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Review Paper

Fish immunity and parasite infections: from innate immunity to immunoprophylactic prospects

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ABSTRACT

The increasing economic importance of fish parasitoses for aquaculture and fisheries has enhanced the interest in the defence mechanisms against these infections. Both innate and adaptive immune responses are mounted by fish to control parasite infections, and several mechanisms described for mammalian parasitoses have also been demonstrated in teleosts. Innate immune initiation relies on the recognition of pathogen-associated molecular patterns (PAMPs) by pathogen recognizing receptors (PRRs). A number of PRRs, mainly Toll-like receptors (TLRs), have been characterized in fish, and some molecules susceptible of functioning as PAMPs are known for some fish parasites. A lectin–carbohydrate interaction has also been described in some host fish–parasite systems, thus probably involving C-type lectin receptors. Inflammatory reactions involving cellular reactions, as phagocytosis and phagocyte activity (including oxidative mechanisms), as well as complement activity, are modulated by many fish parasites, including mainly ciliates, flagellates and myxozoans. Besides complement, a number of humoral immune factors (peroxidases, lysozyme, acute-phase proteins) are also implicated in the response to some parasites. Among adaptive responses, most data deal with the presence of B lymphocytes and the production of specific antibodies (Abs). Although an increasing number of T-cell markers have been described for teleosts, the specific characterization of those involved in their response is far from being obtained. Gene expression studies have demonstrated the involvement of other mediators of the innate and adaptive responses, i.e., cytokines [interleukins (IL-1, IL-8), tumor necrosis factor (TNF), interferon (IFN)], chemokines (CXC, CC), as well as several oxidative enzymes [inducible nitric oxide synthase (iNOS), cyclo-oxygenase 2 (COX-2)]. Information is scarcer for factors more directly linked to adaptive responses, such as major histocompatibility (MH) receptors, T cell receptors (TCRs) and IgM. Expression of some immune genes varied according to the phase of infection, and proinflammatory cytokines were mainly activated in the early stages. Gene expression was generally higher in the target tissues for some skin and gill parasites, as *Ichthyophthirius multifiliis*, *Neoparamoeba* spp. and *Lepeophtheirus salmonis*, thus confirming the relevance of mucosal immunity in these infections. The existence of protective responses has been demonstrated for several fish parasites, both in natural infections and in immunization studies. Most information on the mechanisms involved in protection deals with the production of specific Abs. Nevertheless, their levels are not

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Abbreviations: AGD, amoebic gill disease; APP, acute-phase protein; APR, acute-phase response; CIS, combinatorial immune system; CLRs, C-type lectin receptors; COX2, cyclo-oxygenase 2; CRD, carbohydrate recognition domain; CXC, CC, chemokine subfamilies; EGC, eosinophilic granule cell; GPI, glycosylphosphatidylinositol; HK, head kidney; iNOS, inducible nitric-oxide synthase; LRR, leucine-rich repeat; MMC, melano-macrophage centres; NLR, NOD-like receptor; NOD, nucleotide-binding oligomerization domain; PAMPs, pathogen-associated molecular patterns; PKD, proliferative kidney disease; PRR, pathogen recognizing receptor; RB, respiratory burst; RAG, recombination activation genes; SAA, serum amyloid A; TIR, Toll/interlekin-1 receptor; TLR, Toll-like receptor.

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always correlated to protection, and the precise involvement of immune mechanisms in the response is unknown in many cases. No commercial vaccine is currently available for piscine parasitoses, although experimental vaccines have been assayed against *I. multifiliis*, *Cryptobia salmositica* and scuticociliates. The known information points to the need for integrated studies of the mechanisms involved in protection, in order to choose the optimum antigen candidates, adjuvants and formulations.

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Contents

1. Introduction

There is, currently, a general agreement as to the presence in teleosts of the main immune mechanisms described for mammals. Teleosts possess the elements of both innate and adaptive immunity. As in other vertebrates, the innate immune system of fish provides the first line of immune defence. Adaptive immunity relies on the

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generation of random and highly diverse repertoires of T- and B-lymphocyte receptors encoded by recombinant activation genes (RAGs) and contributes to a more specific and efficient response against infections (McGuinness et al., 2003; Medzhitov, 2007). There is, however, increasing evidence of the integration of the different immune mechanisms into a multilevel network, which challenges the artificial dichotomy between innate and adaptive systems (Flajnik and Du Pasquier, 2004).

Substantial progress has recently been made in the characterization of the fish immune mechanisms and pathways. Nevertheless there are still important gaps in the knowledge of numerous immune mechanisms, and the available information varies according to the fish species. Where knowledge on genome and/or continuous cell lines is available, more information exists. This is the case of zebrafish *Danio rerio* (Trede et al., 2004; Meeker and Trede, 2008), pufferfish *Takifugu rubripes* (Bei et al., 2006) and Japanese flounder *Paralichthys olivaceus* (Aoki and Hirono, 2006). The channel catfish *Ictalurus punctatus* has played an important role as a model species for the study of comparative immunology (Clem et al., 1990). Recently, data on fish immune response is increasing for economically relevant species in aquaculture and fisheries, mainly salmonids (*Salmo salar*, *Oncorhynchus* spp.), cyprinids, flatfish (scophthalmids, pleuronectids), gadoids, ictalurids, moronids or sparids. General reviews on fish immunology have been published, mostly related to the innate immunity (Alexander and Ingram, 1992; Manning and Nakanishi, 1996; Secombes, 1996; Dalmo et al., 1997; Dixon and Stet, 2001; Magor and Magor, 2001; Watts et al., 2001; Holland and Lambris, 2002; Magnadóttir, 2006; Plouffe et al., 2005; Rombout et al., 2005; Zapata et al., 2006; Whyte, 2007). More specific revisions are available for some species, such as Mediterranean sea bass (*Dicentrarchus labrax*) (Chistiakov et al., 2007) and eels (*Anguilla* spp.) (Nielsen and Esteve-Gassent, 2006). Data on cell markers and determinants in fish immunology can be found in the recent review of Randelli et al. (2008). Information available on the fish response to infections deals mainly with bacteria and virus, but knowledge on parasites is limited. Nevertheless, the increasing impact of some parasitoses on fish health and its economic relevance in aquaculture and fisheries has enhanced the need for studies on the fish/parasite relationships, including the immune response. Following the general reviews of Woo (1992), Secombes and Chappell (1996), Jones (2001) and Buchmann et al. (2001a), some specific revisions on flagellates (Woo, 1996, 2001, 2003), ciliates (Dickerson and Clark, 1996; Buchmann et al., 2001b; Matthews, 2005) and monogeneans (Buchmann, 1999; Buchmann and Lindenstrøm, 2002) have been published, as well as compilations on the immune evasion mechanisms (Buchmann, 2000; Sitjà-Bobadilla, 2008). In addition, recent studies on fish immunity to parasite infections have provided some clues for a better understanding of the mechanisms involved in the immune recognition and response to these pathogens.

In the present paper, the most relevant information on the fish immune response to parasite infections is reviewed. The main innate and adaptive immune mechanisms are briefly introduced, with special emphasis on teleosts, and selected examples of their involvement in fish parasitoses are given. Reference is made to the inter-relationship between the different factors and to the connection of innate and adaptive mechanisms. The information on expression of immune genes in response to parasitoses is considered in a separate section. Finally, data on protective immunity is analysed in relation to the immune response and vaccination prospects. The parasites mentioned in this review and the immune mechanisms/factors involved in the host fish responses are listed in Table 1.

2. Parasite/host fish interactions: the initiation of the immune response

2.1. Pathogen recognizing receptors (PRRs) (Toll-like receptors, TLRs and other PRRs)

Two types of host-defence mechanisms have been traditionally accepted, innate and adaptive (also known as acquired). The main distinction between them is the receptor types used to recognize pathogens (Medzhitov, 2007). Innate immune recognition relies on a growing number of receptors (PRRs), with a broad specificity, which are germline encoded and have evolved to recognize pathogen-associated molecular patterns (PAMPs) (Medzhitov and Janeway, 2002; Janeway and Medzhitov, 2002). One unifying feature of PRRs is their highly conserved structures, which are invariant between microorganisms of a given class. In contrast, adaptive immune recognition is mediated by antigen (Ag) receptors, with random but narrow specificities. The different PRRs are involved in performing specific tasks, including opsonization, activation of complement cascade, phagocytosis, etc. (Pasare and Medzhitov, 2004).

There are several functionally distinct classes of PRRs, but the best characterized are Toll-like receptors (TLRs), which are type-I transmembrane proteins with extracellular leucine-rich repeat (LRR) motifs and intracellular Toll/interleukin-1 receptor (TIR) domain. Members of the TLR family contribute both to cell–cell interaction and to signalling. Toll, the founding member of the family, was initially implicated in the establishment of dorsoventral polarity in the early *Drosophila melanogaster* embryo (reviewed in Leulier and Lemaitre, 2008). The study of TLR genes in several teleost species (*D. rerio*, *T. rubripes*, *C. auratus*, *Oncorhynchus mykiss, P. olivaceus*) has demonstrated the evolutionary conservation of the key components of TLR signalling in vertebrates (reviewed in Roach et al., 2005; Purcell et al., 2006). Following these reviews, new data on TLR-signalling molecules or TIR domains has been obtained for some of the above cited species (Rebl et al., 2007; Takano et al., 2006, 2007; Chen et al., 2008), and also for gilthead se bream *Sparus aurata* (Franch et al., 2006), Atlantic salmon *Salmo salar* (Tsoi et al., 2006), *I. punctatus* (Baoprasertkul et al., 2007) and five sea breams (Chen et al., 2008).

Two additional families of innate receptors have been described that join the TLRs as key pathogen sensors (Creagh and O'Neill, 2006). They are intracellular receptors

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Table 1
Parasites for which information on immune response is included in the present review, their fish hosts, the activities/factors considered and the corresponding section

Taxonomic group	Parasites	Diseases	Fish hosts	Activity/factor	Sections
Amoebozoa	*Neoparamoeba* spp.	Amoebic gill disease (AGD)	*Oncorhynchus mykiss*	Inflamm./cell. resp.; MHC in lesions	Sections 3.3, 4.3, 6, 7
	N. perurans		*Salmo salar*	Gene expres.; protec. responses/Abs	
Dinoflagellata	*Amyloodinium ocellatum*	Amyloodiniosis	*Dicentrarchus labrax*, *Sparus aurata*, other species	Inflamm./cell. resp.; antimicrob. peptides	Sections 3.3, 3.4, 7
			Morone saxatilis, Oreochromis aureus, Amphiprion frenatus, D. labrax	Protec. responses/Abs	Section 7
Diplomonadida	*Hexamita, Spironucleus*	Hexamitosis/ spironucleosis	Salmonids and other species	Inflamm./cell. resp.;	Section 3.3
	S. barkhanus		*Salvelinus alpinus*	Phagocytosis	Section 3.3
Kinetoplastida	*Cryptobia salmositica*	Cryptobiosis	*O. mykiss*, *Oncorhynchus* spp.	PAMPs; carb. term.; inflamm./cell. resp.; phagoc. act./oxid. mechan.; complement; anti-proteases; protec. responses/Abs; vaccines	Sections 2.1, 3.3, 3.4, 7
	Trypanoplasma borreli		*Cyprinus carpio*	PAMPs; inflamm./cell. resp.; phagoc. act./oxid. mechan; gene expres.; protec. responses/Abs	Sections 2.1, 3.3, 6, 7
	Trypanosoma carassii		Cyprinids	PAMPs; inflamm./cell. resp.; phagoc. act./oxid. mechan.; protec. responses/Abs	Sections 2.1, 3.3, 7
Ciliophora	*Cryptocaryon irritans*	Cryptocaryosis	*Chelon labrosus*, *Epinephelus coloides, Lates calcarifer* and others	Inflamm./cell. resp.; protec. responses/Abs	Sections 3.3, 7
	Ichthyophthirius multifiliis	Ichthyophthiriosis	*O. mykiss, C. carpio, Ictalurus punctatus, Oreochromis niloticus*	PAMPs, lectin/carb.; mucosal immun.: inflamm./cell. resp.; apoptosis; complement; lysozyme; NCCs; gene expres.; protec. responses/Abs; vaccines	Sections 2.1, 3.2, 3.3, 3.4, 4.2, 6, 7
	Scuticociliatida	Scuticociliatosis	*Psetta maxima, Paralychthis olivaceus*	PAMPs; inflamm./cell. resp.; phagoc. act./oxid. mechan.; apoptosis; complement; lysozyme; gene expres.; protec. responses/Abs; vaccines	Sections 2.1, 3.3, 3.4, 6, 7
Microsporidia	*Glugea anomala*	Microsporidiosis	*Platichthys flessus*	Abs	Section 7
	G. plecoglossi		*Plecoglossus altivelis*	Lectin/carb.; phagocytosis; complement; Abs	Sections 2.1, 3.3, 7
	G. epinephelusi		*Epinephelus akaara*	Abs	Section 7
	Loma salmonae		*Salmo salar*	Phagocytosis; complement/lectins;	Section 3.3
				Protec. responses/Abs; vaccine assays	Section 7
	Pleistophora anguillarum		*Anguilla* spp.	Abs	Section 7
	Spraguea lophii		*P. flessus*	Abs	Section 7
	Tetramicra brevifillum		*Psetta maxima*	Inflamm./cell. resp.; phagoc. act./oxid. mechan; complement/lectins; Abs	Sections 3.3, 7
Apicomplexa	*Goussia carpelli*		*C. carpio*	Inflamm./cell. resp., phagoc. act./oxid. mechan.	Section 3.3
Myxozoa	*Ceratomyxa shasta*	Ceratomyxosis	Salmonids	Inflamm./cell. resp.; lysozyme; protec. responses/Abs	Sections 3.3, 3.4, 7
	Enteromyxum leei	Enteromyxosis	*S. aurata*, *Diplodus puntazzo*	Inflamm./cell. resp., phagoc. act./oxid. mechan., EGCs, complement, peroxidases, lysozyme, antiproteases, cytotox. activity, gene expres.	Sections 3.3, 3.4, 4.2, 6
	E. scophthalmi	Enteromyxosis	*P. maxima*	Carb. term.; inflamm./cell. resp.; phagoc. act./oxid. mechan.; complement lysozyme; antiprot.; protec. responses/Abs	Sections 2.1, 3.3, 3.4, 7
	Myxobolus artus		*C. carpio*	Abs	Section 7
	M. cerebralis	Whirling disease	*O. mykiss*	Carb. term.; gene expres.; Abs	Sections 2.1, 6, 7
	M. cyprini		*Leuciscus cephalus*	MMCs	Section 3.3

