes as a consequence of the duplication of the cluster containing the type I interferon receptor genes and Ifngr2 [58]. These remarkable differences with the mammalian IFN-ϒ system results are exciting and open the door to further investigations. It seems evident that this variety of Ifng and Ifngr proteins could provide additional and differential properties to those observed in mammals and in those fish species with only one Ifng. Indeed, Grayfer et al. [59] tested the ability of the recombinant goldfish Ifng and Ifngrel proteins to induce the phosphorylation and translocation of Stat1, and although both proteins were able to alter the phosphorylation state of Stat1, only Ifng allowed its translocation into the nucleus, suggesting different activation pathways (Fig. 2).

Table 2
Summary of *ifngr* genes characterized in teleosts.

Species	*ifng* receptor	Acc. number	Publications
Tetraodon nidroviridis	*ifngr2/crfb6*	Not provided	Lutfalla et al. (2003) [33]; Stein et al. (2007) [54]
Tetraodon nidroviridis	*ifngr1-2/crfb13*	JF773393	Stein et al. (2007) [54]; Lu et al. (2014) [57]
Takifugu rubripes	*ifngr2/crfb6*	Not provided	Stein et al. (2007) [54]
Takifugu rubripes	*ifngr1-2/crfb13*	Not provided	Stein et al. (2007) [54]
Danio rerio	*ifngr2/crfb6*	EF014956	Levraud et al. (2007) [154]; Aggad et al. (2010) [56]
Danio rerio	*ifngr1-2/crfb13*	GQ901864	Stein et al. (2007) [54]; Aggad et al. (2010) [56]
Danio rerio	*ifngr1-1/crfb17*	GQ901865	Stein et al. (2007) [54]; Aggad et al. (2010) [56]
Carassius auratus	*ifngr1-1*	GQ149697	Grayfer & Belosevic (2009) [47]
Carassius auratus	*ifngr1-2*	GQ149698	Grayfer & Belosevic (2009) [47]
Oncorhynchus mykiss	*ifngr1*	EU244876	Gao et al. (2009) [55]
Oncorhynchus mykiss	*ifngr2*	EU244877	Gao et al. (2009) [55]
Carassius auratus langsdorfii	*ifngr1-1*	AB563726	Yabu et al. (2011) [43]
Carassius auratus langsdorfii	*ifngr1-2*	AB563727	Yabu et al. (2011) [43]
Tetraodon nidroviridis	*ifngr1-1*	JF773392	Lu et al. (2014) [57]
Salmo salar	*ifngr2a*	NM_001361121	Sun et al. (2014) [58]
Salmo salar	*ifngr2b*	NM_001361122	Sun et al. (2014) [58]
Acipenser dabryanus	*ifngr1*	MF741650	Luo et al. (2018) [143]
Acipenser dabryanus	*ifngr2*	MF741651	Luo et al. (2018) [155]

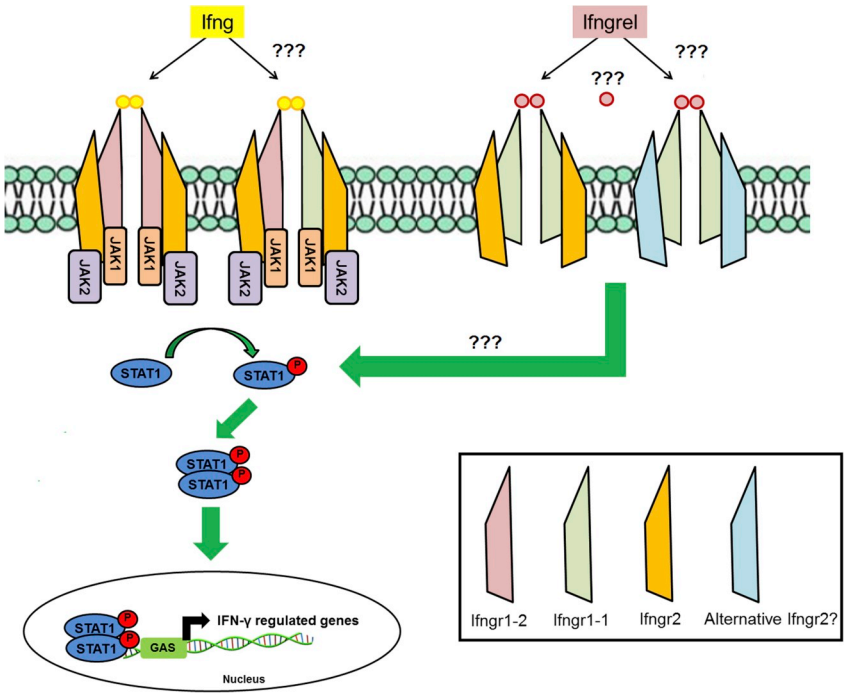

Fig. 2. Hypothetical interaction between type II IFNs and their receptor chains in teleost fish. Ifng homodimers probably interact with a tetrameric receptor composed of two homodimers of Ifngr1-2 and Ifngr2, although an Ifngr1 heterodimer (Ifng1-1 and Ifng1-2) has also been proposed for some fish species. According to the main findings, Ifngrel homodimers/monomers are likely recognized by an Ifngr1-1 homodimer and an Ifngr2 homodimer. However, the existence of an alternative Ifngr2 specific for Ifngrel cannot be discounted. Whereas Ifng signalling seems to be conserved across vertebrates, the exact mechanism of action of Ifngrel is less understood.

4. Immunological properties of teleost Ifng and ifngrel

The pioneering work focused on the bioactivity of Ifng in fish was conducted in rainbow trout in 2005 [35]. Since then, many other studies have tried to describe the properties of teleost Ifng and/or Ifngrel (Fig. 3; Table 3), which were mainly focussed in the potential role of these cytokines in the activation of macrophages.

4.1. Macrophage activation by teleost Ifng and Ifngrel: increase of respiratory burst, nitric oxide production and inflammation

Zou et al. [35] observed that recombinant trout Ifng was able to increase respiratory burst activity in head kidney macrophages. Moreover, the expression of *stat1* was also upregulated in the cell line RTS-11 in a dose-dependent manner, as well as other immune-related genes discussed below [35]. In large yellow croaker, recombinant Ifng enhanced respiratory burst in head kidney macrophages, induced the expression of *nitric oxide synthase* (*inos*) and release of NO, and upregulated the transcription of the pro-inflammatory cytokines *tumour necrosis factor a* (*tnfa*) and *il1b* [60]. The recombinant protein also affected the expression of *stat1*, *il2*, and the Ifng receptor *crfb13* and its own expression [60]. A similar pattern was observed in rohu after transfection of an expression plasmid encoding Ifng in peripheral blood lymphocytes (PBMCs), which induced the expression of *inos*, *il1b* and *ifng* [61]. In this same species, PBMC stimulation with recombinant Ifngrel resulted in increased expression of *inos* [50]. In olive flounder (*Paralichthys olivaceus*), recombinant Ifng treatment of whole kidney leukocytes also increased the transcription of *il1b*, *stat1* and *ifng* [62]. The stimulation of grass carp (*Ctenopharyngodon idella*) macrophages and lymphocytes (isolated from head kidney) with recombinant Ifng induced the production of NO by macrophages but not lymphocytes, and this effect was potentiated by the co-administration of recombinant Il1b [63].

Evidence suggesting that Ifngrel is probably more involved in humoral immunity was found in common carp. In this species, *ifngrel* (IFN-ϒ-1) was predominantly expressed in IgM^+ cell fractions (B lymphocyte-enriched), whereas *ifng* (IFN-ϒ-2) was mainly expressed in IgM^- cell fractions (T lymphocyte-enriched) [39]. In this work, the authors observed an increase in the expression of *ifngrel* in the head kidney and spleen at 3 weeks post-infection with the parasite *Trypanoplasma borreli* and suggested that this belated expression could be linked to antibody production [39]. Indeed, functional studies conducted with

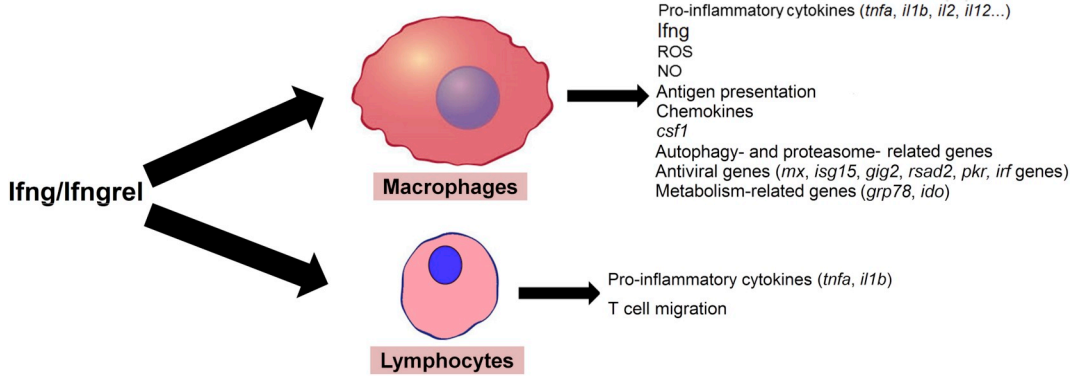

Fig. 3. Effect of fish Ifng and/or Ifngrel on the activation of macrophages. It has been shown that Ifng, and even Ifngrel, are able to induce the typical immune profile of M1 macrophages (inflammation, increased respiratory burst and NO production, synthesis of Ifng, etc.). On the other hand, the effects mediated by Ifng/Ifngrel in Th lymphocytes are still practically unknown in fish.

Table 3
Immune effects mediated by teleost Ifng and/or Ifgnrel both *in vitro* and *in vivo*.

