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A portrait of the immune response to proliferative kidney disease (PKD) in rainbow trout

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Abstract

Proliferative kidney disease (PKD), caused by the myxozoan *Tetracapsuloides bryosalmonae*, is one of the most serious parasitic diseases of salmonids in which outbreaks cause severe economic constraints for the aquaculture industry and declines of wild species throughout Europe and North America. Given that rainbow trout (*Oncorhynchus mykiss*) is one of the most widely farmed freshwater fish and an important model species for fish immunology, most of the knowledge on how the fish immune response is affected during PKD is from this organism. Once rainbow trout are infected, PKD pathogenesis results in a chronic kidney immunopathology mediated by decreasing myeloid cells and increasing lymphocytes. Transcriptional studies have revealed the regulation of essential genes related to T-helper (Th)-like functions and a dysregulated B-cell antibody type response. Recent reports have discovered unique details of teleost B-cell differentiation and functionality and characterized the differential immunoglobulin (Ig)-mediated response. These studies have solidified the rainbow trout *T. bryosalmonae* system as a sophisticated disease model capable of feeding key advances into mainstream immunology and have contributed essential information to design novel parasite disease prevention strategies. In our following perspective, we summarize these efforts to evaluate the immune mechanisms of rainbow trout during PKD pathogenesis.

KEYWORDS

immunoglobulins, immunopathology, lymphocytes, myxozoa, salmonids, *Tetracapsuloides bryosalmonae*

1 | INTRODUCTION

Parasitic infections are responsible for huge economic losses in aquaculture and threaten many freshwater and marine fish.[1-9] The increasing economic and environmental impact of these diseases has enhanced the interest in unravelling the fish immune response to fish parasites to eventually develop novel control strategies against them. It is difficult to provide a single figure for economic losses attributed to parasites, given that they imply costs associated with controlling or managing the infection, indirect loss factors (ie coinfections, reduced growth and fecundity) and downstream socio-economic impacts. A recent report, evaluating the economic impact of disease outbreaks due to protistan and metazoan parasites, estimated that annual costs range from $1.05 billion to $9.58 billion.[10] While an earlier review of the financial impacts of parasitic diseases in the aquaculture industry suggested that myxozoan species such as *Myxobolus cerebralis* (causative agent of whirling disease) and the ectoparasites, *Lepeophtheirus salmonis* (sea lice) and *Gyrodactylus salaris* are often the most reported

and, therefore, arguably the most substantive parasitic diseases in aquaculture. Notable outbreaks and economic constraints caused by other parasitic infections that may have previously gone unreported are also suggested to be on the rise.[11]

Fish are one of the largest groups of vertebrates and the first animal group exhibiting both innate and adaptive immunity.[12] While many immunological elements of the innate and adaptive immune system are common to mammals and fish, there are several differences concerning both elements and functionality. For example, unlike mammals, in teleost fish the kidney is the main hematopoietic tissue in the absence of bone marrow with the spleen representing the only systemic secondary lymphoid organ in the absence of lymph nodes. While as in mammals, fish possess numerous local mucosal-associated lymphoid tissues (gills, nares, gut and skin) as well as lymphoid tissue associated with the liver and thymus. Following the two rounds of whole-genome duplication (WGD) that occurred in the common ancestor of vertebrates, a third genome duplication occurred in the stem lineage of teleost fishes, which largely accounts for fish-specific evolutionary trajectories in both innate and adaptive immunity.[13] Thus, as a consequence, many of the genes involved in immunity are at least duplicated in salmonids.[13] Additionally, many aspects of adaptive immune function appear to have evolved independently in fish, with numerous teleost immune genes being at least duplicated.[13,14] In the past years, a great effort has been made to expand our knowledge of the evolution and diversification of vertebrate immune systems as well as identifying important targets for disease prevention across many species. In light of these advances, how immunity against parasites is organized and regulated is being explored with great progress.

1.1 | Proliferative kidney disease: Impact and life cycle

Proliferative kidney disease (PKD) is one of the most serious parasitic diseases of fish in which outbreaks are linked to global warming, given that incidence and severity of PKD has increased largely owing to seasonal increases in water temperatures.[15,16] PKD outbreaks have resulted in severe economic constraints for freshwater fish farmers throughout Europe and North America. Economic losses in rainbow trout (*Oncorhynchus mykiss*) production attributed to the disease in 2004 in the UK alone were estimated at £2.5 million (~£4 million in the current market).[17] During severe outbreaks, mortality can reach up to 95%-100%.[15,18] PKD has also been implicated in the long-term decline of wild brown trout (*Salmo trutta*) populations in Switzerland.[19,20] Additionally, the parasite has been observed in an increasing number of European countries, reaching as far north as Iceland.[21-23] Recently, the disease was also identified as the cause of a mass mortality event of fish during a heat wave in North America.[24]

PKD is caused by the myxozoan parasite *Tetracapsuloides bryosalmonae*. The *T.bryosalmonae* life cycle exploits two hosts, salmonid fish, the vertebrae host and freshwater bryozoans, the invertebrate host. Infective *T. bryosalmonae* spores are released from bryozoans, into the water, infecting the fish host via the gills. Following attachment of a spore to the fish host, a single sporoplasm invades primarily the skin epithelium. Subsequent parasite stages migrate, via the vascular system, to the primary target organ, the kidney.[25] Additionally, parasites can also colonize other organs, including the spleen and liver. The trout kidney is located ventral to the backbone and has two main regions extending from the base of the cranium (anterior kidney) to the caudal region (posterior kidney). The anterior kidney is interdigitated with adrenal-like tissue, has no renal function and lacks nephrons and is the primary site for lymphohaematopoiesis where B cells develop and where most proliferating B-cell precursors are located.[26] The posterior kidney possesses both renal and immune tissues, hosting substantial populations of partially activated B cells and plasmablasts.[26] *T. bryosalmonae* mature as sporogonic stages in the kidney tubules and collecting ducts (coelozoic sporulation) with mature spores, infective to bryozoans, only released in the urine of native brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*).[27] In Europe, non-native rainbow trout develop severe PKD clinical signs. They do not shed spores infective to bryozoans, which precludes the completion of the parasite life cycle and are, thus, considered as dead-end hosts.[27] The immunosuppressive nature of PKD can increase fish susceptibility to secondary infections.[28] Despite high mortalities in naïve fish stocks, depending on both biotic and abiotic factors, severely infected rainbow trout can on occasion survive the disease, clear the parasite burden, restore kidney structure and develop a form of protective immunity against re-exposure.[29,30]

