Article available at http://www.parasite-journal.org or http://dx.doi.org/10.1051/parasite/2008153420

Fish immune response to Myxozoan parasites

SITJÀ-BOBADILLA A.*

Summary:

Myxozoan parasites are responsible for important economic losses among fisheries and aquaculture industries, and hence the high interest in studying the immune response of fish against them. The most important data available concerning the immune response of fish against myxosporeans are reviewed, with emphasis on the different innate and adaptive immune mechanisms, their relationship with natural and acquired resistance and the strategies to control and prevent myxosporoses. Cellular effectors (lymphocytes, granulocytes, phagocytes, non-specific cytotoxic cells, rodlet cells) and humoral factors (lysozyme, peroxidades, antiproteases, complement, specific antibodies) have been examined for several myxosporoses, and some immune relevant genes have been studied. This information will be crucial for the future development of vaccines and other preventive strategies such as immunomodulation and selection of disease-resistant strains

KEY WORDS : Immune response, fish parasites, Myxosporea.

The Myxozoan phylum gathers more than 2,180 species, being most of them fish parasites, though their presence has also been documented in waterfowl, amphibians, reptiles and even mammals (Lom & Dyková, 2006). Myxozoa are long-known organisms, which still today pose important questions about their origin, phylogenetic relationship and life cycle (Kent *et al.*, 2001; Fiala, 2006). They are responsible for important economic losses among fisheries and aquaculture industries, and hence the high interest in studying the immune response of fish against them. However, research in this group of multicellular organisms is held back by the lack of *in vitro* cultures and the difficulty to set up experimental transmission models. Although the fish immune response has some special features, a functional parallelism between fish and mammalian system is increasingly confirmed by recent findings, and both innate and adaptive immune effectors against parasites do exist in teleosts. This review is focused on the most important data available concerning the immune response of fish against myxosporeans, with emphasis on the different innate and adaptive immune effectors, their relationship with natural and acquired resistance and the strategies to control and prevent myxosporoses.

* Instituto de Acuicultura de Torre de la Sal, Consejo Superior de Investigaciones Científicas, Torre de la Sal s/n, 12595 Ribera de Cabanes, Castellón, Spain.
Correspondence: Dr Ariadna Sitjà-Bobadilla.
Tel.: +34 964 319 500 – Fax: +34 964 319 509.
E-mail: ariadna@iats.csic.es

NON-SPECIFIC IMMUNE RESPONSE

Cellular immune response

Many myxosporean species cause little or no host response, especially coelozoic ones. In some cases, the absence of cellular reaction is due to their development in immunoprivileged sites (such as the central nervous system, eyes or gonads), where the immune system has particular or reduced functions. It has been shown that penetrating triactinomyxon-sporoplams of *Myxobolus cerebralis* reach the cartilage via peripheral nerves and the central nervous system (El-Matbouli *et al.*, 1995). It seems plausible that the actinosporean stage uses this penetrating route to avoid immune response to multiply in high numbers before reaching other hostile sites. Similarly, *Sphaerospora testicularis* exploits efficiently the immune-privileged condition of the seminiferous tubules of European sea bass (*Dicentrarchus labrax*). It proliferates within their lumen without the interference of any cellular reaction (Sitjà-Bobadilla & Alvarez-Pellitero, 1993). On the other hand, some myxosporeans invoke an excessive inflammatory response leading to an immunopathological condition, as in *Tetracapsuloides bryosalmonae*-infected rainbow trout, in which the proliferation of lymphocytes is the major cause of renal tissue hyperplasia (Chilmonczyk *et al.*, 2002). The same happens in *Ceratomyxa shasta*-infected fish, which develop a vigorous host response, consisting of lymphocytes and eosinophilic granular leucocytes (Bartholomew *et al.*, 1989). Some authors have pointed out that severe inflammatory reactions are found in abnormal hosts for the myxosporean (Kent & Hedrick, 1985). The commonest picture observed in histopathological studies is the encapsulation of the parasitic stages by connective, fibrotic and epitheliod tissue layers, which is aimed to isolate the parasite and to prevent its dis-

persal to surrounding tissues (Davies & Sienkoswki, 1988). These granulomata are often accompanied by melanomacrophage centres, and their presence can also be increased in head kidney and spleen. However, the efficacy of this encapsulation could be limited, as in *Myxobolus pendula* in which the capsule does not appear to inhibit diffusion of oxygen and nutrients to the parasite (Koehler *et al.*, 2004), or in *S. testicularis* infected sea bass testis, in which the large granulomata reduce the areas of germinal tissue up to the next spawning season (Sitjà-Bobadilla & Alvarez-Pellitero, 2003).