Species	Isoform	Type of stimulation	Cell type	Effect	Publication
Oncorhynchus mykiss	Ifng	Recombinant protein	Head kidney macrophages	ROS	[35]
			RTS-11 cells	*stat1, mx, cxcl10, mhc2b*	
				cxcl10	[74]
				Cell survival (Salmonid alphavirus)	
			RTG-2 cells	*ifngr1, ifngr2*	[55]
			CHO cell line transfected with plasmids encoding Ifngr genes	Interaction of the Ifngr1/Ifngr2 complex with Ifng Ifngr2 identified as the limiting chain	
			RTS-11 cells	Antigen presentation-related genes	[67]
			TO and ASK cells	Cell survival (infectious pancreatic necrosis virus)	[75]
			TO cells	*mx, isg15, rsad2, pkr, gbp, ifna, irf1, irf3, irf7, irf8, irf9, cxcl10*	
			SHK-1 cells	*cxcl10, cxcl2-like, isg15, gig2, mx*	[76]
				Psma6, Grp78 proteins	[68]
			RTG-2 and RTS-11 cells	*il15*	[97]
			RTS-11 cells	*gbp*	[105]
				ido	[124]
			CHSE-Mx10 cells	Activation of the Mx1 promoter	[108]
Larimichthys crocea	Ifng	Recombinant protein	Head kidney macrophages	ROS	[60]
				NO	
				inos, tnfa, il1b, il2, stat1,t-bet, irf1, crfb13, ifng	
Labeo rohita	Ifng	Expression plasmid	Peripheral blood lymphocytes	*inos, il1b, mx, ifng*	[61]
	Ifngrel			*inos, ifngr1*	[50]
Paralichthys olivaceus	Ifng	Recombinant protein	Whole kidney leukocytes	*il1b, stat1, cxcl13, ifng*	[62]
			HINAE cells	*il1b, tnfa, stat1, cxcl13, ifng*	
			In vivo	Survival (*E. tarda*)	
Ctenopharyngodon idella	Ifng	Recombinant protein	Head kidney macrophages	NO	[63]
			Head kidney lymphocytes	NO =	
				il1b, tnfa	
				ifng =	
Cyprinus carpio	Ifng	Recombinant protein	Head kidney phagocytes	ROS	[64]
				NO	
				cxcb	
				Chemotaxis =	
			Granulocytes	Chemotaxis	
			EPC cells	Cell survival (SVCV)	[116]
			In vivo	Survival (SVCV)	
	Ifngrel		Head kidney phagocytes	*inos* =	[64]
Carassius auratus	Ifng	Recombinant protein	Head kidney macrophages	ROS	[40,59]
				NO	
				Phagocytic response	
				inos, tnfa, il1b, il12, ifng, cxcl8, ccl1, rsad2	[40]
				tlr3 (DOWN)	
				inos, tnfa,il1b, p47, gp91, irf1, irf2, irf8, irf9, ifngr1-1	[59]
				Stat1 phosphorilation	
				Stat1 nuclear translocation	
				csf1	
			Head kidney neutrophils	ROS	[40]
			Head kidney macrophages	*ifngr1-1, ifngr1-2*	[47]
				Interaction with Ifngr1-2	
	Ifngrel			ROS	[59]
				NO	
				Phagocytic response	
				inos, tnfa, il1b, gp91, cxcl8, ifngr1-1, cp, irf2, irf9	
				p40, irf7 (DOWN)	
				Stat1 phosphorilation	
				Stat1 nuclear translocation =	
				Interaction with Ifngr1-1	[47]
C. auratus langsdorfii	Ifng1	Recombinant protein	HeLa cells trasfected with *ifngr1-1* or *ifngr1-2*	Interaction with Ifngr1-1	[43]
				Interactiion between Stat1 and *irf1* and *ido* promoters	
			GTS9 cells	Cell survival (crucian carp hematopoietic necrosis virus)	
	Ifng2		HeLa cells trasfected with *ifngr1-1* or *ifngr1-2*	Interaction with Ifngr1-2	
				Interaction between Stat1 and *irf1* and *ido* promoters	
			GTS9 cells	Cell survival (crucian carp hematopoietic necrosis virus)	
	Ifngrel1		HeLa cells trasfected with *ifngr1-1* or *ifngr1-2*	No intection with Ifngr1-1 or Ifngr1-2	
			GTS9 cells	Cell survival (crucian carp hematopoietic necrosis virus)	
			In vivo skin allograft	Graft rejection	[66]
				T cell migration	
		Supernatant from HEK-293 cells	GTS9 cells	Cell survival (crucian carp hematopoietic necrosis virus)	
				Translocation to nucleus	
	Ifngrel2			Cell survival (crucian carp hematopoietic necrosis virus)	

(*continued on next page*)

Table 3 (*continued*)

Species	Isoform	Type of stimulation	Cell type	Effect	Publication
Tetraodon nigroviridis	Ifng	Recombinant protein	COS7 cells transfected with *fngr1-1* or *ifngr1-2*	Interaction with Ifngr1-1 and Ifngr1-2	[57]
			Head kidney cells	*ifngr1-1, ifngr1-2* *isg15, mx* NO ROS =	[65]
			Spleen cells	NO ROS =	
			In vivo	Survival (*V. parahaemolyticus*) = *tnfa* *ifng, ifngrel* (Up or Down) mir-29b	
	Ifngrel		COS7 cells transfected with *ifngr1-1* or *ifngr1-2*	Interaction with Ifngr1-1 and Ifngr1-2	[57]
			Head kidney cells	*ifngr1-2* *isg15* *mx* (Down) NO ROS =	[65]
			Spleen cells	NO ROS =	
			In vivo	Survival (*V. parahaemolyticus*) (Down) *tnfa, il1b* *ifng, ifngrel* (Up or Down) mir-29b	
Epinephelus coioides	Ifng	Recombinant protein	Blood lymphocytes	ROS NO	[51]
			Head kidney macrophages	mir-146a Traf6 (Down)	
	Ifnrel		Blood lymphocytes	ROS NO	
			Head kidney macrophages	mir-146a Traf6	
Danio rerio	Ifng	mRNA	*In vivo*	Developmental haematopoiesis Stat3 activation	[89]
	Ifngrel			*ifng, ifnphi1, lmp2*	[69]
	Ifng + Ifngrel			*ifngrel, ifnphi1, lmp2* *irge3, irge4*	
	Ifng	Expression plasmid		*irf1, stat1a, stat1b*	[110]
			EPC cells	*irf1, mx* *irf1* promoter activation *irf11* = *irf11 promoter activation* =	
	Ifngrel		*In vivo*	*irf1, stat1a, stat1b*	
			EPC cells	*irf1, mx* = *irf1 promoter activation* = *irf11* = *irf11 promoter activation* =	
	Ifng	Supernatant from HEK-293 cells	ZF4 cells	*mxb, mxc* Cell survival (SVCV)	[111]
			In vivo	Survival (SVCV) = Survival (*S. iniae*) = *ifnphi1, ifnphi3, ifng, stat1, inosa* *ifnphi2, pkr* (Down) *mxb,rsad2, il1b* (Up or Down) *mxc, il12a, il10, inosb* =	
		Expression plasmid		Survival (SVCV)	[117]
		Recombinant protein		Survival (SVCV)	
Oreochromis niloticus	Ifng	Recombinant protein	Head kidney cells	*mx*	[109]
Acanthopagrus schlegelii	Ifng	Expression plasmid	Brain (AsB) cells	*stat1, stat2, irf9, mx1, isg15* Reduction of viral titer (red spotted nervous necrosis virus)	[112]
Scophthalmus maximus	Ifng	Expression plasmid	*In vivo*	*csf1* *marco,mrc1,il18,asc,casp1, nox1,il1b tnfa,il15,* *il17,irf1,ifn1, ifn2,mhc1, mhc2* = *Survival (A. salmonicida)* = *Survival* (VHSV) = Gene modulation during *A. salmonicida* or VHSV infection	[41]

recombinant Ifngrel in common carp revealed its incapacity to induce the expression of *inos* in head kidney phagocytes neither alone nor in combination with LPS [64], whereas Ifng recombinant protein induced the expression of *inos* in combination with LPS and was able to induce the release of NO and ROS to the cell supernatant by itself [64]. This combination of Ifng and LPS also synergistically induced the expression of the pro-inflammatory cytokine genes *il1b*, *il12* (p35 and p40 chains) and *tnfa*, which reflects the classical activation of phagocytes by IFN-ϒ [64].

In goldfish, Grayfer et al. [59] conducted probably one of the most complete studies about the bioactivity of Ifng and Ifngrel. In a preliminary work, the authors found that recombinant Ifng is able to prime goldfish macrophages and neutrophils for enhanced respiratory burst activity, increase the phagocytic activity and NO production of macrophages and increase the expression of *inos* (isoforms a and b) and pro-inflammatory cytokines (*tnfa* – isoforms 1 and 2, *il1b* – isoforms 1 and 2, and *il12* – p35 and p40 chains) as well as its own transcription [40]. Then, the authors identified the goldfish *ifngrel* gene, and by using recombinant proteins, they observed that Ifng has long-lasting effects on the production of reactive oxygen intermediates (ROI) in monocytes, whereas Ifngrel had short-lived effects and even inhibitory activity on the ROI production induced by Ifng and Tnfa2 [59]. Contrary to that observed in common carp by Arts et al. [64], goldfish Ifngrel was the most active protein in induction of the expression of *inos* and NO production and induced significantly higher phagocytosis activity than Ifng [59]. Surprisingly, Ifngrel also induced a higher expression of the pro-inflammatory cytokine *tnfa2* than Ifng [59]. In spotted green pufferfish, the expression of inflammatory cytokines in the spleen was analysed after intraperitoneal injection of recombinant Ifng and Ifngrel [65]. Whereas *il1b* was only significantly upregulated by Ifngrel at 9 dpi, this gene was not affected by Ifng at the tested sampling points [65]. In contrast, *tnfa* was upmodulated by Ifng at 1 and 12 dpi and by Ifngrel at 6 and 9 dpi [65]. Both recombinant cytokines (Ifng and Ifngrel) were able to significantly increase the NO and respiratory burst response in head kidney and spleen cells, although Ifng reached higher levels of induction than Ifngrel [65]. Nevertheless, in the orange-spotted grouper, Ifng and Ifngrel induced a similar NO and respiratory burst response in blood leukocytes [51]. According to these observations, we can conclude that although some differential characteristics are observed for Ifng and Ifngrel, both molecules also possess some overlapping functions.

Therefore, the ability of teleost Ifng to activate macrophages seems to be highly conserved across vertebrates (Fig. 3). However, the effect of Ifng on the activation of T helper lymphocytes to polarize them towards a Th1 profile remains practically unexplored in fish. The increasing tools and cell markers in fish, especially in the model species zebrafish, could provide new methods to study this phenomenon in teleosts. Nevertheless, some works registered immune effects of teleost Ifng cytokines in the lymphoid cells (Fig. 3). In grass carp the media harvested from Ifng-stimulated lymphocytes was able to increase the production of NO in macrophages [63]. When the authors analysed the expression of *il1b*, *tnfa* and *ifng* in those lymphocytes, they observed an overexpression of *il1b* and *tnfa* but not *ifng*, and therefore, the cytokines released by Ifng-stimulated lymphocytes could be favouring the production of NO in macrophages [63]. Interestingly, it has been also shown that ginbuna crucian carp Ifng and Ifngrel forms could be involved on allograft rejection [66], which seems to be mainly mediated T lymphocytes both in mammals and teleost [66]. The four genes (*ifng1*, *ifng2*, *ifngrel1* and *ifngrel2*) increased their expression with the progression of skin allograft rejection [66], but only the administration recombinant Ifngrel1 enhanced graft rejection [66]. The higher Ifngrel1-mediated rejection is probably a consequence of the increased migration of $CD4^+$ and $CD8a^+$ T cells at the site of allografting and the secretion of cytotoxic molecules from T cells [66].

4.2. Antigen presentation

Another broadly described effect of IFN-ϒ in mammals is the potentiation of the antigen presentation processes. The enhancement of this mechanism in teleosts was first suggested for rainbow trout macrophages. Zou et al. [35] found an increase in the transcription of the *major histocompatibility complex class 2* (*mhc2*) *beta chain* in the macrophage cell line RTS-11 at 6 h post-stimulation with Ifng. Microarray analysis of rainbow trout RTS-11 macrophage cells stimulated for 6 h with recombinant Ifng also revealed Gene Ontology (GO) enrichment in "antigen presentation" and "ubiquitin-dependent protein catabolism", among other immune-related terms, compared to that of untreated cells [67]. Among those genes significantly modulated and directly related to antigen presentation were *transporter 1, ATP binding cassette subfamily B member* (*tap1*), *tapasin* and *major histocompatibility complex class 1* (*mhc1*) *heavy chain* [67]. The encoded proteins are associated with the antigen presentation of peptides generated by cytosolic degradation via the proteasome of proteins from intracellular pathogens, such as viruses. Proteins to be degraded are ubiquitinated and directed to the proteasome to generate smaller antigenic peptides. Therefore, it is not unusual to observe the modulation of different components of the proteasome (*lmp2 –psmb9–*, *lmp7 –psmb8–* and *mecl1 –psmb10–*). In contrast, in the Atlantic salmon cell line SHK-1, a proteomic analysis revealed a decreased representation of the proteasome subunit 6 alpha (Psma6) protein at 24 h after stimulation with recombinant Ifng compared to that of unstimulated control cells [68].