1.2 | Pathological and histological changes during PKD pathogenesis

In the kidney, *T. bryosalmonae* penetrates the interstitial tissue, multiplies and differentiates from extrasporogonic to renal sporogenic stages. Due to the immune nature of the organ, parasite development provokes a chronic immunopathology characterized by a lymphocytic hyperplasia, hyperimmunoglobulinaemia and renal atrophy.[15,16] Histopathological changes in the kidney can include thrombus formation, necrotizing vasculitis, a strong hyperplastic response, proliferative and diffuse granulomatous nephrosis, leading to the deterioration of renal tubules.[31,32] Eventually, renal lesions are contained, and proliferation and infiltration in the interstitium become displaced by fibrotic tissue followed by tissue regeneration.[29] Apart from a marked kidney swelling, other gross pathological changes include ascites, gill and liver pallor, systemic anaemia, sub-capsular renal oedema, liver and splenic discolouration and enlargement.[15,16] A similar type of cellular reaction to the kidney can occur in the spleen, and in heavily parasitized fish, a progression to extensive collagen formation has been reported.

Furthermore, in cases when the spleen has been heavily enlarged, patches of greyish mottling beneath the capsule and throughout the stroma can be seen.[18] Despite this strong host reaction in the spleen, almost all immunological studies of PKD pathogenesis have been focused on the kidney and to a much lesser extent on the liver and spleen despite the presence of the parasite in these organs.

1.3 | General fish host immune response to *T. bryosalmonae*

A decrease in myeloid cells and the in situ proliferation of lymphocytes are the most notable cellular aspects of PKD pathogenesis.[33,34] Comprehensive transcriptional studies have revealed the regulation of a number of important genes related to T-helper (Th)-like functions, a dysregulated B-cell antibody type response and also the further inhibition of mechanisms of the innate immune response.[34-36] Recent reports have also disentangled several unique details of rainbow trout B-cell differentiation and functionality, characterizing the differential immunoglobulin (Ig)-mediated response in *T. bryosalmonae*-infected fish.[37] Additional studies have described the role cytokines of the tumour necrosis factor (*tnf*) superfamily, such as B-cell activation factor (*baff*) and a proliferation-inducing ligand (*april*),[38] which are now also known to play a major role in B-cell differentiation and survival in rainbow trout.[39] An overview of these mechanisms as well as those presented in this review is provided in Table 1. Collectively, these studies have solidified the rainbow trout-*T bryosalmonae* system as a sophisticated immunological model for teleost B-cell research capable of feeding major advances into mainstream immunology.

Apart from investigations of rainbow trout, a few transcriptional reports have described the immune response during *T. bryosalmonae* infection of brown trout. For instance, in an earlier study Ig light chain (*igl*) and *igm sec* (secretory form) transcripts were found to be upregulated in the kidney of infected brown trout.[40,41] While a recent study by Bailey et al.[42]expanded on this to measure a panel of genes encoding for Th-1 and Th-2-like cytokines and markers of myeloid, natural killer and B- and T-cell subsets, their findings also detailed a strong B-cell antibody type response in brown trout. However, the authors did describe some subtle differences in sequential aspects of the immune response and of immune genes that correlated with parasite intensity for brown trout, in contrast to rainbow trout, in terms of the B-cell response and Th-like transcripts that may be linked to PKD pathogenesis. Still, further research especially at the functional level is required as nothing currently exists of this in brown trout to clarify whether there are actually specific differences in B-cell diversity in comparison with rainbow trout.[42] Thus, the present review places the emphasis on the efforts to evaluate the cells and pathways involved in the immune response of rainbow trout during PKD pathogenesis. Along the way, we explore the capabilities and discuss possibilities for future research using this disease model.

2 | THE INNATE IMMUNE RESPONSE

2.1 | Toll-like receptors

The innate immune response is the first line of defence against invading pathogens, recognizing highly conserved microbial structures known as pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) through pattern recognition receptors (PPRs). PRRs and their signalling pathways have essential roles in triggering inflammatory responses that eliminate invading microorganisms and damaged cells.[43-45] Lee et al. investigated the regulation of a range of TLRs in the posterior kidney of rainbow trout infected with *T. bryosalmonae* from a natural disease outbreak. Fish in this study, as commonly used in the PKD literature, were classified according to the kidney swelling index (KSI) system devised by Clifton-Hadley et al.[18] An overview of the KSI system, including how samples were obtained for experimental procedures using different approaches, is provided in Box 1. Lee et al[46] investigated fish that exhibited a swelling grade of 1-3 and assessed an extensive panel of TLRs. Among them, *tlr8b1*, *tlr19*, *tlr20a*, *tlr22a1* and *tlr22a2* displayed a similar expression profile in *T. bryosalmonae*-infected fish, in that significant upregulation occurred at grades 1-2 to 3, plateauing at grade 2 in the posterior kidney. *tlr8a1* and *tlr8a2* transcripts were also upregulated at grade 1, in addition to the higher grades. However, *tlr18* and *tlr21* were downregulated at every grade investigated, with *tlr3*, *tlr8b2* and *tlr9* expression inhibited at only the more advanced disease stages. Concerning the nonmammalian TLRs that were elevated during PKD pathogenesis, *tlr19* and *tlr20* have also been reported to play a role in the fish host immune response to ectoparasites. For example, *tlr20* was significantly upregulated in the gill, liver, head kidney, tail fin and spleen of goldfish (*Carassius auratus*) challenged with *Dactylogyrus intermedius*,[47] while *tlr19*, in channel catfish (*Ictalurus punctatus*) challenged with *Ichthyophthirius multifiliis*, was significantly elevated in the skin and gill tissues relative to the controls.[48] The collective expression patterns of nonmammalian *tlr19* and *tlr20* during PKD promote the idea of investigating these molecules at an initial stage at the port of entry for *T. bryosalmonae*, the gills. Additionally, examining markers for DAMPs during host recovery from clinical infection in the kidney, in which fibroblasts and extracellular matrix components as well as dying or stressed immune cells may secrete DAMPs. This represents an important area for future research to determine whether homeostatic recovery processes correlate with parasite clearance and tissue resolution.

