IL10, A Tale of an Evolutionarily Conserved Cytokine across Vertebrates

M Carla Piazzon, Georges Lutfalla, Maria Forlenza

IL10, A TALE OF AN EVOLUTIONARY CONSERVED CYTOKINE ACROSS VERTEBRATES

Running title: IL10, a tale of an evolutionary conserved cytokine

M. Carla Piazzon[a,b], George Lutfalla[c], Maria Forlenza[a]

[a] Cell Biology and Immunology Group, Wageningen University, The Netherlands; maria.forlenza@wur.nl

[b] Fish Pathology Group, Institute of Aquaculture Torre de la Sal (IATS-CSIC), Consejo Superior de Investigaciones Científicas, Castellón, Spain; carla.piazzon@csic.es

[c] CNRS/Université Montpellier, Montpellier, France; lutfalla@univ-montp2.fr

Corresponding authors: correspondence should be addressed to: maria.forlenza@wur.nl; Tel: 0031-317-483336 and carla.piazzon@csic.es; Tel: 0034-964-319500.

Abstract

IL10 was discovered in 1989, and since then has been the subject of intense investigation revealing its potent anti-inflammatory and regulatory activities in most immune processes during infection and disease. It was only in 2003 that the first non-mammalian *IL10* sequence was identified in teleost fish, followed in 2004 by the chicken *IL10* sequence. In this review we summarize the work performed in non-mammalian vertebrates in which the *IL10*, *IL10 receptors* (*IL10Rs*), and the signaling components have been identified. We review the genomic organization, gene and protein structure of *IL10(Rs)* and focus on studies providing a functional characterization of their biological activities. In addition, we describe the activities of viral IL10s identified in viruses infecting non-mammalian hosts. Altogether, such analysis revealed a remarkable conservation of the anti-inflammatory and regulatory activities of (viral) IL10 across vertebrates, confirming the crucial role of IL10 throughout evolution. Interestingly, in some teleost fish, the presence of multiple copies of *IL10(Rs)* adds an additional degree of complexity. In fact, evidence suggests that gene duplication not necessarily implies functional redundancy, leaving teleosts with additional possibilities to fine tune IL10 activities. Finally, we discuss the use of zebrafish as a complementary animal model for the study of IL10 activities in non-mammalian vertebrates.

Key words

(viral) Interleukin-10, Evolution, teleosts, amphibians, reptiles, birds

Abbreviations

Sp1, Sp3: Specificity protein 1 or 3, C/EBPs: CCAAT-enhancer-binding proteins, IRF-1: interferon regulatory factor 1, AP-1: activator protein 1, GATA3: GATA binding protein-3; NF1F: Nuclear factor 1F, ISGF3: Interferon-stimulated gene factor-3, PBX: Pre-B-cell leukemia transcription factor, NFAT: Nuclear factor of activated T-cells, CREBs: cAMP response element-binding. NFκB: Nuclear factor κB; (p)STAT3: (phosphorylated) Signal transducer and activator of transcription 3; JAK1: Janus kinase 1; TYK2: Tyrosine kinase 2; CRFB: Cytokine receptor family B = CRF2: Cytokine receptor family class 2; LPS: lipopolysaccharide; PMA: phorbol myristate acetate; PBMC: Periferal blood mononuclear cell; PBL: Periferal blood leukocyte; BMM: Bone marrow-derived macrophages.

I. INTRODUCTION

Interleukin 10 (IL10) was first discovered in 1989 upon the observation that a factor produced by mouse Th2 clones inhibited the synthesis of several cytokines by Th1 clones.[1] This newly discovered cytokine was first named cytokine synthesis inhibitory factor (CSIF) but the name IL10 was already applied in the follow-up publication from the same group where they described that the Epstein-Barr virus (EBV) gene BCRFI showed extensive homology with IL10.[2] The latter study, describing the hijacking of a host cytokine gene as a viral strategy for survival, brought to light the importance of this cytokine in immune regulation and led to a considerable amount of research describing the importance of IL10 in the immune system.

IL10 is a pleiotropic regulatory cytokine produced by all leukocytes, with $CD4^+$ T cells and monocytes/macrophages being the most important sources.[3] Some non-immune cells such as keratinocytes or epithelial cells can also produce IL10.[4,5] The production of IL10 is tightly regulated and depends among other factors, on the stimulus, affected tissue and phase of the immune response.[3] The overall activity of this cytokine is to limit and terminate the immune response in order to prevent damage caused by the host's inflammatory response. Its ability to downregulate the immune response makes it a valuable target for pathogens. For example, the aforementioned EBV but also cytomegalovirus (CMV) and many more DNA viruses, have hijacked the IL10 gene into their genomes and use it to regulate the response of the host upon infection.

The main biological function of IL10 is exerted on dendritic cells, macrophages and neutrophilic granulocytes, inhibiting MHCII expression, differentiation of monocytes, expression of proinflammatory cytokines, phagocytosis and reactive radical species production.[6,7] IL10 anti-inflammatory activities are not only limited to the innate branch of

the immune system. It also directly inhibits proliferation of $CD4^+$ T cells,[8] IL2 and IFNγ synthesis by Th1 cells and IL4 and IL5 synthesis by Th2 cells.[9,10] The downregulation of proinflammatory activities indirectly has an effect on the resolution of the adaptive immune responses leading to an anti-inflammatory or regulatory state of immunity. IL10 has also stimulatory properties on specific cell types: it activates B cells, promotes their survival and proliferation, and contributes to class switching and antibody secretion;[11,12] IL10 can also stimulate NK cell proliferation and cytotoxic activity[13] as well as proliferation of specific subsets of $CD8^+$ T cells.[14] Altogether, IL10 has an important role in the termination of inflammation and restoration of homeostasis helping the development of long-lived memory cells to face future threats.

Based upon its structure, IL10 has been assigned to the class II helical cytokine family that includes IL10, interferons and all the so-called IL10-related cytokines (IL19, IL20, IL22, IL24, IL26, and IL28). They all share a similar overall 3D structure, are encoded by genes with a similar intron-exon structure and bind to receptors of similar structures (Class II, helical cytokine receptors) that signal through the JAK-STAT pathway.

IL10 acts as a homodimer that signals via the IL10 Receptor complex, constituted by two molecules of IL10 receptor 1 (IL10R1) which, upon binding to the ligand, recruit two molecules of IL10R2.[15] IL10R1 is specific and has high affinity for IL10 while IL10R2 can also act as co-receptor for other cytokines.[7] Both receptors belong to the class II cytokine receptor family (CRFB). Upon activation of the IL10R complex a JAK/STAT signaling pathway is initiated, generally triggered by the activation of JAK1 and TYK2 followed by the subsequent phosphorylation of the transcription factor STAT3. Phosphorylated STAT3 stimulates the transcription of several genes, among which the *suppressor of cytokine*

signaling 3 (*SOCS3*), which is considered the main transcription factor responsible for the inhibitory effects of IL10 on proinflammatory genes expression.[16]

In the past decades, the molecular structure, gene regulation, signaling pathway and bioactivity of mammalian IL10 have been extensively described and comprehensively reviewed.[3,4,7] Research on the biological activities of IL10 in non-mammalian vertebrates such as birds, reptiles, amphibians and fish is much more recent and scarce. In this review, keeping the activities of mammalian IL10 as reference, we aim to compile a comprehensive review of the current knowledge on this molecule in non-mammalian vertebrates. We will not only highlight the similarities between mammalian and non-mammalian IL10, but also bring to the attention of the reader the peculiarities of IL10 gene regulation, signaling pathway and bioactivities in selected non-mammalian species. In addition, we will not only review the activities of host IL10, but whenever possible, we will also include information on the bioactivities of viral IL10 identified in viruses infecting non-mammalian hosts. Finally, we will focus on the potential use of a relatively novel animal model, the zebrafish, as an additional and complementary tool for the study of non-mammalian IL10 activities.

I. NON-MAMMALIAN IL10 HOMOLOGUES: CONSERVATION OF GENES AND PROTEINS STRUCTURE

IL10 sequences, both gene and protein, of hundreds of non-mammalian vertebrates can be found in the databases. Most of them are predicted sequences from the recent explosion in genome sequencing and transcriptome analysis of a large variety of species. What is clear is that IL10 homologs can be found in all vertebrate classes and their sequence is conserved to such a degree that makes it easy to identify and classify them as such (Fig. 1). Despite the abundance in gene sequences, their structural and biological characterization is somewhat

lacking behind. For clarity, throughout the manuscript we will adopt the human nomenclature as birds, reptiles, amphibians and teleost fish all use different nomenclatures and were often difficult to combine in a clear manner in sentences referring to genes common in all species. IL10 gene(s) and protein(s) have been well described in duck,[17] chicken,[18] frog[19] and several teleost fish species (fugu,[20] common carp,[21] rainbow trout,[22] zebrafish,[23,24] sea bass,[25] Atlantic cod,[26] goldfish,[27] Indian major carp[28,29] and grass carp[30]) (Fig. 1). Interestingly, despite several reptile *IL10* sequences can be found as predicted genes in the database (included in the phylogenetic analysis in Fig. 1) no further functional characterization of this molecule has been carried out thus far. Among the annotated, but not yet functionally characterized sequences we also find the shark, coelacanth and lungfish *IL10*-like sequences, confirming that IL10 is an evolutionary 'old' cytokine. Furthermore, duplicate copies of *IL10* genes have been identified in several fish species (Piazzon manuscript in preparation)[31,32] but not in mammals, birds, reptiles and amphibians. As it will be further discussed later, gene duplication might not always imply functional redundancy, providing teleost fish with additional tools to finely tune their IL10-mediated regulatory response.

A. Genomic and structural conservation of the *IL10* gene

The synteny of the mammalian *IL10* locus is extremely conserved as in mammals the *IL10* gene is always found linked to *IL19*, and in the same relative position to *MAPKAPK2*, *DYRK3*, *PRELP* and *FMOD* (Fig. 2A). Like all *IL10* genes described in mammals, all known non-mammalian vertebrate *IL10* genes are composed of five exons and four introns (Fig. 2B). The length of exons is generally conserved and introns are in homologous positions, all in phase 0, therefore introns are not interrupting codons.[25] The size of the

introns however, varies greatly making the overall size of the *IL10* gene different among species.

Both, 5' and 3' untranslated regions (UTR) are also variable among species. Human and murine *IL10* cDNAs contain 7 and 6 AUUUA instability motifs respectively in the 3'UTR before the polyadenilation signal (Fig. 2B). The instability motifs are rapid RNA recognition sites for RNase E activity[33] important for post-transcriptional regulation of genes. In chicken[18] and duck[17] *IL10* transcripts, 11 and 6 AUUUA motifs can be observed in the 3'UTR; trout (a)[22] and grass carp[30] *IL10* transcripts present none, whereas sea bass,[25] common carp (a and b)[21] and Indian major carp[29] *IL10* have three. Goldfish and zebrafish possess 5 instability domains in the 3' UTR and an additional instability motif in the 5' UTR.[27] This differences suggest a tight regulation of this cytokine that varies greatly among species. Altogether we can conclude that the genomic organization (synteny) as well as gene organization of the *IL10* gene is highly conserved among vertebrates, further confirming the important role of this cytokine in the immune response.

1. Regulatory aspects: *IL10* duplications and splice variants

Although not a lot of studies have been performed on the regulation of non-mammalian *IL10* and the information available is partial and fragmented, some studies have focused on interesting cases worth mentioning such as the presence of multiple copies (paralogues) of this gene or different splice variants and their possible biological implications.

a. *When one is not enough! Presence of duplicated genes*

During evolution, after the two rounds of whole-genome duplications (WGD) that occurred in the common ancestor of vertebrates, teleost fish underwent a third duplication event[34]

implying that several genes are present in multiple copies within the fish genomes. These duplicated genes include, among many others, also cytokines, cytokine receptors and transcription factors. In addition, some fish species, including rainbow trout, Atlantic salmon or common carp, underwent an additional round of WGD[35,36] leading to the appearance of additional paralogues within their genome. To illustrate this complexity for the case of the *IL10* gene, rainbow trout and common carp have two paralogues (Fig. 2B), namely *IL10a* (Q6L8N7 and HQ323755) and *IL10b* (FR691804 and HQ323756),[31] (Piazzon, manuscript in preparation) that are very similar at the protein level but show differences in the promoter and 3' UTR, suggesting similar biological activities but differential regulation. The synteny of the paralogues is still difficult to analyze as the genome assemblies in these species are still incomplete or the scaffolds are too short, and are therefore not included in figure 2.

In trout, *IL10b* has a long 3'UTR with seven instability motifs, whereas *IL10a* has a short 3'UTR with no instability domains identified thus far, most likely due to incomplete sequencing of the 3' UTR region. Nevertheless, gene expression analysis shows differential stability and basal expression of the two transcripts in various tissues and cell types. Interestingly, *IL10a* presents an alternative ATG in the 5'UTR that, if translated, encodes for a 29 amino acids peptide and is proposed to be a mechanism used to regulate translation of the full-length protein under certain conditions. The 5' UTR of trout *IL10b* did not extend as far, and it is still to be determined whether such regulation occurs for *IL10b* as well. As expected, the two paralogues were differentially regulated under various conditions. IFNγ stimulation specifically affects *IL10b* expression whereas bacterial

infections induce differential regulation of both paralogues depending on the tissue studied.[31]

In carp, both paralogues showed similar bioactivity when tested in vitro (further discussed later) but have very different promoter regions, hinting again to a differential regulation. Carp *IL10a* is generally higher expressed in basal conditions but its expression levels do not seem to be regulated upon viral and parasitic infections. On the contrary, *IL10b* is significantly upregulated in the late phases of infection with the rhabdovirus Spring Viraemia of Carp Virus (SVCV) and the extracellular blood parasite *Trypanoplasma borreli* (Piazzon, manuscript in preparation) Such a differential expression pattern may confer each of the two isoforms different roles in homeostasis and pathogenesis. In agreement, a single-nucleotide polymorphism in the *IL10a* gene has been associated to resistance to cyprinid herpesvirus-3 infections[32] further highlighting the role of IL10 in fish immunity and disease resistance.

To our knowledge, the presence of duplicated copies of *IL10* (and its associated molecules) in the genome of non-mammalian vertebrates is restricted to teleost fish only, and in particular to those that underwent a 4th WGD event. Despite some amphibians, e.g. *Xenopus laevis*, are polyploid still only one IL10 gene can be found in their genome (Fig. 2A), perhaps suggesting that the IL10 locus in these species is under a certain selective pressure to retain a single *IL10* copy. As expected, common carp and rainbow trout also express two copies of the *IL10 receptors*, transcription factors *(i.e. JAK1* and *STAT3)* as well as *SOCS3* genes. As an example, there are two paralogues of *SOCS3* in zebrafish, *SOCS3a* (NP956244) and *SOCS3b* (NP998469). Each of these genes is then present in duplicate copy in common carp and trout, adding up to a total of four *SOCS3* genes in these species. Which one of these paralogues is more important for IL10 signaling, and whether

these differences have any biological significance is still under investigation. What is certain is that such gene expansion greatly widens the field of study and raises the question as to whether gene duplication implies functional redundancy or sub-functionalization, as well as whether gene expansion provides an evolutionary advantage to the species. All this is currently the focus of intense research in the comparative immunology field.

b. *Post-transcriptional regulation:* IL10 *splice variants*

Splicing-derived isoforms of several cytokines and cytokine receptors have been described but poorly studied in mammals.[37] Regarding *IL10* very few reports exist to that respect. A new *IL10* splice variant lacking the entire exon 3, named IL10δ3, was described in human leukemic cells and was associated with improved response to chemotherapy.[38] Other authors described the presence of two splice variants in human PBMC differing in the 5'UTR. One variant was constitutively expressed in unstimulated cells and contained a longer 5'UTR whereas upon stimulation with LPS the transcription of a variant with a shorter 5'UTR was induced which would have an extended half-life and be more accessible for protein translation.[39] Regarding viral-encoded *IL10s*, human cytomegalovirus was shown to produce several splice variants with different biological activities including the formation of complexes with human IL10 that were shown to interfere with host IL10 signaling.[40,41]

Alternative splicing of the *IL10* transcript has also been described outside mammals. When performing the identification of the Pekin duck IL10, the authors described two novel *IL10* splice variants generated by exon skipping or use of an alternative exon.[17] Compared to the normal duck *IL10*, one of the variants showed alternative splicing in the 3'UTR region

leading to a different number of instability domains and stability of this transcript. The second variant presents a complete deletion of exon 5. The truncated variant retains the contact residues with the IL10R1 but lacks the F helix, possibly affecting its activity by preventing the formation of the intercalated homodimers. The basal gene expression of the truncated variant is lower but mirrors that of the wild type transcript, although its expression is not altered by stimuli that regulated wild type duck *IL10* expression. This suggests differential roles of the splice variants in homeostasis and activation. Interestingly, heterologous protein expression in human cell lines, showed that differently from the wild type protein, the truncated form was not secreted in cell culture supernatants.[17] Besides the aforementioned studies, no reports focused on the possible existence of splice variants of the *IL10* gene in other vertebrates. Research on the post-transcriptional regulation of *IL10* can be crucial in the understanding of the fine tuning of this potent regulatory molecule especially during pathological conditions.

c. *The* IL10 *promoter*

In mammals, the *IL10* promoter and the transcription of the *IL10* gene in different cell types has been studied in detail. Transcription factors such as Sp1, Sp3, STAT3, C/EBPs, IRF-1, c-Maf, AP-1, CREBs and NFκB were found to positively regulate *IL10* transcription in human and mouse and the binding site of each of these transcription factors has been mapped to specific sites in the respective promoters. All this information was extensively reviewed by Mosser and Zhang.[3] Despite the low sequence similarity among the promoter regions of different species, in silico comparative analysis showed several common elements in the various promoter regions. Fugu, zebrafish, cod, common carp, duck and