Next, other works reported the induction of antigen presentation-related molecules by Ifng and even by Ifngrel. In zebrafish, the microinjection of one-cell stage embryos with *ifng* and *ifngrel* mRNA induced an increase in the transcription of the proteasome component *lmp2*, especially in the case of *ifng* mRNA [69]. The intramuscular injection of an expression plasmid encoding *ifng* in turbot did not induce the expression of *mhc1* and *mhc2* in the head kidney after 48 h, but it was able to modulate their expression during infection [41].

4.3. Chemotaxis

In mammals, IFN-ϒ by itself is not a chemotactic molecule, but it is able to induce the expression of numerous chemokines that participate in the recruitment of neutrophils, macrophages, or T lymphocytes [70–72]. In teleost, this family of cytokines has enormously diverged from mammals due to extensive intrachromosomal gene duplications and to contact with a broad array of microorganisms, which have hindered the establishment of their concrete functionalities [73].

Different publications have reported the induction of chemokines in teleosts after stimulation with Ifng or Ifngrel. Trout macrophages overexpress *ip10* (*cxcl10*) after stimulation with Ifng [35], as also occurred in the macrophage cell line of Atlantic salmon (TO cells) [74,75]. Microarray analysis of the response induced by recombinant Ifng in the Atlantic salmon cell line SHK-1 also showed an increase in the expression of *ip10* and a *cxcl2*-like chemokine [76]. Stimulation of kidney leukocytes from olive flounder or non-immune HINAE cells with recombinant Ifng induced the expression of the chemokine *cxcl13* in both cases [62]. In common carp, overexpression of *cxcb* was observed after Ifng stimulation alone but especially in combination with LPS, and this synergistic effect was mainly observed in granulocyte-enriched fractions [64]. In contrast, the recombinant protein inhibited LPS-induced expression of *cxcl8_l2* in macrophage and neutrophilic granulocyte-enriched cell fractions, reflecting a modulatory role on the chemokine production induced by LPS [64]. However, common carp Ifngrel was incapable of stimulating these chemokines [64]. The expression of two chemokine receptors (*cxcr1* and *cxcr2*) was also analysed after Ifng stimulation of carp head kidney phagocytes, but no significant modulations were found for Ifng exposure in combination with LPS [64]. In this same work, the authors tested the induction of the chemotactic response towards zymosan-activated serum, and they

observed that recombinant Ifng treatment did not induce chemotaxis in the macrophage-enriched fraction, but the migratory properties of the granulocytes increased upon Ifng treatment [64].

Contradictory results were obtained for goldfish macrophages. In a first experiment describing the bioactivity of Ifng, the authors observed an increase in the transcription of *cxcl8* (*il8*) and *ccl1* at different times post-stimulation [40]; in a second experiment, *ccl1* was not affected by Ifng or Ifngrel [59]. Moreover, although both recombinant proteins induced an upregulation in the expression of *cxcl8*, the Ifngrel stimulation induced significantly higher expression levels compared to that induced by Ifng [59].

4.4. Cell differentiation, proliferation and apoptosis

It is known that IFN-ϒ is involved in the clonal expansion and apoptosis of immune cells and can even be a repressor of tumour cell growth [77–81]. In this sense, it can be established that this cytokine has dual function in inhibiting or promoting cell proliferation. Nevertheless, this cytokine is mainly considered as a growth inhibitor of many cell types and its anti-proliferative activity is principally mediated by the activation of the transcription factor STAT1 [78,82]. Activated STAT1 can inhibit proliferation by inducing cell cycle arrest or different forms of cell death [83].

Cell proliferation and cell death were two of the GO enriched terms observed after microarray analysis of the response induced by trout Ifng in the macrophage cell line RTS-11 [67], indicating the potential involvement of fish Ifng in both processes.

IFN-ϒ is also a key cytokine in the differentiation of most haematopoietic progenitor cells (HPCs) during inflammation or infection [84–88]. Indeed, the potential role of zebrafish Ifng (Ifng1-2) and its receptor Crfb17 as positive regulators of developmental haematopoiesis has been confirmed [89].

IFN-ϒ stimulates haematopoiesis during the early multipotent HPC stage and induces myeloid differentiation by inducing the expression of IL-6 [90]. The differentiation of monocytes to macrophages instead of dendritic cells is mediated by IFN-ϒ through the induction of macrophage colony-stimulating factor (M-CSF or CSF-1) and IL-6 [79]. At this point, a clarification should be made, because whereas IFN-ϒ acts locally as a macrophage differentiation, activation and pro-survival factor, it also inhibits monocyte proliferation [1]. On the contrary, CSF-1 is a critical inducer of macrophage proliferation at systemic level [91]. The interplay between both molecules is crucial in the definition of the macrophage populations. In goldfish, the *in vivo* administration of Csf1 increases the number of circulating monocytes in blood, and the addition of recombinant Csf1 to macrophage cultures extended their longevity and functionality [92,93]. The treatment of goldfish macrophages with recombinant Ifng increases the expression of *csf1*, although in a more modest way than recombinant Tnfa [93]. At the same time, compared to control macrophages, macrophages treated with recombinant Csf1 showed an increase in respiratory burst and NO production, higher phagocytosis and chemotaxis, and upmodulation of those pro-inflammatory cytokines typically induced by Ifng, as well as an increase in *ifng* expression [93], reflecting the positive feedback stablished in the IFN-ϒ system. An overexpression in the level of *csf1* was also found two days after intramuscular administration in juvenile turbot of an expression plasmid encoding *ifng* [41].

Interleukin-15 (IL-15) is a cytokine with a pivotal function in the proliferation of T lymphocytes, differentiation, maintenance and activation of NK cells and dendritic cells [94,95], and proliferation and antibody production by B cells [96]. Recombinant trout Ifng induced a powerful expression of *il15* in the rainbow trout-derived cell lines RTG-2 and RTS-11, and in the same way, recombinant trout Il15 was an inducer of Ifng in splenic leukocytes [97].

4.5. Autophagy

IFN-ϒ has also been described as an inducer of autophagy. The effects of this cytokine on the autophagy process are mainly mediated by two independent pathways: the Jak1-2/Stat1 signalling cascade and the PI3K/AKT/mTOR pathway, although a third route mediated by JAK1/2 and p38 MAPK has been described [98]. Through the classical JAK/STAT signalling pathway, the translocation of STAT1 into the nucleus induces the expression of members of the immunity-related guanosine triphosphatase (GTPase) family (IRG proteins; p47 GTPases) and the 65 kDa guanylate-binding protein family (GBP), which are directly involved in resistance against intracellular pathogens by inducing autophagy [99–101]. Nevertheless, taking into account the conserved functions of IFN-ϒ along vertebrates and the different autophagy activation pathways mediated by IFN-ϒ, this approach is probably a mechanism conserved in fish.

In recent years, the importance of autophagy as a defence mechanism in teleosts has been well documented, especially against intracellular bacteria and viruses [102–104]. Although the induction of autophagy by Ifng has not been analysed in teleosts, Sieger et al. [69] reported the induction of several members of the IRG family in zebrafish embryos injected with *ifng* and/or *ifngrel* mRNA, whereas members of the GBP family were not apparently affected. In rainbow trout, GBP transcripts were induced in RTS-11 cells stimulated with recombinant Ifng [105], and a strong overexpression of GBP was also observed in Atlantic salmon TO cells [75].

4.6. Antiviral mechanisms and protection against viral diseases

Although the main IFNs coordinating the antiviral response are those included in the category of type I IFNs, the effects mediated by IFN-ϒ are also pivotal for the control of viral infections [106]. Indeed, some redundant activities between type I and type II IFNs are mediated via the JAK/STAT signalling cascade, inducing the expression of an overlapping set of genes [107]. Therefore, IFN-ϒ is able, among other things, to induce the expression of multiple interferon-stimulated genes (ISGs), which are typically overexpressed by type I IFNs [107], revealing that both IFNs work together to combat viral infections. Nevertheless, it cannot be discounted that these inductions could be mediated by the expression of type I IFNs mediated by IFN-ϒ.

Several lines of evidence indicate that *myxovirus resistance* (*mx*) genes are commonly induced by Ifng in teleosts. Upregulation of the expression level of *mx* genes by recombinant trout Ifng stimulation was found in the trout cell line RTS-11 [35] and in the Atlantic salmon cell lines SHK-1 [76] and TO Ref. [75]. Moreover, the stimulation of a recombinant CHSE-214 cell line expressing an Mx1 promoter-luciferase system with this cytokine demonstrated that Ifng can initiate *mx* expression in teleosts [108]. In Nile tilapia (*Oreochromis niloticus*), the incubation of head kidney primary cells with the corresponding recombinant Ifng upregulated the transcription of an *mx* gene [109]. The transfection of an expression plasmid encoding Ifng in rohu PBMCs induced the expression of *mx* [61]. This effect also occurred with a plasmid encoding zebrafish Ifng in the EPC cell line, although this gene was not affected by the expression plasmid encoding Ifngrel [110]. In the zebrafish cell line ZF4, *mxb* and *mxc* were also induced after incubation with supernatants from HEK-293 cells transfected with an expression plasmid encoding Ifng [111], and in a black seabream (*Acanthopagrus schlegelii*) brain cell line overexpressing *ifng*, the gene *mx1* was also upregulated [112]. Interestingly, incubation of spotted green pufferfish kidney leukocytes with Ifng and Ifngrel showed an opposite *mx* induction, and whereas Ifng significantly increases the expression of this gene, Ifngrel inhibited its expression [65]. In these last two works, the *interferon-stimulated gene 15* (*isg15*) was also upregulated by the effect of Ifng overexpression [112] or Ifng and Ifngrel addition to head kidney cells [65]. This gene was modulated by recombinant rainbow trout Ifng in Atlantic salmon TO cells after 24 h of

stimulation [75], and a microarray analysis of the transcriptome of SHK-1 cells revealed induction of the *isg15* gene at 6 h post-stimulation [76]. In this microarray, another ISG involved in resistance against viruses was also induced by Ifng, *grass carp haemorrhagic virus (GCHV)-induced gene 2* (*gig2*) [76], which seems to be specific to non-amniote vertebrates [113,114]. Another example of an ISG modulated by Ifng in fish is *radical S-adenosyl methionine domain containing 2* (*rsad2*), also known as *viperin*, which was induced by Ifng in goldfish kidney-derived macrophages [40] and in Atlantic salmon TO cells [75]. Sun et al. [75] also found upregulation of the transcription of *interferon-induced, double-stranded RNA-activated protein kinase* (*pkr*).