BOX 1 Experimental techniques to study PKD pathogensis

(1) *Natural exposure*—Sampling infected fish from a natural outbreak source in either a fish farm, lake or river.[35,36,49] Infected fish are maintained and studied in aquarium facilities, or by taking fish directly at a *T. bryosalmonae*-infected fish farm thus transporting them once infected to an aquarium facility.[25,50] Or by exposing specific pathogen-free fish to such enzootic waters. In this context, samples might not always be studied chronologically as it is difficult to determine the precise start of exposure, and immune parameters are often

TABLE 1 An overview of the main immune features described in the present review focusing on the immune mechanisms investigated in rainbow trout during PKD pathogensis. The abbreviations in the table are consistent with those mentioned in the text

Immune mechanism	Section	Brief summary of response during *T.bryosalmonae infection* in rainbow trout	Study conditions	Supporting literature
TLRs	2.1.	*tlr8b1*, *tlr19*, *tlr20a*, *tlr22a1* and *tlr22a2* transcripts were upregulated with kidney swelling grades 1-2. *tlr8a1* and *tlr8a2* were upregulated with kidney swelling grade 1. *tlr18* and *tlr21* were unresponsive.	Natural outbreak	46
Pro-inflammatory cytokines	2.2.	*TNF-α*, *iil-1β* and *cox-2* were all downregulated or transiently expressed.	Natural outbreak and laboratory infection	34-36
Anti-inflammatory cytokines	2.2.	Strong upregulation during the course of PKD progression, namely *il-6*, *il-10a/b*, *il-11* and *nil-1f*. Hyper-expression of *il-10*.	Natural outbreak and laboratory infection	34-36
AMPs	2.2.	*cath-1*, *cath-2*, *hepcidin-1* and *leap 2* were upregulated at all kidney swelling grades during infection. In particular, *cath1* and *cath2* were strongly upregulated.	Natural outbreak	36
Macrophage activity	2.3.	Downregulation and general unresponsiveness of *mcsf* and its receptor *mcsf-r* and arginase paralogues. Decrease of myeloid cells described in the kidney as well as a depression in phagocytosis and oxidative burst in the kidney.	Natural outbreak and laboratory infection	33,34,36
Chemokines	2.4.	The expression of ligands *ccr4lc1* and *ccr4lc2* decreased in infected fish, while *ccrla* was unresponsive. Concerning the receptors *ccr11* exhibited only a minor increase, while *cmklr3* was significantly downregulated and generally refractory at all kidney swelling grades.	Natural outbreak	67,68
SOCS	2.5.	Significant up-regulation of *socs1* and *socs3* has been observed correlating with parasitic burden and pathology progression.	Natural outbreak and laboratory infection	36,73,74
T cell markers	3.1.	*cd4*, *cd8α* and *cd8β* transcripts were positively correlated with parasite intensity in a natural outbreak study. In an experimental infection trial, there was a significant decrease in the number of $CD8^-$ T cells in the anterior kidney relative to the control at week 4 PE before a significant increase was detected in these T-cell subsets in the posterior kidney at week 6 PE	Natural outbreak and laboratory infection	34,36
Th1-like cytokines	3.2.1.	Th1-like cytokines *t-bet*, *ifnγ* and *il-2* transcripts have been shown to be upregulated in various disease stages in infected fish	Natural outbreak and laboratory infection	34,36,84
Th-2-like cytokines	3.2.1.	*gata3*, *il-4/13a*, *il-4/13b1*, *il4/13b2* and *il-10* were upregulated in the posterior kidney of fish with advanced clinical PKD	Natural outbreak and laboratory infection	34,36,85
Th-17-like cytokines	3.2.2.	*il-17c-1* was shown to be significantly upregulated at early clinical stages. *il-21* expression correlated with parasite prevalence and significantly, although to a lesser extent, with kidney swelling grade, whereas *il-22* correlated only with parasite prevalence. RORγ transcripts, on the other hand, did not correlate with either.	Natural outbreak and laboratory infection	34,36,88

(Continues)

TABLE 1 (Continued)

Immune mechanism	Section	Brief summary of response during *T.bryosalmonae infection* in rainbow trout	Study conditions	Supporting literature
Treg-associated transcripts	3.2.2.	*foxp3a* and *foxp3b* were significantly upregulated and their expression levels correlated with parasite burden	Natural outbreak and laboratory infection	34,36
B-cell markers	3.4.	Transcription of secreted *igt* and *igm* but not *igd* correlated with kidney pathology. *blimp1*, *pax5*, *baff*, *april*, *balm*, *baff-r* and *taci* were all upregulated in infected fish. A significant increase in the number of igm^+ B cells was detected through flow cytometry in the posterior kidney during advanced clinical stages. Fish that have recovered from PKD, when re-exposed, exhibited an increase of igm^+ B cells in the blood relative to naïve-infected fish. In addition, an increase in total plasma Igs relative to control fish was observed at the start of a natural outbreak study.	Natural outbreak and laboratory infection	30,34,36-38,49

correlated with (a) a pathological index, that is the kidney swelling index system devised by Clifton-Hadley et al (1987). In which, grade 0 is a healthy unaffected kidney; grade 1 is low level pathology; 2 is moderate, 3 and 4 are when most clinical and internal changes occur in the fish kidney[31] and (b) the parasite burden quantified by a chosen qPCR method.[36] In addition, there is a risk of coinfections using these techniques.