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Table 1 (*Continued*)

Taxonomic group	Parasites	Diseases	Fish hosts	Activity/factor	Sections
	Sphaerospora dicentrarchi	Sphaerosporosis	*D. labrax*	Phagoc. act./oxid. mechan.; complement; lysozyme; Ab secreting cells	Sections 3.3, 3.4, 7
	Tetracapsuloides bryosalmonae	Proliferative kidney disease (PKD)	*O. mykiss*	Carb. term.; inflamm./cell. resp.; phagoc. act./oxid. mechan.; cytotox. Activity; gene expres.; protec. responses	Sections 2.1, 4.2, 6, 7
Monogenea	*Diplectanum aequans*	Diplectanosis	*D. labrax*	Inflamm./cell. resp.; gene expres.; Abs	Sections 3.3, 6, 7
	Discocotyle sagittata		*O. mykiss*	Complement; protec. responses/Abs	Sections 3.3, 7
	Gyrodactylus derjavini	Gyrodactylosis	*O. mykiss*	Lectin/carb.; complement; gene expres.	Sections 2.1, 3.3, 6
	G. salaris		*S. salar*	Complement; gene expres.	Sections 3.3, 6
	Hetrobothirum okamotoi		*Takifugu rubripes*, *P. olivaceus*	Carb. term.; protec. responses/Abs	Sections 2.1, 7
	Microcotyle sebastis	Microcotylosis	*Sebastes schlegeli*	Abs	Section 7
	Neobenedenia girellae		*Seriola dumerili*, *P. olivaceus*	Inflamm./cell. resp.;phagocytosis	Section 3.3
	Neoheterobothrium hirame		*P. olivaceus*	Gene expres.	Section 6
	Pseudodactylogyrus bini, *P. anguillae*		*Anguilla* spp.	Inflamm./cell. resp.; Abs	Sections 3.3, 7
Digenea	*Cryptocotyle lingua*		Several species	Abs	Section 7
	Diplostomum spathaceum	Diplostomosis	Several species	Inflamm./cell. resp.; rodlet cells; Abs	Sections 3.3, 7
	Rhipidocotyle fennica		*Rutilus rutilus*	Protec. responses/Abs	Section 7
	Ribeoroia marini		*Carassius auratus*	Inflamm./cell. resp.; EGCs	Section 3.3
	Cardicola fosteri		*Thunnus maccoyii*	Protec. responses/Abs	Section 7
	Sanguinicola inermis	Sanguinicolosis	*C. carpio*	Inflamm./cell. resp.; EGCs; complement; Abs	Sections 3.3, 7
Cestoda	*Bothriocephalus scorpi*			C-reactive protein	Section 3.4
	Diphyllobothrium spp.		*O. mykiss*	Inflamm./cell. resp.; Abs	Sections 3.3, 7
Nematoda	*Anguillicola crassus*		*Anguilla spp.*	Oxid. mechan.; protec. responses/Abs	Sections 3.3, 7
	Contracaecum osculatum, *Pseudoterranova decipiens*		Antarctic fish	Abs	Section 7
Mollusca	*Utterbrackia imbecilis*		*Lepomis macrochirus*	Protec. responses/Abs	Section 7
Crustacea	*Argulus japonicus*		*S. salar*	Gene expression	Section 6
	Lepeophtheirus salmonis	Sea lice disease	*S. salar*	Inflamm./cell. resp.; phagoc. act./oxid. mechan.; Gene expres., protec. responses/Abs, vaccine studies	Sections 3.3, 6, 7
	Sinergasilus major		*Ctenopharyngodon idella*	Gene expression	Section 6

The name of the disease is indicated when it is recognised as pathologically important.

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including NOD-like receptors (NLRs) and RIG-I (retinoic-acid-inducible gene I)-like proteins (RLRs). All NLRs contain a nucleotide-binding oligomerization domain (NOD) followed by a LRR at the carboxy terminus.

Another important group of PRRs is represented by C-type lectins, which are best known for their ability to recognise specific pathogen-associated carbohydrate structures. These are characterized by C-type lectin receptors (CLRs), proteins that contain carbohydrate recognition domains (CRDs) (McGreal et al., 2004). Some CLRs are produced as transmembrane proteins present on dendritic cells and macrophages (MΦs)(Medzhitov, 2007; Cambi and Figdor, 2005), or are secreted as soluble proteins. The involvement of a mannose receptor in phagocytosis has been suggested for gilthead sea bream (Rodriguez et al., 2003). Amongst soluble PRRs, the mannan-binding lectins (MBLs) and ficolins bind *N*-acetyl-glucosamine and mannose structures common among microbes (Fujita et al., 2004) (see Section 3.4.4).

In mammals, the involvement of determined TLRs in the recognition of certain bacterial, viral, and fungi PAMPs has been described. In addition, NLRs recognize bacterial and viral pathogens. Other receptors recognising virus have been also reported (reviewed in Medzhitov, 2007). Data on the involvement of PRRS in the recognition of PAMPs of parasites is scarcer. Components of human protozoan pathogens, such as *Trypanosoma* spp., *Toxoplasma gondi*, *Leishmania major* and *Plasmodium falciparum* are recognised by TLRs. The involved PAMPs include dominant surface glycolipids [glycosylphosphatidylinositol (GPI) anchors recognised by TLR2 and TLR4], structural proteins (profiling-like proteins recognised by TLR11) and genomic DNA that activates TLR9 (reviewed in McGuinness et al., 2003; see also Akira et al., 2006; Gazinelli and Denkers, 2006). However, information on the function of fish TLRs and NLRs is very scarce. TLRs are involved in the response of zebra fish upon *Aeromonas salmonicida* and *Staphylococcus aureus* infection (Lin et al., 2007). The expression of TLR5 and TLR9 was involved in the response of purified phagocyte populations of *S. aurata* to different PAMPs (Sepulcre et al., 2007). As for piscine parasites, some molecules susceptible of functioning as PAMPS have been found in several fish flagellates. Lischke et al. (2000) identified and partially characterized GPI-anchored, mucin-like surface glycoproteins (containing sialic acid in the carbohydrate moiety) from blood-stream forms of *Trypanosoma carassii*. This surface is thus similar to that of non-Salivarian trypanosomes, such as *Trypanosoma cruzi*, with subsequent limitation in antigenic variation. GPI-anchored proteins are also probably present in *Trypanoplasma borreli*. The ciliate *Ichthyophthirius multifiliis* (Ich) expresses immobilization Ags that are GPI-anchor proteins (Clark et al., 2001). In addition, a cysteine protease in *Cryptobia salmositica* might also function as a PAMP (reviewed in Wiegertjes et al., 2005). Proteases have also been characterized in other piscine parasites, such as Myxozoans (Martone et al., 1999; Kelley et al., 2004) and the ciliate *Philasterides dicentrarchi* (2006). Other molecules susceptible of functioning as PAMPs are probably present in many fish parasites.

C-type lectins are also involved in the recognition of PAMPs. The transmembrane lectin-1 is implicated in antifungal defence (Medzhitov, 2007). In addition, the interaction of other transmembrane or soluble lectins domains with mannose, fucose or galactose structures in different parasites plays a crucial role in the host/parasite interaction and invasion, as well as in the immune evasion. In addition, the formation of mixed molecular receptor assemblages (for example between CLRs and TLRs, complement receptors) might further extend the PAMP profile recognition (Cambi and Figdor, 2005). Such specific lectin–carbohydrate interaction has been proven for several fish parasites, such as *I. multifiliis* (Xu et al., 2001), *Glugea plecoglossi* (Kim et al., 1999), *Gyrodactylus derjavivni* (Buchmann, 2001; Buchmann and Lindenstrøm, 2002) and *Hetrobothrium okamotoi* (Tsutsui et al., 2003). Carbohydrate terminals with possible functional significance were also detected in *Cryptobia salmositica* (Woo, 2001, 2003; see also Section 7). The presence of carbohydrate terminals specifically detected by lectins at the parasite membrane and host–parasite interface of the myxozoans *Tetracapsuloides bryosalmonae*, *Myxobolus cerebralis* and *Enteromyxum scophthalmi* suggests a role in host–parasite interactions (Morris and Adams, 2004; Knaus and El-Matbouli, 2005; Redondo et al., 2008).

Besides their role in innate immunity, PRRs can also participate in the induction of adaptive response, as commented below (Section 4).

2.2. Inflammation: apoptosis

In relation to innate immunity, TLRs and some NLRs have essential roles in the inflammatory response. They activate tissue-resident MΦs to produce inflammatory cytokines, including tumour-necrosis factor (TNF), interleukin-1β (IL-1β) and IL-6. These cytokines activate hepatocytes to produce acute-phase proteins (APPs), which, in turn, activate complement and opsonize pathogens for phagocytosis by MΦs and neutrophils. TLRs also directly induce MΦs to produce antimicrobial proteins and peptides, the enzyme inducible nitric-oxide synthase (iNOS) and other oxidative factors. In addition, members of the TLR family can induce apoptosis or programmed cell death (Salaun et al., 2007). The proteins of the NOD subfamily are involved in sensing bacterial peptidoglycans, which also trigger the production of pro-inflammatory cytokines and chemokines and the recruitment of neutrophils (reviewed in Medzhitov, 2007). Important interactions occur between TLRs and certain NLRs for inducing the pro-inflammatory IL-1β, and further priming of a multisubunit complex called the "inflammasome", in which caspases are activated to respond to bacterial products and products of damaged cells (Pétrilli et al., 2007; Creagh and O'Neill, 2006). The caspase pathway is also one of the main routes involved in apoptosis (Schaumburg et al., 2006). Several caspases have been characterized in teleosts, including pro-inflammatory caspases, such as caspase-1 (López-Castejón et al., 2008) and caspases related to apoptosis, such as caspase-9 (Reis et al., 2007a) or caspase-3 (Reis et al., 2007b).

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Apoptosis is crucial for maintaining social order among the cells comprising metazoans and is also an important effector mechanism of innate and adaptive host responses to pathogens (Schaumburg et al., 2006). In addition, the existence of apoptosis in single-celled organisms implies a certain degree of interaction between individuals and could perform the role of an "immune" response. Apoptosis plays an important role in the host response to infections. Parasitic protozoa and helminths are able to modulate host apoptotic pathways to survive within their hosts, preventing apoptosis in host cells that are inhabited by parasites and promoting apoptosis in host immune cells programmed to attack them (James and Green, 2004). Thus, apoptosis is an important mechanism for immune evasion. Apoptotic processes can be divided into intrinsic and extrinsic pathways. The best characterized interaction involved in the extrinsic pathway is the C95 (Fas)–C95 ligand (FasL) [members of the (TNF)-α and TNF-α receptor proteins]. The key feature of the intrinsic apoptotic pathway is the breakdown in the integrity of the mitochondrial outer membrane with release of cytochrome *c* and other components to the cytosol. In addition, some alternative routes can induce caspase activation, such as the products of cytotoxic T cells granzyme and perforine. Other pathways involving PAMPs, TLRs and NOD proteins can also interact with the apoptotic mechanisms (reviewed in James and Green, 2004). Some factors involved in apoptosis have been identified in teleosts. Soluble apoptosis regulatory factors were detected in stressed fish (reviewed in Evans et al., 2001). The potential participation of the FasL–FasR system was demonstrated in fish non-specific cytotoxic cells (NCCs) (Jaso-Friedmann et al., 2000; Bishop et al., 2002). In addition, a FasL-like molecule is present in leucocytes of gilthead sea bream (Cuesta et al., 2003). The cellular apoptosis susceptibility (CAS) gene involved in multiple cellular mechanisms including apoptosis, is expressed in tilapia (*Oreochromis niloticus*) NCCs (Praveen et al., 2006a). The apoptotic responses related to piscine parasites are reviewed in Section 3.3.