The induction of these ISGs is probably mediated by other family of ISGs, the interferon-regulatory factors (IRFs), which can act in a type I IFN-dependent or -independent way to rapidly allow ISG expression before IFN itself can be produced [115]. Five IRF genes (*irf1*, *irf3*, *irf7*, *irf8* and *irf9*) were significantly overexpressed in Atlantic salmon TO cells at 24 h post-stimulation with trout Ifng [75]. In zebrafish embryos, microinjection of expression plasmids encoding Ifng or Ifngrel enhanced the expression of *irf1*, which was more pronounced in the case of Ifng than for Ifngrel [110]. By using luciferase plasmids containing different regions of the zebrafish *irf1* promoter, it was demonstrated that this upregulation was due to GAS-dependent *irf1* promoter activation, although Ifngrel barely activated the *irf1* promoter [110]. Enhanced expression of *irf1* by Ifng was also found in large yellow croaker primary kidney leukocytes [60]. HeLa cells transfected with ginbuna crucian carp *ifngr1-1* or *ifngr1-2* and incubated with recombinant Ifng1 or Ifng2, respectively, showed immunoprecipitation of the promoter coding for human IRF-1 after STAT1 antibody addition [43]. In black seabream brain cells, *irf9* was induced by the transfection of an expression plasmid encoding Ifng [112].

As mentioned above, ISGs are mainly induced by type I IFNs, and IFN-ϒ induces the expression of type I IFNs. This induction of type I IFNs by teleost Ifng was confirmed in different species [41,75,111]. However, in spotted green pufferfish, the intraperitoneal inoculation of recombinant Ifng and Ifngrel generated contradictory results in the expression of two type I IFNs in the spleen [65], provoking inhibition or overexpression in the mRNA level of both genes depending on the sampling time point [65]. To determine whether ISG overexpression after Ifng administration is due to a previous increase in the levels of type I IFNs or to a direct effect of Ifng, Sun et al. [75] used an anti-Ifna1 antibody to study its effect on the expression of *mx*, *isg15* and *irf1* induced by recombinant trout Ifng in Atlantic salmon TO cells. The authors found a reduction in the expression of *mx* and *isg15* at the transcriptomic and protein levels but not in the levels of *irf1* mRNA [75]. These results suggested that the induction of some ISGs by Ifng is partially dependent on the induction of type I IFNs [75].

Considering the induction of this repertoire of ISGs, the involvement of Ifng in antiviral immunity seems evident, although it is assumed that its antiviral effect is weaker compared to that induced by type I IFNs. The *in vitro* inductions mentioned above were not observed during *in vivo* trials. Several *in vitro* experiments clearly demonstrated the antiviral abilities of fish Ifng by reducing the viral replication or viral titre [43,74,75,111,112], even for ginbuna crucian carp Ifngrel [43,49]. Nevertheless, these effects were not as evident in the *in vivo* assays, where neither protection nor prominent immune modulations were observed [41,111].

Type I IFNs and Ifng seem to act synergistically to inhibit viral proliferation. Although *in vitro* assays revealed the ability of fish Ifng or Ifngrel to reduce the replication of different viruses, including salmonid alphavirus 3 (SAV3) in TO cells [75], it was shown that this antiviral effect was potentiated in the presence of Ifna1, and vice versa [75]. A very recent work reported that common carp recombinant Ifng significantly increased the resistance to the spring viremia of carp virus (SVCV) both in EPC cells and *in vivo* [116]. In zebrafish, the *in vivo* co-administration of Ifn1 (Ifnphi1) and Ifng did not ameliorate the survival achieved by Ifn1 alone after SVCV challenge, although the combination of both cytokines induced a more rapid induction of antiviral and pro-inflammatory genes compared to that of the administration of Ifng1 alone [111]. However, Álvarez-Rodríguez et al. [117] observed an increased resistance to SVCV in zebrafish larvae previously inoculated with an expression plasmid encoding *ifng* or in adult zebrafish co-inoculated with the recombinant protein. However, intramuscular injection of an expression plasmid encoding Ifng in juvenile turbot did not reduce the mortality caused by viral haemorrhagic septicaemia virus (VHSV) or viral replication, but it potentiated the expression of type I IFNs and pro-inflammatory cytokines, among other immune genes, induced by the viral challenge [41].

4.7. Protection against bacterial diseases

The antibacterial effects mediated by Ifng in fish were first documented in zebrafish [56,69]. Simultaneous morpholino-mediated knockdown of *ifng* and *ifngrel* increased the mortality of zebrafish embryos after an *E. coli* or *Yersinia ruckeri* challenge, whereas individual blockages had a lower or null effect on mortality, reflecting a certain redundancy in the elicited responses [56,69]. This effect was also found for the simultaneous blockage of the receptor genes *crfb13* and *crfb17* during an infection with *Y. ruckeri* [56].

Protective results against bacterial infections were achieved in olive flounder by intraperitoneally injecting a mixture of recombinant Ifng and *Edwardsiella tarda* [62]. In this case, the survival rate was 0% for the individuals inoculated with the bacteria alone, but 60% was reached in the presence of Ifng [62]. In contrast, the injection of recombinant Ifng in adult zebrafish did not ameliorate the survival after an infection with *Streptococcus iniae*, whereas the type I IFN Ifn1 significantly increased resistance against this bacterium [111]. The absence of protection was also observed in turbot, where intramuscular injection of an expression plasmid encoding Ifng did not alter the mortality caused by *Aeromonas salmonicida* [41]. Interestingly, the pretreatment of spotted green pufferfish with recombinant Ifng and Ifngrel by intraperitoneal administration did not ameliorate the survival rate after a *Vibrio parahaemolyticus* infection, and even higher mortalities were achieved than those for control fish, especially in the case of Ifngrel [65]. Taking these results into consideration, we cannot rule out the possibility that Ifng is more effective at protecting organisms from certain bacteria than from others. In addition, the absence of established stimulation protocols could also affect the results obtained.

5. Metabolic changes induced by Ifng in teleosts

In mammals, the metabolic alterations induced by IFN-ϒ on immune cells have been described in recent years. As detailed above, the activation of macrophages and Th cells implies not only the induction of immune factors but also a metabolic reprogramming of these cells [18–20,27,28].

The immune changes mediated by Ifng in teleosts are practically unknown. Nevertheless, the first evidence suggesting a metabolic reprogramming of immune cells following Ifng stimulation was found in a proteomic analysis conducted in the Atlantic salmon head kidney cell line SHK-1 [68]. In this work, a higher level of glucose-regulated protein 78 (Grp78) was obtained in SHK-1 cells incubated for 24 h with recombinant trout Ifng compared to that of the control cells. In mammals, GRP78 is considered an endoplasmic reticulum (ER) chaperone induced by the ER stress response and is involved in several tumour properties, such as promotion of the Warburg effect in cancer cells [118–120]. Therefore, a similar effect could be mediated by this protein in teleosts.

Tryptophan catabolism, which has relevant consequences for the host immune state, is favoured by IFN-ϒ. Indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) are the enzymes that catalyse the first step of tryptophan degradation through the tryptophan-kynurenine pathway [121]. Tryptophan depletion implies the

suppression of the activity of the nutrient-sensing receptor mTOR [19]. IFN-ϒ induces the synthesis of IDO, and therefore, this cytokine acts as an inhibitor of mTOR activity [121]. Because mTOR is involved in diverse functions of APCs and is pivotal in the activities of T cells [122], tryptophan degradation directly impairs several aspects of the immune response that condition the response against pathogens (discussed below).

The existence of IDO orthologs in non-mammalian vertebrates was well described by Yuasa et al. [123]. The authors observed that, whereas mammals possess IDO and a paralog gene (proto-IDO) tandemly positioned on their genomes, in non-mammalian vertebrates only the proto-IDO gene is observed [123]. Phylogenetic analysis revealed that mammalian IDOs and proto-IDOs clustered separately, suggesting that IDO genes derived from proto-IDOs by gene duplication [123]. The study of the enzymatic properties of proto-IDOs (including fish proto-IDO) revealed a significantly lower tryptophan degradation ability compared to mammalian IDOs [123]. This could indicate that tryptophan is not the main substrate of these proto-IDOs.

However, is the induction of IDO (proto-IDO) by IFN-ϒ conserved in teleosts? To our knowledge, only two works addressed this issue. A chromatin immunoprecipitation assay was conducted to elucidate whether both ginbuna crucian carp Ifng (Ifng1 and Ifng2) ligands are able to promote the interaction of STAT1 with the GAS element in the promoter of human IDO in HeLa cells expressing the *ifngr1-1* or *ifngr1-2* gene [43]. The results showed that both Ifng proteins are able to induce the interaction of STAT1 with the promoter region of IDO only when each cytokine interacts with its corresponding receptor [43]. Recently, the stimulation of the rainbow trout cell line RTS-11 wit recombinant Ifng revealed the overexpression of the trout proto-IDO gene [124]. Therefore, although the exact function of fish proto-IDOs is unknown, it seems that their induction by Ifng is conserved.

6. Duality of IFN-ϒ

IFN-ϒ is mainly considered a pro-inflammatory cytokine, although some anti-inflammatory properties have been attributed to this molecule [125,126], acting as an immunomodulatory protein on both innate and adaptive immunity. Several works describe the effect of IFN-ϒ during different types of infection in vertebrates, with a special focus on viruses [75,127] and bacteria [128,129]. Nevertheless, evidence about its dual function depending on the type of pathogen is very scarce in both mammals and fish. We found a completely differential activity of turbot Ifng depending on the infection [41]. Ifng overexpression had an inhibitory effect on the expression of macrophage-related markers (*marco*, *csf1* and *nox1*) and *mhc2* expression (involved in the presentation of antigens from extracellular pathogens) but potentiated the expression of both pro-inflammatory cytokines (*il1b*, *il15*, and *il17*) and type I IFN system genes (*ifn1* and *ifn2*) during an infection with VHSV [41]. The completely opposite effect was observed during an infection with the bacterium *A. salmonicida*, promoting an increase in the macrophage markers (*marco* and *mrc1*) but dampening both the inflammatory (*casp1*, *il1b*, and *il17*) and type I IFN response (*irf1* and *ifn1*), as well as the transcription of *mhc1* (involved in the presentation of intracellular pathogens, such as viruses) [41].

This duality is probably a consequence of the modulatory effects of this cytokine on the different immune cell types already activated in each infection. The activation of PRRs by different PAMPs initiates a specific signalling pathway with the objective of inducing an adequate response to the existing stimulus that activates them, which is also variable depending on the cell population that is activated [130,131]. This effect, together with the preference of each pathogen for different tissues or cells, generates a pathogen-specific response [132].

The induction of IDO by IFN-ϒ also has an anti-inflammatory effect due to the immunosuppression and tolerization state generated as a consequence of tryptophan depletion and mTOR inhibition. Intermediates of the kynurenine pathway or kynurenine itself induce both metabolic and immune modulations that alter the immune state of the animal [133–137]. Classically, the tryptophan-kynurenine pathway has been considered a bactericidal and sepsis-protective mechanism by leading to an immunosuppressive status, but in the last decade, this pathway has been shown to be pernicious when the animal fights a viral infection [138], representing a double-edged sword in the defence against pathogens. Indeed, the use of TDO and IDO inhibitors increases the survival of adult zebrafish during a challenge with SVCV [117]. Therefore, due to the induction of IDO by IFN-ϒ we need to consider once again the duality of this cytokine in the response against different pathogens. Nonetheless, as was mentioned above, the function of teleost proto-IDOs in tryptophan catabolism is not fully clear.

In zebrafish, a single intraperitoneal dose of β-glucans increased mortality after infection with the rhabdovirus SVCV at 35 days post-immunostimulation [117]. The immunosuppressive or immunotolerant status induced by β-glucans seems to be mediated by the interplay of lipid metabolism, the tryptophan-kynurenine pathway and IFN-ϒ signalling [117]. In this work, an increased expression of TDO was found in those animals previously inoculated with β-glucans, and although IDO was not significantly affected by the treatment, the increased expression of *ifng* could be a prelude of this effect [117].