(2) *Laboratory controlled exposure*—This is carried out using parasites cultured in the laboratory obtained from either maintaining *T. bryosalmonae*-infected bryozoa [27] or from parasites collected directly from the field (via sampling infected bryozoa) for infection.[34] Either exposure procedure requires an initial source of infected bryozoans and also the methodology to confirm and quantify parasite stock for infection. Successful fish infection is confirmed by the presence of the parasite in the host tissues using qPCR or histology. The laboratory-based infection model enables the same evaluation by infection read outs as the natural exposure approach mentioned above, but also allows a clearly defined sequential analysis in terms of parameters taken at a precise post-exposure time period. However, an earlier study performed a successful exposure with an experimental intraperitoneal (i.p.) injection with parasitic cells from infected donor fish.[33]

Advantages and disadvantages

Both technical approaches result in clinical PKD in fish; the major difference is that fish sampled at the infection source are constantly exposed to other pathogens primary or opportunistic or an additional stressor in the environment (dependant on the source) vs. a once-off exposure in a controlled laboratory environment. This provides a major advantage for the laboratory-based study as it allows to compare with true control noninfected fish under the same conditions, from the same fish stock. Alternatively, it could be also argued that fish exposed in the laboratory are not a true reflection of what happens in nature to fish that are continuously exposed to the parasite. Moreover, in an initial study by Gorgoglione et al. performed using a natural exposure approach, when many of the same genes were measured again in a later laboratory infection set up by Bailey et al. in a different country under different conditions, the major expression profiles were shared.[34,36] Clearly, the most suitable approach depends on the research question time and available facilities and sources of fish or parasites.

2.2 | Pro-inflammatory cytokines and antimicrobial peptides

After an invading pathogen is recognized, PRRs activate signalling cascades leading to induction of pro-inflammatory cytokines, antimicrobial responses and chemotactic cytokines.[36,51] In *T. bryosalmonae*-infected fish, transcriptional studies have demonstrated the putative lack or transient response of pro-inflammatory cytokines in both a natural infection and a laboratory challenge scenario.[34-36] The establishment of a study model and background information comparing these methods (natural infection and laboratory challenge) for obtaining samples of *T. bryosalmonae*-infected fish is the focus of Box 1. Concerning pro-inflammatory cytokines, tumour necrosis factor-alpha (*tnf-α*), interleukin-1β (*il-1β*) and cyclooxygenase (*cox*)-2 isoforms in rainbow trout were found to be unresponsive during *T. bryosalmonae* infection, reflecting the skewing of pro-inflammatory mechanisms towards an anti-inflammatory phenotype.[36] Illustrating this, Gorgoglione et al. reported a strong upregulation of several anti-inflammatory molecules during the course of PKD progression, namely *il-6*, *il-10a/b*, *il-11* and *nIL-1F*. In particular, marked upregulation of *il-10* has been noted in several studies concerning PKD.[30,34-36] *il-10* is known to inhibit pro-inflammatory cytokines, phagocytosis and respiratory burst activity,[52] all of which are suppressed by *T. bryosalmonae*.[33] Similarly, other myxozoan parasites of fish have been described to elicit either weak transient expression

or downregulation of pro-inflammatory cytokines as part of a global downregulation of innate response genes (summarized in [53]).

In contrast, several anti-microbial peptides (AMPs), considered as key components of innate immunity (cathelicidins—*cath-1*, *cath-2*, *hepcidin-1* and liver-enriched antimicrobial peptide-2 (*leap-2*) were upregulated at all kidney swelling grades during *T. bryosalmonae* infection. In particular, *cath-1* and *cath-2* were strongly upregulated.[26] Given that AMPs also have an immunoregulatory function preventing an excessive inflammatory response, they might also contribute to the anti-inflammatory phenotype observed during the course of PKD progression.[36,54,55]

2.3 | Macrophage activity

Further evidence for the lack of a pro-inflammatory response to *T. bryosalmonae* is generated by the downregulation and general unresponsiveness of macrophage marker genes such as macrophage stimulating colony factor (*mcsf*), its receptor (*mcsf-r*) and arginase paralogues.[34,36,56] Exemplifying this is a study in which arginase 1 (*arg1*) and arginase 2 (*arg2*) were analysed during *T. bryosalmonae* infection; *arg1* and *arg2* are commonly identified as markers for M2-like macrophages that heal and repair tissue injuries in contrast to M1-like macrophages that focus on killing infectious organisms.[57] In this study, arg1a and arg1b transcripts were downregulated with increasing kidney swelling index, while *arg2a* and *arg2b* transcripts were either transient or downregulated.[56] Moreover, the general unresponsiveness of the immune cells that express these molecules has been correlated with a sequential decrease in myeloid cells in the kidney as well as a depression in phagocytosis and oxidative burst in *T. bryosalmonae* laboratory challenges.[33,34] Furthermore, a reduction in the number of melanomacrophage aggregates is also quite drastic during PKD pathogenesis.