281 chicken *IL10* promoters present, among others, an NFκB site, interferon response elements
282 (IREs), STAT3, GATA3, AP-1 and several Sp1 elements (Piazzon, manuscript in
283 preparation).[17,18,26]
284 Interestingly, in common carp analysis of the putative promoter region of the two *IL10*
285 paralogues showed several common binding element (e.g. for STAT1 and IRF4) but also
286 the presence of potentially crucial differences: the *IL10a* promoter contained NF1F, ISGF3
287 and SP1 binding sites that were not present in the *IL10b* promoter region, whereas *IL10b*
288 had STAT6, PBX and STAT5 binding sites that were not found in the *IL10a* promoter.
289 Altogether this could explain the differential expression of the *IL10a* and *IL10b* transcripts
290 and suggests a potentially different function of the proteins as they are differentially
291 regulated (Piazzon, manuscript in preparation). In mammals, the transcription factor
292 GATA3 has been assigned a central role in activating *IL10* transcription.[42,43] It is also
293 known that IL10 induces STAT3 expression and the presence of STAT3 binding sites in
294 the IL10 promoter suggests that IL10 regulates its expression in a positive feedback loop.[44]
295 As a difference, while the human *IL10* promoter presents several C/EBP-β binding sites,
296 the chicken and cod promoters only contain one, the carp promoters contains between two
297 and four, depending on the paralogue, whereas the duck and zebrafish promoters present
298 none.[17,26]
299 The presence of several common regulatory elements in the promoter regions shows that
300 the regulation of *IL10* is somehow conserved. Nevertheless it is important to note that all
301 above described binding sites are derived from in silico analyses and only two studies have
302 been conducted addressing the real involvement of these transcription factors in the
303 regulation of *IL10* transcription in non-mammalian vertebrates.[29,20] In Indian major carp

304 cells, the use of Bay 11-7082, a potent inhibitor of NFκB, blocked the expression of *IL10*
305 induced by LPS suggesting that the NFκB sites found in teleost have a real regulatory
306 function on this gene.[29] In fugu, the characterization of the *IL10* promoter was performed
307 by a series of deletion mutants on the promoter region using a luciferase reporter system in
308 trout RTG2 cell line. In this study it was shown that the binding element for NFAT,
309 situated 92 bp upstream the TATA box, was involved in TNFα-mediated induction of *IL10*.
310 The authors also characterized two regions in the fugu *IL10* promoter, one closer to the
311 TATA box which would contain activating elements, and another further upstream
312 containing inhibitory elements.[20] Although the study was performed in trout rather than
313 fugu cells, it provides preliminary functional evidence of the conserved regulation of the
314 *IL10* gene at least in teleost fish.
315 In general, little is known about the regulation of *IL10* expression in non-mammalian
316 vertebrates. Analysis of the *IL10* promoter region in cartilaginous fish, coelacanth,
317 amphibians and reptiles has not been conducted. Nevertheless, based on the aforementioned
318 presence of highly conserved regulatory elements in the promoter region of the known *IL10*
319 sequences, together with the patterns of expression in various tissues and cell types further
320 highlighted below, it is safe to suggest that the regulation of *IL10* might be conserved
321 across vertebrates.
322
323 **B. Structural conservation of the IL10 protein**
324 The IL10 proteins described in non-mammalian vertebrates range from 172 to 184 amino
325 acids (aa) with molecular weights between 15-21 kDa, and signal peptides of 16-22 aa long.
326 These proteins have an aa identity with their mammalian counterpart of 30-55%, with

Xenopus being the most similar, followed by birds and then fish. The degree of conservation of this cytokine among species seems low but is much higher than the interspecies conservation of other cytokines of the same structural family.[45]

IL10 is a homodimer formed by two intertwined but non-covalently bound monomers each with six alpha-helices and two intra-chain disulphide bridges.[46] All the non-mammalian IL10 proteins studied present the same 6-helix structure with the four conserved cysteine residues to form the two prototypical disulphide bridges (Fig. 2C).[17–19,22,23,25] A single study in goldfish, using *in vitro* binding studies between recombinant IL10 and IL10R1, provided experimental evidence that also in fish IL10 might be present as non-covalently bound homodimer.[47] Differences in the secondary structure when compared to mammals exist but are minimal. For instance, *Xenopus* IL10 presents shorter helix A and C and longer AB and CD loops than mammalian IL10;[19] in sea bass the CD loop is longer than in humans and helix E is smaller;[25] Indian major carp IL10 has helices A and F of different length.[28] In general, sites and motifs essential for the bioactivity of IL10 are well preserved. The ion pair, the many hydrogen bonds and the extensive hydrophobic core to stabilize the domain structure is conserved among species. The amino acids predicted to interact with IL10R1 are highly conserved or modified by similar amino acids (Fig. 2C), while the ones predicted to interact with IL10R2 are not well conserved.[17–23,25–31,48]

The residue I69 of human IL10, key for IL10 immunostimulatory functions[49] can be identified in most species in a similar position and the IL10 family signature motifs are generally conserved in all investigated species.[18–20,23,27,30] Trout and sea bass IL10 have one potential N-glycosylation site[22,25], fugu has two[20] and chicken and zebrafish IL10[18,23] have none. Human IL10 possesses one potential glycosylation site but is actually not

glycosylated while murine IL10 is glycosylated in its two potential sites. Nevertheless, glycosylation is not essential for IL10 bioactivity.[49,50]

All fish IL10 present two extra conserved cysteine residues that were believed to form an additional disulphide bridge specific for fish IL10. A 3D modeling study performed on Indian major carp showed that these two cysteines do not form any significant bond involved in structural stabilization or protein-receptor interaction.[28] It is therefore speculated that this residues mutated during evolution in higher vertebrates.

Altogether, we can conclude that across vertebrate species the structure of the IL10 protein has been extremely conserved (Fig. 2D), particularly the residues necessary for receptor-ligand interaction. As it will be further discussed below, this supports the evolutionary conservation of the regulatory functions of IL10 in non-mammalian vertebrates.

II. IL10 RECEPTORS AND SIGNALING PATHWAY

IL10 exerts its functions upon binding to the IL10 receptor complex on the cell surface. The IL10 receptor complex is constituted by two class II cytokine receptor (CRF2 or CRFB) family members, one belonging to the R1 type with a long intracellular domain (IL10R1 or CRFB7 in fish), and the other to the R2 type with a short intracellular domain (IL10R2 or CRFB4 in fish) (Fig. 3A).[51,52] Binding of the IL10 homodimer to two IL10R1 molecules induces a conformational change in the cytokine allowing the association of two IL10R2 molecules.[53] The latter activates the Janus kinases Jak1 and Tyk2 associated with the cytoplasmic tails of IL10R1 and IL10R2 respectively.[54,55] All this leads to phosphorylation of STAT3 or other latent transcription factors depending on the cell type.[56,57]

The components of the IL10 signaling pathway have been well characterized in mammals and there are only a few studies dealing with their functional characterization in non-

mammalian vertebrates. While IL10 genes have been easily identified using whole genome sequences, identification of its receptor chain in non-mammalian vertebrates, in particular in teleost fish, has been more challenging due to higher sequence divergence. Based upon the first high quality whole genome sequences from fish species, a repertoire of genes encoding class II helical cytokine receptors in fish has been established. They have been called CRFB1 to CRFB17 (Fig. 3B).[58,59] Due to high sequence divergence, sequence similarities are not a sufficient criterion to assign a function to most of these CRFBs in fish. Furthermore, some fish species (e.g. common carp, rainbow trout, and Atlantic salmon) have duplicated copies of some of these genes (Fig. 3C). Additional criteria such as synteny may be used, but functional identification based upon biological activity in at least one fish species is necessary.

A. The IL10 receptor 1

Several *IL10R1* sequences (such as those for chicken (AM049243), turkey (XP_003212786), finch (XP_002189322), Chinese softshell turtle (ENSPSIG00000002111) and frog (XP_002932948)) can be found in the databases as automatic predictions and genome annotations. Functional studies on non-mammalian species were performed only very recently in Pekin duck[52], zebrafish[47], and goldfish;[47] in fish, CRFB7 was identified as being *IL10R1*. Compared to their ligand, the *IL10R1* sequences have diverged to a larger degree throughout evolution. Nevertheless, the genomic organization (synteny) and gene structure of the CRFB family members that include the *IL10R1* homologues is highly conserved (Fig. 4) and allowed for a relatively straightforward identification of the IL10R1 (CRFB7) in non-mammalian vertebrates.

In the human, chicken, frog and zebrafish genomes the gene(s) is always flanked by *TMPRSS13*, *FXYD6* and *DSCAML1* upstream, and by *TMPRSS4*, *SCN4B* and *SCN2B* downstream (Fig. 4A) making it easier to identify the *IL10R1* sequences by synteny analysis.[47] Like most other class II helical cytokine receptors in vertebrates, IL10R1 (CRFB7) is encoded by a gene composed of 7 exons and 6 introns of respective phases 1, 2, 1, 0, 1 and 0 (Fig. 4B).

The mammalian, avian, amphibian and fish *IL10R1* genes encode proteins with conserved residues particularly in the regions that are needed for the formation of the hydrophobic patches where ligand binding occurs.[47,52] With respect to the intracellular portion of the receptor, JAK1-binding motive (PXXL) has been highly conserved and can be found within the first cytoplasmic residues in all species studied (Fig. 4C).[47,52] Two conserved peptide motifs containing a conserved tyrosine residue (GYXXQ) predicted to be involved in the recruitment of STAT3 can be found after the JAK1 binding site in avian[52] and most mammalian sequences (Fig. 4C). In some mammalian species such as mouse and rat as well as in birds, an additional STAT3 recruitment site can be found upstream of the canonical sites. Fish and frog sequences present one very conserved STAT3 recruitment site,[47] identified as GYXXQ, and a second non-canonical site identified as DYLLQ in frog and GYRSG in fish. In fish and birds but also in rabbit and horse a third tyrosine residue can be found downstream of the other two (canonical) STAT3 recruitment sites (Fig. 4C). Finally, in fish species where the ligand was found to be duplicated (e.g. common carp) also the receptor is present in two copies, adding an additional degree of complexity to the understanding of IL10 signaling in fish. A report in rainbow trout described one CRFB7 molecules,[60] but this might also be due to the preliminary assembly of the genome. Furthermore, the exact contribution of each of the canonical as well as additional (potential)

421 STAT3 recruitment sites in the cytoplasmic tail of the IL10R1 of fish and frog has not been
422 systematically addressed and awaits further investigation.
423 IL10R1 is typically expressed on immune cells and in immune organs.[4] Avian and fish
424 IL10R1 are most expressed in spleen and thymus followed by bursa, lung and cecal tonsil
425 in the case of birds and gills, kidney and gut in fish.[47,52,61] In general, highest expression is
426 detected in hematopoietic (fish kidney, avian bursa) and immune organs, especially in
427 mucosal immune tissues such as gut, lung and gills. In carp and goldfish, IL10R1 is highest
428 expressed in macrophages, considered the main cellular target of IL10, followed by
429 neutrophils, B cells and thymocytes.[47,61] In goldfish monocytes, IL10R1 is specifically
430 downregulated by inflammatory signals, such as bacterial or parasite antigens, but is
431 marginally regulated by poly I:C or zymosan.[47] Duck PBMCs stimulated with PMA exhibit
432 a rapid upregulation of the receptor in the first 2 hours, falling even below the basal levels
433 after 8 hour stimulation.[52] Not much more is known about the regulation of the expression
434 of IL10R1 besides mammals. The data so far indicate that inflammatory stimuli would
435 generally downregulate the expression of this anti-inflammatory-related molecule and that
436 IL10 itself is also able to downregulate its own receptor, hinting at a conserved negative
437 feedback loop in the IL10 system.[47]
438
439 **B. The IL10 receptor 2**
440 The IL10 receptor 2 belongs to the R2 type subunits of CRFB receptors. These subunits
441 contain shorter intracellular domains and act as co-receptors for the R1 subunits after
442 binding of the latter to the ligand.[62] IL10R2 is not exclusive for IL10 and, in mammals, also
443 serves as a co-receptor for other IL10 family members (i.e. IL22, IL26) and for type III
444 interferon signaling.[63–65] In mammals, the gene cluster *IFN-α receptor-2* (*IFNAR2*),

445 *IL10R2, IFNAR1* is a very conserved group of synteny.[66] The first non-mammalian *IL10R2*
446 sequence was identified in chicken using a hybridization probe against human *IL10R2;* by
447 synteny analysis it led to the identification of *IFNAR1* and *IFNAR2* (Fig. 5A).[51] In the same
448 study, the hybridization approach failed to identify the *IL10R2* gene in a fish genome.
449 Owing to the first available high quality fish genomes, and using protein similarities, gene
450 structure similarities and synteny, the fish homologues of the mammalian *IL10R2* gene
451 have been identified in 2003.[58] They are named *CRFB4* and *CRFB5* and are present in all
452 fish genomes analyzed so far. They are most probably derived from a recent duplication
453 event. As it can be easily appreciated in figure 5A, the gene cluster *IFNAR2, IL10R2,*
454 *IFNAR1* is highly conserved not only in mammals, but also in birds, reptiles and
455 amphibians. Such conservation however is completely lost when it comes to fish genomes
456 (Fig. 5A);[67] also when comparing several fish genomes, many differences can be found in
457 the locus organization of most of the *CRFBs* homologous to the genes involved in these
458 gene cluster. For example, all fish express two *IFNAR2* homologues named *CRFB1* and
459 *CRFB2* (Fig. 5A)[68] but they are often found in regions very distant from, rather than in
460 proximity of, the putative *IL10R2* genes (i.e. *CRFB4* and *CRFB5*). Furthermore, a fish-
461 specific *CRFB3* gene is present only in some fish species, but when present, it is found in
462 the gene cluster neighboring the potential *IL10R2* genes. To complicate matters, the *CRFB6*
463 gene (previously confirmed to be the *IFNGR2* homologue)[59] is present in all fish species,
464 but only in some it is found neighboring the *CRFB4* or *CRFB5* gene; similarly to *CRFB3*,
465 *CRFB4* and *CRFB5*, it encodes a protein with a short cytoplasmic tail. Altogether, solely
466 based on *CRFB4* and *CRFB5* protein structure (both encoding for a co-receptor with short
467 cytoplasmic tail), or on the genomic organization of the locus, it was not possible to
468 unequivocally determine which of the two would be the functional equivalent of *IL10R2*.

469 The question about which between CRFB4 and CRFB5 could act as the actual co-receptor
470 of IL10R1 (CRFB7) was recently addressed in grass carp using a functional approach.
471 Grass carp kidney cell lines were transfected with a pSTAT3-luciferase reporter plasmid
472 together with a vector encoding for the IL10R1 in combination with either CRFB4 or
473 CRFB5. After stimulation with recombinant IL10 an increase in the luciferase activity was
474 observed only in cells transfected with the CRFB7+CRFB4 combination, providing the first
475 functional indication that CRFB4 is the likely co-receptor for the IL10R complex in fish.[69]
476 Previous functional studies in zebrafish on the characterization of the type I IFN receptor
477 complex indicate that CRFB5 acts as the co-receptor for CRFB1 and CRFB2 involved in
478 type I IFN signaling.[59] Based on the functional work performed in grass carp and zebrafish,
479 and despite the high sequence similarity between CRFB4 and CRFB5, it is unlikely that the
480 type I IFN and the IL10 system would share common co-receptor subunits. This leaves
481 indeed CRFB4 as the most likely co-receptor of CRFB7 in IL10 signaling. Nevertheless,
482 only a systematic functional approach using both, IL10 and type I IFN ligands would give
483 us a definite answer.
484 It is very important to mention that in databases such as ensembl.org automatic gene
485 annotation assigned the name *IL10R2* (or *IL10Rb*) to all *CRFB5* present in fish genomes.
486 When the *IL10R2* GeneTree is generated in ensembl
487 (http://www.ensembl.org/Multi/GeneTree/Image?gt=ENSGT00530000063449) two main
488 clusters are clearly generated: one containing the *IFNAR1* sequences clustering together
489 with the fish *CRFB5* (here wrongly named *IL10R2*/*b*) and a second branch containing fish
490 *CRFB4* grouping together with the *IL10R2* sequences in other species. Therefore, in this
491 example, phylogenetic analyses already hint at the incorrect annotations of the *CRFB4* and

492 *CRFB5* sequences in the database, and stress the confusion that can be generated by
493 automated annotations.
494 With respect to gene structure and expression, in all investigated vertebrates, including fish
495 *CRFB4* and *CRFB5*, the genes present seven exons of conserved length and six introns of
496 variable length (Fig. 5B). Like in mammals, gene expression studies in frog and duck
497 (*IL10R2*) and fish (*CRFB4* and *CRFB5)* show that these molecules are constitutively
498 expressed in all tissues examined being highest expressed in immune organs and lowest in
499 muscle, heart and brain.[58,69–71] The expression levels remain stable in most cells even after
500 activation.[65,71] With respect to protein structure, chicken, duck and frog *IL10R2* genes
501 encode for proteins that have about 40% amino acid identity to the human counterpart,
502 while fish proteins are only 30% identical to the human homologue. IL10R2 proteins from
503 fish and amphibian share the 4 conserved cysteine residues important for the linkage of the
504 extracellular β-strands, but chicken and duck proteins only present 3 of these 4 conserved
505 residues.[70]
506 Altogether, in teleost fish *CRFB* genes have evolved rapidly and independently not only
507 from their mammalian counterpart but also from homologous genes in other tetrapods. This
508 is especially reflected in the poor conservation of the *IFNAR2*, *IL10R2*, *IFNAR1* genomic
509 locus in teleosts. The approach taken to identify CRFB4 as the functional IL10R2, shows
510 how functional analysis, together with genomic and gene structure analysis, have all been
511 instrumental to unravel the role especially of this fast evolving gene. The incorrect
512 annotation in the database of CRFB5 as IL10R2, further confirms how automated analysis,
513 not supported by functional data, can lead to incorrect conclusions. Finally, considering that
514 in some species, such as common carp (unpublished observation), Atlantic salmon[72] and