Several studies have demonstrated the promising immunostimulant properties of β-glucans in teleosts when these are administered in the diet by immersion or via injection, with increased resistance against bacteria [139–142]. However, contradictory results were obtained with β-glucans in viral diseases, probably due, among other things, to the doses used in immunostimulation protocols conducted in the different experiments. Whereas sustained diet administration of β-glucans increases the resistance of teleosts to viruses [143–145], a single dose injection of this compound usually increases their susceptibility [117,142,146]. Whether Ifng is involved in these differences of protection observed between bacterial and viral infections and/or depending on the β-glucan stimulation protocol is a question that needs to be further elucidated.

7. MicroRNA induction by type II IFNs in fish

MicroRNAs (miRNAs) are a family of conserved small (~21–25-nucleotides) non-coding RNAs that negatively regulate gene expression by interacting with the 3' untranslated region (UTR) of target mRNAs [147]. Consequently, miRNAs can alter a multitude of biological processes, including the immune response.

In recent years, knowledge about this kind of molecule has enormously increased, and some studies have even been conducted in fish. Some of these publications reported the miRNA profile induced by type II IFNs. In spotted green pufferfish, spleen cells were treated with recombinant Ifng and Ifngrel, and Solexa high-throughput sequencing revealed the miRNA profile [148]. Differential expression analysis identified 438 and 398 miRNAs differentially expressed after Ifng or Ifngrel treatment, respectively, compared to those of the untreated cells [148]. When the targets of these miRNAs were predicted and analysed by GO and KEGG analyses, numerous immune-related terms were observed, including the Jak-Stat signalling pathway, TLR signalling pathway, NOD-like receptor signalling pathway and antigen processing and presentation [148]. The expression of one of these miRNAs induced by Ifngrel stimulation, miR-29, was analysed in the spleen after Ifng and Ifngrel *in vivo* inoculation in the absence or presence of *V. parahaemolyticus* infection [65]. The results showed that Ifng is able to induce the overexpression of miR-29 at 1 dpi, whereas Ifngrel increased its expression after 9 days [65]. Interestingly, at those time points where both cytokines did not alter the level of miR-29 (3, 6, and 12 dpi), pre-treatment with Ifng or Ifngrel before challenge with *V. parahaemolyticus* synergistically potentiated the transcription of miR-29 [65]. Because it has been described that IFN-ϒ is a direct target of the miR-29 family [149,150], the interplay between fish type II IFNs and miR-29 could provide new interesting routes of investigation. In

orange-spotted grouper, the incubation of head kidney monocytes with Ifng increased the expression of miR-146a [51]. This mi-RNA potentially targets TNF receptor-associated factor 6 (*traf6*), which is a key adapter of the TLR receptor signalling pathway; therefore, a potential novel regulatory mechanism of Ifng in the TLR pathway via mir-146a-TRAF6 could be suggested [51].

8. Conclusions and future perspectives

Numerous aspects of mammalian IFN-ϒ were found to be perfectly conserved in teleosts, especially those related to macrophage activation (induction of respiratory burst, NO production and inflammation). The existence of an *ifngrel* gene, and even duplicated *ifng* and/or *ifngrel* genes, in some teleost species hinders the establishment of an equivalent functionality of this cytokine between teleosts and mammals. Indeed, although some overlapping functions could be assumed between Ifng and Ifngrel, certain differential characteristics were also observed.

In mammals, in addition to the typical immune modulations conducted by IFN-ϒ, recent investigations shed light on the immunometabolomic reprogramming of immune cells induced by this molecule. This reprogramming includes alteration of glucose metabolism and induction of tryptophan catabolism, among others. Although some evidence suggested an effect of recombinant Ifng on the modulation of these processes in teleosts, these findings are very preliminary, and their interpretation is highly speculative. Notwithstanding, the increasing knowledge obtained from mammals could open the door to new research in fish, especially in the model species zebrafish, due to the increased availability of research tools for this organism.

Another interesting line of investigation is to study the differential effects mediated by IFN-ϒ depending on the type of infection the animal is dealing with. Only a few publications have reported this potential duality, which seems to be completely compatible with the pleiotropic nature of this cytokine. Due to the effect of some immunostimulants used in aquaculture on the levels of Ifng, the study of the collateral effects mediated by this cytokine if the animal suffers an infection is a very important issue to be addressed. This could also be of great importance for biomedical studies since human IFN-ϒ is used in the treatment of chronic granulomatous disease and osteopetrosis. Therefore, it is fundamental to understand the potential consequences of this treatment if the patient contracts an infectious disease.