The potentially dangerous and toxic entities, such as apoptotic cells, must be cleared by the innate system. In analogy with PAMPs, some authors have proposed that innate immune molecules recognize apoptotic cell-associated molecule patterns (APAMPs) (reviewed in Gasque, 2004). Ultimately professional and non-professional phagocytes recognise PAMPs and APAMPs to eliminate the pathogen and the noxious apoptotic cells, with the participation of different mechanisms, including complement.

3. Modules of the innate system

In his elegant review, Medzhitov (2007) refers to the mammalian innate immune system as a collection of distinct subsystems or modules, which are activated by primary sensors of infection, in most cases PRRs. These modules include the mucosal epithelia, phagocytes, acute-phase proteins and complement, inflammasomes, natural killer (NK) cells, type I IFNs and IFN-induced proteins; eosinophils, basophils and mast cells. Some modules are co-induced by an infection, and thus they tend to develop functional links and are usually co-regulated by the same inducing signals, most commonly cytokines.

In this section we analyse the available data on the different modules of the innate defence in the fish hosts and in response to parasite infections. As the knowledge on some of these modules is still scarce for teleosts, some of them are analysed together, thus allowing their interrelationship to become evident. A brief reference to the development of fish immune cells, with emphasis on differences with mammals is also included.

3.1. Development of fish immune cells

The different leucocyte types known from mammals have also been recognised in teleost fish, including cells that are morphologically and functionally equivalent to mammalian MΦs, neutrophils, monocytes, thrombocytes, B cells, plasma cells, T cells, natural killer cells and eosinophils (Manning and Nakanishi, 1996; Secombes, 1996; Whyte, 2007). Some recent data support the role of certain leucocytes as Ag-presenting or dendritic-like cells (Cuesta et al., 2006a; Lovy et al., 2006; Araki et al., 2008). However, fish leucocytes are generally poorly characterized, partly due to the lack of specific tools.

Teleosts do not have a bone marrow or lymph nodes. Myelopoiesis generally occurs in the head kidney (HK) and/or spleen, whereas thymus, kidney and spleen are the major lymphoid organs (Zapata et al., 2006). Next to the thymus as the primary T cell organ, HK is considered the primary B cell organ. High expressions of certain genes considered to be lymphoid cell lineage specific, such as TdT, RAG 1 and 2 and Ikaros have been demonstrated in thymus and HK of teleosts (Rombout et al., 2005). HK exhibits morphological similarities with the bone marrow of higher vertebrates and also serves as a second lymphoid organ (a lymph node analogue), and in the clearance of soluble and particulate Ags from the circulation. Furthermore, it is also the major site of Ab production (reviewed in Whyte, 2007). The spleen of teleosts has also been implicated in the clearance of blood-borne antigens and immune complexes in splenic ellipsoids, and also has a role in the Ag presentation and the initiation of the adaptive immune response (Chaves-Pozo et al., 2005; Whyte, 2007). HK and spleen present MΦ aggregates, also known as melano-macrophage centres (MMCs). MMCs may also develop in association with chronic inflammatory lesions in other organs. In higher teleosts they often exist as complex discrete centres, containing lymphocytes and MΦs (Agius and Roberts, 2003). The hypothesis that MMCs play a role analogous to the germinal centres of lymph nodes in mammals was confirmed by the labelling of free melano-macrophages and MMCs from the kidney and spleen of three teleost species by CNA-42, an Ab usually employed for labelling follicular dendritic cells of higher vertebrates (Vigliano et al., 2006).

The thymus, a primary lymphoid organ and a major site of T-cell development in mammals, is poorly known in teleosts and the information is mainly based on histological studies. Basically, thymus can be considered as an encapsulated aggregation of MΦs that processes the proliferation of T cells. Differentiation of the structure is highly variable in teleosts (reviewed in Bowden et al., 2005). Knowledge has increased from the study of some

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model fish, such as zebrafish, in which a demarcation between cortex and medulla has been described (Trede et al., 2004; Meeker and Trede, 2008). In cyprinids and sea bass, the thymus is the first lymphoid organ and T cells appear to be selected there much earlier than the first detection of T cell-dependent Ab responses (Rombout et al., 2005).

The gut-associated lymphoid tissue (GALT) of teleosts consists principally of different sized lymphocytes, plasma cells and MΦs, as well as several types of granulocytes, PAS positive cells and eosinophil granular cells (Zapata and Amemiya, 2000). In teleosts, gut intraepithelial lymphocytes are largely considered T cells, whereas lymphoid cells in the lamina propria are mainly B lymphocytes (Zapata et al., 2006). Recent data indicates an extra-thymic origin of some T cells in mammals (Rocha, 2007). According to Rombout et al. (2005), a similar process takes place in bony fish, as suggested by a very early (prethymic) appearance of T-like cells in the gut.

3.2. Mucosal epithelia

According to Medzhitov (2007), the receptors involved in the response of mucosal epithelia are TLRs and NODs. Recognition of pathogens occurs not only through the specialised Ag presenting cells (APCs), but also by epithelial cells which constitute the primary cellular barrier (Fritz et al., 2007). Thus, both types of cells can activate the corresponding signals leading to the different mechanisms and pathways (phagocytosis, complement, acute-phase reaction, cytokine activation), so that the epithelial response is finally integrated into the whole immune response. The epithelial cell/T cell relationship plays an important role in the immune regulation of the gut (Shao et al., 2001).

The mucosal epithelia play a significant role in fish immunology. When it encounters the host fish, the parasite must overcome the first barrier: skin and tegument. Mucus not only acts as a barrier, but also contains several components with a role in the host–parasite interaction. Immunoglobulin, complement, C-reactive protein (CRP), lectins, lysozyme, proteolytic enzymes, alkaline phosphatase and esterase, antimicrobial peptides, and hemolysine are among the substances present with biostatic or biocidal activities (reviewed in Jones, 2001; see also Alexander and Ingram, 1992; Palaksha et al., 2008). Some skin and gill infections illustrate this situation, such as the ichthyophthiriosis produced by the ciliate *I. multifilis*, one of the best studied parasitosis regarding immune response (see reviews of Dickerson and Clark, 1996; Buchmann et al., 2001b; Matthews, 2005 and Sections 3.3, 6 and 7). Different elements of innate and adaptive immunity are involved in the host response to this ciliate, mainly in the skin, the target organ. In the case of ectoparastic monogeneans the role of mucus in limiting the parasite load has been demonstrated (Buchmann, 1999; Buchmann and Lindenstrøm, 2002). Mucosal reaction is also involved in the response to some crustaceans, such as *Lepeophtheirus salmonis* (reviewed in Jones, 2001). Gene expression studies in the target organs of these ectoparasites have confirmed the role of mucosal components in the response to their infections (see Section 6).

The mucosal intestinal system is essential in the immune modulation of the gut. As in the skin mucus, several substances involved in the host–parasite interaction are present in the intestinal mucosa. In addition, all cells necessary for the mucosal immune system are present in the fish hindgut, which also appears to be an important Ag transport site (Rombout and Joosten, 1998). The digestive tract is the target organ of some piscine parasites, such as certain flagellates, apicomplexans and myxozoans. Their adhesion and penetration involve interactions thus far poorly known and also induce changes in the immune status of epithelia and lamina propria, such as leucocytic infiltration or variations in the amount and types of leucocytes. Some available information on myxozoans is commented below (Section 3.3).

3.3. Inflammation and some related pathways: cellular responses—complement

3.3.1. Inflammation

As indicated above, a combination of innate mechanisms (with the contribution of some adaptive ones) leads to an inflammatory reaction, and such response has frequently been described in fish parasitoses. Until the last two decades, the information on the cellular response to the parasites had been limited to the description of the histopathological lesions produced by the parasites, and changes in leucocytes in blood or infected sites. On many occasions the inflammatory response leads to the formation of granulomas, in which the parasite and their products are encapsulated. This is one of the recognised mechanisms of immune evasion (see reviews of Buchmann, 2000; Sitjà-Bobadilla, 2008). Thus inflammatory reactions involving cellular responses, either accompanied by the formation of granulomas or not, have been reported for numerous piscine parasites belonging to different groups, such as amoebas, dinoflagellates, diplomonads, kinetoplastids, microsporidians, apicomplexans, ciliates, myxozoans, monogeneans, digeneans, cestodes, nematodes, acanthocephalans and crustaceans (see Bartholomew et al., 1989; Lom and Dyková, 1992; Secombes and Chappell, 1996; Dick and Choudhury, 1999a,b; Dickerson and Dawe, 1999; Dyková, 1999; Lom, 1999; Lom and Dyková, 1999; Molnár, 1999; Nickol, 1999; Paperna, 1999; Adams and Nowak, 2001; Buchmann et al., 2001a,b; Alvarez-Pellitero et al., 2004a; Ferguson, 2006; Dyková and Lom, 2007; Alvarez-Pellitero, 2008). Information on the cellular responses has been provided on some occasions, as illustrated by the examples given below. MΦs and neutrophils were the most abundant leucocytes identified in *Salmo salar* with amoebic gill disease (AGD) (Adams and Nowak, 2003). Cell-mediated responses were demonstrated in salmonids infected with *Cryptobia salmositica* by the presence of MΦs that have engulfed parasites, and by using delayed-type hypersensitivity and the MΦ inhibition migration test (Woo, 2003). Inflammatory and cellular reactions are produced in systemic hexamitosis/spironucleosis of several salmonids (reviewed in Alvarez-Pellitero, 2008). By contrast, the slight damage produced in

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Salvelinus alpinus, is considered to be related to the clearance of parasites by phagocytosis (Sterud et al., 2003). Infection of *Paralichthys olivaceus* by the monogenean *Neoheterobothirum hirame* produced a rapid increase of monocyte/macrophages and granulocytes in the blood and infected sites. Some infiltrated cells adhered to the parasites and phagocytosed them (Nakayasu et al., 2005). The blood fluke *Sanguinicola inermis* elicits cellular reactions in carp *Cyprinus carpio* (Richards et al., 1994a, 1996). Rainbow trout MΦs can kill diplosotmules of *Diplostomum spathaceum* (Whyte et al., 1989). In experimental infection of goldfish *C. auratus* with metacercaria of the digenean *Ribeoroia marini*, an acute inflammatory response, involving granulocytes and MΦs was observed (Huizinga and Nadakavukaren, 1997). An inflammatory response was induced by *Diphyllobothrium* spp. in natural infections of rainbow trout (Sharp et al., 1989) and host MΦs were able to kill the cestodes *in vitro* (Sharp et al., 1991). MMCs are also frequently associated to inflammation, and the retention of parasite spores and Ag processing during the immune response have been described among the MMC functions. Myxozoan stages are frequently found in association with MMCs (Lom and Dyková, 1999). Holzer and Schachner (2001) found a closely association of *Myxobolus cyprini* with MΦ aggregation in *Leuciscus cephalus*. Sitjà-Bobadilla et al. (2006) reported higher abundance of MMC in *Enteromyxum scophthalmi*-infected turbot (*Psetta maxima*), which also showed melanization of spleen MMC associated to the presence of parasite stages or debris engulfed in MΦs; in addition, the presence of numerous apoptotic cells suggested an induction of apoptosis by the parasite. Apoptosis was also induced in turbot HK leucocytes by cysteine proteases of the ciliate *Philasterides dicentrarchi*, and an increase in caspase-3-like activity was observed (Paramá et al., 2006). The fish host can also induce apoptosis as a mechanism of pathogen killing. Thus, apoptosis of *I. multifiliis* theronts was detected in the presence of *I. punctatus* skin fluid containing cutaneous Abs (Xu et al., 2005). In further studies, FasR was detected on the surface of *I. multifiliis* theronts and the theront apoptosis induced by immune cutaneous Ab appeared to be positively correlated with the expression of Fas on the surface (Xu et al., 2006a).

Further research has provided more specific data on the involvement of different factors related to inflammation, including cellular components (phagocytes, other leucocytes) and complement in the response against parasites.