Several cellular types are involved in the response against myxosporeans. Rodlet cells (RC) are among them, as they are generally increased in infected tissues or have been observed in close vicinity to myxosporeans, some times even discharching their rods (Leino, 1996; Palenzuela *et al.*, 1999; Muñoz *et al.*, 2000a). By contrast, RC are very scarce in *D. puntazzo*, even when infected by *E. leei* (Alvarez-Pellitero *et al.*, 2007). RC are thought to be connected with inflammatory cells and host defence against parasites (Reite, 2005), but their function is still controversial and their specific role in myxosporean infections is unknown. In addition, different types of leucocytes and their functions have been studied in several myxosporoses. Mononuclear inflammatory cells were present in perichondral and periosteal connective tissue foci, but were notably absent in areas of cartilage necrosis due to *Myxobolus hyborhynchi* (Cone & Frasca, 2002). Phagocytes and granulocytes were present in the ascitic fluid of European sea bass infected by *S. testicularis* (Sitjà-Bobadilla & Alvarez-Pellitero, 1993). Eosinophilic granular cells and other granulocytes can be increased at the site of the infection (intestine) (Alvarez-Pellitero *et al.*, 2007) or in blood (Sitjà-Bobadilla *et al.*, 2006) in enteromyxosis, but its presence can be decreased in haematopoietic organs such as head kidney and spleen (Cuesta *et al.*, 2006b, Alvarez-Pellitero *et al.*, 2007). Also the number and distribution of lymphocytes can be altered by enteromyxosis in turbot, with a significant decrease of the percentage of circulating lymphocytes (Sitjà-Bobadilla *et al.*, 2006), and in the number of Ig^+ cells in head kiney and spleen (Bermúdez *et al.*, 2006), whereas its number was increased at intestine (Bermúdez *et al.*, 2006).

The respiratory burst of phagocytes plays an important role in the fight against parasites, and their activity can be modulated by myxosporean infections. Some enteromyxosis induce an increase in the respiratory burst of circulating phagocytes (Alvarez-Pellitero *et al.*, 2007; Sitjà-Bobadilla *et al.*, 2006), but a decrease in the activity of head kidney phagocytes (Cuesta *et al.*, 2006b). Furthermore, it has been demonstrated *in vitro* that the addition of a myxosporean parasite induces the production of reactive oxygen species (Muñoz *et al.*, 2000b). By constrast, other myxosporean infections invoke a depressed function of phagocytes (Chilmonczyk *et al.*, 2002) or lymphocytes (Densmore *et al.*, 2004). Reactive nitrogen intermediates are also produced by activated phagocytes and are also cytotoxic effector molecules against fish pathogens. In *E. leei*-infected sharpsnout sea bream, serum nitric oxide values were gradually increased after exposure to the parasite (Golomazou *et al.*, 2006). In addition, cell-mediated cytotoxicity due to non-specific cytototoxic cells was also increased in *E. leei*-exposed gilthead seabream (Cuesta *et al.*, 2006b).