Despite this, a few lines of evidence exist that do not fully support the absence of a macrophage response. Several molecules indicative of macrophage activity (eg *il-6*, *il-12*, and *il-34*) have been reported to be upregulated in *T. bryosalmonae*-infected fish.[36,58,59] Furthermore, macrophages have been observed to be as numerous as lymphocytes in granulomatous lesions that develop during the resolving stages of PKD.[16,29] M2-like macrophages are commonly featured in the mammalian anti-parasitic response to helminth parasites.[60] Therefore, it may be possible that M2-like macrophages play a more important role in the late stages of PKD pathogenesis relative to M1-like classically activated macrophages. M2-like macrophages function in tissue healing processes and are alternatively activated by exposure to certain cytokines (*il-4*, *il-10* or *il-13*) all of which are markedly expressed during an advanced PKD pathogenesis. The common denominator of all three types of M2-like macrophages: M2a, M2b and M2c is high *il-10* production accompanied by low production of *il-12* and *arg1*.[61,62] Despite high *il-10* production during *T.bryosalmonae* infection, the mRNA levels of *il-12* (upregulated) and arginase genes (unresponsive) do not indicate an M2-like phenotype.[58] However, arginase gene expression has only been investigated from early (grade 1) to advanced (grade 3) stages of disease pathology. Thus, given that M2-like macrophages have a role in tissue repair, an fuller assessment of M2 macrophage marker expression would be needed that would cover advanced to resolving stages of disease pathogenesis. It must also be noted that two trout recombinant *il-12* molecules have been characterized, namely *p35p40b* and *p35p40c*. Importantly, during PKD pathogenesis only a moderate (*ca.* 4- to 7-fold) transcriptional increase in the *p35* and *p40c* subunits reported with *p40b* transcription remaining unaffected.[58] Thus, limited expression, especially of the *p35* subunit, may not translate into an increase in *il-12* at the protein level.

2.4 | Chemokines

In mammalian literature, chemokines are generally referred to as cytokines with chemotactic capacities that regulate the migration of leukocytes to tissues under inflammatory, pathogenic and normal physiological conditions.[63] Many chemokine families have greatly expanded challenging the ascription of true mammalian homologues among fish (reviewed in 64). Although a functional role has not yet been investigated for many of the fish chemokines reported to date, some extra functions in addition to their chemotactic activity have been described for a few of them, including antimicrobial activity[65] and a role in neuroendocrine regulation.[66] As part of the studies characterizing the chemokine receptor ligands (*cc* = chemokine, *m* = motif, *r* = receptor and *l* = ligand) *ccr4la*, *ccr4lc1* and *ccr4lc2* and chemokine receptors *cmklr3* and *ccr11* in rainbow trout, the expression profiles of these genes were examined in the posterior kidney of *T. bryosalmonae*-infected fish.[67,68] The expression of ligands *ccr4lc1* and *ccr4lc2* decreased in infected fish, while *ccrla* was unresponsive. Concerning the receptors, *ccr11* exhibited only a minor increase, while *cmklr3* was significantly downregulated and generally refractory at kidney swelling grades 1-3. The expression profiles of these chemokines are consistent with the abovementioned lack of macrophage pro-inflammatory responses during *T. bryosalmonae* infection.[67,68] The future investigation of chemokines during PKD pathogenesis might include those now known to modulate B lymphocyte functions in rainbow trout, such as *ck9* or *ck12*. *Ck9* has been demonstrated to regulate B lymphocyte trafficking (igm^+ and igt^+ B cells) as well as antigen-presenting cells (APCs) (major histocompatibility complex class II – *mhc ii* + cells) in rainbow trout blood, spleen and kidney,[69] whereas *ck12* has been revealed to recruit igm^+ B cells within the rainbow trout spleen.[70] Finally, it would also be interesting to determine the functional role of chemokines in PKD pathogenesis, particularly *ck11* that is known to have antimicrobial activity to a wide range of pathogens, including parasites.[65]

2.5 | SOCS: suppressors of chemokine signalling

Suppressors of cytokine signalling (SOCS) are physiological suppressors of cytokines and of the signalling pathways that regulate the

growth and development of an organism.[71] SOCS can be induced by pathogens, PAMPs and chemokines.[72] Selective genes involved in the JAK-STAT-SOCS (janus kinases (*jak*), signal transducers and activators of transcription (*stat*) and SOCS) signalling pathway have been measured in several *T. bryosalmonae* infection studies, namely *socs1*, *socs3*, *socs5b*, *socs7*, cytokine-inducible SH2-containing protein (*cish*), *jak1* and *stat3*. Significant upregulation of *socs1* and *socs3* has been observed correlating with parasitic burden and pathology progression in the posterior kidney of *T. bryosalmonae*-infected rainbow trout.[73,74] The expression of these molecules during *T. bryosalmonae* infection has been suggested to contribute to the general immunosuppression of the fish host, proposing it as a parasitic strategy to evade the immune system like that of protozoan parasites.[61,74] For example, *Toxoplasma gondii* (*socs1*)[75] and *Leishmania donovani* (*socs3*)[76] induce SOCS expression that subsequently inhibits interferon gamma (*ifnγ*)-mediated macrophage activation as an immune evasion tactic. In *T. bryosalmonae*-infected rainbow trout, although there is a lack of macrophage response during PKD pathogenesis, *ifnγ* expression was only moderately upregulated at all swelling grades in a natural outbreak study in the posterior kidney.[36] Furthermore, *ifnγ* was downregulated in the anterior kidney at weeks 4, 6 and 7 post-exposure (PE) correlating with a significant decrease in myeloid cells.[34] Thus, while macrophage activity may be suppressed during PKD pathogenesis, whether this down-regulation is related to *ifnγ* production requires further investigation.