3.3.2. Phagocytes and phagocytosis: oxidative mechanisms

Phagocytosis is one of the main mechanisms involved in the host protective responses leading to the clearance of pathogens. As in mammals, the leucocytes involved in phagocytosis in teleosts include mainly neutrophils, acidophilic granulocytes and monocyte–macrophages (Dalmo et al., 1997; Sepulcre et al., 2002). The increase of phagocytic cells in response to fish parasitoses has frequently been reported, as well as the role of phagocytosis and other indices of phagocyte activity, i.e. oxidative mechanisms, as defence mechanisms for the elimination of parasites. Inducible antimicrobial responses such as the respiratory burst (RB) and nitric oxide (NO) have been demonstrated in fish phagocytes, and display biochemical and physiological similarities to homologous responses induced in mammalian phagocytes (Neumann et al., 2001; Whyte, 2007). In experimental infections of turbot with the myxosporean *E. scophthalmi*, the RB of circulating leucocytes increased soon after exposure, in congruence with the rise in the percentages of granulocytic cells in blood (Sitjà-Bobadilla et al., 2006) and with the presence of blood MΦs harbouring stages of the myxosporean (Redondo et al., 2004). In *Enteromyxum leei*-exposed *D. puntazzo*, an initial rise in RB of circulating leucocytes was followed by a further decrease (Alvarez-Pellitero et al., 2008). Other studies rely on the use of leucocytes isolated (mostly from HK) from *in vivo* parasitized or non-parasitized fish to study their *in vitro* phagocytic ability and capability as well as other indices of their response. There are several examples of depression of oxidative burst and/or impaired phagocytic activity, such as in *E. leei*-exposed *S. aurata* (Cuesta et al., 2006b); rainbow trout *O. mykiss* infected by *Tetracapsuloides bryosalmonae* (Chilmonczyk et al., 2002) or *Lepeophtheirus salmonis* (Fast et al., 2002; Mustafa et al., 2000); salmon infected by *Neoparamoeba* sp. (Gross et al., 2004a,b); and turbot inoculated with the ciliate *Philasterides dicentrarchi* (Leiro et al., 2004) or the microsporidians *Tetramicra brevifilum* (Leiro et al., 2001) and *Glugea plecoglossi* (Kim et al., 1998). Live *Philasterides dicentrarchi* also induced a ROS scavenging activity of phagocytes (Leiro et al., 2004), in a similar way as *Uronema marinum* in *Paralichthys olivaceus* phagocytes (Kwon et al., 2002). These mechanisms can help the parasite to evade the host immune response, and a similar role as can be attributed to the detoxifying enzyme of the nematode *Anguillicola crassus* (see Buchmann, 2000). In contrast, the coccidian *Goussia carpelli* induced a response in common carp phagocytes, including the RB (Steinhagen and Hespe, 1997). RB also increased in MΦs of rainbow trout infected or vaccinated with *Cryptobia salmositica* (Mehta and Woo, 2002; Chin and Woo, 2005), and was higher in phagocytes of sea bass parasitized by *Sphaerospora dicentrarchi* than in non-parasitized fish (Muñoz et al., 2000a). In *Trypanoplasma borreli*-infected carps, up-regulation or down-regulation of some immune functions (including phagocytic activity, NO production and neutrophil chemotaxis) have been reported (Scharsack et al., 2003a,b; Saeij et al., 2002; Stakauskas et al., 2007). The NO-inducing activity of *Trypanoplasma borreli* in carp may be an adaptation developed to ensure survival and immune evasion in the fish host, and could explain the pathology associated with these infections. In contrast, *Trypanosoma carassii* has apparently adopted another strategy by deactivating the specific function of phagocytes (Saeij et al., 2002).

Fish flagellates have also been used in studies to demonstrate the involvement of the different activation pathways of MΦs known in mammals. Depending on the cytokine environment, MΦs can differentiate into distinct subsets that perform specific immunological roles. Classically activated MΦs (caMf), triggered by interferon (IFN)-γ, play an important role in the type-I immune responses (Noël et al., 2004). CaMf are characterized by their NO

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production from L-arginine and are relatively well studied in teleost fish (Joerink et al., 2006a). It has recently been recognised that MΦs exposed to cytokines generated by Th2 cells exert an alternative activation programme. Although the role of these alternatively activated macrophages (aaMF) is still poorly defined, they may down regulate inflammation and can be essential for host survival in infections such as schistosomiasis (Herbert et al., 2004; Noël et al., 2004). Recently, Joerink et al. (2006a) demonstrated differential polarization of carp MΦs depending on the flagellate infection. *Trypanoplasma borreli*- or *Trypanosoma carassii*-infected carps are more prone to increase NO production by caMF, or arginase activity by aaMF, respectively. However, in contrast to mammals, fish arginase 2 and not arginase 1, is differentially regulated and probably involved in the alternative activation of MΦs (Joerink et al., 2006b). The study of type I versus type II responses in fish deserves more attention (Wiegertjes et al., 2005). As suggested by Joerink et al. (2006a), some granulome-forming parasites (such as *Sanguinicola inermis* in carps) could be suitable models for the study of aaMf.

3.3.3. Eosinophilic granule cells, mast cells, rodlet cells

Eosinophils are frequently involved in the response to parasite diseases. However, the terminology and nature of fish eosinophilic cells have been controversial and still remain confusing. The term eosinophilic granule cells (EGCs) was introduced by Roberts et al. (1971) to designate mononuclear eosinophilic granule-containing cells distributed in the connective tissues of various teleosts. EGCs have been suggested to be mast cell analogous or equivalent (Reite, 1998), similar to intestinal Panneth cells (Sveinbjornsson et al., 1996) or representing a cell type originating from the evolutionary precursors of both Panneth cells and mast cells (Paulsen et al., 2001). Their heterogeneity and staining diversity have been stressed (Sveinbjornsson et al., 1996; Reite and Evensen, 2006; Rocha and Chiarini-Garcia, 2007), and some of them produce anti-microbial peptides or lysozyme. Recently, Mulero et al. (2007) found also histamine in mast cells of several perciform fish. Important differences can also occur between fish species, and thus, the characterization of these cells using specific markers is needed. Changes in EGC abundance and distribution can occur in response to parasites. Adams and Nowak (2003) reported EGCs adjacent to lesions produced by amoebas in *Salmo salar* with AGD. Alvarez-Pellitero et al. (2008) found two apparently different types of EGCs involved in the response of *Diplodus puntazzo* to the myxozoan *E. leei*. EGC1 (very similar to the EGC of salmonids), degranulated in this infection and their number decreased with the progression of the infection, in parallel with an increase in EGC2-type cells. The releasing of bioactive granules from eosinophils, causing the expulsion of most metacercaria, was also reported in *C. auratus* infected by the digenean *Ribeoroia marini* (Huizinga and Nadakavukaren, 1997). Degranulating eosinophils were also involved in the cellular response elicited by *S. inermis* in *C. carpio* (Richards et al., 1994b)

Another enigmatic cellular type in teleosts is the rodlet cell, commonly associated with the epithelial tissues of virtually every fish species, with a high variability in abundance and distribution. The precise function of rodlet cells has been controversial, and they were even initially considered to be parasites. However, recent findings have confirmed their endogenous origin and it is thought that they are a type of inflammatory cell akin to other piscine inflammatory cells (reviewed in Manera and Dezfuli, 2004; Reite, 1998; Reite and Evensen, 2006). Their function is probably related with the release of rodlets due to the contractile capability of the fibrous layer, in which the S100 protein (related to the contraction mechanism) has recently been identified (Vigliano et al., 2008). Antimicrobial peptides have also been detected in the rodlet cells of some fish (see Section 3.4.4). Some parasites, including protozoans, myxozoans and helminths, seem to induce the recruitment of rodlet cells or changes in their abundance and distribution, as well as the releasing of rodlets (Manera and Dezfuli, 2004; Alvarez-Pellitero and Sitjà-Bobadilla, 2002; Alvarez-Pellitero et al., 2004a). Rodlet cells were the only type of inflammatory cell found in response to *Diplostomum spathaceum* in *Phoxinus phoxinus* (Dezfuli et al., 2007). The precise role of this enigmatic cells in relation to fish immune response deserves further investigation.

3.3.4. Complement

Complement is amongst the main mechanisms involved in the initiation of the innate response and further mounting of an adaptive response. The complement cascade is part of the phylogenetically ancient innate immune response and is crucial to our natural ability to ward off infection (Gasque, 2004). Complement include a combination of three pathways, the alternative, lectin and classical. The classical pathway is initiated by a complex between an Ag and an Ab. It is triggered by binding of the Fc portion of the IgG to the C1q component of the C1 complex. In the alternative pathway, the spontaneous activation of C3 is amplified upon the covalent binding of C3(H_2O) to various microbial surfaces (Boshra et al., 2006). The lectin pathway requires the interaction of lectins such as mannose-binding lectin (MBL) and ficolins with sugar moieties found on the surface of microbes (Fujita et al., 2004). However, Lutz et al. (2007), proposed the redrawing of the complement system, putting the amplification loop of the alternative pathway in the centre of the complement system, as a means to increasing C3/C5 activation. Thus, C3b molecules generated by any pathway can stimulate complement amplification, so this scheme may help illustrate the interplay of pathways. Complement involves numerous soluble and membrane-bound proteins that are produced not only by host liver cells but also by MΦs (Dalmo et al., 1997). Among other functions in innate response and in its connection with adaptive response, complement plays important roles in the killing of pathogens, through opsonization and activation of phagocytes, and in inflammation.

Complement constitutes a clear example of the link between both innate and adaptive immune responses (Carroll, 2004; Hawlisch and Köhl, 2006). Apart from the involvement of Ag/Ab complexes in the classical pathway, such connections are mainly made through CD21 (C

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receptor type 2), a receptor for CD3d activation via "natural" IgM, C-reactive protein, collectins or alternative pathway. C1q also acts as a bridge following binding to natural IgM complexed to Ags, and C4b through CD40 receptor in B cells (reviewed in Gasque, 2004). The different components of complement have been demonstrated in fish and the three recognised pathways are all involved. Teleosts possess a large repertoire of genes encoding the complement components, especially in the case of C3, which can have several isoforms in a single species (reviewed in Holland and Lambris, 2002; Boshra et al., 2006; Whyte, 2007).

Most data on the implication of complement in piscine parasitic diseases was obtained by measuring the alternative activity in serum or the effect of the serum addition on the activity of phagocytes. In this way, such alternative activity increased in serum of gilthead sea bream exposed to *E. leei* (Cuesta et al., 2006c) and oscillated over the post-exposure period in turbot exposed to *E. scophathlmi* (Sitjà-Bobadilla et al., 2006). An initial increase followed by a decrease occurred in sea bass injected with *S. dicentrarchi* spores (Muñoz et al., 2000b). Injection of live scuticociliates induced a significant increase of such activity in turbot (Sitjà-Bobadilla et al., 2008a) and a rise was also observed in *C. carpio* injected with cercariae of the *S. inermis* (Roberts et al., 2005). The monogeneans *Gyrodactylus derjavini* (Buchmann, 1998), *G. salaris* (Harris et al., 1998) and *Discoctyle sagittata* (Rubio-Godoy et al., 2004) can be killed *in vitro* by the alternative pathway. This route also seems be involved in the immobilisation and lysis of *I. multifiliis* theronts in non-immune fish serum (see Buchmann et al., 2001b), in the response of *Dicentrarchus labrax* HK cells to spores of *S. dicentrarchi* (Muñoz et al., 2000b), and of turbot phagocytes to spores of *T. brevifilum* (Leiro et al., 2001). For *Trypanosoma carassii*, *in vitro* studies demonstrated the involvement of the alternative pathway in the lysis of enzyme treated parasites and the role of surface proteins for the resistance to lysis (Plouffe and Belosevic, 2004). Both the classical Ab-dependent pathway and the alternative pathway are involved in the response of salmonids to *Cryptobia salmositica*, with the alternative pathway being the mechanism of resistance in refractory fishes (Woo and Poynton, 1999). *Trypanoplasma borreli* can be killed presumably by the complement-mediated classical pathway (Scharsack et al., 2004). The lectin/carbohydrate interaction, and thus the probable involvement of the lectin complement pathway, has been demonstrated in the phagocytosis of microsporidian spores (*T. brevifilum*, *Glugea caulleryi*, *G. plecoglossi*, *Loma salmonae*) by phagocytes of the corresponding hosts (Leiro et al., 1996; Kim et al., 1999; Shaw et al., 2001).

Little data is available on the involvement of determined complement factors in the response to parasites, and mainly includes variations in gene expression (see Section 6).

3.4. Cytokines and other humoral factors

Other components of the innate system playing a role in the mounting of innate response are produced, and can be released as humoral factors. Besides cytokines and chemokines, they include peroxidases, antiproteases, lysozyme, acute-phase proteins and antimicrobial peptides, among others. Some of these factors can change in response to parasite infections.

3.4.1. Cytokines, chemokines and cytokine-induced enzymes

As a result of the orchestrated immune responses involving the different components and pathways commented above, inflammatory factors, cytokines, chemokines, etc. are produced. Cytokines are related to both innate and adaptive responses. The main inflammatory cytokines TNFα, IL-1β and IL-6, elicited after injury, and those further released in the downstream cascade, are present in teleosts (reviewed in Secombes et al., 1999a,b, 2001; Huising et al., 2004; Whyte, 2007). New classes of IL families have recently been identified in fish (Igawa et al., 2006; Bei et al., 2006), including some of those mediating Th2 responses, such as IL-10 and a molecule related to IL-4 referred as a "Th2 type cytokine" (Li et al., 2007) (reviewed in Randelli et al., 2008). IFNs are cytokines that play a major role in the defence against virus infection of vertebrates. Type I IFNs have been cloned from several fish, as well as putative IFN receptor genes and Mx protein (Igawa et al., 2006; Robertsen, 2006), and the interferon regulatory factor (IRF-1) (Ordás et al., 2006). Thus, available data points to the existence of Th1 and Th2 responses in fish. A third lineage of effector T cells, Th17, has been recently discovered (reviewed in Weaver et al., 2007). The cytokines IL-17 (one of the most ancient cytokines) and IL-22 secreted by these Th17 cells are known from teleosts (Gunimaladevi et al., 2006; Igawa et al., 2006). In addition, three different isoforms of transforming growth factor (TGF) have also been demonstrated in fish (Randelli et al., 2008). Different chemokines (small secreted cytokines that direct the migration of immune cells to sites of infection), have also been characterized from fish, including members of the CXC and CC subfamilies. In addition, two cytokine-induced enzymes, playing a key role in inflammation, the COX (responsible for prostaglandin production) and the iNOS, have been sequenced from teleosts (Secombes et al., 2001; Laing and Secombes, 2004). The available knowledge in the involvement of fish cytokines, chemokines and related enzymes in the response to parasites is limited to the study of their expression in some infections (see Section 6).