HUMORAL IMMUNE RESPONSE

Some humoral innate factors such as peroxidases (PO), lysozyme (LY) or complement can partake in direct fish pathogen elimination, and the data obtained in myxosporean infections are quite variable depending on the host-parasite model. Concerning serum PO, in *E. leei*-exposed sharpsnout sea bream, the levels were higher than in non-exposed ones (Muñoz *et al.*, 2007), whereas serum and HK leucocyte PO depletion was reported in *E. leei*-exposed gilthead sea bream (Cuesta *et al.*, 2006a,b). Regarding LY activity, there was a significant depletion in gilthead sea bream naturally infected by *Polysporoplasma sparis* (Karagouni *et al.*, 2005) and in *E. leei*-exposed gilthead sea bream (author's unpublished observations). However, in sharpsnout sea bream no LY could be detected in either infected or healthy animals (Golomazou *et al.*, 2006; Alvarez-Pellitero *et al.*, 2007), and it has been suggested that its absence could contribute to the high pathogenicity of this myxosporean in this host (Alvarez-Pellitero *et al.*, 2007). In contrast, in other myxosporean infections or immunizations, LY levels can be increased (Muñoz *et al.*, 2000a; Sitjà-Bobadilla *et al.*, 2006; Foott *et al.*, 2004). The information on fish serum complement in myxosporean infections is scarce and it can be quite variable depending on the host-parasite model and the timing of the infections. Plasma complement activity of infected salmon was not altered until ceratomyxosis was quite advanced (Foott *et al.*, 2004). In *Enteromyxum* spp. infections, the activity of the complement alternative pathway is initially increased and/or unaltered in response to the parasite exposure, but later on consumed to fight it (Cuesta *et al.*, 2006a; Sitjà-Bobadilla *et al.*, 2006; Alvarez-Pellitero *et al.*, 2007). It has also been suggested that some parasite-specific glycans may activate the complement system through the lectin pathway (Kaltner *et al.*, 2007).

α-2M is a versatile anti-protease capable of trapping and functionally silencing all classes of microbial and parasite proteases. Increased serum total antiproteases and serum α-2M were found in *E. leei*-parasitized sharpsnout sea bream (Muñoz *et al.*, 2007) and *E. scoph-*

thalmi-parasitized turbot (Sitjà-Bobadilla *et al*., 2006), respectively. These data suggest a role in counteracting the putative action of parasite proteases at the local level.

SPECIFIC IMMUNE RESPONSE

During decades, it was thought that fish were unable to mount an adaptive or specific immune response against Myxosporea, as the first works failed to detect specific antibodies (Pauley, 1974; Halliday, 1974; Siau, 1980; Bartholomew *et al*., 1989), and a theory of the antigenic mimicry of the parasite was even proposed (McArthur & Sengupta, 1982). However, nowadays, the presence of specific antibodies has been unambiguously reported in fish infected by the myxosporeans *Myxobolus cerebralis* (Hedrick *et al*., 1998), *Myxobolus artus* (Furuta *et al*., 1993), *Tetracapsuloides bryosalmonae* (Saulnier & Kinkelin, 1996), *Ceratomyxa shasta* (Bartholomew, 2001) and *Enteromyxum scophthalmi* (Sitjà-Bobadilla *et al*., 2004). The speed of antibody production is relatively low in comparison with available data for other fish immunized or vaccinated with different parasites. Thus, in rainbow trout exposed to *M. cerebralis*, specific antibodies were not present until 12 weeks after exposure (Ryce, 2003), whereas anti-PKX antibodies were detected as early as six weeks p.e. (Hedrick *et al*., 1991). When turbot were challenged with *E. scophthalmi*, specific antibodies were detected as soon as 48 days p.e. if fish belonged to previously exposed stocks (Sitjà-Bobadilla *et al*., 2007b), whereas if fish belonged to naïve stocks, they developed the disease and died without producing antibodies at 40-49 days p.e. (Redondo *et al*., 2002; Sitjà-Bobadilla *et al*., 2006).