515 possibly trout, the genes encoding for IL10 and its receptors are duplicated, we can expect
516 that unique features and regulatory mechanisms might be unraveled by the study of
517 duplicated genes in teleost fish.
518
519 **C. Downstream signaling**
520 In mammals, upon binding of IL10, the IL10 receptor complex activates the Janus tyrosine
521 kinases, JAK1 and TYK2, associated with IL10R1 and IL10R2 respectively. The
522 cytoplasmic tail of IL10R1 is phosphorylated leading to the recruitment and subsequent
523 phosphorylation of STAT3 by the kinases.[3] What happens downstream the IL10 receptor is
524 not very well documented in non-mammalian species, with only a few reports in fish
525 dealing with the prototypical signaling cascade of STAT3 phosphorylation and activation
526 of the *SOCS3* gene. By use of cross-reacting antibodies recognizing phosphorylated
527 STAT3, it was possible to show that goldfish, common carp and grass carp IL10 induce
528 STAT3 phosphorylation and translocation to the nucleus.[27,30,61] Phosphorylation of
529 cytoplasmic STAT3 occurs in the first 15 minutes after stimulation even though the cellular
530 association of IL10 with the receptor persists for more than 90 minutes.[27] *SOCS3*
531 expression, in fish as in mammals, is also upregulated within the first hours of exposure to
532 IL10[27,30,61] and this effect can be abolished by a STAT3 inhibitor.[30] What remains to be
533 studied, in fish and in other vertebrate species, is the significance of the various canonical
534 and non-canonical STAT3 binding sites and how this, together with serine-rich stretches of
535 residues, might affect the downstream signaling. Furthermore, in human and mice it has
536 been observed that not all STAT3-inducing receptors, e.g. IL6R, trigger anti-inflammatory
537 responses. This implies that activation of STAT3 might not be the only mechanism
538 required for the anti-inflammatory activity of IL10. Inhibition of NFκB activation,

translocation as well as DNA binding have all been shown to occur in various cell types following IL10 stimulation. The inhibition of NFκB activity by IL10 would explain the large number of immune response genes that are less responsive to stimuli or are downregulated following IL10 treatment (reviewed by Mosser and Zhang).[3] SOCS3 activation is a hallmark of IL10 (and not IL6) induced gene expression, possibly suggesting that SOCS3 might play a unique role in the IL10-specific response. Detailed analysis of the IL10 signaling pathway in various cell types, besides the activation of STAT3, has not been systematically addressed in non-mammalian species. As mentioned above, the cytoplasmic tail of the IL10R1 presents various canonical and non-canonical STAT3-binding sites. This, together with the presence of additional tyrosine and serine-rich motives in the cytoplasmic tail, leaves open the possibility that, also in non-mammalian species, IL10 might act through signaling mechanisms other than STAT3.

III. BIOACTIVITY

The conservation of the IL10 protein, signaling pathway and the expression dynamics upon stimulation or infection hint to a conservation of bioactivity when compared to mammals. But actual bioactivity studies of non-mammalian IL10 on different cells of the immune system are very scarce and have been conducted only in a few avian and fish species.

A. Bioactivity on phagocytes

Monocytes, macrophages and neutrophilic granulocytes are among the main targets of IL10. This cytokine is known to strongly inhibit phagocytes by downregulating the production of toxic radicals, phagocytosis, antigen presentation and expression of proinflammatory cytokines.[3,6]

563 The only study in chicken addressing the inhibitory activity of IL10 on macrophages made
564 use of neutralizing antibodies against chicken IL10.[73] Chicken bone marrow-derived
565 macrophages (BMMs) were shown to produce nitrogen radicals upon LPS stimulation and
566 to readily express IL10 protein as early as 2 h after stimulation. Under the same conditions,
567 the addition of IL10 neutralizing antibodies led to a significant increase in nitrogen radical
568 production by BMMs.
569 The effect of IL10 on phagocytes of other avian species, reptiles or amphibians has not
570 been investigated thus far. Nevertheless, a substantial amount of data is available from
571 studies in various teleost fish species. Recombinant goldfish IL10 was shown to
572 significantly reduce the respiratory burst induced in goldfish monocytes by *Aeromonas*
573 *salmonicida* or IFNγ stimulation as well as the expression of several pro-inflammatory
574 genes including *TNFα1*, *TNFα2*, *IL10*, *CXCL8* and the NADPH oxidase component
575 $p47^{phox}$. Under the same conditions, goldfish splenocytes showed downregulation of the
576 expression of *IFNγ*.[27] In mammals, the inhibition of the respiratory burst in macrophages by
577 IL10 is mainly attributed to an indirect effect of IL10 acting through the downregulation of
578 TNFα rather than directly on radical production and release.[74,75] In the case of goldfish,
579 besides downregulation of *TNFα1* and *TNFα2*, a direct effect of IL10 on the respiratory
580 burst was demonstrated due to the direct downregulation of NADPH oxidase components.[27]
581 Recombinant carp IL10, similarly to goldfish IL10, significantly inhibited the PMA and
582 LPS induced production of toxic oxygen and nitrogen radicals in carp macrophages and
583 neutrophils.[61,76] The effect was dose dependent and very rapid, again pointing towards a
584 direct inhibitory effect of IL10 on fish phagocytes. Carp IL10 also inhibited the LPS-
585 induced expression of proinflammatory genes in macrophages and neutrophils. More

specifically, *IL1β*, *TNFα*, *iNOS* and *IL6* were downregulated in both cell types and the *p35* gene was downregulated only in macrophages. Carp IL10 also showed inhibitory effects on genes involved in antigen presentation in carp neutrophils, but not macrophages, as it downregulated the expression of *MHCI* and *MHCII* genes[61] and the surface expression of MHCI protein.[76] Interestingly, as mentioned above, common carp and trout present two copies of the *IL10* gene both encoding for potentially functional proteins. While the biological activity of both isoforms was not compared in trout, functional studies in common carp, using recombinant IL10a and IL10b, clearly indicate that the two proteins have identical biological activities. Nevertheless, as discussed in section I.A.1.a., the transcriptional regulation of the paralogues is different under various conditions, consistently with their different promoter regions. This indicates that although they might bind to the same receptor complex and trigger the same signaling in carp leukocytes, they might not be expressed under the same circumstances and at the same level. This points towards a possible sub-functionalization, rather than functional redundancy, of paralogous genes, further increasing the level of regulation and fine tuning of the immune system in those species presenting multiple gene copies.

The direct effect of fish IL10 on phagocytes was also studied in grass carp. Recombinant grass carp IL10 inhibits the LPS-induced transcription of *TNFα*, *IL1β*, *IL8* and *iNOS* in monocytes/macrophages.[77] On the same cells the authors also tested the effect that endogenous IL10 had on TGFβ1 expression, another important regulatory cytokine. LPS was found to induce proinflammatory gene expression in monocytes/macrophages after 6 h and the upregulation was reduced at 12 h when endogenous IL10 and TGFβ1 mRNA and protein levels increased. When IL10 and TGFβ1 blocking antibodies were used, the

609 stimulatory effects of LPS were still significantly high at 12 h, confirming the inhibitory
610 activity exerted by the endogenously produced anti-inflammatory cytokines. The inhibitory
611 activity exerted by grass carp IL10 and TGFβ1 on LPS-induced NFκB activation was also
612 investigated. The protein IκBα, which inhibits NFκB by blocking its ability to bind DNA,
613 is degraded in grass carp monocytes/macrophages upon LPS stimulation. Both, IL10 and
614 TGFβ1 showed the ability to block LPS-induced IκBα protein degradation thereby
615 attenuating the pro-inflammatory effect of LPS.[77]
616 Altogether we can conclude that the prototypical anti-inflammatory activities of IL10 on
617 phagocytes are generally conserved also in non-mammalian vertebrates. What perhaps still
618 needs to be further investigated is the ability of IL10 to also inhibit antigen presentation by
619 macrophages. The studies performed so far in fish on the regulation of antigen presentation
620 do not show a significant effect of IL10 on macrophages.[78] The study however only
621 focused on *MHCII* transcription rather than protein expression, leaving open the possibility
622 that IL10 might directly affect MHCII protein expression on macrophages thereby lowering
623 their antigen presentation capacity.
624
625 **B. Bioactivity on lymphocytes**
626 The effect of IL10 on B and T lymphocytes is diverse. On the one hand IL10 is known to
627 induce proliferation, antigen presentation, differentiation and antibody secretion in B
628 lymphocytes[11,12] and to promote proliferation of subsets of $CD8^+$ T lymphocytes.[14] On the
629 other hand, it directly inhibits cytokine synthesis and proliferation of $CD4^+$ Th1 and Th2
630 lymphocytes, indirectly affecting the progression or the resolution of the adaptive immune
631 responses.[8–10] The paucity of tools available to study B and T cell biology in non-

632 mammalian vertebrates makes the characterization of these cells and their function very
633 difficult. Only few markers are available to separate different cell populations and the
634 different lymphocyte responses known in mammals have not been fully characterized in all
635 non-mammalian vertebrate species. Nevertheless, some advances have been made in the
636 last years, especially in chicken and in a few teleost fish species owing to the development
637 of B and T cell-specific monoclonal antibodies or to the identification of cross-reactive
638 antibodies against mammalian transcription factors.

639 In chicken, recombinant IL10 inhibits IFNγ transcription and protein expression in mitogen
640 stimulated lymphocytes from spleen. IL10 also inhibits the ability of the supernatants of
641 these stimulated lymphocytes to induce nitrogen radicals, probably due to the lower
642 concentrations of IFNγ, indirectly affecting the activity of the phagocytes.[18] Duck
643 recombinant IL10 inhibits the expression of IL2 induced by mitogen stimulation of
644 PBMCs.[17]

645 In teleost fish, recombinant carp IL10 inhibited the IL2-induced proliferation of
646 thymocytes.[76] This is in contrast with the activity of mammalian IL10 on the same cell
647 type[79] but the biological implications of this difference remain to be studied. Interestingly,
648 only in immunized carp, IL10 showed to enhance proliferation of a subpopulation of T cells
649 when administered with the immunizing antigen.[61] Under the same conditions IL10 had no
650 effect on proliferation of naïve T cells, suggesting that the stimulatory effect of IL10 is
651 restricted to a subpopulation of memory T cells. Due to the lack of antibodies against T
652 cell surface markers, the class of T cells involved in this response was characterized only
653 by real time-quantitative PCR and the results indicated that IL10 inhibited the Th1 and Th2
654 responses induced by the immunizing antigen while promoting the proliferation of a subset

655 of $CD8^+$ T cells. Further characterization of the specific T cells populations stimulated by
656 IL10 is expected soon owing to newly developed antibodies specific for various subsets of
657 carp T cells.
658 In carp, the availability of an anti-IgM antibody[80] allowed for the study of the effect of
659 IL10 specifically on IgM^+ B cells. Recombinant carp IL10 directly promoted IgM^+ B cell
660 proliferation in sorted cells and in mixed PBL cultures; the stimulatory effect was further
661 enhanced by LPS or *Trypanoplasma borreli* antigens, both known to induce a polyclonal
662 activation of carp IgM^+ B cells.[61,76] Contrary to what was found in neutrophils, IL10
663 increased the surface expression of MHCI molecules in IgM^+ B cells possibly improving
664 antigen presentation by these cells.[76] Regretfully, the lack of specific antibodies to detect
665 MHCII left this characterization incomplete, but what is clear is that carp IL10 exerts
666 differential and cell type-specific effects on MHCI protein expression with possible
667 consequences on antigen presentation. In carp head kidney leukocyte cultures IL10 induced
668 an increase in secreted total and antigen specific IgM, which also correlated with an
669 increase in differentiation of plasmablasts to plasma cells.[61] These studies in carp show well
670 conserved bioactivity of IL10 on B cells when compared to mammals but focus only on
671 IgM^+ B cells. To complete these studies, the effect of fish IL10 on IgT^+ and IgD^+ B cells
672 should be conducted.
673 In grass carp IL10 enhanced cell viability of PBLs. Although the specific cell type affected
674 was not characterized, this activity resembled the prototypical effect of IL10 on B cells.
675 This effect was also shown upon incubation with TGFβ1, but further studies using blocking
676 antibodies against IL10 and TGFβ1, confirmed that the actual induction of proliferation is
677 due to the endogenous IL10 whose expression and secretion is activated by the TGFβ1.[30]

678 In general, besides the work performed in fish, in particular in common carp, not much is
679 known about the activities of IL10 on lymphocytes in non-mammalian species.
680 Nevertheless, based on the work in teleost fish, on the indirect data in chicken and duck,
681 and considering the structural conservation of the protein discussed above, it could be safe
682 to speculate that IL10 activities on lymphocytes might be conserved also in non-
683 mammalian vertebrates.
684

685 **IV. VIRAL HOMOLOGS**

686 A common strategy used by DNA viruses to counteract the host immune system is the
687 expression of homologs of host genes, in particular cytokines, chemokines, growth factors
688 and cytokine receptors.[81] IL10 homologs have been identified in multiple members of the
689 Poxviridae and Herpesvirales and, although they share relatively low amino acid identity
690 with their host counterpart, they can still bind to the IL10R complex, effectively mimicking
691 at least part of the biological activities of the host protein.[82,83] Among the most studied
692 IL10 viral homologs are those produced by the human Cytomegalovirus (CMV)[84] and
693 Epstein-Barr virus (EBV),[2] although more than 20 cytokine homologs have been described
694 in viruses infecting mammals including horse,[85] monkeys,[86] sheep,[87,88] cow,[89] goat,[90]
695 camel[91] and even bats.[92] This phenomenon is not restricted to mammals, as several viruses
696 infecting birds (pigeon pox virus, penguin pox virus[93] and canary pox virus),[94] reptiles
697 (testudinid herpesvirus)[95] and fish (anguillid herpesvirus 1[96] and cyprinid herpesvirus 3)[97]
698 present IL10 homologs in their genomes. Sequence analysis of these homologs showed
699 again low sequence identity but conservation of the essential residues required for receptor-
700 binding. Nevertheless, uncharacterized biological functions for these proteins cannot be
701 excluded. Besides studies on $_{CMV}$IL10 and $_{EBV}$IL10, functional studies on the biological

activities of viral cytokine homologues have been conducted only on the cyprinid herpesvirus 3 IL10 homologue ($_{CyHV3}$IL10).