3 | ADAPTIVE IMMUNITY

3.1 | T-cell markers

T cells are lymphocytes that express a surface T-cell receptor (*tcr*) which recognizes antigens in the context of MHC, along with cluster of differentiation (*cd*)3 and co-stimulatory (eg *cd28*) and co-inhibitory (eg cytotoxic T-lymphocyte-associated protein 4 (*ctla-4*) surface molecules).[77] T cells are categorized into two general populations according to their function: 1) cytotoxic T cells which express *cd8* and interact with *mhc* class I and 2) Th cells which express *cd4* and interact with *mhc ii*.[77] Naive $CD4^+$ T cells undergo massive proliferation and differentiation into at least four distinct T helper cell subsets upon activation by APCs: Th1, Th2, Th17 and T-regulatory (Treg) cells.[78-80] Th1 cells lead to an increased cell-mediated immune response usually against intracellular pathogens (viruses and intracellular bacteria or parasites). Th2 cells mediate the humoral response usually against extracellular pathogens (extracellular bacteria or parasites). While Th17 cells participate in inflammation, tissue damage and autoimmune process with Tregs mediating immune tolerance.[81]

During *T. bryosalmonae* infection of rainbow trout *cd4*, *cd8α* and *cd8β* transcripts were positively correlated with parasite intensity in a natural outbreak study.[36] In contrast, in an experimental infection trial, there was a significant decrease in the number of $CD8^-$ T cells in the anterior kidney relative to the control at week 4 PE before a significant increase was detected in these T-cell subsets in the posterior kidney at week 6 PE[34] In general, there is a lack of functional T-cell studies during PKD pathogenesis despite the fact that while myeloid cells are downmodulated, lymphocytes are proliferating especially in comparison with what is currently known regarding the B-cell response (see section 3.3). The most comprehensive data concerning T-cell responses are based on transcriptional studies classifying Th-like cytokines that will be reviewed in the subsequent sections.

3.2 | T helper-like immunity

3.2.1 | Th1/Th2 polarization

Although it is not conclusively demonstrated that Th-like functionality exists in fish, homologs of most of the surface markers, cytokines and transcription factors associated with T-cell responses have been identified in many teleost species enabling a more holistic molecular characterization of fish immune responses (reviewed in 78,82,83). Generally, fish transcriptional studies describe a Th1-like profile consisting of increased mRNA levels of *il-2*, *ifnγ*, *tnfα* and the Th1 master transcription factor, *t-box 21* (*t-bet*), while a Th2-like profile consists of increased mRNA levels of *il-4/13*, *il-10* and the master transcription factor, GATA binding protein 3 (*gata3*). A gene expression profile reminiscent of a Th1-like bias was initially reported in rainbow trout infected with *T. bryosalmonae* in which *t-bet*, *ifnγ* and *il-2* transcripts were increased, while only a small change in the expression of *gata3* was observed.[84] However, in later studies investigating samples from a natural outbreak[36] and from a laboratory challenge model[34] reported expression of Th2-like cytokines. For example, Wang et al.[85] while investigating the three trout *il-4/13* isoforms *il-4/13a*, *b1* and *b2* found very marked upregulation of *il-4/13b1* and *il4/13b2* during advanced PKD pathogenesis (kidney swelling grade 3, while Bailey et al.[34] reported an upregulation of *gata3*, *il-4/13a*, *il-10* in the posterior kidney at week 6 P.E in fish with clinical PKD. Though, in the same study *il-2*, *t-bet*, *ifnγ*, *tnfα 1 + 2* (Th1) and *gata3*, *il4/13a*, and *il-10* (Th2) were all simultaneously upregulated at week 2 P.E in both the anterior and posterior kidneys, suggestive of an early imbalance of Th-like cytokines during infection.[34]

Given that PKD pathogenesis is a chronic immunopathological disorder which resembles classical aspects of a lymphoproliferative autoimmune disease, a mode of complete Th1- or Th2-like polarization may not take place as seen in other immune responses that are not directly against a pathogen, such as those against autoantigens and environmental antigens.[86] Alternatively, an aspect of parasite virulence could be to prevent a clear Th polarization that enables effective parasite clearance.

3.2.2 | Th17/Treg-associated transcripts

Th17 cells and Treg cells share a common precursor cell and require a common transforming growth factor β (*tgfβ*) signal for their initial

differentiation, although they fulfil almost opposite functions.[87] Many features of mammalian Th17 cell differentiation have been described in fish, for example *il-6*, *tgf-β-1*, *il-21*, *il-22*, *il-23*, *il-17*, and the Th17 master transcription factor Retinoic acid-related orphan receptor-gamma (*rorγ*).[78] All of these genes have been investigated in rainbow trout during *T. bryosalmonae* infection.[34,36,88] *il-17* family members *il-17a/f2a*, *il-17c-2* and *il-17d* exhibited weak or no correlation with parasite burden and no correlation with kidney swelling in the posterior kidney.[36] In contrast, *il-17c-1* was shown to be significantly upregulated at early clinical stages in several studies.[88] *il-21* expression correlated with parasite prevalence and significantly, although to a lesser extent, with kidney swelling grade, whereas *il-22* only correlated with parasite burden with *rorγ* transcripts not correlating with parasite burden or swelling grade.[36] Regarding the Treg response, the master Treg transcription factor, forkhead box transcripts were significantly expressed in both a laboratory trial (*foxp3a*) [34] and a natural outbreak (*foxp3a* and *foxp3b*).[36] These results, together with reported upregulation of *tgf-β1* by Gorgoglione et al,[36] suggest that both Treg-like and Th17-like cytokines could play key roles in PKD pathogenesis. Given the above-mentioned elements of an autoimmune disease-like profile of PKD, the expression signatures of Th17-like markers would support this observation. As Treg cells have been suggested to maintain immune homoeostasis,[87] expression changes of these T-reg-associated markers during PKD pathogenesis may imply the counterbalancing of these effects to maintain tolerance.[34]

3.3 | B-cell response

Several studies have described a massive activation of B cells during PKD pathogenesis and induced hyperimmunoglobulinaemia in response to the parasite's extra-sporogonic histozoic proliferation.[30,34,36-38] For instance, Gorgoglione et al.[36] established marked upregulation of secreted *igt* and *igm* but not *igd* that correlated with kidney pathology. Likewise, Bailey et al[89] noted that the transcription of *igmsec*, membrane bound IgT (*igtmem*) and the B lymphocyte-induced protein-1 (*blimp1*), a master regulator of B-cell differentiation into antibody-secreting plasma cells, were strongly upregulated during PKD progression[34] Similarly, a significant increase in the number of igm^+ B cells was detected in the posterior kidney during advanced clinical stages by flow cytometry.[34] Fish that have recovered from PKD, when re-exposed, exhibited an early upregulation of IgMsec relative to naïve-infected fish and an increase in IgM + B cells in the blood which may have contributed to the lower pathogen burden and reduced kidney swelling observed in these fish in contrast to the fish infected for the first time.[30]