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References

[1] K. Schroder, P.J. Hertzog, T. Ravasi, D.A. Hume, Interferon-gamma: an overview of signals, mechanisms and functions, J. Leukoc. Biol. 75 (2004) 163–189.
[2] J.R. Schoenborn, C.B. Wilson, Regulation of Interferon-γ during innate and adaptive immune responses, Adv. Immunol. 96 (2007) 41–101.
[3] X. Hu, L.B. Ivashkiv, Cross-regulation of signaling pathways by interferon-gamma: implications for immune responses and autoimmune diseases, Immunity 31 (2009) 539–550.
[4] E.A. Bach, M. Aguet, R.D. Schreiber, The IFN gamma receptor: a paradigm for cytokine receptor signaling, Annu. Rev. Immunol. 15 (1997) 563–591.
[5] P.S. Subramaniam, M.M. Green, J. Larkin 3rd, B.A. Torres, H.M. Johnson, Nuclear translocation of IFN-gamma is an intrinsic requirement for its biologic activity and can be driven by a heterologous nuclear localization sequence, J. Interferon Cytokine Res. 21 (2001) 951–959.
[6] M. Marchetti, M.N. Monier, A. Fradagrada, K. Mitchell, F. Baychelier, P. Eid, L. Johannes, C. Lamaze, Stat-mediated signaling induced by type I and type II interferons (IFNs) is differentially controlled through lipid microdomain association and clathrin-dependent endocytosis of IFN receptors, Mol. Biol. Cell 17 (2006) 2896–2909.
[7] R. Sadir, A. Lambert, H. Lortat-Jacob, G. Morel, Caveolae and clathrin-coated vesicles: two possible internalization pathways for IFN-gamma and IFN-gamma receptor, Cytokine 14 (2001) 19–26.
[8] D.S. Green, H.A. Young, J.C. Valencia, Current prospects of type II interferon ϒ signaling and autoimmunity, J. Biol. Chem. 292 (2017) 13925–13933.
[9] F. Kierszenbaum, H. Mejia Lopez, M.K. Tanner, M.B. Sztein, *Trypanosoma cruzi*-induced decrease in the level of interferon-γ receptor expression by resting and activated human blood lymphocytes, Parasite Immunol. 17 (1995) 207–214.
[10] Q. Li, R. Means, S. Lang, J.U. Jung, Downregulation of γ interferon receptor 1 by Kaposi's sarcoma-associated herpesvirus K3 and K5, J. Virol. 81 (2007) 2117–2127.
[11] K. Tominaga, T. Yoshimoto, K. Torigoe, M. Kurimoto, K. Matsui, T. Hada, H. Okamura, K. Nakanishi, IL-12 synergizes with IL-18 or IL-1β for IFN-γ production from human T cells, Int. Immunol. 12 (2000) 151–160.
[12] Y. Ohmori, T.A. Hamilton, Interleukin-4/STAT6 represses STAT1 and NF-κB-dependent transcription through distinct mechanisms, J. Biol. Chem. 275 (2000) 38095–38103.
[13] Y. Ding, D. Chen, A. Tarcsafalvi, R. Su, L. Qin, J.S. Bromberg, Suppressor of cytokine signaling 1 inhibits IL-10-mediated immune responses, J. Immunol. 170 (2003) 1383–1391.
[14] J.T. Lin, S.L. Martin, L. Xia, J.D. Gorham, TGF-beta 1 uses distinct mechanisms to inhibit IFN-gamma expression in CD4+ T cells at priming and at recall: differential involvement of Stat4 and T-bet, J. Immunol. 174 (2005) 5950–5958.
[15] X. Hu, W.P. Li, C. Meng, L.B. Ivashkiv, Inhibition of IFN-γ signaling by glucocorticoids, J. Immunol. 170 (2003) 4833–4839.
[16] P. Italiani, D. Boraschi, From monocytes to M1/M2 macrophages: phenotypical vs. functional differentiation, Front. Immunol. 5 (2014) 514.
[17] F.O. Martinez, S. Gordon, The M1 and M2 paradigm of macrophage activation: time for reassessment, F1000Prime Reports 6 (2014) 13.
[18] X. Hu, A.Y. Chung, I. Wu, J. Foldi, J. Chen, J.D. Ji, T. Tateya, Y.J. Kang, J. Han, M. Gessler, R. Kageyama, L.B. Ivashkiv, Integrated regulation of Toll-like receptor responses by Notch and interferon-gamma pathways, Immunity 29 (2008) 691–703.
[19] X. Su, Y. Yu, Y. Zhong, E.G. Giannopoulou, X. Hu, H. Liu, J.R. Cross, G. Rätsch, C.M. Rice, L.B. Ivashkiv, Interferon-γ regulates cellular metabolism and mRNA translation to potentiate macrophage activation, Nat. Immunol. 16 (2015) 838–849.
[20] F. Wang, S. Zhang, R. Jeon, I. Vuckovic, X. Jiang, A. Lerman, C.D. Folmes, P.D. Dzeja, J. Herrmann, Interferon gamma induces reversible metabolic reprogramming of M1 macrophages to sustain cell viability and pro-inflammatory activity, EbioMedicine 30 (2018) 303–316.
[21] S.E. Keating, V. Zaiatz-Bittencourt, R.M. Loftus, C. Keane, K. Brennan, D.K. Finlay, C.M. Gardiner, Metabolic reprogramming supports IFN-γ production by CD56bright NK Cells, J. Immunol. 196 (2016) 2552–2560.
[22] E.G. Schulz, L. Mariani, A. Radbruch, T. Höfer, Sequential polarization and imprinting of type 1 T helper lymphocytes by interferon-γ and interleukin-12, Immunity 30 (2009) 673–683.
[23] A. Berger, Th1 and Th2 responses: what are they? BMJ 321 (2000) 424.
[24] E. Maggi, P. Parronchi, R. Manetti, C. Simonelli, M.P. Piccinni, F.S. Rugiu, M. De Carli, M. Ricci, S. Romagnani, Reciprocal regulatory effects of IFN-gamma and IL-4 on the in vitro development of human Th1 and Th2 clones, J. Immunol. 148 (1992) 2142–2147.
[25] A. Pernis, S. Gupta, K.J. Gollob, E. Garfein, R.L. Coffman, C. Schindler, P. Rothman, Lack of interferon gamma receptor beta chain and the prevention of interferon gamma signaling in TH1 cells, Science 269 (1995) 245–247.
[26] E.A. Bach, S.J. Szabo, A.S. Dighe, A. Ashkenazi, M. Aguet, K.M. Murphy, R.D. Schreiber, Ligand-induced autoregulation of IFN-gamma receptor beta chain expression in T helper cell subsets, Science 270 (1995) 1215–1218.
[27] L. Almeida, M. Lochner, L. Berod, T. Sparwasser, Metabolic pathways in T cell activation and lineage differentiation, Semin. Immunol. 28 (2016) 514–524.
[28] C.S. Palmer, M. Ostrowski, B. Balderson, N. Christian, S.M. Crowe, Glucose metabolism regulates T cell activation, differentiation, and functions, Front. Immunol. 6 (2015) 1.
[29] J. Zou, C.J. Secombes, Teleost fish interferons and their role in immunity, Dev. Comp. Immunol. 35 (2011) 1376–1387.
[30] P.M. Peters, L.P. Svedersky, M.R. Shalaby, M.A. Palladino Jr., Interferon gamma: more than a MAF, Lymphokine Res. 4 (1985) 265–273.
[31] S. Graham, C.J. Secombes, The production of a macrophage-activating factor from rainbow trout Salmo gairdneri leukocytes, Immunology 65 (1988) 293–297.
[32] S. Graham, C.J. Secombes, Cellular requirements for lymphokine secretion by rainbow trout *Salmo gairdneri* leukocytes, Dev. Comp. Immunol. 14 (1990) 59–68.
[33] G. Lutfalla, H. Roest Crollius, N. Stange-Thomann, O. Jaillon, K. Mogensen, D. Monneron, Comparative genomic analysis reveals independent expansion of a lineage-specific gene family in vertebrates: the class II cytokine receptors and their ligands in mammals and fish, BMC Genomics 4 (2003) 29.
[34] J. Zou, Y. Yoshiura, J.M. Dijkstra, M. Sakai, M. Ototake, C. Secombes, Identification of an interferon gamma homologue in Fugu, *Takifugu rubripes*, Fish Shellfish Immunol. 17 (2004) 403–409.
[35] J. Zou, A. Carrington, B. Collet, J.M. Dijkstra, Y. Yoshiura, N. Bols, C. Secombes, Identification and bioactivities of IFN-gamma in rainbow trout *Oncorhynchus mykiss*: the first Th1-type cytokine characterized functionally in fish, J. Immunol. 175 (2005) 2484–2494.
[36] D. Igawa, M. Sakai, R. Savan, 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, Mol. Immunol. 43 (2006) 999–1009.
[37] I. Milev-Milovanovic, S. Long, M. Wilson, E. Bengten, N.W. Miller, V.G. Chinchar, Identification and expression analysis of interferon gamma genes in channel catfish, Immunogenetics 58 (2006) 70–80.
[38] B. Robertsen, The interferon system of teleost fish, Fish Shellfish Immunol. 20 (2006) 172–191.
[39] E.H. Stolte, H.F. Savelkoul, G. Wiegertjes, G. Flik, B.M. Lidy Verburg-van Kemenade, Differential expression of two interferon-gamma genes in common carp (*Cyprinus carpio* L.), Dev. Comp. Immunol. 32 (2008) 1467–1481.
[40] L. Grayfer, M. Belosevic, Molecular characterization, expression and functional analysis of goldfish (*Carassius auratus* L.) interferon gamma, Dev. Comp. Immunol. 33 (2009) 235–246.
[41] P. Pereiro, G. Forn-Cuni, A. Figueras, B. Novoa, Pathogen-dependent role of turbot (*Scophthalmus maximus*) interferon-gamma, Fish Shellfish Immunol. 59 (2016) 25–35.
[42] M.K. Purcell, K.J. Laing, J.C. Woodson, G.H. Thorgaard, J.D. Hansen, Characterization of the interferon genes in homozygous rainbow trout reveals two novel genes, alternate splicing and differential regulation of duplicated genes, Fish Shellfish Immunol. 26 (2009) 293–304.
[43] T. Yabu, H. Toda, Y. Shibasaki, K. Araki, M. Yamashita, H. Anzai, N. Mano, Y. Masuhiro, S. Hanazawa, H. Shiba, Antiviral protection mechanisms mediated by ginbuna crucian carp interferon gamma isoforms 1 and 2 through two distinct interferon gamma-receptors, J. Biochem. 150 (2011) 635–648.
[44] K.A. Christensen, W.S. Davidson, Autopolyploidy genome duplication preserves other ancient genome duplications in Atlantic salmon (*Salmo salar*), PLoS One 12 (2017) e0173053.
[45] P. Xu, X. Zhang, X. Wang, J. Li, G. Liu, Y. Kuang, J. Xu, X. Zheng, L. Ren, G. Wang, Y. Zhang, L. Huo, Z. Zhao, D. Cao, C. Lu, C. Li, Y. Zhou, Z. Liu, Z. Fan, G. Shan, X. Li, S. Wu, L. Song, G. Hou, Y. Jiang, Z. Jeney, D. Yu, L. Wang, C. Shao, L. Song, J. Sun, P. Ji, J. Wang, Q. Li, L. Xu, F. Sun, J. Feng, C. Wang, S. Wang, B. Wang, Y. Li, Y. Zhu, W. Xue, L. Zhao, J. Wang, Y. Gu, W. Lv, K. Wu, J. Xiao, J. Wu, Z. Zhang, J. Yu, X. Sun, Genome sequence and genetic diversity of the common carp, *Cyprinus carpio*, Nat. Genet. 46 (2014) 1212–1219.
[46] R. Savan, S. Ravichandran, J.R. Collins, M. Sakai, H.A. Younga, Structural conservation of interferon gamma among vertebrates, Cytokine Growth Factor Rev. 20 (2009) 115–124.
[47] L. Grayfer, M. Belosevic, Molecular characterization of novel interferon gamma receptor 1 isoforms in zebrafish (*Danio rerio*) and goldfish (*Carassius auratus* L.), Mol. Immunol. 46 (2009) 3050–3059.
[48] W.Q. Chen, Q.Q. Xu, M.X. Chang, J. Zou, C.J. Secombes, K.M. Peng, P. Nie, Molecular characterization and expression analysis of the IFN-gamma related gene (IFN-gammarel) in grass carp *Ctenopharyngodon idella*, Vet. Immunol. Immunopathol. 134 (2010) 199–207.
[49] Y. Shibasaki, T. Yabu, K. Araki, N. Mano, H. Shiba, T. Moritomo, T. Nakanishi, Peculiar monomeric interferon gammas, IFNγrel 1 and IFNγrel 2, in ginbuna crucian carp, FEBS J. 281 (2014) 1046–1056.
[50] B. Swain, M. Basu, S.S. Lenka, S. Das, P. Jayasankar, M. Samanta, Characterization and inductive expression analysis of interferon gamma-related gene in the Indian major carp, rohu (*Labeo rohita*), DNA Cell Biol. 34 (2015) 367–378.
[51] W. Peng, Y. Sun, G.F. Li, L.G. He, R.Z. Li, Y.S. Liang, X. Ding, X. Yu, Y. Zhang, H.R. Lin, D.Q. Lu, Two distinct interferon-γ in the orange-spotted grouper (*Epinephelus coioides*): molecular cloning, functional characterization, and regulation in toll-like receptor pathway by induction of miR-146a, Front. Endocrinol. 9 (2018) 41.
[52] T. Sareneva, J. Pirhonen, K. Cantell, I. Julkunen, N-glycosylation of human interferon-gamma: glycans at Asn-25 are critical for protease resistance, Biochem. J. 308 (1995) 9–14.
[53] J. Parhi, L. Sahoo, J. Choudhury, T.G. Choudhury, A. Baruah, K. PaniPrasad, M. Makesh, Molecular characterization and expression analysis of interferon γ (IFN-γ) gene in *Labeo rohita* (Ham.), Aquaculture Reports 2 (2015) 97–105.
[54] C. Stein, M. Caccamo, G. Laird, M. Leptin, Conservation and divergence of gene families encoding components of innate immune response systems in zebrafish, Genome Biol. 8 (2007) R251.
[55] Q. Gao, P. Nie, K.D. Thompson, A. Adams, T. Wang, C.J. Secombes, J. Zou, The search for the IFN-gamma receptor in fish: functional and expression analysis of putative binding and signalling chains in rainbow trout *Oncorhynchus mykiss*, Dev. Comp. Immunol. 33 (2009) 920–931.
[56] D. Aggad, C. Stein, D. Sieger, M. Mazel, P. Boudinot, P. Herbomel, J.P. Levraud, G. Lutfalla, M. Leptin, In vivo analysis of Ifn-γ 1 and Ifn-γ 2 signaling in zebrafish, J. Immunol. 185 (2010) 6774–6782.
[57] D.Q. Lu, T.T. Leng, X. Ding, W. Peng, M. Yao, S.S. Li, H.R. Lin, Y. Zhang, Two IFNGR1 homologues in *Tetraodon nigroviridis*: origin, expression analysis and ligand-binding preference, Dev. Comp. Immunol. 44 (2014) 270–279.
[58] B. Sun, L. Greiner-Tollersrud, B.F. Koop, B. Robertsen, Atlantic salmon possesses two clusters of type I interferon receptor genes on different chromosomes, which allows for a larger repertoire of interferon receptors than in zebrafish and mammals, Dev. Comp. Immunol. 47 (2014) 275–286.
[59] L. Grayfer, E.G. Garcia, M. Belosevic, Comparison of macrophage antimicrobial responses induced by type II interferons of the goldfish (*Carassius auratus* L.), J. Biol. Chem. 285 (2010) 23537–23547.
[60] S. Yang, Q. Li, Y. Mu, J. Ao, X. Chen, Functional activities of interferon gamma in large yellow croaker *Larimichthys crocea*, Fish Shellfish Immunol. 70 (2017) 545–552.
[61] M.K. Bedekar, P. Soman, S. Kole, D. Anand, G. Tripathi, M. Makesh, K.V. Rajendran, Evaluation of interferon gamma (IFN-γ) of *Labeo rohita* as an immunomodulator: in vitro expression model, Aquacult. Int. 26 (2018) 1401–1413.
[62] C.Y. Jung, J. Hikima, M. Ohtani, H.B. Jang, C.S. del Castillo, S.W. Nho, I.S. Cha, S.B. Park, T. Aoki, T.S. Jung, Recombinant interferon-γ activates immune responses against *Edwardsiella tarda* infection in the olive flounder, *Paralichthys olivaceus*, Fish Shellfish Immunol. 33 (2012) 197–203.
[63] K. Yang, S. Zhang, D. Chen, A. Zhang, X. Wang, H. Zhou, IFN-γ-activated lymphocytes boost nitric oxide production in grass carp monocytes/macrophages, Fish Shellfish Immunol. 35 (2013) 1635–1641.
[64] J.A.J. Arts, E.J. Tijhaar, M. Chadzinska, H.F.J. Savelkoul, B.M.L. Verburg-van Kemenade, Functional analysis of carp interferon-Ƴ: evolutionary conservation of classical phagocyte activation, Fish Shellfish Immunol. 29 (2010) 793–802.
[65] W. Peng, D.Q. Lu, G.F. Li, X. Zhang, M. Yao, Y. Zhang, H.R. Lin, Two distinct interferon-γ genes in *Tetraodon nigroviridis*: functional analysis during *Vibrio parahaemolyticus* infection, Mol. Immunol. 70 (2016) 34–46.
[66] Y. Shibasaki, C. Hatanaka, Y. Matsuura, R. Miyazawa, T. Yabu, T. Moritomo, T. Nakanishi, Effects of IFNγ administration on allograft rejection in ginbuna crucian carp, Dev. Comp. Immunol. 62 (2016) 108–115.
[67] S.A. Martin, J. Zou, D.F. Houlihan, C.J. Secombes, Directional responses following recombinant cytokine stimulation of rainbow trout (*Oncorhynchus mykiss*) RTS-11 macrophage cells as revealed by transcriptome profiling, BMC Genomics 8 (2007) 150.
[68] S.A. Martin, B.P. Mohanty, P. Cash, D.F. Houlihan, C.J. Secombes, Proteome analysis of the Atlantic salmon (*Salmo salar*) cell line SHK-1 following recombinant IFN-gamma stimulation, Proteomics 7 (2007) 2275–2286.
[69] D. Sieger, C. Stein, D. Neifer, A.M. van der Sar, N. Leptin, The role of gamma interferon in innate immunity in the zebrafish embryo, Disease Models & Mechanisms 2 (2009) 571–581.
[70] J.N. Barker, M.L. Jones, C.L. Swenson, V. Sarma, R.S. Mitra, P.A. Ward, K.J. Johnson, J.C. Fantone, V.M. Dixit, B.J. Nickoloff, Monocyte chemotaxis and activating factor production by keratinocytes in response to IFN-gamma, J. Immunol. 146 (1991) 1192–1197.
[71] R. Bonecchi, N. Polentarutti, W. Luini, A. Borsatti, S. Bernasconi, M. Locati, C. Power, A. Proudfoot, T.N. Wells, C. Mackay, A. Mantovani, S. Sozzani, Up-regulation of CCR1 and CCR3 and induction of chemotaxis to CC chemokines by IFN-gamma in human neutrophils, J. Immunol. 162 (1999) 474–479.
[72] J. Tan, B. Deleuran, B. Gesser, H. Maare, M. Deleuran, C.G. Larsen, K. Thestrup-Pedersen, Regulation of human T lymphocyte chemotaxis in vitro by T cell-derived cytokines IL-2, IFN-gamma, IL-4, IL-10, and IL-13, J. Immunol. 154 (1995) 3742–3752.
[73] S. Bird, C. Tafalla, Teleost chemokines and their receptors, Biology 4 (2015) 756–784.
[74] C. Xu, T.C. Guo, S. Mutoloki, O. Haugland, I.S. Marjara, O. Evensen, Alpha interferon and not gamma interferon inhibits salmonid alphavirus subtype 3 replication in vitro, J. Virol. 84 (2010) 8903–8912.
[75] B. Sun, I. Skjæveland, T. Svingerud, J. Zou, J. Jørgensen, B. Robertsen, Antiviral activity of salmonid gamma interferon against infectious pancreatic necrosis virus and salmonid alphavirus and its dependency on type I interferon, J. Virol. 85 (2011) 9188–9198.
[76] S.A. Martin, J.B. Taggart, P. Seear, J.E. Bron, R. Talbot, A.J. Teale, G.E. Sweeney, B. Høyheim, D.F. Houlihan, D.R. Tocher, J. Zou, C.J. Secombes, Interferon type I and type II responses in an Atlantic salmon (*Salmo salar*) SHK-1 cell line by the salmon TRAITS/SGP microarray, Physiol. Genom. 32 (2007) 33–44.
[77] T.M. Aune, S.L. Pogue, Inhibition of tumor cell growth by interferon-gamma is mediated by two distinct mechanisms dependent upon oxygen tension: induction of tryptophan degradation and depletion of intracellular nicotinamide adenine dinucleotide, J. Clin. Investig. 84 (1989) 863–875.
[78] H. Asao, X.Y. Fu, Interferon-gamma has dual potentials in inhibiting or promoting cell proliferation, J. Biol. Chem. 275 (2000) 867–874.
[79] Y. Delneste, P. Charbonnier, N. Herbault, G. Magistrelli, G. Caron, J.Y. Bonnefoy, P. Jeannin, Interferon-γ switches monocyte differentiation from dendritic cells to macrophages, Blood 101 (2003) 143–150.
[80] D. Stoycheva, K. Deiser, L. Stärck, G. Nishanth, D. Schlüter, W. Uckert, T. Schüler, IFN-γ regulates CD8+ memory T cell differentiation and survival in response to weak, but not strong, TCR signals, J. Immunol. 194 (2015) 553–559.
[81] J.K. Whitmire, J.T. Tan, J.L. Whitton, Interferon-γ acts directly on CD8+ T cells to increase their abundance during virus infection, J. Exp. Med. 201 (2005) 1053.
[82] J.F. Bromberg, C.M. Horvath, Z. Wen, R.D. Schreiber, J.E. Darnell Jr., Transcriptionally active Stat1 is required for the antiproliferative effects of both interferon alpha and interferon gamma, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 7673–7678.
[83] K. Meissl, S. Macho-Maschler, M. Müller, B. Strobl, The good and the bad faces of STAT1 in solid tumours, Cytokine 89 (2017) 12–20.
[84] L. Yang, I. Dybedal, D. Bryder, L. Nilsson, E. Sitnicka, Y. Sasaki, S.E. Jacobsen, IFN-gamma negatively modulates self-renewal of repopulating human hemopoietic stem cells, J. Immunol. 174 (2005) 752–757.
[85] M.T. Baldridge, K.Y. King, N.C. Boles, D.C. Weksberg, M.A. Goodell, Quiescent haematopoietic stem cells are activated by IFN-gamma in response to chronic infection, Nature 465 (2010) 793–797.
[86] K.C. MacNamara, M. Jones, O. Martin, G.M. Winslow, Transient activation of hematopoietic stem and progenitor cells by IFNgamma during acute bacterial infection, PLoS One 6 (2011) e28669.
[87] A.M. de Bruin, S.F. Libregts, M. Valkhof, L. Boon, I.P. Touw, M.A. Nolte, IFNγ induces monopoiesis and inhibits neutrophil development during inflammation, Blood 119 (2012) 1543–1554.