Open Reading Frame 134 (ORF134) of CyHV3 encodes for the $_{CyHV3}$IL10, which was shown to be the second most abundant protein in the virus secretome.[98] It was found to be highly expressed in infected carp tissues during the acute and reactivation phases of viral infection and at lower levels during virus persistence at low temperatures.[99] The predicted three-dimensional structure and residues important for the interaction with the IL10R1 are highly conserved.[48] Indirect evidence of $_{CyHV3}$IL10 signaling via this receptor was provided by a study in zebrafish using a morpholino approach, in which knock-down of the *IL10R1* abrogated the response to both $_{CyHV3}$IL10 and zebrafish IL10.[99] More direct evidence was provided by work in common carp, in which recombinant $_{CyHV3}$IL10 was shown to induce phosphorylation of STAT3 and expression of *SOCS3* in carp leukocytes.[76] Furthermore, recombinant $_{CyHV3}$IL10 was shown to share several activities with its host counterpart, carp IL10: it inhibited the respiratory burst in phagocytes, downregulated the expression of proinflammatory genes in macrophages and promoted proliferation of IgM^+ B cells and of certain subsets of memory $CD8^+$ T cells.[76] In zebrafish, injections of $_{CyHV3}$IL10 mRNA induced an increase in the number of lysozyme-positive cells in zebrafish embryos in a manner similar to zebrafish IL10.[99] Nevertheless, similarly to some mammalian viral cytokines such as $_{EBV}$IL10, it does not mimic the full repertoire of host IL10 activities. $_{CyHV3}$IL10 presented lower effects on the inhibition of proinflammatory cytokines expression in neutrophils, failed to inhibit nitrogen radical production and did not affect expression of molecules involved in antigen presentation and thymocyte proliferation.[76] These differences are most likely due to difference in affinity of the viral IL10 to the receptor, but the possibility of an alternative signaling pathway, depending on the cell type,

726 cannot be excluded. Some effects of CyHV3 infections on the innate immune response of
727 the host, such as inhibition of type I interferons[100] and inhibition of apoptosis,[101] have also
728 been attributed to its ability to express an IL10 homolog among other anti-inflammatory
729 proteins. Interestingly, although $_{CyHV3}$IL10 is highly secreted upon infection and has
730 important anti-inflammatory properties, in vivo studies using recombinant virus strains with
731 a deleted ORF134, suggested that $_{CyHV3}$IL10 is not essential for viral replication in vitro or
732 virulence in vivo.[98] This apparent contrast should be further studied to unravel the
733 biological relevance of this viral homolog. Considering the importance of IL10 in
734 regulating the immune response and the vast number of viruses carrying IL10 homologues,
735 it would be certainly interesting to gather more data on the function of viral IL10s in other
736 non-mammalian species. Furthermore, considering the different environments and body
737 temperature that the various hosts live in, it would be interesting to investigate how and
738 possibly why the same viral IL10 homologue has been retained throughout viral evolution.
739 This will not only give important insight in virus biology, but will certainly help us
740 understand the key features of the host IL10 that have been retained through host and virus
741 evolution.
742

743 **V. IL10 EXPRESSION: WHO, WHERE AND WHEN?**

744 **A. Tissue expression and cellular sources of IL10**

745 In mammals it has been shown that IL10 can be produced by almost all leukocyte subtypes,
746 with $CD4^+$ T cells and monocytes/macrophages being the most important sources.[3]
747 Together with the identification of the sequence, the basal expression of *IL10* in different
748 tissues has been reported for several non-mammalian vertebrates. Chicken and duck *IL10*
749 showed higher expression in bursa and cecal tonsil and moderate expression in thymus,

750 liver and lung; no constitutive expression could be found in chicken spleen and bone
751 marrow as well as in non-lymphoid tissues such as kidney, brain, heart and muscle. In
752 contrast, in duck constitutive expression of *IL10* can be found in spleen and the highest
753 expression is seen in lung.[17,18] In frogs, the highest constitutive expression is found in
754 kidney, spleen and gut, and low expression is seen in liver or heart.[19] In teleost fish, the
755 constitutive expression in different tissues varies among species.[20–23,25–27,29,30] Head kidney,
756 gut and gills showed constitutive high expression in all investigated species; the same was
757 true for spleen with the exception of fugu. The expression in isolated cell types was only
758 determined in carp (Piazzon, manuscript in preparation) and goldfish,[27] where neutrophilic
759 granulocytes and monocytes/macrophages are the cells expressing the highest levels of
760 *IL10*. In rainbow trout the expression of the IL10 paralogues was investigated in a
761 mononuclear/macrophage-like cell line (RTS-11) showing that both paralogues can be
762 expressed and are differentially regulated by various stimuli.[31]
763 In chicken, bone-marrow derived macrophages and the HD11 macrophage cells line were
764 shown to considerably upregulate *IL10* expression and protein production when stimulated
765 with LPS.[73]
766 In fish, other than immune cells, the epithelial cell line from fathead minnow (EPC) is able
767 to express *IL10* and its expression is regulated by poly I:C and ranavirus infections.[102]
768 Similarly, in rainbow trout, the epithelial cell line RTL from liver, the fibroid cell lines
769 RTG-2 from gonad, and RTGill from gills, were all shown to express *IL10* and
770 differentially regulate its expression upon poly I:C, LPS or IFNγ stimulation.[22] It is
771 important to note that observed differences between species can be due to the use of
772 different techniques to measure expression, some used real time-quantitative PCR while
773 others used standard PCR with lower detection limits. Other differences, such as the

774 expression in PBMC (PBL in fish), can be attributed to the different composition of
775 circulating leukocytes that varies greatly among species. Despite this, we can state that in
776 general, there is high expression of *IL10* transcripts in mucosal tissues such as gut, gills or
777 lungs. This expression pattern is expected owing to the homeostatic and tolerogenic role
778 played by IL10 at these surfaces, preventing excessive immune responses against ingested
779 or inhaled antigens as well as microbiota at mucosal sites. Immune tissues such as spleen,
780 avian bursa and cecal tonsil, or fish head kidney also generally present high constitutive
781 expression of this cytokine.
782
783 **B. Kinetics of *IL10* expression**
784 IL10 expression is highly regulated and is generally expected following or concomitantly
785 with the expression of pro-inflammatory mediators. Several studies show that *IL10* is
786 upregulated by proinflammatory molecules such as bacterial and viral PAMPS. For
787 instance, LPS, Poly I:C, bacterial and mitogen stimulations rapidly increase the expression
788 of *IL10* on chicken, duck, frog and fish cells and tissues. The induction of the expression of
789 this cytokine starts quite early; peaks between 6 and 24 h depending on the species, tissue
790 and treatment, and goes down gradually generally lasting longer than the expression of the
791 pro-inflammatory genes. [17,19,21–23,25,26,29,73,103] This early induction has been proposed as a
792 "self-control" mechanism to limit collateral damage caused by exaggerated
793 inflammation.[103,104] TNFα stimulation of goldfish monocytes and macrophages
794 downregulated *IL10* expression corroborating the presence of the TNFα responsive element
795 reported in fugu.[20,27]

IL10 can also be induced by anti-inflammatory mediators such as TGFβ1.[30] LPS-stimulated grass carp monocytes start producing proinflammatory mediators in the first hours and shortly after, the expression of *TGFβ1* and *IL10* starts and remains high controlling the expression of the pro-inflammatory mediators.[77]

To note, most of the aforementioned studies refer to gene expression data although it was already previously mentioned that IL10 is highly regulated also at posttranscriptional levels. Therefore, the development of specific antibodies for the analysis of protein levels would be crucial. This would allow to study the ratio of pro-/anti-inflammatory cytokines in various cell types upon treatment or infection and to obtain valuable information about the regulation of this cytokine at total protein levels.

C. Role of IL10 in infection, inflammation and in stress

The ability of certain cells or tissues to express *IL10* has been related to different pathogenic or stress situations. In some cases, differential expression of IL10 in different genetic backgrounds was associated to disease resistance or susceptibility. For instance, in chicken, susceptible and resistant animals have been described regarding *Eimeria maxima* (protozoan intestinal parasite) infections. Upon infection, susceptible birds show increased *IL10* expression in the small intestine when compared to resistant birds. Although the expression of other proinflammatory mediators such as IFNγ does not seem to be affected, the high levels of IL10 in the susceptible line may counteract inflammation, possibly contributing to the inability of these animals to limit the growth of the parasite. Uninfected animals already show an important difference in the constitutive expression of *IL10*, as

818 spleens of susceptible birds express 43 fold higher amounts of *IL10* when compared to the
819 resistant line.[18]
820 A similar observation was obtained in teleost fish. Two different common carp strains have
821 been described to present different susceptibilities and mortality rates upon infection with
822 the haemoflagellate parasite *Trypanoplasma borreli*. While the resistant strain shows
823 upregulation of *IL10* in later phases of the infection coinciding with the downregulation of
824 proinflammatory genes and increase in specific antibodies, the susceptible line shows an
825 abnormal early expression of *IL10* leading to a reduced inflammatory response and higher
826 mortalities (Fig. 6).[105] Again, in common carp, a single nucleotide polymorphism in the
827 *IL10a* promoter has been strongly associated to resistance against cyprinid herpes virus 3
828 infections.[32]
829 It is clear that also in non-mammalian vertebrates a well-regulated expression of *IL10*
830 during the course of the inflammatory processes is crucial, and dysregulation of the IL10
831 network has been associated with mortalities or higher disease susceptibility. For example,
832 specific chicken breeds with impaired *IL10* expression show prolonged inflammation and
833 infectious symptoms when exposed to *Campylobacter jejuni*, a commensal bacteria in
834 chicken.[106] It is widely accepted that correct IL10 regulation and expression is especially
835 important in maintaining gut homeostasis, and dysregulation of this molecule leads to
836 pathologic situations such as inflammatory bowel disease or ulcerative colitis widely
837 studied in mammals.[107] In Tetraodon, ablation of regulatory T cells through administration
838 of neutralizing anti-CD25 antibodies, led to a decrease in *IL10* expression in the gut and to
839 an increase in pro-inflammatory gene expression as well as intestinal lesions.[108] The data
840 presented in this study closely resemble those seen in mammalian models of gut
841 inflammation, nevertheless, a direct link between IL10 levels and disease outcome needs to

842 be formally proven. Other studies in fish focusing on the enteritis model, tried to find a link
843 between IL10 and intestinal health. In zebrafish, oxazolone-induced enterocolitis was
844 characterized by an increased expression of *IL10* together with *IL1β* and *TNFα*.[109] When
845 common carp are fed with soy containing feeds they develop transient enteritis and recover
846 after 4 weeks. During this process *IL10* upregulation was observed already after 1 week of
847 feeding, more or less coinciding with the peak of inflammation and with the upregulation of
848 *IL1β*. The anti-inflammatory molecule that was upregulated during the recovery phase was
849 *TGFβ*.[110] In the case of the pathogenic enteritis caused by *Enteromyxum leei* in seabream,
850 *IL10* showed the highest upregulation among all the interleukin genes studied in the gut and
851 this upregulation is much prominent in later phases of infection[111] coinciding with the peak
852 of antibody production (unpublished observation). The use of probiotics and
853 immunostimulants in animal feed also showed to regulate IL10 levels. The introduction of
854 *Saccharomyces boulardii* in chicken diets induced a higher IL10 production in gut and at
855 the same time an increased number in IgA positive cells and positive effects on intestinal
856 ultrastructure.[112] The upregulation of an immunosuppressive gene upon stimulatory
857 conditions can be interpreted as a compensatory mechanism to regulate exaggerated
858 responses that can be caused by the immunostimulant.
859 Altogether, when focusing on intestinal infections or inflammation models IL10 seems to
860 have a conserved regulatory role in the gut of mammalian and non-mammalian species.
861 More studies focusing on the kinetics of IL10 expression especially at protein level will be
862 needed to better understand the multifaceted aspects of IL10 function and the role of IL10-
863 producing leukocytes, particularly at mucosal surfaces.

Additional (indirect) evidence of the crucial role of IL10 in the regulation of immune responses to infections comes from several in vitro and in vivo studies mostly using intracellular pathogens, in which the pathogen itself has been shown to possibly manipulate or interfere with *IL10* expression. For instance, the facultative intracellular bacteria *Franciella noatunensis* can infect Atlantic cod macrophages inducing an elevated expression of *IL10*. This has been related to a downregulation of *IL1β, IL6, IL8* and *IFNγ* which in turn has been proposed as a mechanism of the pathogen to regulate the host immune response.[113,114] Mammalian mycobacterial species have been shown to increase SOCS3 levels as a strategy to downregulate inflammation.[115] In *Mycobacterium marinum*-infected goldfish elevated expression of *IL10* and *SOCS3* has also been observed but whether upregulation of *SOCS3* is caused directly by the bacteria or by increased levels of IL10 is yet to be determined.[116,117] Upon infection with infectious pancreatic necrosis virus (IPNV) Atlantic salmon spleen, head kidney and liver increase the expression of *IL10* and this is also proposed as a pathogen strategy to control the inflammatory response induced by IFNγ and favor a switch towards an anti-inflammatory state.[118] Actually, the strategy of regulating the cytokine network of the host, and more specifically, the use of anti-inflammatory molecules such as IL10, is well known for several pathogens, including viruses.[82,83] As seen before, some viruses encode their own IL10 homologs to regulate the host immune responses further highlighting the pivotal role of IL10 in protection against infections.

Finally, the expression of this cytokine has also been used as a marker for animal welfare as its expression has been linked to certain stressful conditions or to the presence of specific pollutants. It is known that tributylin, a wide spread marine pollutant, cause

887 immunosuppressive effects in some fish species. This immunosuppressive effects have
888 been linked to an increased expression of *IL10* and *TGFβ1* caused by this pollutant in
889 Atlantic salmon.[119] An increase in serum IL10 was found in an experimental handling stress
890 model in goldfish. In this study IL10 levels are proposed as an additional stress indicator
891 together with cortisol and glucose levels.[120]
892 Altogether, the data accumulated so far, mostly in chicken and several teleost fish species,
893 point towards a strong conservation of the regulatory role of IL10 during infection and
894 inflammation. Even in non-mammalian vertebrates, manipulation of the IL10 network has
895 to be approached carefully as exaggerated expression of this cytokine can lead to an
896 immunosuppresive state facilitating pathogen invasion, whereas impaired expression can
897 lead to excessive inflammation and damage.
898
899 **VI. ZEBRAFISH MODEL AS A TOOL TO STUDY IL10**
900 Over the past years the zebrafish model has established itself as a great tool to study
901 fundamental questions in developmental biology but most of all, it has recently emerged as
902 a suitable tool to investigate biomedical questions also related to human diseases.[121]
903 Zebrafish is currently being used as a model to study genes involved in tumor
904 progression,[122] stem cell development and differentiation,[123] several infection models of
905 host-pathogen interaction,[124,125] drug discovery[126] as well as metabolic disease.[127,128]
906 Owing to the availability of an ever growing number of transgenic zebrafish lines
907 expressing reporter fluorescent proteins under the control of several immune cell-specific
908 promotors, there is no doubt that the zebrafish model will serve as an additional tool to help
909 dissect IL10 biology in fish as well. Where antibodies are not available, the use of

transgenic reporter zebrafish lines expressing fluorescent proteins under the control of the IL10 promotor can help elucidate the source(s) of IL10 expression in immune and non-immune cells. Since cells can be imaged in vivo at various time points without sacrificing the animal or sorting the cells, the kinetics of IL10 expression can be concomitantly imaged in various cell types.

Most importantly, very recently, IL10 knockout mutants became available (http://www.sanger.ac.uk/sanger/Zebrafish_Zmpgene/ENSDARG00000078147) and can help to further elucidate the role of IL10 during infection and diseases. For example, the possibility to image in real-time the kinetics of cell recruitment during tumor progression or host-pathogen interaction during infections in an IL10 transgenic or in an IL10 knockout background, will allow for a complementation and refinement of the approaches used to date to investigate IL10 functions. Finally, through the use of knockdown or knockout approaches for the candidates of the IL10 receptor complex, in a manner similar to the one used for the discovery of the type I IFN receptor complex,[129] it will be possible to unequivocally ascertain the role of CRFB4 in the formation of the fish IL10 receptor complex with CRFB7. Altogether, we think that the zebrafish model will provide numerous possibilities to expand, complement and validate the study of this (and other cytokines), not only in fish, but in all vertebrate species.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

In this review we have shown that from the data accumulated thus far, there is strong evidence suggesting that the structure, intracellular signaling, and overall biological functions of IL10 are strongly conserved throughout vertebrate evolution. The functional studies, performed mainly in chicken and teleost fish, point towards a conservation of the

anti-inflammatory activities of IL10 on phagocytes and to a crucial regulatory role of IL10 in gut homeostasis. Knowledge on the role of IL10 on lymphocytes is still scarce and only addressed in fish; the association of IL10 with disease susceptibility or resistance has also been partly addressed, again confirming the pivotal role of this cytokine in the regulation of exacerbated inflammatory responses also in non-mammalian vertebrates. Finally, the identification and functional characterization of the first non-mammalian viral IL10 homologue in a cyprinid herpesvirus, $_{CyHV3}$IL10, supports the various observations suggesting that manipulation of the IL10 pathway can be sufficient to tip the balance between disease susceptibility and resistance.

Despite the many advances made in the last years in the understanding of IL10 biology and function in non-mammalian vertebrates, still a few pieces of the puzzle remain open. For example, the presence in some teleost species of duplicated genes, for both ligands and receptors, together with the observation that gene duplication does not necessarily imply functional redundancy (Piazzon, manuscript in preparation),[31] certainly increases the level of complexity of IL10 regulation in Teleosts and the role of each of the paralogues still needs to be investigated in detail. Progress, although substantial, has been greatly slowed down by the lack of tools (recombinant proteins, antibodies) in most non-mammalian vertebrates. Nevertheless, cross-reactive inhibitors or antibodies can be found, especially against transcription factors. The latter are usually well conserved molecules, and it has been relatively easy to find cross-reactive antibodies, as for example against STAT3 and phosphorylated STAT3.[27,61] Nowadays, the genomes and transcriptomes of hundreds of species are available in the databases. This increased enormously the possibilities to perform in silico analyses and comparative studies in almost any vertebrate class. Regardless, it is important to be aware that most of the molecules found in these databases

958 are automatic predictions and their automatic annotation should always be supported by
959 functional analysis. Finally, functional data on the biological activities of IL10 in reptiles,
960 amphibians and modern bony fish (coelacanth or lungfish) are completely lacking and
961 would certainly add important pieces to the evolutionary puzzle of IL10 evolution.
962
963 **Acknowledgments**
964 MCP was supported by the European Community's 7th Framework Programme (FP7/2007-
965 2013) under Grant FISHIL10 (PIEF-GA-2011-302444) and by the Spanish grant
966 Formacion postdoctoral 2013, MINECO (FPDI-2013-15741). GL and MF were supported
967 by the FP7 Programme under Grant FishForPharma (PITN-GA-2011-289209); MF was
968 also supported by FP7 Programme under Grant TARGETFISH (311993), as well as by the
969 Netherlands Organisation for Scientific Research under Veni Project 11200.
970