3.4.2. Peroxidases

Peroxidases (PO), released from the cytoplasmatic granules of phagocytes, can participate in the oxidative responses against pathogens. Serum PO levels increased in *E. leei*-exposed *S. aurata* (Cuesta et al., 2006a) (in connection with a decrease in HK levels, see above). However, a subsequent fall occurred at later post-exposure times. This result is in accordance with the decrease in PO in *S. aurata* chronically infected by the same parasite (Sitjà-Bobadilla et al., 2008a,b). In *E. leei*-exposed *D. puntazzo*, the clear increase in PO level observed was maintained throughout the experiment (Muñoz et al., 2007), which could be related to the reported increase in NO level (Golomazou et al., 2006) and to the cellular response observed in the intestinal submucosa (Alvarez-Pellitero et al., 2008; also see Section 3.3).

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3.4.3. Lysozyme

Lysozyme is an important defence molecule of the innate immune system, playing a role in mediating protection against microbial invasion. It is a mucolytic enzyme produced by leucocytes, especially monocytes, MΦs and neutrophils. Fish lysozyme possesses lytic activity against bacteria and can activate complement and phagocytes. It is present in mucus, lymphoid tissue, plasma and other fluids and is also expressed in a wide variety of tissues (reviewed in Saurab and Sahoo, 2008). Serum lysozyme levels can change in fish in response to parasite infections. An increase was detected in rainbow trout following immunisation with live theronts of *I. multifilis* (Alishahi and Buchmann, 2006), in *D. labrax* immunized with *S. dicentrarchi* spores (Muñoz et al., 2000a) and in fish infected by *Ceratomyxa shasta* (Foott et al., 2004); in contrast, a decrease followed by an increase occurred in *E. scophthalmi* exposed turbot (Sitjà-Bobadilla et al., 2006). The low serum lysozyme levels found in *D. puntazzo*, could be involved in the higher susceptibility of this species to *E. leei* (Alvarez-Pellitero et al., 2008). Lysozyme was significantly depleted in *S. aurata* after chronic exposure to *E. leei* (Sitjà-Bobadilla et al., 2008b).

3.4.4. Acute-phase proteins

As reviewed in Bayne and Gerwick (2001), the acute-phase response (APR) is a pervasive physiological response of the body to injury, trauma or infection. In its broadest context, the APR involves changes in at least the hepatic, neuroendocrine, hematopoietic, musculo-skeletal and immune systems, and is induced by pro-inflammatory cytokines such as IL-1, IL-6 and TNFα. Among the humoral components increasing their concentrations in APR in mammals are included complement system, clotting system, anti-proteases, metal-binding proteins, lectins, lysozymes, antimicrobial peptides and opsonins. In Section 3.4.3 we have referred to lysozyme, and complement has been reviewed in Section 3.3. Other factors directly or indirectly related to APR are commented below.

3.4.4.1. Lectins. In Section 2.1 we have made reference to the role of C-type lectins as PRRs. Among soluble PRRs, MBL is well known for its role as initiator of the primary immune response and its participation in the lectin complement pathway (Turner, 2003; Klein, 2005). MBL or its homologous have also been demonstrated in teleosts (Russell and Lumsden, 2005). In *S. salar*, MBL can recognise bacterial pathogens (Ewart et al., 1999). The direct implication of fish MBL in the recognition of parasites has not so far been reported, but several fish parasites exhibit mannose residues which could putatively interact with this lectin; moreover, another mannose-binding lectin, the pufflectin, present in the mucosal tissues of skin and digestive tract, binds specifically to the monogenean *Heterobothrium okamotoi* (Tsutsui et al., 2003, 2004). The pentraxins CRP and serum amyloid P (SAP), as well as transferrin and thrombin have been identified in teleosts (reviewed in Bayne and Gerwick, 2001). However, information on changes in relation to parasite infections is scarce. CRP is known to inhibit egg production in *Bothriocephalus scorpii* cultured *in vitro*, via phosphorylcholine/complement (Fletcher et al., 1980). A role of serum amyloid A (SAA) in the APR of carp against *I. multifiliis* (Gonzalez et al., 2007a) and *Trypanoplasma borreli* has recently been demonstrated in expression studies (Saeij et al., 2003a) (see Section 6).

3.4.4.2. Antimicrobial peptides. Antimicrobial peptides (AMPs) are widespread in nature as defence mechanisms in plant and animals (Zasloff, 2002). Increasing numbers of them have been identified from teleosts in recent years and some can be activated against bacteria (Cuesta et al., 2008; Maier et al., 2008) though many are yet unidentified (reviewed in Whyte, 2007). Besides the four previously known different classes of antimicrobial peptides, a fifth class, NK-lysines has been discovered in mammalian NK and T cells. Three distinct transcripts of NK-lysine exist in channel catfish and they seem to be similarly arranged in zebra fish (Wang et al., 2006). When comparing the APR of zebrafish with mammals, both similar APPs and a similar pattern for their induction were identified, although some novel APPs were discovered (Lin et al., 2007). Piscidines, α-helical haemolytic AMPs were detected in EGCs (named as mast cells) and rodlet cells of several teleosts (Silphaduang and Noga, 2001; Silphaduang et al., 2006). Information on the involvement of antimicrobial peptides in response to fish parasitosis is scarce. Some histone-like proteins isolated from fish tissues may be important for defence as they are able to kill the dinoflagellate *Amyloodinium ocellatum* (Noga et al., 2001, 2002). In addition, piscidice 2 isolated from mast cells of hybrid striped bass was lethal to the infective stages of *I. multifiliis*, whereas marine parasites (*A. ocellatum*, *Cryptocaryon irritans*) were affected at a lesser extent (Colorni et al., 2008).

3.4.4.3. Anti-proteases. Host protease inhibitors modulate protease activities and control a variety of critical protease-mediated processes, including the resistance to invasion by infectious agents. Variations in antiproteases, mainly α-2-macroglobulin have been observed in several parasite infections. The best characterized function of α-2-macroglobulin family is the clearance of active proteases from the tissue fluids (Armstrong and Quigley, 1999). They can, however, also interact with innate and adaptive mechanisms (Dalmo et al., 1997). In fish resistant to cryptobiosis, α-2-macroglobulin can neutralise the metalloprotease involved in *Cryptobia salmositica* virulence (Woo, 2001). In *E. scophthalmi*-exposed turbot and *E. leei*-exposed *D. puntazzo*, total antiproteases and α-2-macroglobulin levels oscillated after exposure, but they were generally higher than in control fish (Sitjà-Bobadilla et al., 2006; Muñoz et al., 2007). Some data is available on the expression of α-2-macroglobulin gene in fish in response to parasites (see Section 6).

4. Immune mechanisms in the connection between innate and adaptive immunity

The integration of innate and adaptive responses in a single immune system was suggested more than a decade

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ago. New findings are confirming the links between both innate and adaptive mechanisms. These two components are connected in different ways and both form part of an integrated and efficient immune system (Fearon and Locksley, 1996; Medzhitov and Janeway, 1997; Dixon and Stet, 2001; Medzhitov, 2007).

Certain elements of the immune response clearly illustrate the connection and mutual influence of innate and adaptive responses. Some references to PRRs and complement have been made in the previous sections. Here we comment specifically about some elements clearly involved in such connections, such as innate-like lymphocytes (with reference to the role of some PRRs), natural killer cells, and the major histocompatibility (MH) receptors.

4.1. Innate-like lymphocytes

According to Medzhitov (2007), there are two types of lymphocytes that express Ag receptors: conventional lymphocytes (see Section 5) and innate-like lymphocytes, that is B1 cells, marginal-zone B cells, natural-killer T cells and subsets of γδ T cells. For these innate-like lymphocytes, the diversity of Ag receptors is restricted and not entirely random. In fact, B1 cells can be activated directly by PRRs and are programmed to produce Abs with a wide specificity for common bacterial Ags. Innate T-like cells recognize microbial Ags presented by non-classical MHC molecules.

In addition to the activation of innate host-defence mechanisms, some PRRs are coupled to the induction of adaptive immune responses. The basic principle of innate control of adaptive immunity is based on establishing an association between the Ags recognised by lymphocytes and the PAMPs recognised by PRRs (Janeway, 1989). For T cells, this association is interpreted by dendritic cells, which monitor the tissue environment using various PRRs. For B cells, the association between an Ag and a PAMP can be established directly, or, in the extreme case, a TLR ligand is itself recognised by the B cell receptor and by a corresponding TLR expressed by a B cell. Ags of this class, which combine ligands for both innate and adaptive immune recognition, are called T-independent Ags (Medzhitov, 2007).

Another function of innate-like lymphocytes deserves to be mentioned. A normal counterpart of the malignant B/macrophage cells reported for decades has been identified, contradicting the current paradigms of haematopoietic lineage relationship. This cell expresses traits common to CD5+ B cells (B1-a subset) and MΦs (Borrello and Phipps, 1996). The recent identification of phagocytic B lymphocytes in teleosts and amphibians supports the idea of an evolutionary relationship between B-1 lymphocytes and MΦs of mammals, and that B cells might have evolved from ancient phagocytic cell (Li et al., 2006). Further evidence for the relatedness of myeloid and lymphoid potentials has been obtained, as well as examples of how extracellular signals can dramatically influence lineage outcomes (reviewed in Brown et al., 2007). The use of some model fish can help to identify other innate-like lymphocytes in fish.

4.2. Non-specific cytotoxic cells

Killer cells, including specific and unspecific cytotoxic cells, have been identified in teleost fish, but some of them are poorly characterized. They are morphologically variable, ranging from agranular small monocyte-like cells to a mixture of lymphocytes, acidophilic granulocytes and monocyte MΦs (see Whyte, 2007). The discrimination according to the mammalian CD nomenclature is difficult, partly due to the lack of a number of tools to characterise them (Fischer et al., 2006). A novel cytotoxic population, unique in its ability to lyse various transformed human and mouse cell lines was identified in catfish and called non-specific cytotoxic cells (NCCs). They have also been found in other teleost species and may represent an evolutionary precursor of mammalian NK cells (reviewed in Plouffe et al., 2005). Constitutive expression of TNFα has been demonstrated in tilapia NCCs (Praveen et al., 2006b).

Mammalian NK cells differ from lymphocytes in that they do not express Ag-specific clonally distributed receptors but display two receptor classes, the killer cell Ig-like receptors (KIRs) and the killer cell C-type lectin receptors (KLRs). A small number of other KLRs have been identified in fish. Recent studies have shown that teleosts also possess NK-like cells distinct from NCCs, characterized by novel NK cell receptors, named novel immune-type receptors (NITRs) (members of the Ig superfamily) that have no homologous in mammals (reviewed in Plouffe et al., 2005). Channel catfish NK-like cells are armed with IgM via a putative Fcμ receptor that enables them to kill targets by an Ab-dependent cell-mediated mechanism (Shen et al., 2003).

NCCs appear to participate in the immune response against protozoan parasites, such as *Tetrahymena pyriformis* and *I. multifiliis* (Graves et al., 1985). Fish NCCs also play a role in the induction of apoptosis (se Section 2.2), which was also demonstrated using a model with the ciliate *Tetrahymena* (Jaso-Friedmann et al., 2000). Cytotoxic activity has been reported in other parasite infections, but information on the cells involved has not been provided. Cell-mediated cytotoxicity (evaluated as the tumoricidal activity of HK cells) was significantly enhanced in *E. leei*-exposed *S. aurata* and is considered one of the main immune factors involved in the cellular defence of this fish against this infection (Cuesta et al., 2006b). In contrast, in *E. leei*-exposed *D. puntazzo*, no significant change of this activity was found (Muñoz et al., 2007). A reduction of NCC activity was reported in rainbow trout infected by *Tetracapsuloides bryosalmonae*, although the high inter-fish variability rendered the observed reduction non-significant (Chilmonczyk et al., 2002).