[88] A.M. de Bruin, O. Demirel, B. Hooibrink, C.H. Brandts, M.A. Nolte, Interferon-gamma impairs proliferation of hematopoietic stem cells in mice, Blood 121 (2013) 3578–3585.
[89] S. Sawamiphak, Z. Kontarakis, D.Y.R. Stainier, Interferon gamma signaling positively regulates hematopoietic stem cell emergence, Dev. Cell 31 (2014) 640–653.
[90] C.M. Schurch, C. Riether, A.F. Ochsenbein, Cytotoxic CD8+ T cells stimulate hematopoietic progenitors by promoting cytokine release from bone marrow mesenchymal stromal cells, Cell Stem Cell 14 (2014) 460–472.
[91] F.J. Pixley, E.R. Stanley, CSF-1 regulation of the wandering macrophage: complexity in action, Trends Cell Biol. 14 (2004) 628–638.
[92] P.C. Hanington, S.J. Hitchen, L.A. Beamish, M. Belosevic, Macrophage colony stimulating factor (CSF-1) is a central growth factor of goldfish macrophages, Fish Shellfish Immunol. 26 (2009) 1–9.
[93] L. Grayfer, P.C. Hanington, M. Belosevic, 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 (2009) 406–413.
[94] T.A. Waldmann, Y. Tagaya, The multifaceted regulation of interleukin-15 expression and the role of this cytokine in NK cell differentiation and host response to intracellular pathogens, Annu. Rev. Immunol. 17 (1999) 19–49.
[95] M. Mohamadzadeh, F. Berard, G. Essert, C. Chalouni, B. Pulendran, J. Davoust, G. Bridges, A.K. Palucka, J. Banchereau, Interleukin 15 skews monocyte differentiation into dendritic cells with features of Langerhans cells, J. Exp. Med. 194 (2001) 1013–1020.
[96] R.J. Armitage, B.M. Macduff, J. Eisenman, R. Paxton, K.H. Grabstein, IL-15 has stimulatory activity for the induction of B cell proliferation and differentiation, J. Immunol. 154 (1995) 483–490.
[97] T. Wang, J.W. Holland, A. Carrington, J. Zou, C.J. Secombes, 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 (2007) 1475–1488.
[98] T. Matsuzawa, B.H. Kim, A.R. Shenoy, S. Kamitani, M. Miyake, J.D. Macmicking, IFN-γ elicits macrophage autophagy via the p38 MAPK signaling pathway, J. Immunol. 189 (2012) 813–818.
[99] M.G. Gutierrez, S.S. Master, S.B. Singh, G.A. Taylor, M.I. Colombo, V. Deretic, Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages, Cell 119 (2004) 753–766.
[100] J.D. MacMicking, G.A. Taylor, J.D. McKinney, Immune control of tuberculosis by IFN-gamma-inducible LRG-47, Science 302 (2003) 654–659.
[101] B.H. Kim, A.R. Shenoy, P. Kumar, R. Das, S. Tiwari, J.D. MacMicking, A family of IFN-γ-inducible 65-kD GTPases protects against bacterial infection, Science 332 (2011) 717–721.
[102] A.H. Meijer, M. van der Vaart, DRAM1 promotes the targeting of mycobacteria to selective autophagy, Autophagy 10 (2014) 2389–2391.
[103] P. García-Valtanen, MdelM. Ortega-Villaizán, A. Martínez-López, R. Medina-Gali, L. Pérez, S. Mackenzie, A. Figueras, J.M. Coll, A. Estepa, Autophagy-inducing peptides from mammalian VSV and fish VHSV rhabdoviral G glycoproteins (G) as models for the development of new therapeutic molecules, Autophagy 10 (2014) 1666–1680.
[104] P. Pereiro, A. Romero, P. Díaz-Rosales, A. Estepa, A. Figueras, B. Novoa, Nucleated teleost erythrocytes play an Nk-lysin- and autophagy-dependent role in antiviral immunity, Front. Immunol. 8 (2017) 1458.
[105] B. Robertsen, J. Zou, C. Secombes, J.A. Leong, Molecular and expression analysis of an interferon-gamma-inducible guanylate-binding protein from rainbow trout (*Oncorhynchus mykiss*), Dev. Comp. Immunol. 30 (2006) 1023–1033.
[106] L.G. Guidotti, F.V. Chisari, Noncytolytic control of viral infections by the innate and adaptive immune response, Annu. Rev. Immunol. 19 (2001) 65–91.
[107] A.J. Lee, A.A. Ashkar, The dual nature of type I and type II interferons, Front. Immunol. 9 (2018) 2061.
[108] J.B. Jorgensen, A. Johansen, M.N. Hegseth, J. Zou, B. Robertsen, B. Collet, C.J. Secombes, A recombinant CHSE-214 cell line expressing an Mx1 promoter–reporter system responds to both interferon type I and type II from salmonids and represents a versatile tool to study the IFN-system in teleost fish, Fish Shellfish Immunol. 23 (2007) 1294–1303.
[109] J. Velázquez, J. Acosta, N. Herrera, A. Morales, O. González, F. Herrera, M.P. Estrada, Y. Carpio, Novel IFNγ homologue identified in Nile tilapia (*Oreochromis niloticus*) links with immune response in gills under different stimuli, Fish Shellfish Immunol. 71 (2017) 275–285.
[110] B.Y. Ruan, S.N. Chen, J. Hou, B. Huang, Z.A. Laghari, L. Li, P. Nie, Two type II IFN members, IFN-gamma and IFN-gamma related (rel), regulate differentially IRF1 and IRF11 in zebrafish, Fish Shellfish Immunol. 65 (2017) 103–110.
[111] A. López-Muñoz, F.J. Roca, J. Meseguer, V. Mulero, New insights into the evolution of IFNs: zebrafish group II IFNs induce a rapid and transient expression of IFN-dependent genes and display powerful antiviral activities, J. Immunol. 182 (2009) 3440–3449.
[112] Y. Xiang, W. Liu, P. Jia, Y. Li, Y. Jin, L. Chen, J. Zhang, K. Jia, M. Yi, Molecular characterization and expression analysis of interferon-gamma in black seabream *Acanthopagrus schlegelii*, Fish Shellfish Immunol. 70 (2017) 140–148.
[113] Y.B. Zhang, J.F. Gui, Identification of two novel interferon-stimulated genes from cultured CAB cells induced by UV-inactivated grass carp hemorrhage virus, Dis. Aquat. Org. 60 (2004) 1–9.
[114] Y.B. Zhang, T.K. Liu, J. Jiang, J. Shi, Y. Liu, S. Li, J.F. Gui, Identification of a novel gig2 gene family specific to non-amniote vertebrates, PLoS One 8 (2013) e60588.
[115] J.W. Schoggins, C.M. Rice, Interferon-stimulated genes and their antiviral effector functions, Current Opinion in Virology 1 (2011) 519–525.
[116] J. Fu, Z. Yi, H. Cui, C. Song, M. Yu, Y. Liu, Intein-mediated expression and purification of common carp IFN-γ and its protective effect against spring viremia of carp virus, Fish Shellfish Immunol. 88 (2019) 403–406.
[117] M. Álvarez-Rodríguez, P. Pereiro, F.E. Reyes-López, L. Tort, A. Figueras, B. Novoa, Analysis of the long-lived responses Induced by immunostimulants and their effects on a viral infection in zebrafish (*Danio rerio*), Front. Immunol. 9 (2018) 1575.
[118] Z. Li, Z. Li, Glucose regulated protein 78: a critical link between tumor microenvironment and cancer hallmarks, Biochim. Biophys. Acta 1826 (2012) 13–22.
[119] U.K. Misra, S.V. Pizzo, Activated α2-macroglobulin binding to human prostate cancer cells triggers insulin-like responses, J. Biol. Chem. 290 (2015) 9571–9587.
[120] U. Gopal, S.V. Pizzo, Cell surface GRP78 promotes tumor cell histone acetylation through metabolic reprogramming: a mechanism which modulates the Warburg effect, Oncotarget 8 (2017) 107947–107963.
[121] A.A. Badawy, Kynurenine pathway of tryptophan metabolism: regulatory and functional aspects, Int. J. Tryptophan Res. 10 (2017) 1–20.
[122] A.W. Thomson, H.R. Turnquist, G. Raimondi, Immunoregulatory functions of mTOR inhibition, Nat. Rev. Immunol. 9 (2009) 324–337.
[123] H.J. Yuasa, M. Takubo, A. Takahashi, T. Hasegawa, H. Noma, T. Suzuki, Evolution of vertebrate indoleamine 2,3-dioxygenases, J. Mol. Evol. 65 (2007) 705–714.
[124] J. Cortés, C. Alvarez, P. Santana, E. Torres, L. Mercado, Indoleamine 2,3-dioxygenase: first evidence of expression in rainbow trout (*Oncorhynchus mykiss*), Dev. Comp. Immunol. 65 (2016) 73–78.
[125] H. Mühl, J. Pfeilschifter, Anti-inflammatory properties of pro-inflammatory interferon-gamma, Int. Immunopharmacol. 3 (2003) 1247–1255.
[126] J. Zhang, Yin and yang interplay of IFN-gamma in inflammation and autoimmune disease, J. Clin. Investig. 117 (2007) 871–873.
[127] B. Shrestha, T. Wang, M.A. Samuel, K. Whitby, J. Craft, E. Fikrig, M.S. Diamond, Gamma interferon plays a crucial early antiviral role in protection against West Nile virus infection, J. Virol. 80 (2006) 5338–5348.
[128] H. Beekhuizen, J.S. van de Gevel, Gamma interferon confers resistance to infection with *Staphylococcus aureus* in human vascular endothelial cells by cooperative proinflammatory and enhanced intrinsic antibacterial activities, Infect. Immun. 75 (2007) 5615–5626.
[129] B. Nandi, S.M. Behar, Regulation of neutrophils by interferon-γ limits lung inflammation during tuberculosis infection, J. Exp. Med. 208 (2011) 2251–2262.
[130] K. Newton, V.M. Dixit, Signaling in innate immunity and inflammation, Cold Spring Harbor Perspectives in Biology 4 (2012) a006049.
[131] O. Takeuchi, S. Akira, Pattern recognition receptors and inflammation, Cell 140 (2010) 805–820.
[132] A. Rivera, M.C. Siracusa, G.S. Yap, W.C. Gause, Innate cell communication kick-starts pathogen-specific immunity, Nat. Immunol. 17 (2016) 356–363.
[133] A. Kimura, T. Naka, T. Nakahama, I. Chinen, K. Masuda, K. Nohara, Y. Fujii-Kuriyama, T. Kishimoto, Aryl hydrocarbon receptor in combination with Stat1 regulates LPS-induced inflammatory responses, J. Exp. Med. 206 (2009) 2027–2035.
[134] J.D. Mezrich, J.H. Fechner, X. Zhang, B.P. Johnson, J. William, An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells, J. Immunol. 185 (2010) 3190–3198.
[135] J.P. Routy, B. Routy, G.M. Graziani, V. Mehraj, The kynurenine pathway is a double-edged sword in immune-privileged sites and in cancer: implications for immunotherapy, Int. J. Tryptophan Res. 9 (2016) 67–77.
[136] S.-H. Seok, Z.-X. Ma, J.B. Feltenberger, H. Chen, H. Chen, C. Scarlett, Z. Lin, K.A. Satyshur, M. Cortopassi, C.R. Jefcoate, Y. Ge, W. Tang, C.A. Bradfield, Y. Xing, Trace derivatives of kynurenine potently activate the aryl hydrocarbon receptor (AHR), J. Biol. Chem. 293 (2017) 1994–2005.
[137] L.Z. Agudelo, D.M.S. Ferreira, I. Cervenka, G. Bryzgalova, S. Dadvar, P.R. Jannig, A.T. Pettersson-Klein, T. Lakshmikanth, E.G. Sustarsic, M. Porsmyr-Palmertz, J.C. Correia, M. Izadi, V. Martínez-Redondo, P.M. Ueland, Ø. Midttun, Z. Gerhart-Hines, P. Brodin, T. Pereira, P.O. Berggren, J.L. Ruas, Kynurenic acid and Gpr35 regulate adipose tissue energy homeostasis and inflammation, Cell Metabol. 27 (2018) 378–392.
[138] S.V. Schmidt, J.L. Schultze, New insights into Ido biology in bacterial and viral infections, Front. Immunol. 5 (2014) 384.
[139] V. Selvaraj, K. Sampath, V. Sekar, Administration of yeast glucan enhances survival and some non-specific and specific immune parameters in carp (*Cyprinus carpio*) infected with *Aeromonas hydrophila*, Fish Shellfish Immunol. 9 (2005) 293–306.
[140] I. Rodríguez, R. Chamorro, B. Novoa, A. Figueras, β-Glucan administration enhances disease resistance and some innate immune responses in zebrafish (*Danio rerio*), Fish Shellfish Immunol. 27 (2009) 369–373.
[141] M.E. El-Boshy, A.M. El-Ashram, F.M. Abdelhamid, H.A. Gadalla, Immunomodulatory effect of dietary *Saccharomyces cerevisiae*, beta-glucan and laminaran in mercuric chloride treated Nile tilapia (*Oreochromis niloticus*) and experimentally infected with *Aeromonas hydrophila*, Fish Shellfish Immunol. 28 (2010) 802–808.
[142] M. Librán-Pérez, M.M. Costa, A. Figueras, B. Novoa, β-glucan administration induces metabolic changes and differential survival rates after bacterial or viral infection in turbot (*Scophthalmus maximus*), Fish Shellfish Immunol. 82 (2018) 173–182.
[143] W.M. Sealey, F.T. Barrows, A. Hang, K.A. Johansen, K. Overturf, S.E. LaPatra, R.W. Hardy, Evaluation of the ability of barley genotypes containing different amounts of β-glucan to alter growth and disease resistance of rainbow trout *Oncorhynchus mykiss*, Anim. Feed Sci. Technol. 141 (2008) 115–128.
[144] J. Beaulaurier, N. Bickford, J.L. Gregg, C.A. Grady, A.L. Gannam, J.R. Winton, P.K. Hershberger, Susceptibility of Pacific herring to viral hemorrhagic septicemia is influenced by diet, J. Aquat. Anim. Health 24 (2012) 43–48.
[145] J.H. Lee, J.W. Kim, Y.J. Kang, D.W. Ko, J.M. Kim, S.H. Choi, K.H. Park, Effects of