REFERENCES

1. Fiorentino DF, Bond MW, Mosmann TR. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. J Exp Med. 1989; 170(6): 2081–2095.
2. Moore KW, Vieira P, Fiorentino DF, Trounstine ML, Khan TA, Mosmann TR. Homology of cytokine synthesis inhibitory factor (IL-10) to the epstein-barr virus gene BCRFI. Science. 1990; 248(4960): 1230–1234.
3. Mosser DM, Zhang X. Interleukin-10: New perspectives on an old cytokine. Immunol Rev. 2008; 226(1): 205–218.
4. Moore KW, De Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol. 2001; 19: 683–765.
5. Williams LM, Ricchetti G, Sarma U, Smallie T, Foxwell BMJ. Interleukin-10 suppression of myeloid cell activation--a continuing puzzle. Immunology. 2004; 113(3): 281–292.
6. Fiorentino DF, Zlotnik A, Mosmann TR, Howard M, O'Garra A. IL-10 inhibits cytokine production by activated macrophages. J Immunol. 1991; 147(11): 3815–3822.
7. Sabat R, Grütz G, Warszawska K, Kirsch S, Witte E, Wolk K, Geginat J. Biology of interleukin-10. Cytokine Growth Factor Rev. 2010; 21(5): 331–344.
8. Brooks DG, Walsh KB, Elsaesser H, Oldstone MBA. IL-10 directly suppresses CD4 but not CD8 T cell effector and memory responses following acute viral infection. Proc Natl Acad Sci U S A. 2010; 107(7): 3018–3023.
9. Del Prete G, De Carli M, Almerigogna F, Giudizi MG, Biagiotti R, Romagnani S. Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. J Immunol. 1993; 150(2): 353–360.
10. Groux H, Bigler M, De Vries JE, Roncarolo M-G. Interleukin-10 induces a long-term antigen-specific anergic state in human CD4+ T cells. J Exp Med. 1996; 184(1): 19–29.
11. Go NF, Castle BE, Barrett R, Kastelein R, Dang W, Mosmann TR, Moore KW, Howard M. Interleukin 10, a novel B cell stimulatory factor: Unresponsiveness of X chromosome-linked immunodeficiency B cells. J Exp Med. 1990; 172(6): 1625–1631.
12. Rousset F, Peyrol S, Garcia E, Vezzio N, Andujar M, Grimaud J-A, Banchereau J. Long-term cultured CD40-activated B lymphocytes differentiate into plasma cells in response to IL-10 but not IL-4. Int Immunol. 1995; 7(8): 1243–1253.
13. Carson WE, Lindemann MJ, Baiocchi R, Linett M, Tan JC, Chou - CC, Narula S, Caligiuri MA. The functional characterization of interleukin-10 receptor expression on human natural killer cells. Blood. 1995; 85(12): 3577–3585.
14. Emmerich J, Mumm JB, Chan IH, LaFace D, Truong H, McClanahan T, Gorman DM, Oft M. IL-10 directly activates and expands tumor-resident CD8+ T cells without De Novo infiltration from secondary lymphoid organs. Cancer Res. 2012; 72(14): 3570–3581.
15. Josephson K, Logsdon NJ, Walter MR. Crystal structure of the IL-10/IL-10R1 complex reveals a shared receptor binding site. Immunity. 2001; 15(1): 35–46.
16. Riley JK, Takeda K, Akira S, Schreiber RD. Interleukin-10 receptor signaling through the JAK-STAT pathway. Requirement for two distinct receptor-derived signals for anti-inflammatory action. J Biol Chem. 1999; 274(23): 16513–16521.
17. Yao Q, Fischer KP, Tyrrell DL, Gutfreund KS. Genomic structure, molecular characterization and functional analysis of Pekin duck interleukin-10. Dev Comp Immunol. 2012; 38(1): 30–43.
18. Rothwell L, Young JR, Zoorob R, Whittaker CA, Hesketh P, Archer A, Smith AL, Kaiser P. Cloning and characterization of chicken IL-10 and its role in the immune response to Eimeria maxima. J Immunol. 2004; 173(4): 2675–2682.
19. Qi Z, Zhang Q, Wang Z, Zhao W, Gao Q. Cloning of interleukin-10 from African clawed frog (Xenopus tropicalis), with the Finding of IL-19/20 homologue in the IL-10 locus. J Immunol Res. 2015; 2015: ID462138.
20. Zou J, Clark MS, Secombes CJ. Characterisation, expression and promoter analysis of an interleukin 10 homologue in the puffer fish, Fugu rubripes. Immunogenetics. 2003; 55(5): 325–335.
21. Savan R, Igawa D, Sakai M. Cloning, characterization and expression analysis of interleukin-10 from the common carp, Cyprinus carpio L. Eur J Biochem. 2003; 270(23): 4647–4654.
22. Inoue Y, Kamota S, Ito K, Yoshiura Y, Ototake M, Moritomo T, Nakanishi T. Molecular cloning and expression analysis of rainbow trout (Oncorhynchus mykiss) interleukin-10 cDNAs. Fish Shellfish Immunol. 2005; 18(4): 335–344.

23. Zhang DC, Shao YQ, Huang YQ, Jiang SG. Cloning, characterization and expression analysis of interleukin-10 from the zebrafish (Danio reriori). J Biochem Mol Biol. 2005; 38(5): 571–576.
24. Hamming OJ, Lutfalla G, Levraud J-P, Hartmann R. Crystal structure of zebrafish interferons I and II reveals conservation of type I interferon structure in vertebrates. J Virol. 2011; 85(16): 8181–8187.
25. Pinto RD, Nascimento DS, Reis MI, do Vale A, Dos Santos NM. Molecular characterization, 3D modelling and expression analysis of sea bass (Dicentrarchus labrax L.) interleukin-10. Mol Immunol. 2007; 44(8): 2056–2065.
26. Seppola M, Larsen AN, Steiro K, Robertsen B, Jensen I. Characterisation and expression analysis of the interleukin genes, IL-1β, IL-8 and IL-10, in Atlantic cod (Gadus morhua L.). Mol Immunol. 2008; 45(4): 887–897.
27. Grayfer L, Hodgkinson JW, Hitchen SJ, Belosevic M. Characterization and functional analysis of goldfish (Carassius auratus L.) interleukin-10. Mol Immunol. 2011; 48(4): 563–571.
28. Sahoo BR, Swain B, Basu M, Panda P, Maiti NK, Samanta M. 3D modeling and molecular dynamics simulation of an immune-regulatory cytokine, interleukin-10, from the Indian major carp, Catla catla. J Mol Model. 2012; 18(5): 1713–1722.
29. Swain B, Samanta M, Basu M, Panda P, Sahoo BR, Maiti NK, Mishra BK, Eknath AE. Molecular characterization, inductive expression and mechanism of interleukin-10 gene induction in the Indian major carp, catla (Catla catla). Aquac Res. 2012; 43(6): 897–907.
30. Wei H, Yang M, Zhao T, Wang X, Zhou H. Functional expression and characterization of grass carp IL-10: An essential mediator of TGF-β1 immune regulation in peripheral blood lymphocytes. Mol Immunol. 2013; 53(4): 313–320.
31. Harun NO, Costa MM, Secombes CJ, Wang T. Sequencing of a second interleukin-10 gene in rainbow trout Oncorhynchus mykiss and comparative investigation of the expression and modulation of the paralogues in vitro and in vivo. Fish Shellfish Immunol. 2011; 31(1): 107–117.
32. Kongchum P, Sandel E, Lutzky S, Hallerman EM, Hulata G, David L, Palti Y. Association between IL-10a single nucleotide polymorphisms and resistance to cyprinid herpesvirus-3 infection in common carp (Cyprinus carpio). Aquaculture. 2011; 315(3-4): 417–421.
33. Wennborg A, Sohlberg B, Angerer D, Klein G, von Gabain A. A human RNase E-like activity that cleaves RNA sequences involved in mRNA stability control. Proc Natl Acad Sci. 1995; 92(16): 7322–7326.
34. Opazo JC, Butts GT, Nery MF, Storz JF, Hoffmann FG. Whole-genome duplication and the functional diversification of teleost fish hemoglobins. Mol Biol Evol. 2013; 30(1): 140–153.
35. Berthelot C, Brunet F, Chalopin D, Juanchich A, Bernard M, Noël B, Bento P, Da Silva C, Labadie K, Alberti A, Aury J-M, Louis A, Dehais P, Bardou P, Montfort J, Klopp C, Cabau C, Gaspin C, Thorgaard GH, Boussaha M, Quillet E, Guyomard R, Galiana D, Bobe J, Volff J-N, Genêt C, Wincker P, Jaillon O, Roest Crollius H, Guiguen Y. The rainbow trout genome provides novel insights into evolution after whole-genome duplication in vertebrates. Nat Commun. 2014; 5: 3657.
36. David L, Blum S, Feldman MW, Lavi U, Hillel J. Recent duplication of the common carp (Cyprinus carpio L.) Genome as revealed by analyses of microsatellite loci. Mol Biol Evol. 2003; 20(9): 1425–1434.
37. Sahoo A, Im S-H. Interleukin and interleukin receptor diversity: role of alternative splicing. Int Rev Immunol. 2010; 29(1): 77–109.
38. Wu S, Gessner R, Taube T, von Stackelberg A, Henze G, Seeger K. Expression of interleukin-10 splicing variants is a positive prognostic feature in relapsed childhood acute lymphoblastic leukemia. J Clin Oncol. 2005; 23(13): 3038–3042.
39. Forte GI, Scola L, Bellavia D, Vaccarino L, Sanacore M, Sisino G, Scazzone C, Caruso C, Barbieri R, Lio D. Characterization of two alternative Interleukin(IL)-10 5'UTR mRNA sequences, induced by lipopolysaccharide (LPS) stimulation of peripheral blood mononuclear cells. Mol Immunol. 2009; 46(11-12): 2161–2166.
40. Lin YL, Chang PC, Wang Y, Li M. Identification of novel viral interleukin-10 isoforms of human cytomegalovirus AD169. Virus Res. 2008; 131(2): 213–223.
41. Jenkins C, Abendroth A, Slobedman B. A Novel Viral Transcript with Homology to Human Interleukin-10 Is Expressed during Latent Human Cytomegalovirus Infection. J Virol. 2004; 78(3): 1440–1447.
42. Chang HD, Helbig C, Tykocinski L, Kreher S, Koeck J, Niesner U, Radbruch A. Expression of IL-10 in Th memory lymphocytes is conditional on IL-12 or IL-4, unless the IL-10 gene is imprinted by

GATA-3. Eur J Immunol. 2007; 37(3): 807–817.
43. Shoemaker J, Saraiva M, O'Garra A. GATA-3 directly remodels the IL-10 locus independently of IL-4 in CD4 + T cells. J Immunol. 2006; 176(6): 3470–3479.
44. Murray PJ. Understanding and exploiting the endogenous interleukin-10/STAT3-mediated anti-inflammatory response. Curr Opin Pharmacol. 2006; 6(4): 379–386.
45. Secombes CJ, Wang T, Bird S. The interleukins of fish. Dev Comp Immunol. 2011; 35(12): 1336–1345.
46. Windsor WT, Syto R, Tsarbopoulos A, Zhang R, Durkin J, Baldwin S, Paliwal S, Mui PW, Pramanik B, Trotta PP, Tindall SH. Disulfide bond assignments and secondary structure analysis of human and murine interleukin 10. Biochemistry. 1993; 32(34): 8807–8815.
47. Grayfer L, Belosevic M. Identification and molecular characterization of the interleukin-10 receptor 1 of the zebrafish (Danio rerio) and the goldfish (Carassius auratus L.). Dev Comp Immunol. 2012; 36(2): 408–417.
48. van Beurden SJ, Forlenza M, Westphal AH, Wiegertjes GF, Haenen OLM, Engelsma MY. The alloherpesviral counterparts of interleukin 10 in European eel and common carp. Fish Shellfish Immunol. 2011; 31(6): 1211–1217.
49. Ding Y, Qin L, Kotenko SV., Pestka S, Bromberg JS. A Single Amino Acid Determines the Immunostimulatory Activity of Interleukin 10. J Exp Med. 2000; 191(2): 213–224.
50. Moore KW, O'Garra A, De Waal Malefyt R, Vieira P, Mosmann TR. Interleukin-10. Annu Rev Immunol. 1993; 11: 165–190.
51. Reboul J, Gardiner K, Monneron D, Uzé G, Lutfalla G. Comparative genomic analysis of the interferon/interleukin-10 receptor gene cluster. Genome Res. 1999; 9(3): 242–250.
52. Yao Q, Fischer KP, Tyrrell DL, Gutfreund KS. cDNA cloning, genomic structure, molecular characterization and mRNA expression analysis of the Pekin duck interleukin-10 receptor 1. Int J Immunogenet. 2012; 39(1): 55–67.
53. Yoon S Il, Logsdon NJ, Sheikh F, Donnelly RP, Walter MR. Conformational changes mediate interleukin-10 receptor 2 (IL-10R2) binding to IL-10 and assembly of the signaling complex. J Biol Chem. 2006; 281(46): 35088–35096.
54. Schindler C, Darnell JE. Transcriptional responses to polypeptide ligands: The JAK-STAT pathway. Annu Rev Biochem. 1995; 64: 621–651.
55. Decker T, Meinke A. Jaks, Stats and the immune system. Immunobiology. 1997; 198(1-3): 99–111.
56. Finbloom DS, Winestock KD. IL-10 induces the tyrosine phosphorylation of tyk2 and Jak1 and the differential assembly of STAT1α and STAT3 complexes in human T cells and monocytes. J Immunol. 1995; 155(3): 1079–1090.
57. Wehinger J, Gouilleux F, Groner B, Finke J, Mertelsmann R, Maria Weber-Nordt R. IL-10 induces DNA binding activity of three STAT proteins (Stat1, Stat3, and Stat5) and their distinct combinatorial assembly in the promoters of selected genes. FEBS Lett. 1996; 394(3): 365–370.
58. Lutfalla G, Crollius HR, Stange-Thomann N, Jaillon O, Mogensen K, Monneron D. 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. 2003; 4
59. Aggad D, Stein C, Sieger D, Mazel M, Boudinot P, Herbomel P, Levraud J-P, Lutfalla G, Leptin M. In vivo analysis of Ifn-γ1 and Ifn-γ2 signaling in zebrafish. J Immunol. 2010; 185(11): 6774–6782.
60. Monte MM, Wang T, Collet B, Zou J, Secombes CJ. Molecular characterisation of four class 2 cytokine receptor family members in rainbow trout, Oncorhynchus mykiss. Dev Comp Immunol. 2015; 48(1): 43–54.
61. Piazzon MC, Savelkoul HFJSJ, Pietretti D, Wiegertjes GF, Forlenza M. Carp Il10 Has Anti-Inflammatory Activities on Phagocytes, Promotes Proliferation of Memory T Cells, and Regulates B Cell Differentiation and Antibody Secretion. J Immunol. 2015; 194(1): 187–199.
62. Monte MM, Wang T, Collet B, Zou J, Secombes CJ. Molecular characterisation of four class 2 cytokine receptor family members in rainbow trout, Oncorhynchus mykiss. Dev Comp Immunol. 2015; 48(1): 43–54.
63. Kotenko S V, Krause CD, Izotova LS, Pollack BP, Wu W, Pestka S. Identification and functional characterization of a second chain of the interleukin-10 receptor complex. EMBO J. 1997; 16(19): 5894–5903.
64. Trivella DBB, Ferreira-Júnior JR, Dumoutier L, Renauld J-C, Polikarpov I. Structure and function of interleukin-22 and other members of the interleukin-10 family. Cell Mol Life Sci. 2010; 67(17):

2909–2935.
65. Qi Z, Nie P, Secombes CJ, Zou J. Intron-containing type I and type III IFN coexist in amphibians: refuting the concept that a retroposition event gave rise to type I IFNs. J Immunol. 2010; 184(9): 5038–5046.
66. Cheng S, Lutfalla G, Uze G, Chumakov IM, Gardiner K. GART, SON, IFNAR, and CRF2-4 genes cluster on human Chromosome 21 and mouse Chromosome 16. Mamm Genome. 1993; 4(6): 338–342.
67. Stein C, Caccamo M, Laird G, Leptin M. Conservation and divergence of gene families encoding components of innate immune response systems in zebrafish. Genome Biol. 2007; 8(11): R251.
68. Aggad D, Mazel M, Boudinot P, Mogensen KE, Hamming OJ, Hartmann R, Kotenko S, Herbomel P, Lutfalla G, Levraud J-P. The two groups of zebrafish virus-induced interferons signal via distinct receptors with specific and shared chains. J Immunol. 2009; 183(6): 3924–3931.
69. Wei H, Wang X, Zhang A, Du L, Zhou H. Identification of grass carp IL-10 receptor subunits: Functional evidence for IL-10 signaling in teleost immunity. Dev Comp Immunol. 2014; 45(2): 259–268.
70. Yao Q, Fischer KP, Arnesen K, Lorne Tyrrell D, Gutfreund KS. The Pekin duck IL-10R2 common chain: cDNA cloning, genomic structure, molecular characterization and mRNA expression analysis. Int J Immunogenet. 2013; 40(5): 386–395.
71. Lutfalla G, Gardiner K, Uzé G. A new member of the cytokine receptor gene family maps on chromosome 21 at less than 35 kb from IFNAR. Genomics. 1993; 16(2): 366–373.
72. Sun B, Greiner-Tollersrud L, Koop BF, Robertsen B. 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. 2014; 47(2): 275–286.
73. Wu Z, Hu T, Rothwell L, Vervelde L, Hume DA, Kaiser P. Analysis of the function of IL-10 in chickens using specific neutralising antibodies and a sensitive capture ELISA. Dev Comp Immunol. 2016; doi:10.1016/j.dci.2016.04.016
74. Oswald IP, Wynn TA, Sher A, James SL. Interleukin 10 inhibits macrophage microbicidal activity by blocking the endogenous production of tumor necrosis factor α required as a costimulatory factor for interferon γ-induced activation. Proc Natl Acad Sci U S A. 1992; 89(18): 8676–8680.
75. Flesch IEA, Hess JH, Oswald IP, Kaufmann SHE. Growth inhibition of Mycobacterium bovis by IFN-γ stimulated macrophages: Regulation by endogenous tumor necrosis factor-α and by IL-10. Int Immunol. 1994; 6(5): 693–700.
76. Piazzon MC, Wentzel AS, Tijhaar EJ, Rakus KŁ, Vanderplasschen A, Wiegertjes GF, Forlenza M. Cyprinid Herpesvirus 3 Il10 Inhibits Inflammatory Activities of Carp Macrophages and Promotes Proliferation of Igm+ B Cells and Memory T Cells in a Manner Similar to Carp Il10. J Immunol. 2015; 195(8): 3694–3704.
77. Wei H, Yin L, Feng S, Wang X, Yang K, Zhang A, Zhou H. Dual-parallel inhibition of IL-10 and TGF-β1 controls LPS-induced inflammatory response via NF-κB signaling in grass carp monocytes/macrophages. Fish Shellfish Immunol. 2015; 44(2): 445–452.
78. Thibodeau J, Bourgeois-Daigneault M-C, Huppé G, Tremblay J, Aumont A, Houde M, Bartee E, Brunet A, Gauvreau M-E, de Gassart A, Gatti E, Baril M, Cloutier M, Bontron S, Früh K, Lamarre D, Steimle V. Interleukin-10-induced MARCH1 mediates intracellular sequestration of MHC class II in monocytes. Eur J Immunol. 2008; 38(5): 1225–1230.
79. MacNeil IA, Suda T, Moore KW, Mosmann TR, Zlotnik A. IL-10, a novel growth cofactor for mature and immature T cells. J Immunol. 1990; 145(12): 4167–4173.
80. Secombes CJ, Van Groningen JJM, Egberts E. Separation of lymphocyte subpopulations in carp Cyprinus carpio L. by monoclonal antibodies: Immunohistochemical studies. Immunology. 1983; 48(1): 165–175.
81. Alcami A. Viral mimicry of cytokines, chemokines and their receptors. Nat Rev Immunol. 2003; 3(1): 36–50.
82. Slobedman B, Barry PA, Spencer J V, Avdic S, Abendroth A. Virus-encoded homologs of cellular interleukin-10 and their control of host immune function. J Virol. 2009; 83(19): 9618–9629.
83. Ouyang P, Rakus K, van Beurden SJ, Westphal AH, Davison AJ, Gatherer D, Vanderplasschen AF. IL-10 encoded by viruses: A remarkable example of independent acquisition of a cellular gene by viruses and its subsequent evolution in the viral genome. J Gen Virol. 2014; 95(PART 2): 245–262.
84. Kotenko S V, Saccani S, Izotova LS, Mirochnitchenko O V, Pestka S. Human cytomegalovirus