4.3. Major histocompatibility complex (MHC)

The MH receptors are immunoglobulin superfamily member proteins that interact with T-cells through a specific T-cell receptor (TCR) in order to initiate immune responses. Recent discoveries have demonstrated their role in the integration of innate and adaptive responses (Dixon and Stet, 2001). In tetrapods and sharks, MHC genes

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are linked in a complex on a single chromosome, whereas in teleosts MH genes are not linked and are even located in different chromosomes (reviewed in Dixon and Stet, 2001; Chistiakov et al., 2007). Both types of MH receptors, class I and class II receptors, are present in teleosts, and their function is to display foreign peptides (Ags) to T cells (usually class I for intracellular pathogens and class II for extracellular pathogens). Class I and II MH genes are highly polymorphic, particularly in the peptide-binding-encoded region, and such polymorphism has been found in some fish species (Chistiakov et al., 2007). Granulocytes of *S. aurata* express MHC II genes, suggesting a role as APCs (Cuesta et al., 2006a). A highly regulated expression of MH II in *S. salar* inoculated with *Neoparamoeba* sp. was suggested by the numerous positive cells detected within AGD lesions, and thus such cells could contribute to Ag presentation (Morrison et al., 2006). Dendritic-like cells were described in the gills of Chinook salmon *O. tshawytscha* infected by *Loma salmonae* (Lovy et al., 2006). The scarce available information on the gene expression of fish MH genes in response to parasites is reviewed in Section 6.

5. Adaptive immunity: lymphocytes and receptors

Adaptive immunity arose early in vertebrate evolution, between the divergence of cyclostomes (lampreys) and cartilaginous fish (sharks). All jawed vertebrates possess the genetic elements essential for the function of the adaptive/combinatorial immune response (Marchalonis et al., 2006). The combinatorial immune system (CIS) consists of Ag-recognizing lymphocytes, immunoglobulins (Abs and Ig-family TCR), MHC products, and recombination-activating (RAG) 1 and 2 genes. The overall shape of the molecules and the recombination mechanisms that create junctional diversity in TCRs and Igs are similar in fish and mammals (Du Pasquier, 2001). Naturally occurring Abs whether in serum or on lymphocytes as receptors for Ag are also essential to the selective basis of combinatorial/adaptive vertebrate immunity (Marchalonis et al., 2006).

Ag receptors are clonally distributed on T and B lymphocytes, which allows clonal selection of pathogen-specific receptors and is the basis of immunological memory. In the case of conventional lymphocytes (mostly αβT cells and B2 cells), Ag receptors are essentially assembled randomly (not predetermined). There are two types of conventional αβT cells: Th cells, which are marked by the co-receptor CD4 on the cell surface; and cytotoxic T cells, which express CD8. These cells recognize antigenic peptides bound to the major histocompatibility complex (MHC) class II and class I molecules, respectively. In mammals, in addition to the effects of natural regulatory T cells, some T cells can also have a role in tempering the initial innate responses (Kim et al., 2007). Conventional B cells can recognize almost any Ag by binding to a specific three-dimensional molecular determinant (epitope). T and B types of conventional lymphocytes have been demonstrated in teleosts, although analysis of lymphocyte subsets is only just beginning in fish (Fischer et al., 2006).

5.1. T cells and TCRs

The first evidence of the presence of T cells in teleosts was obtained *in vitro* using proliferation assays. Presently, the availability of some tools has allowed the detection of some specific T cell markers (reviewed in Randelli et al., 2008). Genes corresponding to the TCR-α, -β, -γ, and -δ subunits have been characterized. Other putative T cell markers in fish, such as CD3, CD4 and CD8α are known for a few species (Hordvik et al., 2004; Taylor et al., 2005; Randelli et al., 2008). In fugu, in addition to $CD8^+$ lymphocytes/thrombocytes (probably $CD8^+$ T cells), neutrophils and monocyte/macrophages from an inflammatory site expressed CD8α, similar to mammalian dendritic cells (Araki et al., 2008). T cells have also been characterized from the gut of rainbow trout, as they express transcripts of T cell marker homologs of CD8, CD4, CD28, CD3ε, TCR-ζ, TCR-γ, and TCR-β and lacked IgM. Thus, a highly diverse αβ TCR repertoire is maintained in fish IEL in absence of Peyer's patches and mesenteric lymph nodes (Bernard et al., 2006). The involvement of specific T cell responses in piscine parasitosis is limited to little data on the TCR expression (see Section 6).

5.2. B cells and Igs

B cells are characterized by the expression of B cell receptor, a surface immunoglobulin receptor (sIG). The expression of surface sIG-related receptors have been conserved in phylogenetically distinct species as a critical checkpoint in B cell development (Pike and Ratcliffe, 2002). Fish B cells, like those of mammals, have been demonstrated to show Ig H-chain rearrangement and allelic exclusion (reviewed in Miller et al., 1998). Germline VH and VL elements, as well as the joining (J) segments are highly conserved among distinct vertebrate species (Marchalonis et al., 2006).

The Ab repertoire in teleosts is more limited than in mammals. As reviewed in Solem and Stenvik (2006), the most prevalent immunoglobulin in serum of teleosts is an IgM tetramer. Some teleosts have the monomer (H2L2) of IgM in serum, and a dimmer has been observed in secretions (Lobb and Clem, 1981). Other Ig classes have been found in fish, namely IgD, IgT and IgZ (reviewed in Randelli et al., 2008). The number of VH families varies tremendously between different teleost species. In addition, a mechanism to generate structural diversity in the tetrameric Ab, through a random polymerisation of constituent monomeric – likely to be important in the generation of functional diversity – has been suggested for salmonids to compensate the reduced isotypy (Kaattari et al., 1999).

IgM can be present in serum and secretions of fish, including cutaneous and gut mucus. The Ab response and serum concentration of IgM may vary between teleost species. The salmonids are high responders that produce a relatively large amount of specific Abs to a variety of Ags, but strongly regulate the response toward certain epitopes. On the contrary, a characteristic feature of *Gadus morhua* is that no, or only a low, increase in Ab levels are seen after

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immunisation with hapten-carriers or bacteria (Solem and Stenvik, 2006).

Teleost B cells have been directly defined in a number of species due to the development of monoclonal Abs (mAbs) to fish immunoglobulins and the identification of IgH and IgL chains genes. In sea bass, several mAbs have been generated and used to study the distribution of Ab-producing cells and to enrich immunoreactive cells with B lymphocytes (reviewed in Chistiakov et al., 2007). Polyclonal Abs have also been employed to detect IgM+ cells in fish, such as Atlantic cod (Ronneseth et al., 2007), halibut (Grove et al., 2006) and turbot (Fournier-Betz et al., 2000).

The production of specific Abs in response to parasites has been reported for several piscine parasitoses, on some occasions in relation to protection. The available information, mainly dealing with humoral Abs, but also with mucosal Abs and with the presence of Ab-bearing cells, is commented below (Section 7).

6. Expression of immune genes in fish parasitoses

As reviewed above, a substantial number of fish immune genes have been characterized at the molecular level. Although information on their expression in parasitoses is still scarce, some data has recently been obtained that allows their role during these infections to be deduced. Most studies are based on the use of real time quantitative PCR (rtqPCR), but semiquantitative PCR and hybridisation to microarrays were employed in some cases.

6.1. Ciliophora

The most extensively studied infection regarding the expression of immune genes is that produced by *I. multifiliis* in experimentally infected fish. Proinflammatory cytokines, IL-1β and TNF-α were up-regulated in infected *O. mykiss* (Sigh et al., 2004a) and *Cyprinus carpio* (Gonzalez et al., 2007b), and this response was produced in the early phases of infection. IL-8 and the type II IL-1 receptor were also expressed in the former host (Sigh et al., 2004a). Expression of the enzyme iNOS was slightly increased in the skin of *O. mykiss* (Sigh et al., 2004b) and clearly up-regulated in *C. carpio* (Gonzalez et al., 2007b). In this fish, the chemokine receptor CXCR1 and CXCa gene were also up-regulated, mainly in skin but also in blood; in addition, CXCR2 and the arginase 2 genes were specifically induced in blood. Up-regulation of C3 complement occurred in both hosts, whereas MHCII and IgM genes were up-regulated in head kidney and skin of infected *O. mykiss* (Gonzalez et al., 2007c; Sigh et al., 2004b). The role of the skin as a main player in APR was demonstrated by the early and dramatic up-regulation of factorB/C2-A and SAA in carp skin (Gonzalez et al., 2007a). New studies in *C. carpio* have demonstrated down-regulation of prostaglandin D2 synthase (PGDS) and microglobulin (β2-m)-2 and up-regulation of complement factor 7 (C7) in skin, and the CC chemokine molecule SCYA 103 in the liver of infected fish (González et al., 2007d). These studies demonstrated that expression of immune genes was generally higher in the skin, the target tissue (which plays an important role in modulating the local inflammation) and that mucosal Abs can be produced at the site of infection. Expression and regulation of evaluated genes in blood confirmed the important role of migrated leucocytes in the carp immune response against *I. multifiliis*. In addition, a differential transcription of the four isoforms of the antiprotease α2-macroglobulin was seen in the liver of carp infected with *I. multifiliis* (Onara et al., 2008).

Some information has been also obtained for the scuticociliate *Philasterides dicentrarchi*. Cysteine proteases of this ciliate increased the expression levels of IL-1β in turbot HK cultured in vitro (Paramá et al., 2007).

6.2. Amoebozoa

Several studies have recently been performed on the host immune response to *Neoparamoeba perurans*, the etiological agent of AGD (Young et al., 2007). IL-1β and iNOS were significantly up-regulated in the gills and IL-8 in the liver of infected *O. mykiss* (Bridle et al., 2006a). In *Salmo salar*, up-regulation of IL-1β occurred in the AGD-affected gill tissue without changes in the expression of either TNFα or iNOS (Bridle et al., 2006b; Morrison et al., 2007). Recent microarray and qRT-PCR analysis confirmed that the majority of the transcriptional response to AGD in *S. salar* was restricted to lesions, and no evidence of coordinated innate or adaptive immune responses was observed. In fact, a down-regulation of IFN-γ and MHC genes may inhibit the development of acquired immunity and could explain the high susceptibility of Atlantic salmon to AGD (Young et al., 2008). In addition, a recent study by Wynne et al. (2007) suggests the association of salmon resistance to AGD to MHC polymorphisms, as AGD severity was associated with the presence of determined MH alleles. However, the need for further studies was stressed, considering the small sampling size.

6.3. Kinetoplastida

The available information is related to *Trypanoplasma borreli* infection in carps. Saeij et al. (2003a) found up-regulated expression of TNFα, IL-1β and mRNAs for APPs (complement factor 3, SAA and α2-macroglobulin) in fish injected with this flagellate. In addition, an association between one TNF2 isoform and resistance was found (Saeij et al., 2003b), as well as a differential transcription of the four isoforms of the antiprotease α2-macroglobulin (Onara et al., 2008).

6.4. Myxozoa

Modulation of expression of some immune genes has also been studied for some myxozoans infections. TNF-α2, COX-2 and, to a lesser extent, TGF-β1 genes were up-regulated in *O. mykiss* during a natural outbreak of proliferative kidney disease (PKD) produced by *Tetracapsuloides bryosalmonae* (Holland et al., 2003). Some proin-

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flammatory and oxidative pathway-related genes (IL-1β, TGF-β and COX-2) were also up-regulated in *O. mykiss* after exposure to *Myxobolus cerebralis* and some expression differences were detected between susceptible and non-susceptible rainbow trout strains (Severin and El-Matbouli, 2007). In the same host/parasite model, the expression of natural resistance-associated MΦ proteins (Nramp α and β) decreased in infected susceptible rainbow trout (Rucker and El-Matbouli, 2007). In the intestine of *Sparus aurata* chronically exposed to *E. leei*, a down-regulation of IL-1β, TNF-α, glutathione peroxidase-1 (GPX-1), and an up-regulation of α-2M and the heat shock protein GRP-75 (mortalin) were observed using rtqPCR, but no significant changes were seen in HK (Sitjà-Bobadilla et al., 2008b). However, up-regulation of the proinflammatory IL-1β was detected soon after exposure by Cuesta et al. (2006c), using semiquantitative PCR.

6.5. Monogenea

Some data is also available for monogenean infections. In the skin of *O. mykiss* parasitized by *Gyrodactylus derjavini*, a clear induction of IL-1β expression was observed during the initial phases of primary infections, whereas the type II IL-1β receptor was expressed later (Lindenstrøm et al., 2003). In addition, up-regulation of TNF-α, iNOS and COX-2 were detected, whereas no clear parasite related changes in transcript levels of TCR-β and MHC IIβ could be observed (Lindenstrøm et al., 2004). In *S. salar* infected by *Gyrodactylus salaris* Mc1-1 (related to IL-1β) was up-regulated (Matejusová et al., 2006). Further studies indicated that expression of MHC I, Mx, IFN-γ and CD8α genes increased in susceptible responding Baltic *S. salar*, whereas changes were slight in highly susceptible non-responding Danish salmon, and no increase in Ig genes was detected in any of the strains (Kania et al., 2007). Using microarrays, a change in the expression levels of several immune-related genes [(matrix metalloproteinases)-9 and 13, leukotrine-B4 receptor, CD20 receptor, MHC class I and class II β chain, Ig H and L chains] was demonstrated in peripheral blood leucocytes of *Paralichthys olivaceus* during the infection by *Neoheterobothrium hirame* (Matsuyama et al., 2007). The pro-inflammatory cytokines IL-1β (spleen and gills) and TGF-β (gills) were up-regulated in *Diplectanum aequans*-infected *Dicentrarchus labrax*, but no relationship was found between TCR-β expression and the parasite infection (Faliex et al., 2008).