3.3.1 | BAFF/APRIL axis

In mammals, cytokines of the *tnf* superfamily play a key role in the modulation of B-cell responses.[77] Among them, *baff* and *april* produced by cells of the innate immune system are known to modulate the homoeostasis, activation and differentiation of B cells.[90] In fish, an additional *baff* and *april*-like molecule (*balm*) closely related to both *april* and *baff* is also present in this family.[29] As B-cell function appears dysregulated during PKD, Granja et al.[38] investigated the role of the *baff/april* axis during *T. bryosalmonae* infection of rainbow trout. The study identified homologues to mammalian *baff* and *april* receptors, namely transmembrane activator and *caml* interactor (*taci*), B-cell maturation antigen (*bcma*) and the *baff* receptor (*baff-r*) that constituted the first report of these receptor sequences in fish. Thereafter, the transcription levels of these receptors and their potential ligands were investigated in kidney samples from a natural *T bryosalmonae* infection. *baff*, *april*, *balm*, *baff-r* and *taci* were all significantly upregulated in parasite-exposed fish, with transcription levels correlating with disease development.[38] The expression signatures of *baff*, *april*, *balm*, *baff-r* and *taci* significantly correlated with total *igm* mRNA levels, whereas *baff*, *balm*, *baff-r* and *taci* transcription correlated with secreted *igm* and *igt* mRNA levels.[38] In addition, Granja et al.[38] investigated the impact of *baff*, *april* and *balm* on isolated posterior kidney leukocytes with all three cytokines having a positive effect on *igm* transcription. These results point to the *baff/april* axis being at least partly responsible for the alteration of B-cell functionality during PKD pathogenesis.

3.3.2 | Immunoglobulins

Fish have three major Ig isotypes, *igm*, *igd* and *igt*.[91-93] *igm* and *igd* are equivalent to the mammalian counterparts. Thus, *igm* is the main Ig produced and is known to be the primary responding Ig at a systemic level. *igt*, on the other hand, is a fish-specific Ig, expressed by an independent B-cell lineage, that is considered to be specialized in mucosal immunity.[94] Therefore, the surface expression of these Igs defines different B-cell subsets. igm^+igd^+ B cells constitute the main B-cell subset in lymphoid and systemic organs, while igt^+ cells although present in all tissues are more prevalent in mucosal surfaces. Additionally, when igm^+igd^+ cells are activated, they lose surface *igd* and thus become igm^+igd^- cells.[95] Finally, a subset of B cells exclusively expressing *igd* on the cell surface has been identified in catfish blood [96] and rainbow trout gills,[97] although the precise contribution of *igd* to the immune response is still unknown in both fish and mammals. Building on the transcriptional evidence that demonstrated an increase of *igm* and *igt* during PKD pathogenesis,[34,36] an in-depth analysis of the regulation all three fish Igs in *T. bryosalmonae*-infected rainbow trout was performed by Abos et al[37] The study demonstrated that all three Ig isotypes are increased at the protein level in the kidney in response to the parasite and that four distinct B-cell subsets coexist in the infected kidney according to the expression profiles of different Ig isotypes (namely, igm^+igd^+, igm^+igd^-, igd^+igm^- and igt^+ cells). Interestingly, this was the first report of *igd* regulation in response to a pathogen in teleost fish.[37] The study also demonstrated a prevailing role for *igt* during clinical PKD in the posterior kidney, contributing to

the evidence of a broader role for *igt* outside of its suggested mucosal specialization. In addition, a repertoire sequencing analysis (RepSeq) of the variable heavy chain (VH) domain of the three Ig subtypes in fish with no clinical signs of disease in comparison with fish with a clear pathology was undertaken in this study.[37] The results revealed significant changes in VH family usage, clonal expansion and mutation rate. These findings demonstrated that the host response to *T. bryosalmonae* does not involve the clonal selection of a specific B-cell subset, but that it involves a polyclonal activation of different B-cell subsets, which may actually be an evasion strategy induced by the parasite.[37]

4 | THE INFLUENCE OF COINFECTIONS ON THE IMMUNE RESPONSE DURING PKD PATHOGENESIS

T. byrosalmonae infection can increase fish host susceptibility to secondary infections.[22,98-100] Kotob et al., for example, examined the SOCS/JAK/STAT signalling pathway in rainbow trout exposed to *T bryosalmonae* and another myxozoan parasite, *Myxobolus cerebralis* (the causative agent of whirling disease).[73,101] Rainbow trout infected with *M. cerebralis* and *T. bryosalmonae* had the highest mortality rate, highest loads of both parasites in the posterior kidney and cranial cartilage (the target site of *M. cerebralis*) and also the highest expression levels of *socs1* and *socs3* in both the posterior kidney and the cranial cartilage.[73,101] A further study examined the immune response at the transcriptomic level of brown trout infected with *T. bryosalmonae* and viral haemorrhagic septicaemia virus (VHSV). In this study, a more pronounced upregulation pro-inflammatory and Th1-like cytokines, as well several antiviral marker genes, were observed in these fish.[28] This was in contrast to single infected fish and in contrast to what has been reported within this review. Given the drastic immunosuppressive effects on myeloid cells and the apparent dysregulation of lymphoid cells that takes place during PKD, these findings suggest that the way in which *T. bryosalmonae* interacts with other pathogens could be specific to the type of secondary agent, with different consequences in each case.