harbors its own unique IL-10 homolog (cmvIL-10). Proc Natl Acad Sci U S A. 2000; 97(4): 1695–1700.
85. Wilkie GS, Kerr K, Stewart JP, Studdert MJ, Davison AJ. Genome sequences of equid herpesviruses 2 and 5. Genome Announc. 2015; 3(2): e00119–15.
86. Lockridge KM, Zhou S-SS, Kravitz RH, Johnson JL, Sawai ET, Blewett EL, Barry PA. Primate cytomegaloviruses encode and express an IL-10-like protein. Virology. 2000; 268(2): 272–280.
87. Tulman ER, Afonso CL, Lu Z, Zsak L, Kutish GF, Rock DL. Genome of lumpy skin disease virus. J Virol. 2001; 75(15): 7122–7130.
88. Fleming SB, McCaughan CA, Andrews AE, Nash AD, Mercer AA. A homolog of interleukin-10 is encoded by the poxvirus orf virus. J Virol. 1997; 71(6): 4857–4861.
89. Rziha H-J, Bauer B, Adam K-H, Röttgen M, Cottone R, Henkel M, Dehio C, Büttner M. Relatedness and heterogeneity at the near-terminal end of the genome of a parapoxvirus bovis 1 strain (B177) compared with parapoxvirus ovis (Orf virus). J Gen Virol. 2003; 84(Pt 5): 1111–1116.
90. Haig DM, Thomson J, McInnes CJ, Deane DL, Anderson IE, McCaughan CA, Imlach W, Mercer AA, Howard CJ, Fleming SB. A comparison of the anti-inflammatory and immuno-stimulatory activities of orf virus and ovine interleukin-10. Virus Res. 2002; 90(1-2): 303–316.
91. Nagarajan G, Swami SK, Dahiya SS, Sivakumar G, Narnaware SD, Tuteja FC, Patil N V. Comparison of virokine from camel pseudocowpoxvirus (PCPV) with interleukin 10 of the Dromedary camel (Camelus dromedarius). Cytokine. 2013; 61(2): 356–359.
92. Host KM, Damania B. Discovery of a Novel Bat Gammaherpesvirus. mSphere. 2016; 1(1): e00016–16.
93. Offerman K, Carulei O, van der Walt AP, Douglass N, Williamson A-L. The complete genome sequences of poxviruses isolated from a penguin and a pigeon in South Africa and comparison to other sequenced avipoxviruses. BMC Genomics. 2014; 15(1): 463.
94. Tulman ER, Afonso CL, Lu Z, Zsak L, Kutish GF, Rock DL. The Genome of Canarypox Virus. J Virol. 2003; 78(1): 353–366.
95. Gandar F, Wilkie GS, Gatherer D, Kerr K, Marlier D, Diez M, Marschang RE, Mast J, Dewals BG, Davison AJ, Vanderplasschen AFC. The Genome of a Tortoise Herpesvirus (Testudinid Herpesvirus 3) Has a Novel Structure and Contains a Large Region That Is Not Required for Replication In Vitro or Virulence In Vivo. J Virol. 2015; 89(22): 11438–11456.
96. Van Beurden SJ, Bossers A, Voorbergen-Laarman MHA, Haenen OLM, Peters S, Abma-Henkens MHC, Peeters BPH, Rottier PJM, Engelsma MY. Complete genome sequence and taxonomic position of anguillid herpesvirus 1. J Gen Virol. 2010; 91(4): 880–887.
97. Aoki T, Hirono I, Kurokawa K, Fukuda H, Nahary R, Eldar A, Davison AJ, Waltzek TB, Bercovier H, Hedrick RP. Genome sequences of three koi herpesvirus isolates representing the expanding distribution of an emerging disease threatening koi and common carp worldwide. J Virol. 2007; 81(10): 5058–5065.
98. Ouyang P, Rakus K, Boutier M, Reschner A, Leroy B, Ronsmans M, Fournier G, Scohy S, Costes B, Wattiez R, Vanderplasschen A. The IL-10 homologue encoded by cyprinid herpesvirus 3 is essential neither for viral replication in vitro nor for virulence in vivo. Vet Res. 2013; 44(1): 53.
99. Sunarto A, Liongue C, McColl KA, Adams MM, Bulach D, Crane MSJ, Schat KA, Slobedman B, Barnes AC, Ward AC, Walker PJ. Koi herpesvirus encodes and expresses a functional interleukin-10. J Virol. 2012; 86(21): 11512–11520.
100. Adamek M, Rakus KŁ, Chyb J, Brogden G, Huebner A, Irnazarow I, Steinhagen D. Interferon type I responses to virus infections in carp cells: In vitro studies on Cyprinid herpesvirus 3 and Rhabdovirus carpio infections. Fish Shellfish Immunol. 2012; 33(3): 482–493.
101. Miest JJ, Adamek M, Pionnier N, Harris S, Matras M, Rakus KŁ, Irnazarow I, Steinhagen D, Hoole D. Differential effects of alloherpesvirus CyHV-3 and rhabdovirus SVCV on apoptosis in fish cells. Vet Microbiol. 2015; 176(1-2): 19–31.
102. Holopainen R, Tapiovaara H, Honkanen J. Expression analysis of immune response genes in fish epithelial cells following ranavirus infection. Fish Shellfish Immunol. 2012; 32(6): 1095–1105.
103. He H, Genovese KJ, Swaggerty CL, MacKinnon KM, Kogut MH. Co-stimulation with TLR3 and TLR21 ligands synergistically up-regulates Th1-cytokine IFN-γ and regulatory cytokine IL-10 expression in chicken monocytes. Dev Comp Immunol. 2012; 36(4): 756–760.
104. O'Garra A, Vieira P. T(H)1 cells control themselves by producing interleukin-10. Nat Rev Immunol. 2007; 7(6): 425–428.

105. Forlenza M, Fink IR, Raes G, Wiegertjes GF. Heterogeneity of macrophage activation in fish. Dev Comp Immunol. 2011; 35(12): 1246–1255.
106. Humphrey S, Chaloner G, Kemmett K, Davidson N, Williams N, Kipar A, Humphrey T, Wigley P. Campylobacter jejuni is not merely a commensal in commercial broiler chickens and affects bird welfare. MBio. 2014; 5(4): e01364–14.
107. Paul G, Khare V, Gasche C. Inflamed gut mucosa: downstream of interleukin-10. Eur J Clin Invest. 2012; 42(1): 95–109.
108. Wen Y, Fang W, Xiang LX, Pan RL, Shao JZ. Identification of Treg-like cells in Tetraodon: insight into the origin of regulatory T subsets during early vertebrate evolution. Cell Mol Life Sci. 2011; 68(15): 2615–2626.
109. Brugman S, Liu K-Y, Lindenbergh-Kortleve D, Samsom JN, Furuta GT, Renshaw SA, Willemsen R, Nieuwenhuis EES. Oxazolone-induced enterocolitis in zebrafish depends on the composition of the intestinal microbiota. Gastroenterology. 2009; 137(5): 1757–1767.
110. Urán PA, Gonçalves AA, Taverne-Thiele JJ, Schrama JW, Verreth JAJ, Rombout JHWM. Soybean meal induces intestinal inflammation in common carp (Cyprinus carpio L.). Fish Shellfish Immunol. 2008; 25(6): 751–760.
111. Pérez-Cordón G, Estensoro I, Benedito-Palos L, Calduch-Giner JA, Sitjà-Bobadilla A, Pérez-Sánchez J. Interleukin gene expression is strongly modulated at the local level in a fish-parasite model. Fish Shellfish Immunol. 2014; 37(2): 201–208.
112. Rajput IR, Li LY, Xin X, Wu BB, Juan ZL, Cui ZW, Yu DY, Li WF. Effect of Saccharomyces boulardii and Bacillus subtilis B10 on intestinal ultrastructure modulation and mucosal immunity development mechanism in broiler chickens. Poult Sci. 2013; 92(4): 956–965.
113. Bakkemo KR, Mikkelsen H, Bordevik M, Torgersen J, Winther-Larsen HC, Vanberg C, Olsen R, Johansen LH, Seppola M. Intracellular localisation and innate immune responses following Francisella noatunensis infection of Atlantic cod (Gadus morhua) macrophages. Fish Shellfish Immunol. 2011; 31(6): 993–1004.
114. Ellingsen T, Inami M, Gjessing MC, Van Nieuwenhove K, Larsen R, Seppola M, Lund V, Schrøder MB. Francisella noatunensis in Atlantic cod (Gadus morhua L.); waterborne transmission and immune responses. Fish Shellfish Immunol. 2011; 31(2): 326–333.
115. Narayana Y, Balaji KN. NOTCH1 up-regulation and signaling involved in Mycobacterium bovis BCG-induced SOCS3 expression in macrophages. J Biol Chem. 2008; 283(18): 12501–11.
116. Grayfer L, Hodgkinson JW, Belosevic M. Analysis of the antimicrobial responses of primary phagocytes of the goldfish (Carassius auratus L.) against Mycobacterium marinum. Dev Comp Immunol. 2011; 35(11): 1146–1158.
117. Redpath S, Ghazal P, Gascoigne NR. Hijacking and exploitation of IL-10 by intracellular pathogens. Trends Microbiol. 2001; 9(2): 86–92.
118. Ingerslev HC, Rønneseth A, Pettersen EF, Wergeland HI. Differential expression of immune genes in Atlantic salmon (Salmo salar L.) challenged intraperitoneally or by cohabitation with IPNV. Scand J Immunol. 2009; 69(2): 90–98.
119. Pavlikova N, Arukwe A. Immune-regulatory transcriptional responses in multiple organs of Atlantic salmon after tributyltin exposure, alone or in combination with forskolin. J Toxicol Environ Health A. 2011; 74(7-9): 478–493.
120. Dror M, Sinyakov MS, Okun E, Dym M, Sredni B, Avtalion RR. Experimental handling stress as infection-facilitating factor for the goldfish ulcerative disease. Vet Immunol Immunopathol. 2006; 109(3-4): 279–287.
121. Lieschke GJ, Currie PD. Animal models of human disease: zebrafish swim into view. Nat Rev Genet. 2007; 8(5): 353–367.
122. Blackburn JS, Langenau DM. Zebrafish as a model to assess cancer heterogeneity, progression and relapse. Dis Model Mech. 2014; 7(7): 755–762.
123. Bertrand JY, Traver D. Hematopoietic cell development in the zebrafish embryo. Curr Opin Hematol. 2009; 16(4): 243–8.
124. Torraca V, Masud S, Spaink HP, Meijer AH. Macrophage-pathogen interactions in infectious diseases: new therapeutic insights from the zebrafish host model. Dis Model Mech. 2014; 7(7): 785–797.
125. Cronan MR, Tobin DM. Fit for consumption: zebrafish as a model for tuberculosis. Dis Model Mech. 2014; 7(7): 777–784.

126. MacRae CA, Peterson RT. Zebrafish as tools for drug discovery. Nat Rev Drug Discov. 2015; 14(10): 721–731.
127. Seth A, Stemple DL, Barroso I. The emerging use of zebrafish to model metabolic disease. Dis Model Mech. 2013; 6(5): 1080–1088.
128. Asaoka Y, Terai S, Sakaida I, Nishina H. The expanding role of fish models in understanding non-alcoholic fatty liver disease. Dis Model Mech. 2013; 6(4): 905–914.
129. Levraud JP, Boudinot P, Colin I, Behmansour A, Peyrieras N, Herbomel P, Lutfalla G. Identification of the zebrafish IFN receptor: Implications for the origin of the vertebrate IFN system. J Immunol. 2007; 178(7): 4385–4394.

FIG. 1. The IL10 protein is present and conserved in all vertebrate species. Phylogenetic tree analysis of full length IL10 protein sequences from selected species within each relevant group. The tree was constructed using the neighbor joining method within the MEGA 6 package and bootstrapped 10000 times. Bootstrap values over 50% are shown. Duplicated genes in rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*) are indicated as (a) and (b) next to the species name. IL10 protein sequences can be found in all vertebrate species and it groups within each class of vertebrate. The low boostrap values obtained are due to high sequence similarity but the tree is supported by the perfect grouping of each class of vertebrate. The accession numbers of the sequences used to perform the analysis are the following: NP_000563 *Homo sapiens*, NP_036986 *Rattus norvegicus*, NP_034678 *Mus musculus*, NP_999206 *Sus scrofa*, NP_776513 *Bos taurus*, NP_001003077 *Canis lupus familiaris*, XP_006922887 *Pteropus alecto*, XP_006754445 *Myotis davidii*, NP_001075514 *Oryctolagus cuniculus*, ALG04628 *Lepus europaeus*, XP_007523171 *Erinaceus europaeus*, XP_004610114 *Sorex araneus*, ELW47753 *Tupaia chinensis*, ABQ40392 *Dasypus novemcinctus*, XP_003410325 *Loxodonta africana*, AIA08972 *Elephas maximus*, AAD01799 *Trichosurus vulpecula*, AFY22677 *Phascolarctos cinereus*, XP_007668455 *Ornithorhynchus anatinus*, XP_010402880 *Corvus cornix cornix*, XP_014728054 *Sturnus vulgaris*, XP_010304693 *Balearica regulorum gibbericeps*, XP_010158678 *Eurypyga helias*, XP_009646203 *Egretta garzetta*, XP_009463847 *Nipponia nippon*, NP_001004414 *Gallus gallus*, BAL02992 *Coturnix japonica*, NP_001297297 *Anas platyrhynchos*, XP_013045032 *Anser cygnoides domesticus*, XP_005230381 *Falco peregrinus*, XP_011591578 *Aquila chrysaetos canadensis*, XP_009325615 *Pygoscelis adeliae*, XP_009271033 *Aptenodytes forsteri*, KQL51993 *Amazona aestiva*, XP_005143250 *Melopsittacus undulatus*,

XP_009956868 *Leptosomus discolor*, XP_008936084 *Merops nubicus*, XP_014803968 *Calidris pugnax*, XP_009886505 *Charadrius vociferus*, XP_010086506 *Pterocles gutturalis*, XP_009581167 *Fulmarus glacialis*, EMC81973 *Columba livia*, XP_009895472 *Picoides pubescens*, XP_009995817 *Chaetura pelagica*, XP_009940291 *Opisthocomus hoazin*, XP_009562150 *Cuculus canorus*, XP_010165026 *Caprimulgus carolinensis*, XP_008498919 *Calypte anna*, ADU34193 *Carassius auratus*, AAW78362 *Danio rerio*, cypCar_00007086 *Cyprinus carpio*(a), cypCar_00012555 *Cyprinus carpio*(b), XP_015227932 *Cyprinodon variegatus*, XP_014868952 *Poecilia mexicana*, BAD20648 *Oncorhynchus mykiss*(a), FR691804 *Oncorhynchus mykiss*(b), ABM46995 *Salmo salar*, XP_004545126 *Maylandia zebra*, XP_005924770 *Haplochromis burtoni*, AAP57415 *Tetraodon nigroviridis*, CAD62446 *Takifugu rubripes*, KKF31567 *Larimichthys crocea*, XP_006628630 *Lepisosteus oculatus*, AHX22596 *Paralichthys olivaceus*, XP_008318394 *Cynoglossus semilaevis*, XP_010872914 *Esox lucius*, XP_010786179 *Notothenia coriiceps*, AJO68021 *Epinephelus coioides*, XP_004069312 *Oryzias latipes*, AJA39866 *Seriola dumerili*, XP_007247805 *Astyanax mexicanus*, XP_008294254 *Stegastes partitus*, CAK29522 *Dicentrarchus labrax*, AGS55345 *Sparus aurata*, ABV64720 *Gadus morhua*, XP_013911813 *Thamnophis sirtalis*, XP_007437603 *Python bivittatus*, XP_003224060 *Anolis carolinensis*, XP_015283261 *Gekko japonicus*, EMP30816 *Chelonia mydas*, XP_005306530 *Chrysemys picta bellii*, XP_006267889 *Alligator mississippiensis*, XP_006024846 *Alligator sinensis*, CAE92388 *Xenopus laevis*, NP_001165400 *Xenopus tropicalis*, XP_007897740 *Callorhinchus milii*, XP_006000454 *Latimeria chalumnae*.