6.6. Crustacea

The little data known for crustacean infections deals with copepods. In low-level *Lepeophtheirus salmonis* infections of *Salmo salar*, differential MH gene expression (decreasing in MH I and increasing in MH II), as well as up-regulation of IL-1β were observed, but no changes occurred in COX-2 expression (Fast et al., 2006a). In contrast, after repeated exposure to the parasite, which also induces an stress response, IL-1β, TNF-α, TGF-β, COX-2 and MH II genes were up-regulated at 9 days post-exposure, but most of them returned to control levels by day 21 p.i., when MH I gene showed the highest expression level; new increases in the expression of some genes occurred later, but the immunological stimulation did not reduce parasite numbers (Fast et al., 2006b). Chang et al. (2005) found up-regulation of C3 complement, MHC I, α-2-macroglobulin, source of immunodominant MHC-associated peptides (SIMP) and TNF receptor-associated factor 2 binding protein (T2BP) in the liver, and down-regulation of SIMP in the gills of *Ctenopharyngodon idella* infected by *Sinergasilus major*. Recently, an up-regulation of CxCa, CXCR1 and TNF-α was observed at the skin level in *C. carpio* exposed to the branchiuran *Argulus japonicus* (Forlenza et al., 2008).

In conclusion, differences in the gene expression according to the considered tissue were noticed in some cases, with the effect being generally higher in target tissues, as in the case of *I. multifiliis* or AGD. The expression of some genes can also change according to the phase of infection. Genes related to the proinflammatory response are generally up-regulated in the first steps, as illustrated by *I. multifiliis* infections. Their expression can persist after repeated exposure, as in *L. salmonis* infected salmon, or decrease in non-target organs, as in chronic *E. leei* infections of gilthead sea bream.

7. Protective responses in fish parasitoses and prospects for immunoprophylaxis: data on vaccination against parasites of fish

Some vaccines are routinely used in aquaculture production of some species, such as rainbow trout, salmon, turbot, gilthead sea bream, Mediterranean sea bass and tilapias, and are mainly bacterial vaccines (Gudding et al., 1999; Midtlyng, 2000; Biering et al., 2005; Håstein et al., 2005; Sommerset et al., 2005). Molecular approaches to obtain fish vaccines have also been assayed, but most information deals with antiviral vaccines (Winton, 1998; Biering et al., 2005; Lorenzen and LaPatra, 2005; Tonheim et al., 2008).

The initial evidence of the existence of protection in piscine parasitoses was deduced from empirical observations on resistance to re-infection following epizootics, which was the basis for further studies with vaccination purposes. The obtaining of parasite vaccines is impaired, among other factors, by the lack of a continuous source of the parasites, as *in vitro* culture is available only for a few of them. Nowadays, no commercial or routinely used vaccine is available for any fish parasite, although some experimental vaccines exist for certain flagellates and ciliates (see below). However, studies on immunization with the further purpose of vaccine preparation have been performed for several parasites. The available information is summarised in this section. Studies on the mechanisms involved in protection are scarce and were mainly focused on the search of specific Abs. Most data were obtained in experimental infections or immunizations, but some information on natural infections is also included.

7.1. Ciliophora

The protective response to *Ichthyophthirius multifiliis* (Ich) and the vaccine development have recently been

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reviewed by Matthews (2005). It is sufficient here, therefore, to cover only some relevant aspects and new information. Acquired protective immunity against ichthyophthiriosis is well recognised in teleosts (Buchmann et al., 2001b; Matthews, 2005). Data on protective immunity have been obtained from naturally and experimentally infected fish and mainly after immunization with live or killed parasite stages (mainly using *O. mykiss*, *I. punctatus* and tilapias). Apart from the non-specific cellular and humoral factors activated in Ich infections (see Section 3), specific Abs are produced against this ciliate and they can play a marked role in immunity to the ciliate. Some Abs react with immobilization Ags (i-antigens), that are well characterized for *I. multifiliis* and different serotypes are recognised (Dickerson and Clark, 1996; Matthews, 2005). High serum levels of immobilizing Abs are a characteristic feature of the immune response to Ich. However, cutaneous Abs are involved in parasite clearance (Clark and Dickerson, 1997) and can contribute to the response elicited at the mucosal skin. As suggested by Buchmann et al. (2001a,b), the activation of the classical complement pathway can neither be excluded.

Immunization assays have generally demonstrated a better protection after exposure to live ciliates than when killed ciliates are inoculated (Xu et al., 2004). In addition, exposure to live theronts is more effective than i.p. inoculation and induce cross-protections in *I. punctatus* against different serotypes (Swennes et al., 2006, 2007; Xu et al., 2006b), which also points to a role for skin mucosal immunity. The mechanisms involved in protection are yet to be fully elucidated. Serum Ab titres, as detected by ELISA, do not always correlate with protection. In addition, immobilization activity and specific Abs in serum are not clearly correlated (Sigh and Buchmann, 2001). Thus, other factors besides i-antigens could be involved in the immobilization process and clearance of the parasite, and in the initiation of protective immunity (Matthews, 2005). Recent data of Xu et al. (2008) also point to protection of *Oreochromis niloticus* not only depending on serum Ab response. Available information points to the involvement of several immune mechanisms, mainly at the skin, the target organ, as corroborated by recent studies on expression of immune genes (see Section 6).

The situation of vaccine development against Ich has been thoroughly reviewed by Matthews (2005). Although acquired protective immunity is established in fish following administration of live ciliates or killed/sonicated preparations, the obtaining of a commercial vaccine is impaired by the lack of a continuous and standardized source of the parasite, as continuous *in vitro* culture has yet to be developed. Therefore, several studies aiming at the obtaining of recombinant vaccines have been performed. One approach is based on the use of *Tetrahymena termofila*, a ciliate closely related to *I. multifiliis* and readily cultured *in vitro*, as a delivery system of an i-antigen gene (Lin et al., 2002). Nevertheless, the toxic residues of cadmium originating from the gene promoter can accumulate in fish epidermis, raising serious health and environmental issues (see Matthews, 2005). In spite of some promising results, no recombinant vaccine is yet available. One important limitation of these Ags is that protection is directed against the homologous serotypes of the parasite.

Studies on fish immune response to histophagous *scuticociliates* have recently increased due to the pathological impact of this ciliatosis in fish-farming, mainly for turbot (*Psetta maxima*) and Japanese flounder (*Paralichthyis olivaceus*). These ciliates can be readily maintained *in vitro* in continuous culture, and thus a source of the parasite is guaranteed (Iglesias et al., 2003a; Alvarez-Pellitero et al., 2004a,b). Empirical data from field observations pointed to the acquisition of disease resistance in fish surviving scuticociliate epizootics. Serum-specific Abs were found in turbot using ELISA, both in natural infections and in fish inoculated with different formulations of killed ciliates, and certain protection against challenge was obtained (Iglesias et al., 2003a,b; Sanmartín et al., 2008; Sitjà-Bobadilla et al., 2008a,b; Palenzuela et al., unpublished results). Partial protection and production of specific Abs were also obtained in olive flounder immunised with scuticociliates (Jung et al., 2004). According to Iglesias et al. (2003a,b), *Philasterides dicentrachi* expresses immobilisation Ags that could be involved in turbot protective responses. However, in several cases, the Ab levels were not correlated with protection, and little or no correspondence was observed between the agglutinating activity of serum and the levels of specific Abs evaluated by ELISA (Iglesias et al., 2003b; Palenzuela et al., unpublished results). A change of i-antigens after agglutination as a mechanism of immune evasion, explaining the absence of lysis and further escape of the parasite, has been suggested, as well as the presence of other surface Ags besides the i-antigens (Iglesias et al., 2003b; Lee and Kim, 2008). As in the case of *I. multifiliis*, other immune factors could be involved in protection against scuticociliates. Some innate immune factors were studied in a small scale immunization trial using a formalin-killed trivalent formulation (a mix of three isolates from different origins) with the adjuvant Montanide ISA 763A (Sitjà-Bobadilla et al., 2008a,b). Significant increases in lysozyme and complement were only detected after challenge with live ciliates and also occurred in control fish. A relationship between different immune factors was suggested by their correlation within individual fish, mainly between Ab levels and complement.

In medium-scale vaccination trials using killed ciliates in different vaccine formulations, variable levels of protection were observed (Sanmartín et al., 2008; Palenzuela et al., unpublished results). Promising results were obtained with a trivalent Ag, in comparison with monovalent and pentavalent Ags as a certain protection was obtained even without adjuvant. The obtained results also suggested antigenic differences between isolates. Thus, further studies should address large-scale experiments insisting on optimum adjuvants, dosages and polyvalent Ags, in order to obtain a combined effect on the different immune response pathways allowing cross-protection without induction of tolerance. Standardization of the challenge procedure is also needed.

Burgess and Matthews (1995) reported acquired protection to the ciliate *Cryptocaryon irritans* in *Chelon labrosus* surviving a controlled infection, and the same ciliate produced humoral Abs in serum of immunized *Lates calcarifer* (Bryant et al., 1999). Some experimental data on the immunization of grouper *Epinephelus coloides* against

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C. irritans with live or formalin-killed theronts indicates the acquisition of protection and a good correlation with Ab titres in skin mucus and serum (Yambot and Song, 2006; Luo et al., 2007).

7.2. Kinetoplastida

Cryptobia salmositica is the best studied species amongst flagellates regarding protective immunity and vaccination. The available knowledge has been extensively reviewed by Woo and Poynton (1999) and Woo (2001, 2003). The first observations indicated that fish that had recovered from the infection were resistant to challenge. Specific Abs (agglutinating, neutralizing and complement fixing), detected in sera of recovered and experimentally infected fish, are involved in protective immunity. Specific Abs fix complement to lyse the parasite by the classical pathway and their titres rise significantly after *Cryptobia salmositica* challenge. This classical anamnesic response also confirms that protective immunity is in part due to a humoral response in infected fish. The effectiveness of the immune response has allowed the developing of immunoprophylactic strategies.

Cryptobia salmositica can be cultured *in vitro.* Minimum essential medium (MEM) supplemented with serum vitamins and nutrients is the medium of choice, as the parasite multiplies rapidly and can be repeatedly subcultured (reviewed in Woo and Poynton, 1999). A strain that has been cultured for several years produces low parasitaemias and does not cause disease in fish but protects them. This avirulent strain has remained infective, non-pathogenic and protective for well over a decade, and thus it can be used as a live attenuated vaccine: experimentally vaccinated *Oncorhynchus* spp. remained protected for at least 2 years with a single dose (Woo, 2001, 2003). This attenuated strain does not produce an important disease-causing factor, a metalloprotease, and does not multiply readily in rainbow trout. Prolonged *in vitro* culture also induces some antigenic changes in *Cryptobia salmositica*. The virulent strain was found to have a more negative surface charge than the avirulent strain. In addition, the increase in surface carbohydrate residues in the latter coincides with its loss of virulence. A monoclonal Ab (mAb-001) was produced against the 200-kDa glycoprotein, whose epitope (Cs-gp200) has a phosphatidylinositol residue which anchors the conformational polypeptide to the surface membrane. Cs-gp200 has high mannose components and it appears as a doublet in the pathogenic strain and as a single band in the attenuated strain. The mAb-001 cross reacts with the carbohydrate moieties of the metalloprotease and the cysteine protease of the parasite and inhibits their enzymatic activities. Thus, mAb-001 reduces multiplication, infectivity and survival of the parasite, and is also therapeutic when inoculated in juvenile fish (Woo, 2003). A recombinant cysteine protease (49 kDa) has been produced and could be the base for a future recombinant vaccine (reviewed in Woo, 2001, 2003).

Susceptibility to cryptobiosis varies between individuals of the same species and between different species. In the *Cryptobia*-tolerant charrs *Salvelinus fontinalis*, the immune system readily controls the infection, thus allowing a rapid recovery, and resistance seems to be associated to an antiprotease (see Section 3.4.4). Chin et al. (2004) found differences in susceptibility and Ab production against both the pathogenic and the vaccine strains of *Cryptobia salmositica* between different full-sib families of *Salmo salar*, with increased susceptibility being associated with a delayed Ab response.

Adaptive immune response was demonstrated in carps inoculated with live *Trypanoplasma borrelli* by the presence of parasite-specific Abs. Moreover, Ab production seemed to correlate with clearance of infection (Jones et al., 1993). According to Saeij et al. (2003a), specific Abs against *Trypanoplasma borreli* are able to lyse the parasite in the presence of complement, by the classical pathway. Differences in susceptibility of fish hosts to infection have been reported, and resistance can be associated to differences in the production of specific Abs and to TNF2 polymorphism (Wiegertjes et al., 1995; Saeij et al., 2003b). Scharsack et al. (2004) found differences in the spectrum of immunomodulatory mediators produced by lymphocyte cells between susceptible and resistant carps, and suggested the involvement of a strong polyclonal activation in susceptible lines.