β-1,3-glucan on innate immunity responses and mortality induced by *Vibrio harveyi*, hemorrhagic septicemia virus, or *Miamiensis avidus* in the olive flounder *Paralichthys olivaceus*, Aquacult. Int. 26 (2018) 743–756.
[146] S.E. LaPatra, K.A. Lauda, G.R. Jones, W.S. Shewmaker, C.J. Bayne, Resistance to IHN virus infection in rainbow trout is increased by glucan while subsequent production of serum neutralizing activity is decreased, Fish Shellfish Immunol. 8 (1998) 435–446.
[147] L. He, G.J. Hannon, MicroRNAs: small RNAs with a big role in gene regulation, Nat. Rev. Genet. 5 (2004) 522–531.
[148] S. Yi, D. Lu, W. Peng, T. Wang, Y. Zhang, H. Lin, Differential expression profiling of spleen microRNAs in response to two distinct type II interferons in *Tetraodon nigroviridis*, PLoS One 9 (2014) e96336.
[149] F. Ma, S. Xu, X. Liu, Q. Zhang, X. Xu, M. Liu, M. Hua, N. Li, H. Yao, X. Cao, The microRNA miR-29 controls innate and adaptive immune responses to intracellular bacterial infection by targeting interferon-gamma, Nat. Immunol. 12 (2011) 861–869.
[150] A. Liston, A.S. Papadopoulou, D. Danso-Abeam, J. Dooley, MicroRNA-29 in the adaptive immune system: setting the threshold, Cell. Mol. Life Sci. 69 (2012) 3533–3541.
[151] C. Furnes, M. Seppola, B. Robertsen, Molecular characterisation and expression analysis of interferon gamma in Atlantic cod (*Gadus morhua)*, Fish Shellfish Immunol. 26 (2009) 285–292.
[152] T. Matsuyama, A. Fujiwara, T. Sakai, C. Nakayasu, Molecular cloning and expression analysis of interferon gamma gene in Japanese flounder *Paralichthys olivaceus*, Fish. Sci. 75 (2009) 253–255.
[153] R.N. Chen, Y.Q. Su, J. Wang, M. Liu, Y. Qiao, Y. Mao, Q.Z. Ke, K.H. Han, W.Q. Zheng, J.S. Zhang, Molecular characterization and expression analysis of interferon-gamma in the large yellow croaker *Larimichthys crocea*, Fish Shellfish Immunol. 46 (2015) 596–602.
[154] J.P. Levraud, P. Boudinot, I. Colin, A. Benmansour, N. Peyrieras, P. Herbomel, G. Lutfalla, Identification of the zebrafish IFN receptor: implications for the origin of the vertebrate IFN system, J. Immunol. 178 (2007) 4385–4394.
[155] K. Luo, S. Zhang, D. Tang, L. Xia, W. Gao, G. Tian, Z. Qi, Q. Xu, W. Zhang, Analysis of the expression patterns of the cytokine receptor family B (CRFB) and interferon gamma receptor (IFNGR) in Dabry's sturgeon (*Acipenser dabryanus*), Dev. Comp. Immunol. 84 (2018) 420–426.