FIG. 2. Genomic organization of the *IL10* locus, the *IL10* gene structure and the IL10 protein are conserved across vertebrates. **A)** Schematic organization of the *IL10* locus using the gene orders on the human chromosome as reference. The information of the gene order was retrieved from ensemble (http://www.ensembl.org/) using the following genome assemblies: Human GRCh38.p5, Mouse GRCm38.p4, Chicken Galgal4, Chinese softshell turtle PelSin_1.0, Frog JGI4.2, Coelacanth LatCha1, Fugu FUGU4.0 and Zebrafish GRCz10. The syntenic conserved orthologs or gene blocks are shown in matching colors. Asterisks (*) next to the chromosome (Ch) or scaffold (scaf) name indicates that the orientation was inverted to optimize the alignment. Note the overall syntenic conservation of the *IL10* locus across vertebrate species. **B)** Intron/Exon organization and length of the *IL10* gene in various vertebrate species in which the *IL10* sequence was characterized in detail. The schematics were constructed using http://wormweb.org/exonintron. When the gene is present in duplicate copy, the paralogues are indicated as (a) or (b) next to the species name. The numbers on the introns of the human gene denote the phase of the intron. Red vertical lines indicate the sites and number of instability motifs (ATTTA) in the untranslated regions. Note the conservation of the gene structure with 5 exons and 4 introns. While exons retained the same length, intron size varied greatly among species. In amphibians, introns are a little longer than in human[19] while birds present shorter introns when compared to mammals.[18] The bird *IL10* gene is still 1.5-2 times longer than the fish *IL10*, making the fish gene the most compact one,[21,23,25] with the exception of trout[22] that presents introns of similar size to the mammalian counterparts. **C)** Amino acid sequence alignment of IL10 from several species (accession numbers in Fig.1) performed with PROMALS3D (http://prodata.swmed.edu/promals3d) using the crystal structure of human IL10 (PDB ID: 2H24) as a reference. Conserved cysteine residues are marked in black and

the IL10R1 binding sites are indicated by the squares. The 27 residues predicted to make contact with the human IL10R1 are color coded as follows: blue (complete conservation), green (1-2 differences), yellow (3 differences), pink (50% conservation) and red (low conservation). Consensus amino acid (aa) symbols at the bottom of the alignment are: highly conserved aa are in bold and uppercase letters; aliphatic: *l*; aromatic: @; hydrophobic: *h*; alcohol: o; polar residues: p; tiny: t; small: s; bulky residues: b; positively charged: +; negatively charged: -; charged: c. Consensus secondary structure symbol "h" points to the position of the conserved alpha helices. Numbers above the alignment indicate the most conserved amino acids as compared to the human sequence, with 9 being the highest conservation score as calculated by the AL2CO sequence conservation analysis server http://prodata.swmed.edu/al2co/al2co.php. **D)** The sequences included in **C** were modeled with Swiss-Model (http://swissmodel.expasy.org/) and all automatically fitted the structure of human IL10 with good quality scores. The PDB files obtained were manipulated with Jmol 14.6.0 to obtain the 3D representations of the IL10 homodimers colored by domain. It is easily appreciated that all 3D structures are very similar and only slight differences can be observed.

FIG. 3. The IL10 receptor complex in mammalian and non-mammalian vertebrates. A) Schematic representation of the prototypical IL10R complex in mammals: one IL10 molecule binds to two molecules of the IL10R1 which in turn recruits two molecules of the IL10R2. This leads to the activation of the JAK1 and TYK2 kinases present in the cytoplasmic tails of the receptors and subsequent phosphorylation of the tyrosine (Y) residues at specific sites in the cytoplasmic tail of the IL10R1. Phosphorylated tyrosines represent the docking sited for cytosolic STAT3, which in turn will be phosphorylated and

will translocate to the nucleus. The binding site for JAK1 (PxxL) is highly conserved in all investigated species, similarly to the TYK2 binding site, whereas the number of potential phosphorylation sites in the IL10R1 tail varies among mammals and also between mammals, birds and amphibians, indicated by dashed arrows (see further details in Fig. 4C). Although not investigated in detail at the functional level, orthologues of the IL10R complex in amphibians can be identified based on conserved synteny (see also Fig. 4 and 5). In reptiles a conserved IL10R2 and an incomplete IL10R1 can be found at conserved genomic locations. Therefore it is likely that the IL10R complex also in reptiles would have a conserved structure although it still needs to be formally proven. **B) Schematic representation of the putative IL10R complex in teleost fish**: identification of the orthologues of the IL10R complex in teleost fish has proven more challenging due to the lack of sequence conservation and weak preservation of genomic (synteny) structure. Nevertheless, based on structural features and *in vitro* functional studies,[47] class II cytokine receptor family-7 (CRFB7) has been without doubt annotated as IL10R1. Annotation of the IL10R2 has proven more difficult due to the presence of two paralogues, CRFB4 and CRFB5, which most likely are derived from a recent tandem duplication. Functional work in grass carp[69] however, indicates that CRFB4 is most likely the co-receptor of the IL10R complex in teleost whereas work in zebrafish supports the idea that CRFB5 is rather involved in the type I IFNR complex (not shown). **C) Duplicated copies of all members of the IL10R complex in some teleost fish**: in common carp, Atlantic salmon (and most likely rainbow trout), duplicate copies of all members of the IL10R complex can be found. This adds an extremely higher degree of complexity when considering all possible ligand-receptor combinations. In the scheme a hypothetical complex has been depicted, but to date it cannot be excluded that all combinations of subunits are possible.

FIG. 4. Genomic organization of the *IL10R1* locus and the *IL10R1* gene structure are conserved across vertebrates. **A)** Schematic organization of the *IL10R1* locus using the gene orders on the human chromosome as reference. The information of the gene order was retrieved from ensembl (http://www.ensembl.org/) using the following genome assemblies: Human GRCh38.p5, Mouse GRCm38.p4, Chicken Galgal4, Green anole AnoCar2.0, Frog JGI4.2, Fugu FUGU4.0 and Zebrafish GRCz10. The syntenic conserved orthologs or gene blocks are shown in matching colors. Asterisks (*) next to the chromosome (Ch) or scaffold (scaf) name indicate that the orientation was inverted to optimize the alignment. **B)** Intron/Exon organization and length of the *IL10R1* gene in various vertebrate species. The Green anole sequence (as well as the one of other reptile *IL10R1*) is still incomplete in the database and was not included. The schematics were constructed using http://wormweb.org/exonintron and the intron/exon length information was retrieved from ensembl. The numbers on the introns of the human gene denote the phase of the intron, which is conserved in all species (not shown). Note the conservation of the gene structure with 7 exons and 6 introns. While exons retained the same length, intron size varied among species with chicken and fugu in particular, being the most compacted. Common carp expresses two copies of the CRFB7 gene, but the genome assembly is still incomplete to provide synteny information on the position of these genes in the carp genome. **C)** Amino acid alignment of the cytoplasmic tails of various IL10R1/CRFB7 sequences in vertebrates. Green highlights at the beginning indicate transmembrane regions; in light blue is the very conserved JAK1 binding site (PxxL). In yellow are the two canonical STAT3-binding sites (GYXXQ) found in all species, including the two non-canonical sites in frog (DYLLQ) and in most fish species (GYRSG). Tetraodon is an exception to all species as it presents two non-canonical sites (dark green) with substantially diverged sequences but at conserved

positions with respect to the ones found in other vertebrates. In grey are the additional STAT3-binding sites found upstream of the canonical ones in some mammalian and avian sequences. In almost all sequences, an additional tyrosine (Y) residue is found downstream of the canonical STAT3-binding sites (light grey), the function of which is still unknown.

FIG. 5. The *IL10R2* locus and gene structure are not that well conserved among mammals and become complicated in teleost. A) Schematic organization of the *IL10R2* locus using the gene orders on the human chromosome as reference. The information of the gene order was retrieved from ensembl (http://www.ensembl.org/) using the following genome assemblies: Human GRCh38.p5, Mouse GRCm38.p4, Chicken Galgal4, Chinese softshell turtle PelSin_1.0, Frog JGI4.2, Stickleback BROAD S1, Tetraodon TETRAODON 8.0, Zebrafish GRCz10 and Atlantic salmon.[72] The syntenical conserved orthologs or gene blocks are shown in matching colors. Asterisks (*) next to the chromosome (Ch) or scaffold (scaf) name indicate that the orientation was inverted to optimize the alignment. Teleost fish express two homologues of the mammalian IFNAR2 gene, named CRFB1 and CRFB2, thus the color gradient in the IFNAR2 block; CRFB6 is homologous to mammalian IFNGR2 and, as discussed in the text, CRFB5 seems to act as the IFNAR1 functional equivalent while CRFB4 functions as IL10R2. Note the conserved synteny of the IFNAR2, IL10R2 and IFNAR1 gene cluster between mammals and birds, reptiles and amphibians. Such conservation is completely lost in and among fish genomes, making it more difficult to identify functional equivalent solely based on genome organization. Atlantic salmon presents multiple copies of several CRFB in this gene cluster, and similar to the Tetraodon expresses a CRFB3 gene not present in other fish species. **B)** Intron/Exon organization and length of the *IL10R2* gene in various vertebrate species. The

schematics were constructed using http://wormweb.org/exonintron and the intron/exon length information was retrieved from ensembl. The numbers on the introns of the human gene denote the phase of the intron, which is conserved in all species (not shown). Note the conservation of the gene structure with 7 exons and 6 introns. While exons retained the same length, intron size varied among species.

FIG. 6. Kinetics of IL10 expression in resistant and susceptible carp lines during a *Trypanoplasma borreli* infection. In resistant strains, the peak of pro-inflammatory molecules expression (*iNOS, IFNγ* and *TNFα*) closely follows the increase in parasitaemia (black line). Upregulation of *IL10* (blue line) occurs in a later phase of the infection, followed by a downregulation of pro-inflammatory genes, an increase in specific antibodies, and ultimately by a reduced parasite burden. In contrast, in susceptible lines, an early expression of *IL10* is observed, prior to a very modest upregulation of pro-inflammatory genes. This leads to an uncontrolled parasite replication and increased mortalities; (Modified from Forlenza et al 2011[105] and unpublished data from our group).

FIG 1:

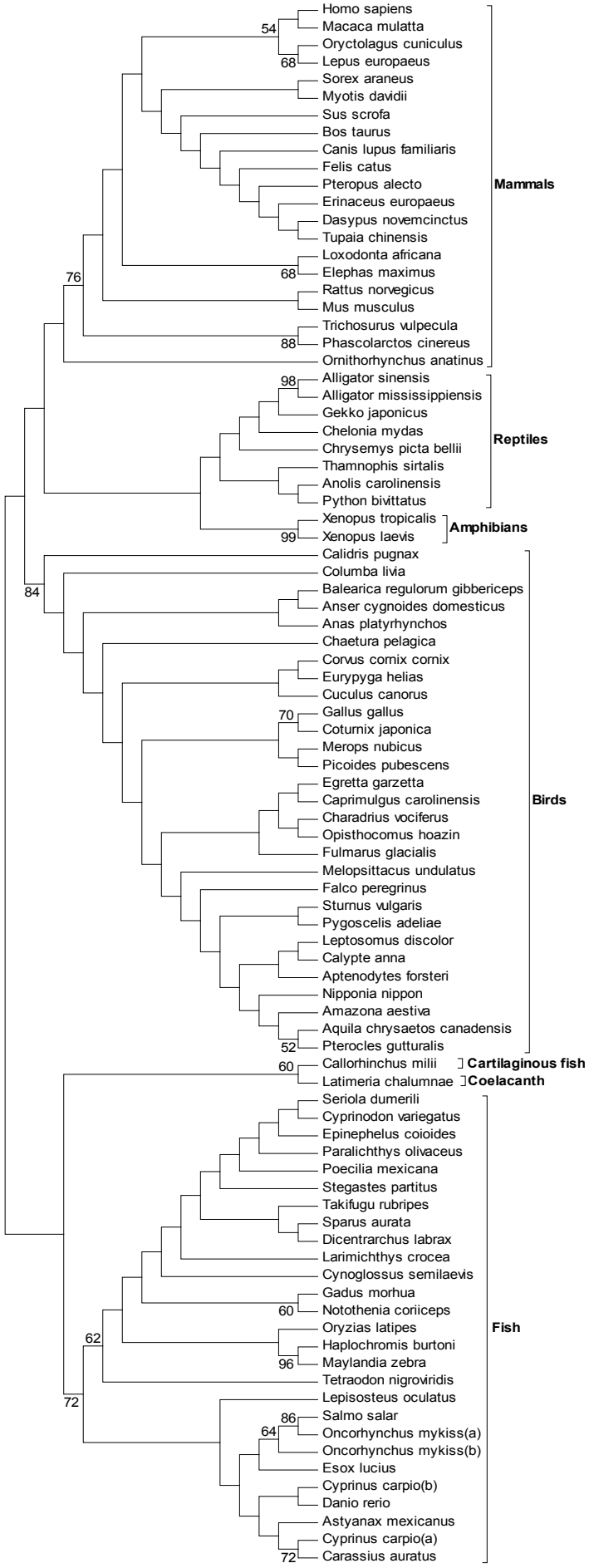
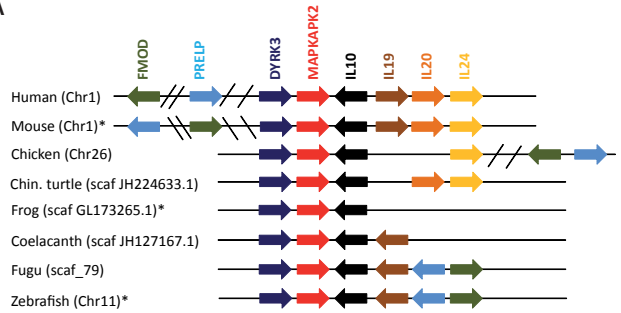
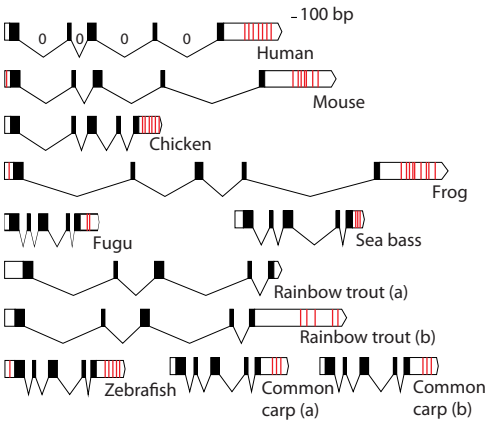

Homo sapiens
Macaca mulatta
Oryctolagus cuniculus
Lepus europaeus
Sorex araneus
Myotis davidii
Sus scrofa
Bos taurus
Canis lupus familiaris
Felis catus
Pteropus alecto
Erinaceus europaeus
Dasypus novemcinctus
Tupaia chinensis
Loxodonta africana
Elephas maximus
Rattus norvegicus
Mus musculus
Trichosurus vulpecula
Phascolarctos cinereus
Ornithorhynchus anatinus
Mammals
Alligator sinensis
Alligator mississippiensis
Gekko japonicus
Chelonia mydas
Chrysemys picta bellii
Thamnophis sirtalis
Anolis carolinensis
Python bivittatus
Reptiles
Xenopus tropicalis
Xenopus laevis
Amphibians
Calidris pugnax
Columba livia
Balearica regulorum gibbericeps
Anser cygnoides domesticus
Anas platyrhynchos
Chaetura pelagica
Corvus cornix cornix
Eurypyga helias
Cuculus canorus
Gallus gallus
Coturnix japonica
Merops nubicus
Picoides pubescens
Egretta garzetta
Caprimulgus carolinensis
Charadrius vociferus
Opisthocomus hoazin
Fulmarus glacialis
Melopsittacus undulatus
Falco peregrinus
Sturnus vulgaris
Pygoscelis adeliae
Leptosomus discolor
Calypte anna
Aptenodytes forsteri
Nipponia nippon
Amazona aestiva
Aquila chrysaetos canadensis
Pterocles gutturalis
Birds
Callorhinchus milii
Cartilaginous fish
Latimeria chalumnae
Coelacanth
Seriola dumerili
Cyprinodon variegatus
Epinephelus coioides
Paralichthys olivaceus
Poecilia mexicana
Stegastes partitus
Takifugu rubripes
Sparus aurata
Dicentrarchus labrax
Larimichthys crocea
Cynoglossus semilaevis
Gadus morhua
Notothenia coriiceps
Oryzias latipes
Haplochromis burtoni
Maylandia zebra
Tetraodon nigroviridis
Lepisosteus oculatus
Salmo salar
Oncorhynchus mykiss(a)
Oncorhynchus mykiss(b)
Esox lucius
Cyprinus carpio(b)
Danio rerio
Astyanax mexicanus
Cyprinus carpio(a)
Carassius auratus
Fish
54
68
76
68
88
98
99
84
70
52
60
60
62
96
72
86
64
72