Goldfish that have recovered from previous *Trypanosoma carassii* infection are protected against subsequent homologous challenge. The involvement of an adaptive immune response was demonstrated by the presence of neutralizing Abs in the plasma of immune fish (reviewed in Woo and Poynton, 1999). Passive immunization was also obtained by injecting fish with IgM purified from serum of recovered carp (Overath et al., 1999). Immunization of goldfish with excretory/secretory (ES) products of the parasite conferred protection against infection only when ES were administered with Freund's complete adjuvant (FCA), suggesting the involvement of cell-mediate response (Bienek et al., 2002). Partial Ab-mediated protection of *C. auratus* against a challenge infection with *Trypanosoma carassii*, was obtained after administration of recombinant-β-tubulin + FCA (Katzenback et al., 2008).

7.3. Amoebozoa

Resistance of Atlantic salmon to AGD after secondary exposure was reported by Findlay and Munday (1998) on the basis of gill pathology. In contradiction with this result, an absence of resistance to challenge in exposed fish was further observed (Gross et al., 2004a). Anti-*Neoparamoeba* sp. Abs were detected in salmon farmed in Tasmania (Australia), but their presence was not correlated with protection (Gross et al., 2004b). However, further studies demonstrated that Atlantic salmon exposed and subsequently challenged with AGD are more resistant than naïve control fish, and such resistance was associated with anti-*Neoparamoeba* sp. Abs in serum (Vincent et al., 2006).

7.4. Dinoflagellata

Some observations had suggested a resistance of fish to amyloodioniosis following repeated challenges. The existence of an acquired immunity was demonstrated in *Oreochromis aureus* immunised with *Amyloodinium ocella-*

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tum, as specific Abs were detected in an ELISA assay (Smith et al., 1992). Anti-*A. ocellatum* Abs were also found in sera of cultured stripped bass having recovered from an amyloodiniosis outbreak (Smith et al., 1994). Further observations of Cobb et al. (1998a,b) in *Amphiprion frenatus* confirmed the association of acquired immunity with the Ab response. Cecchini et al. (2001) also found specific Abs in Mediterranean sea bass naturally infected by amyloodiniosis.

7.5. Microsporidia

Specific Abs against several microsporidians have also been found in natural infections, though in most cases were detected after immunization with purified spores, such as in turbot injected with *Tetramicra brevifillum* (Leiro et al., 1993) and in *Platichthys flessus* inoculated with *Spraguea lophii* and *Glugea anomala* (Pomport-Castillon et al., 1999). In *Plecoglossus altivelis*, Abs were detected after oral administration of *G. plecoglossi* intact spores, but the Ab production played no protective role against infection (Kim et al., 1996). Specific Abs were observed in serum of Japanese eels naturally and experimentally infected by *Pleistophora anguillarum* (Hung et al., 1997). A specific humoral response was elicited in natural infections of *Epinephelus akaara* with *G. epinephelusis*, though the intensity of infection was not correlated with the Ab level (Zhang et al., 2005). A low virulent strain of *Loma salmonae* has been used to develop a whole-spore vaccine to limit microsporidian gill disease in trout. When tested in an experimental infection, i.p. injection of the vaccine was effective, and the addition of adjuvant did-not improve vaccine performance against the disease-causing microsporidian (Sanchez et al., 2001; Speare et al., 2007).

7.6. Myxozoa

Fish recovered from previous infection by *Tetracapsuloides bryosalmonae* are relatively more resistant to challenge infections (Ferguson and Ball, 1979). Salmonids develop some resistance against *Ceratomyxa shasta* (Bartholomew, 1998). Humoral specific Abs have been found in several infections by myxozoans, such as *C. shasta* (Bartholomew, 2001), *Myxobolus cerebralis* (Griffin and Davies, 1978; Hedrick et al., 1998), *M. artus* (Furuta et al., 1993) and *Enteromyxum scophthalmi* (Sitjà-Bobadilla et al., 2004). The acquired protection of turbot that had survived enteromyxosis appeared to be related with the production of specific anti-*E. scophthalmi* Abs (Sitjà-Bobadilla et al., 2007). In contrast, no humoral Abs were detected after immunization of sea bass with *Spharospora dicentrarchi* spores, although Ab secreting cells were observed using ELISPOT (Muñoz et al., 2000a). In turbot experimentally infected with *E. scophthalmi*, Ig+ cells increased in the intestine, the target organ, but decreased in kidney and spleen (Bermúdez et al., 2006).

7.7. Monogenea

Some data on the Ab responses and acquisition of acquired protection against monogeneans is available (see Buchmann et al., 2001a,b). Abs against *Pseudodactylogyrus bini* and *P. anguillae* (Mazzanti et al., 1999) were detected in eels (Buchmann, 1993; Mazzanti et al., 1999), but the significance of this humoral response in protection has not been clarified (Nielsen and Esteve-Gassent, 2006). Specific Abs against *Diplectanum aequans* Ags were detected in sera of sea bass infected by the monogenean using Western Blot analysis (Monni and Cognetti-Varriale, 2001). Abs also seem to be involved in the partial immunity observed in *O. mykiss* immunized with *Discocotyle sagittata* extracts (Rubio-Godoy et al., 2003a). High Ab titres were also detected in farmed trout, although no correlation was found between infection intensity and Ab titres in individual fish and thus the precise role of Igs could not be established (Rubio-Godoy et al., 2003b). *Seriola dumerili* and *Paralichthys olivaceus* recovered from *Neobenedenia girellae* infection acquired partial protection against re-infection. However, the involved factors were not investigated (Ohno et al., 2008). As for polyopisthocotyleans, Kim et al. (2000) reported protection of rockfish *Sebastes schlegeli* after immunization with *Microcotyle sebastes* Ag, but the involved immune factors were not determined. Tiger puffer produces Abs against *Heterobothirum okamatoi* (Wang et al., 1997), though no clear relationship could be established between the acquired protection induced by persistent infection and the serum Ab levels (Nakane et al., 2005).

7.8. Digenea

Some digenean infecting fish in the metacercarial phase induce protective or Ab responses in fish. Abs are produced in several fish hosts against *Cryptocotyle lingua* and *Diplostomum pathaceum*. Some protection against challenge infection of the latter has been recorded, and the participation of an antibody-dependent cell-mediated cytotoxic mechanism has been proposed (Whyte et al., 1990), but other immune mechanisms are likely to be involved (reviewed in Buchmann et al., 2001a). Specific Abs against *Rhipidocytle fennica* were detected in wild roach *Rutilus rutilus*, and also in roach immunized with trematode cercariae. The response was stronger when infecting fish with living cercariae and also depended on the route of immunization. Previous infection gave some protection against this digenean (Aaltonen et al., 1997). Specific Abs were also found in *Cyprinus carpio* injected with cercariae of *Sanguinicola inermis* but not in fish exposed to the parasite (Roberts et al., 2005). In contrast, an Ab response and the development of acquired resistance were found in *Thunnus maccoyii* naturally infected with the sanguinicolid *Cardicola fosteri* (Aiken et al., 2008).

7.9. Cestoda

Scarce information is available on protective and Ab responses of fish to cestodes. Sharp et al. (1989) reported that specific Abs are elicited in the response of rainbow trout to natural infections of *Dyphyllobothrium* spp.

7.10. Nematoda

Anguilla spp. produce specific Abs against the swim bladder *Anguillicola crassus* Abs were directed against

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surface Ags and also against the detoxifying enzyme glutathione-*S*-transferase, which could thus be a possible target for a vaccine (reviewed in Buchmann et al., 2001a; Nielsen and Esteve-Gassent, 2006). Recently, experiments of vaccination of eels with irradiated L_3 of *A. crassus* suggested that *A. japonica*, the original host, is able to mount efficient protective responses against this nematode, whereas the newly acquired host, *A. anguilla*, seems to lack this ability (Knopf and Lucius, 2008). Antarctic fish produce specific Abs against anisakids. Anti-*Contracaecum osculatum* Abs were detected in plasma and anti-*Pseudoterranova decipiens* Abs in bile and plasma of naturally infected fish (Coscia and Oreste, 1998, 2000).

7.11. Mollusca

Acquired resistance of *Lepomis macrochirus* against the glochidia of the mollusc *Utterbrackia imbecillis* was observed after four sequential infection periods, and it was associated with humoral and mucosal Ab primary and secondary responses (Rogers-Lowery et al., 2007).

7.12. Crustacea

Sea lice, *Lepeophtheirus salmonis*, is an economically important parasite of farmed salmonids. In the search for preventive measures, some studies on the immune response to this infection have been carried out. Farmed Atlantic salmon developed a serum Ab response following natural infection by the crustacean (Grayson et al., 1991). In addition, injection of extracts of the parasite induced the production of specific Abs in the same fish (see Reilly and Mulcahy, 1993). However, the Ab responses were not protective when fish were challenged with adult and preadult stages. Grayson et al. (1995) demonstrated partial protection and an effect on louse fecundity, but the protective Ags were not identified and there was not replication of treatment groups. Raynard et al. (2002) reviewed the present knowledge about the vaccination of salmon against this crustacean. One of the novel approaches was based on targeting the louse gut immunologically and some recombinant Ags were assayed. However, this approach requires the identification and evaluation of critical Ags. The authors concluded that research toward such vaccines is still in its infancy; Abs could target critical host–parasite interactions that are amenable to disruption, but such targets have not been identified. Recent expression studies have demonstrated the stimulation of some immune factors (see Section 6), but such response did not protect fish against re-infection (Fast et al., 2006b).

In conclusion, basic studies are still required to obtain the information on protective immune responses which could allow the further development of immunprophylactic measures. Even for the most studied parasites, *I. multifiliis*, scuticociliates and *Cryptobia salmositica*, new studies are necessary to guarantee a standardized source of Ag, and the obtaining and delivery of appropriate formulations. Although scuticociliates and *Cryptobia salmositica* can be cultured *in vitro*, the standardization needed for a commercial vaccine is lacking. The use of live vaccines, as in the case of *Cryptobia salmositica*, has important practical and legal restrictions. The characterization of Ags susceptible of being used in recombinant vaccines is an interesting approach, already initiated for some parasites, which should be developed. In addition, provided scientific challenges could be overcome, the development of commercial piscine vaccines is constrained by economic and practical difficulties. Once a technology and a product have been selected, a development plan is necessary to assure that formulations are safe, stable and efficacious. Disease risk, vaccine efficacy and market price must be evaluated to assess the feasibility of vaccination as a management tool (Ellis, 2001; Thorarinsson and Powell, 2006).

Other strategies based on genetic selection of resistant strains and on immunomodulation, which have also been suggested, are beyond the scope of this review. Some examples of differences in susceptibility to parasitoses between fish species and strains, on some occasions linked to immune genes have been exposed (see also Sitjà-Bobadilla et al., 2007). They should, in any case, be considered in the context of the integrated knowledge of the protective responses.

8. Concluding remarks

The available information indicates that fish respond to parasite infections by activating different innate and adaptive immune mechanisms. Studies have been mainly focused on some innate activities, such as inflammatory and cellular reactions, phagocytic activities, complement and other humoral factors (mainly including lysozyme and molecules involved in APR), most of which were demonstrated to be involved in the immune response to parasitoses. In relation to adaptive immunity, most information relies on the production of specific Abs, though Ab titres are not always correlated to protection. Gene expression studies have allowed the confirmation of the involvement of some innate immune mechanisms, mainly those related to inflammation and some oxidative pathways, even though changes according to the phase of infection and the considered organ were observed. These expression studies confirmed the relevance of mucosal immunity, mainly at the target organ, in the response to *I. multifiliis* and other gill and skin parasites, as *Neoparamoeba* sp. and *Lepeophtheirus salmonis*. Information is scarce for those genes more directly related to adaptive immunity; some results suggest the involvement of MH genes in AGD and lepeophtheiriosis of Atlantic salmon, and in the infections of *O. mykiss* by *Gyrodactylus derjavini*, and *Ctenopharyngodon idella* by *Sinergasilus major*, but no clear changes could be detected in the limited studies on the expression of TCR or IgM.

Therefore, in spite of the increasing research into the immune response to fish parasites in recent years, there are yet important gaps in the knowledge of the mechanisms involved in protection. An integrated approach is necessary and should start with the characterization of PAMPs which could be involved in both innate and adaptive responses. More studies of the role of NCCs, MHC and TCRs are also necessary, although these possible

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studies are impaired by the lack of adequate tools and markers. Despite the fact that Abs are produced in response to a number of parasites, the characterization of the Ags involved in the response is lacking in most cases, and additionally the production of specific Abs against parasites can take a long time in fish, due to certain particularities of their adaptive responses, including their poikilotherm nature. Thus, an effective application of immunoprophylactic measures in piscine parasitoses is far to be established, and basic studies are still required for most species. Even for the most studied parasites regarding vaccination, as *I. multifiliis*, *Cryptobia salmositica* and scuticociliates, only experimental vaccines are available. An integrated study is necessary to obtain the adequate knowledge of the mechanisms involved in protection. The stimulation of innate immune factors is necessary, but often insufficient to pathogen clearance. As Medzhitov (2007) has pointed out, "what matters to the host organism is not the induction of an immune response but whether the immune response protects against a given infection". The necessary knowledge on the pathogen features and the host effector mechanisms is far to be obtained even for many human pathogens, and such information is in its infancy for most fish parasites.

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