5 | THE INFLUENCE OF ENVIRONMENTAL STRESSORS ON THE IMMUNE RESPONSE DURING PKD PATHOGENESIS

5.1 | Temperature

Elevated water temperatures can increase PKD incidence and severity but can also modulate the rainbow trout immune response during *T. bryosalmonae* infection. Bailey et al.[34] investigated whether a subtle change in water temperature, reflecting a realistic environmental scenario, could influence the immune response of rainbow trout exposed to *T. bryosalmonae*. At the lower temperature (12°C), fish exhibited an asymptomatic version of the disease and lower parasite burden. This was associated with a cytotoxic-like immune response characterized by a subtle increase in lymphocytes, expression of Th1-like cytokines and strong upregulation of natural killer enhancement factor (*nkef*).[34] At the warmer temperature (15°C), *T bryosalmonae* infection was consistent with the phenotype reported within this review, a chronic immunopathology driven by increasing lymphocytes/decreasing myeloid cells in both the anterior and posterior kidneys. Furthermore, a strong B-cell antibody response and a dysregulated Th1/Th2-like cytokine response were observed.[34] The increase in only 3°C appeared to impact parasite proliferation and the biological and biochemical processes of the host, with the lower temperature being less detrimental to host immunocompetence.[34]

5.2 | Anthropogenic contaminants

Given the strong interference of human activities on freshwater fish, the impact of anthropogenic factors on *T bryosalmonae* infection has also been studied and shown to modulate the host response. Earlier reports described the impact of wastewater effluents upon *T bryosalmonae* infection of salmonids.[102,103] Recent studies have investigated the combined impact of ethinylestradiol (EE2), an oestrogenic endocrine disrupting compound found ubiquitously in the aquatic milieu,[104] upon *T. bryosalmonae* infection in rainbow trout.[105,106] EE2 is a known immunomodulator and can influence aspects of the innate and adaptive immune response in various fish species.[107,108] In this context, Von Siebenthal et al., in an experimental infection trial using environmentally relevant concentrations of EE2, determined that *igmsec*, *blimp1*, *nkef* transcription and kidney somatic indices were increased in *T. bryosalmonae*-infected fish relative to *T. bryosalmonae*-infected EE2-exposed fish at the plateau of parasite intensity.[105] As expected, however, hepatic vitellogenin (*vtg*) mRNA levels, commonly used to assess exposure of fish to oestrogens, were significantly elevated in fish exposed to *T. bryosalmonae* and EE2 relative to *T bryosalmonae* only infected fish. In addition, the liver somatic index was increased and levels of insulin-like growth factor (*igf*) decreased in the same organ, indicating the impact of EE2 in these fish.[105] Bailey et al.,[106] as part of a long-term chronic study (130 days), used RNA-Seq to assess the posterior kidney transcriptome in rainbow trout exposed to *T bryosalmonae* and EE2 and all combinations thereof. This study compared the following four experimental groups: (a) unexposed controls; (b) chemical exposure-EE2 only; (c) parasite exposure-*T bryosalmonae* only; and (d) multiple stressor exposure-parasite and chemical-*T. bryosalmonae* and EE2.[106] This study reported a greater number of differentially expressed genes (DEGs) regulating immunological processes in the multiple stressor group (*T. bryosalmonae* and EE2) relative to all other treatments. Moreover, the multiple stressor group had lower parasite intensity and reduced pathological alterations in the posterior kidney in comparison with the *T. bryosalmonae*-only treatment.[106] These findings corroborated an earlier microarray study by Burki et al.[109] who reported a dominating influence of

the parasite infection over EE2 on parasite loads and mortality. Additionally, in the study by Bailey et al.[106] several markers associated with various aspects of T-cell immunity (*t-bet*, *cd8α*, *foxp3a*, *foxp3b*, *cd3* and *mhcii*) were significantly downregulated in the multiple stressor group. This might indicate that *T. bryosalmonae* and EE2 combined could be having a greater immunosuppressing role than in *T. bryosalmonae*-infected fish in the absence of EE2. Specifically, reduced PKD-associated immunopathology and attenuated disease impact. Alternatively, since it was observed that a greater number of immune genes were upregulated in these fish, this may be consistent with decreased parasite intensity and reduced pathological alterations.[106] Thus, while EE2 modulates the immune response during infection, the effects appear somewhat paradoxical in that they exert both immunosuppressive and immunoenhancing actions as reported in mammals.[106]

6 | CONCLUDING REMARKS AND FUTURE AVENUES FOR RESEARCH

A plethora of studies have contributed to this review of the rainbow trout immune response during PKD pathogenesis. It must also be mentioned that beyond the scope of this review, several reports exist regarding brown trout immune responses.[40-42] Several studies have adopted broader techniques such as predictive modelling,[110] quantitative genetics[111] and gut intestinal tract microbiome[112] that have greatly contributed to disentangling the impact of PKD in wild salmonids. In rainbow trout, identifying suitable targets for immunotherapy through elucidating host immune mechanisms to fish parasites in general is a topic of growing urgency in fisheries science given the lack of suitable preventative measures. In particular, the impact of PKD on fish B-cell biology has highlighted this disease model as an invaluable tool to study the evolution and function of the adaptive immune response in fish. However, future research using this model should also address the lack of knowledge concerning T-cell functionality during *T. bryosalmonae* infection. In addition, since most of our knowledge of the immune response to this parasite is based on kidney studies, a greater exploration of the host immune responses at disease stages in different tissues could also unravel new information pertaining to PKD pathogenesis. While not addressed in this review, studies characterizing the host immune response in other susceptible fish species could be informative and are, therefore, warranted given the host range of the parasite. Finally, technological advances, such as dual RNA-Seq, may enable new possibilities to disentangle the transcriptional crosstalk between parasites and fish hosts. Such approaches are critical to understanding disease pathogenesis that will aid the design of future immunotherapies in aquaculture and provide a greater knowledge of parasitic diseases in wild fish.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

AUTHOR CONTRIBUTIONS

CB and CT wrote the main body of the manuscript, with contributions from JWH and CJS. All authors reviewed and approved the final version.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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