FIG 2:

A



B



C

```
Conservation:        9                  55                  7  5   67  96599     7  666  9  6   99        77   796   7 6 99
Homo sapiens         MH-------SSALL--CCLVL-LTGVRA-SPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKD-QLDNLLLKESLLEDFKGYLGCQALSEMIQFY
Gallus gallus        M---------QTCCQALLLLL-AACTLP-AHC----LEPTCLHFSELLPARLRELRVKFEEIKDYFQSRDDDLNIQLLSSELLDEFKGTFGCQSVSEMLRFY
Thamnophis sirtalis  M---------NALI--AYMLL-SLVLKN-AHS----QLIDCRHFSHNLPLRLRELRATFNKIKDYFQDRDSDLDTVLLKQDLLEDFKSYLGCQSVGEMIHFY
Xenopus tropicalis   M---------KFCL--LLTLF-FFTCKT-VKCQSGDAEGNCSRVVNIFPAKLKELRATFQKIKNFFQMKDNALEIVLLQDDLLQEFKGNLGCQSVSETIRFY
Latimeria chalumnae  MPTTLTGQHLARSA--ASLAK-YSTYRKTSWAE-HSAEDGCLLFANAFPGMLKELRIAFKKIQEFFQTKDDNIDIALLDEDLLYGFKDHFGCQLLKEMLKFY
Takifugu rubripes    MT--------PGSL--LSVLL-LLCCACTVWCA-ALCNNRCCSFVEGFPARLKMLRENYSQIRDYYEANDDL-DIVLLDQSIVDTFKTPFACCHLMDGILRFY
Danio rerio          MI-------FSGVI--LSALLTLLLCDCAQSRR-VECKTDCCSFVEGFPIRLRELRSAYKEIQKFYESNDDL--EPLLNEDIKHNINSPYGCHVMNEILHFY
Callorhinchus milii  MQ-------RATVV--LWMLL-TLFNIQCCCCS-HRQAAGLCLSTSVLHIHLRELRTANQKISSYFQRRDGNIRVSLLRGKFQHEFKGRLGCQFLKEMLDFY
Consensus aa:        M.....h..h.hlh.hhhh...s.t.....ssC..hsp.hP..L+-LR.s@pcIpp@@p.pDs.lc..LLppplbppFKs.hGCp.hpEhlcFY
Consensus ss:                hhhh  hhhhh hhh              hhhhhhhhhhhhhhhhhhhhh          hhhhhh   hhhhhhhhhhhhh

Conservation:        7  659 7                65  6  9      9   6 9          6   5  6 96 659 99977967 6  577 77  6
Homo sapiens         LEEVMPQAENQD----PDIKAHVNSLGENLKTLRLRLRRCHRFLPCE----NKSKAVEQVKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTMKIRN------
Gallus gallus        TDEVLPRAMQTS----TSHQQSMGDLGNMLLGLKATMRRCHRFFTCE----KRSKAIKQIKETFEKMDENGIYKAMCEFDIFINYIEEYLLMRRRK------
Thamnophis sirtalis  LEDVLPKVTTDN----P-LKQDVDFLGNMLLDLRQLIRRCHRFFICE----KKNKTVKAIKDTYDKLQDKGIYKAICEFDIFVDHIEQYLLMKMKS------
Xenopus tropicalis   LEEVLPQANHY--------KMNVSFLKDKLLDLKHTLRRCHNFLPCE----RKSKAIKQIKQTYNKMHEQGIYKAMCEFDILIDYIEDYLMSRKK-------
Latimeria chalumnae  LEEVLPKAQDKLDIQPTIQTAVSHIGEMLFNLKQKITLCQNFFACS----NKSRAVKRIKETYNKLQEKGVYKAMCELDIFIDVIEDYLIAKKK-------
Takifugu rubripes    LDSVLPRALATVTAETRNLKPHVESIQQIFDQLKIEVTNCKHYFACK----NRF-DINVLNSTYTKMEDKGLYKAMCELDLLFNYIENYLASKRRRNVA---
Danio rerio          LETILPTALQKNP--LKHSTTPIDSIGNIFQELKRDMVKCKRYFSCQ----NPF-EVNSLKNSYEKMKEKGVYKAMCELDLLFRYIEQYLASKRVKH-----
Callorhinchus milii  LSHIIPAAKTQS----KAYNTHISKIGNTLFELQQNIENCHQLFNCDTCNCNRSNYIEEIYNMYKKLQDKGIYKAMCELDLLFTFIDMLDRFLSMKLKGANKKRK
Consensus aa:        L-pllPpA.pps.....shp..ls.l.p.hbpL+.phppC+p@hsCp....p+.p.lp.lpph@pKhp-pGlYKAMGEhDlhhs@IEpYLh.+.+.......
Consensus ss:        hhhh  hhh        hhhhhhhhhhhhhhhhhhhhhhhhhh          hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhh
```

D

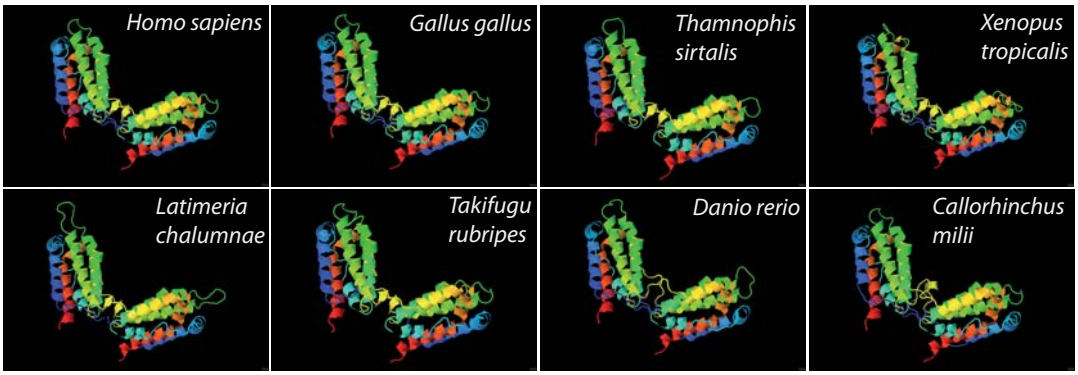


FIG 3:

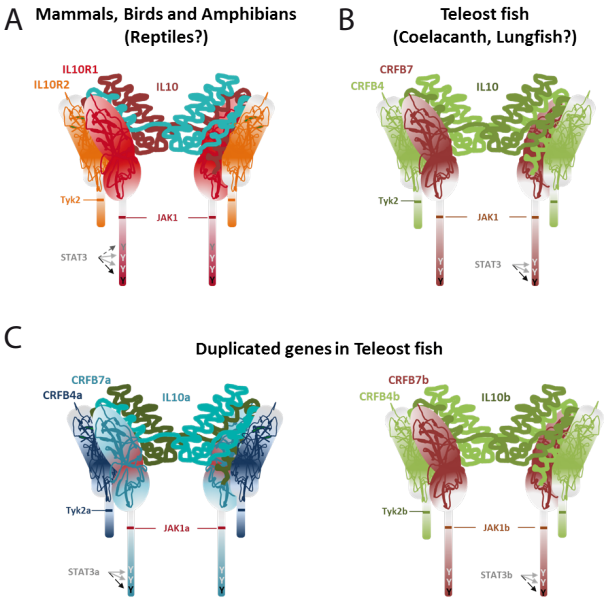

A
Mammals, Birds and Amphibians
(Reptiles?)
IL10R1
IL10R2
IL10
Tyk2
JAK1
STAT3
B
Teleost fish
(Coelacanth, Lungfish?)
CRFB7
CRFB4
IL10
Tyk2
JAK1
STAT3
C
Duplicated genes in Teleost fish
CRFB7a
CRFB4a
IL10a
Tyk2a
JAK1a
STAT3a
CRFB7b
CRFB4b
IL10b
Tyk2b
JAK1b
STAT3b

FIG 4:

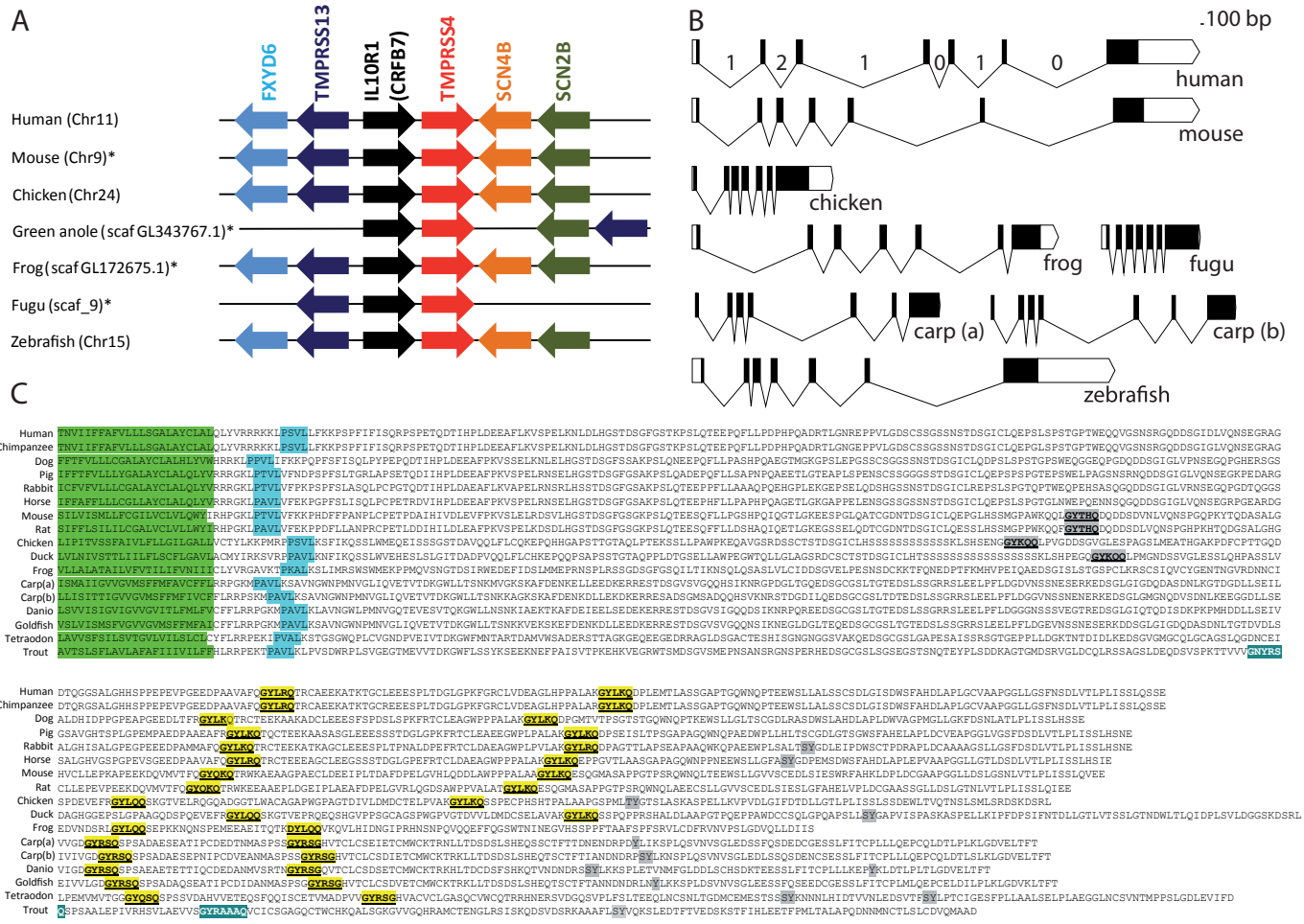

A
FXYD6
TMPRSS13
IL10R1 (CRFB7)
TMPRSS4
SCN4B
SCN2B
Human (Chr11)
Mouse (Chr9)*
Chicken (Chr24)
Green anole (scaf GL343767.1)*
Frog (scaf GL172675.1)*
Fugu (scaf_9)*
Zebrafish (Chr15)
B
.100 bp
1
2
1
0
1
0
human
mouse
chicken
frog
fugu
carp (a)
carp (b)
zebrafish
C
Human
Chimpanzee
Dog
Pig
Rabbit
Horse
Mouse
Rat
Chicken
Duck
Frog
Carp(a)
Carp(b)
Danio
Goldfish
Tetraodon
Trout

FIG 5:

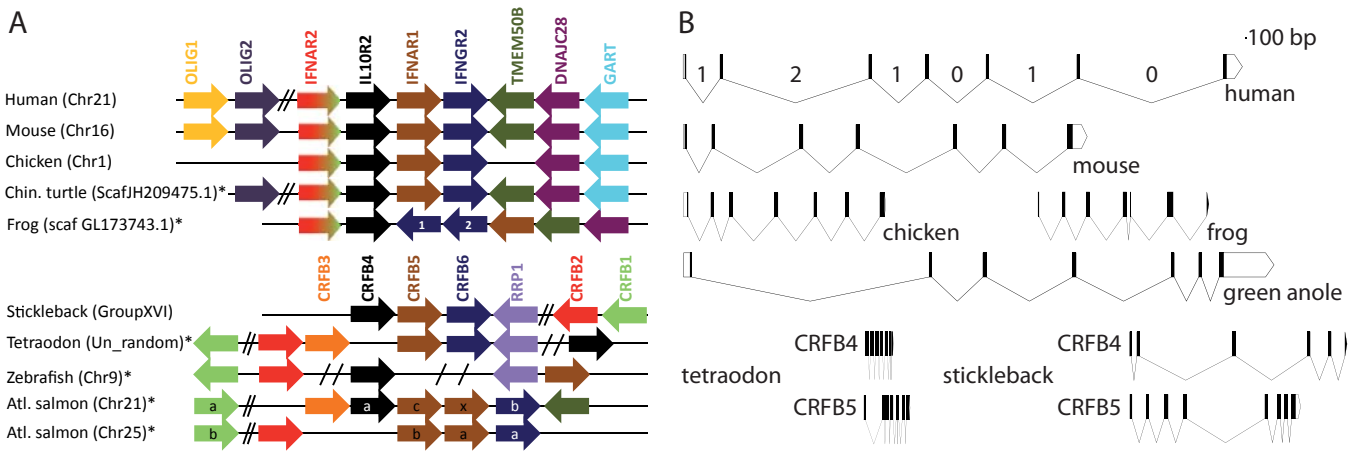
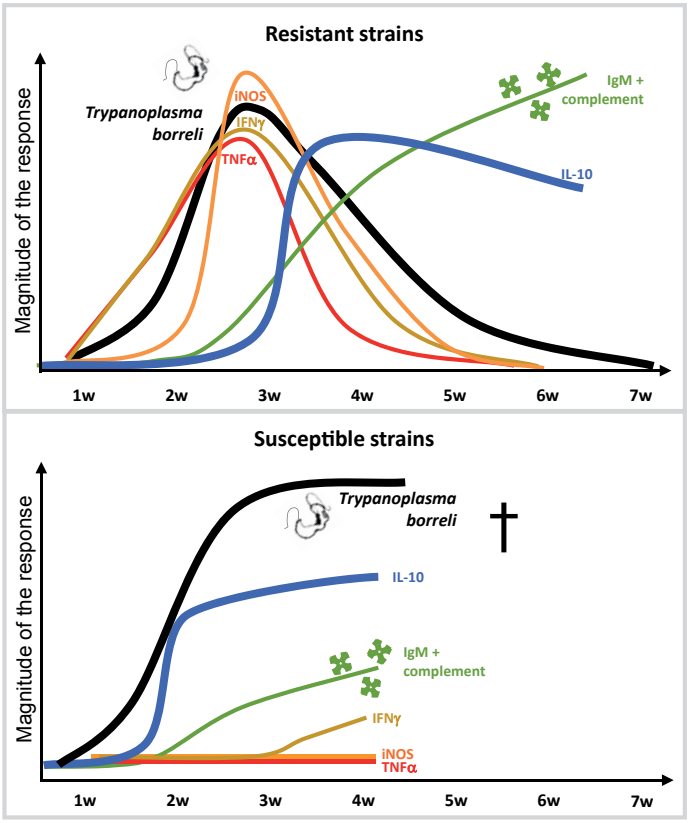

A
OLIG1
OLIG2
IFNAR2
IL10R2
IFNAR1
IFNGR2
TMEM50B
DNAJC28
GART
Human (Chr21)
Mouse (Chr16)
Chicken (Chr1)
Chin. turtle (ScafJH209475.1)*
Frog (scaf GL173743.1)*
CRFB3
CRFB4
CRFB5
CRFB6
TRRP1
CRFB2
CRFB1
Stickleback (GroupXVI)
Tetraodon (Un_random)*
Zebrafish (Chr9)*
Atl. salmon (Chr21)*
Atl. salmon (Chr25)*
B
100 bp
human
mouse
chicken
frog
green anole
tetraodon
CRFB4
CRFB5
stickleback
CRFB4
CRFB5

FIG 6:


Resistant strains
Magnitude of the response
Trypanoplasma borreli
iNOS
IFNγ
TNFα
IgM + complement
IL-10
1w
2w
3w
4w
5w
6w
7w
Susceptible strains
Magnitude of the response
Trypanoplasma borreli
†
IL-10
IgM + complement
IFNγ
iNOS
TNFα
1w
2w
3w
4w
5w
6w
7w