INNATE AND ACQUIRED RESISTANCE TO MYXOSPOROSIS

Natural or innate resistance of certain fish species and strains against Myxosporea has been reported, but the mechanisms involved in such complex phenomenon have not been elucidated in most cases. Concerning inter-specific differences, they have been documented for *C. shasta* (Bartholomew, 1998) and for *M. cerebralis* (Hedrick *et al*., 1998; Blazer *et al*., 2004) among salmonid species. Based on epizootiological data, Sugiyama *et al*. (1999) indicated that amberjack (*Seriola dumerili*) was less susceptible to *Kudoa amamiensis* than yellowtail (*Seriola quinqueradiata*). Data obtained from proliferative kidney disease (PKD) experimental infections indicate that the development of the parasite and the severity of the disease may vary with host salmonid species (Arkush & Hedrick, 1990). For *E. leei,* some aquarium (Padrós *et al*., 2001) and fresh water (Diamant *et al*., 2006) fish are refractory to infection, and among susceptible species, a range of pathogenic effect has also been documented. Thus, in gilthead sea bream this enteromyxosis can be considered a slow-progressing disease, as external symptoms and mortality usually begin two months or later on after exposure to the pathogen (Sitjà-Bobadilla *et al*., 2006). By contrast, in other susceptible species, such as sharpsnout sea bream (*Diplodus puntazzo*) (Golomazou, *et al*., 2006; Alvarez-Pellitero *et al*., 2007) and Japanese flounder (*Paralichthys olivaceus*) initial signs of infection and mortality were detected earlier (Yasuda *et al*., 2005). At the other end of the range, European sea bass stands as a low susceptible species, since the infection progresses even more slowly than in gilthead sea bream and fish did not die nor show any of the typical disease signs of enteromyxosis (Sitjà-Bobadilla *et al*., 2007a). Similarly, *E. scophthalmi* which is highly pathogenic for turbot, seems to be less harmful for sole (*Solea senegalensis*), as experimentally infected fish and cultured stocks have much lower infection levels than turbot ones and infected fish did not show typical emaciative signs (Palenzuela *et al*., 2007). Regarding intra-specific differences in susceptibility, turbot stocks of different origin exhibited different susceptibility to *E. scophthalmi* in natural (Quiroga *et al*., 2006) and experimental (Sitjà-Bobadilla *et al*, 2006) infections. Similarly, field and experimental data suggest that some gilthead sea bream stocks are partially resistant to *E. leei* (Jublanc *et al*., 2006; Sitjà-Bobadilla *et al*., 2007a). Differences in resistance to *C. shasta* within strains of some salmonid species are documented (Bartholomew, 1998), as well as to *M. cerebralis* within different strains of rainbow trout (Hedrick *et al*., 2003), cutthroat trout (Wagner *et al*., 2002) and steelhead (Densmore *et al*., 2001). However, strains of *O. mykiss* resistant to *C. shasta* are susceptible to *M. cerebralis*, suggesting that different mechanisms might be involved in the resistance to each myxosporean (Hedrick *et al*., 2001). Based on epizootiological data, some *Salmo salar* stocks appear to be more resistant to PKD (Quigley & McArdle, 1998), and some carp species have been suggested to be less susceptible to *Thelohanellus nikolskii* (Molnár, 2002).

The association between the production of specific antibodies and acquired immunity in fish has been demonstrated for several protozoan and metazoan parasites. However, acquired resistance has been linked to specific immune response only for two myxosporeans. In turbots surviving *E. scophthalmi* epizootic outbreaks, when experimentally challenged for a second time, a negative relationship between the development of the disease and the presence of antibodies was found

(Sitjà-Bobadilla *et al.*, 2007b). By contrast, for *M. cerebralis,* acquired immunity was found only among previously exposed fish that developed active infections (Hedrick *et al.*, 1998). Similarly, trout which had recovered from clinical PKD infections were found to be resistant to reinfection. Resistant was induced by active infection and not just previous exposure (Foott & Hedrick, 1987). *D. puntazzo* recovered from *E. leei* infection, when challenged with the parasite again were refractive to the disease, indicating possibly the development of immunity against the parasite (Golomazou *et al.*, 2006)

This protective specific immune response is probably accompanied by a repertoire of non-specific immune factors. In fact, they have been suggested as the main mechanism limiting the establishment of ceratomyxosis (Bartholomew, 1998), and contributing to the resistance of rainbow trout *M. cerebralis* (Ryce, 2003). In turbot enteromyxosis, antibody response takes about 50-100 days to appear (Sitjà-Bobadilla *et al.*, 2005; 2007b), whereas some cellular and humoral effectors can be affected from day 20 p.e. (Sitjà-Bobadilla *et al.*, 2006). Thus, the activation of a cascade of events involving different innate immune factors in a first line of combat may lead to the production of specific antibodies in a further step.

FUTURE RESEARCH PERSPECTIVES

Characterization of the fish immune system and its regulation is crucial for the development of vaccines and other preventive strategies such as immunomodulation and selection of disease-resistant strains. As shown above, some aspects of the humoral and cellular immune responses against myxosporeans have been studied, but information on the molecular mechanisms involved in such immune defence is almost null. Recently, data on the expression of immunorelevant genes in fish experimentally infected by the myxosporeans *T. bryosalmonidae* (Holland *et al.*, 2003), *M. cerebralis* (Severin & El-Matbouli, 2007) and *E. leei* (Cuesta *et al.*, 2006a) has been published, but still many questions wait to be answered.

The exploitation of the immune system through breeding selection programmes is a promising future strategy to control myxosporoses, but much work is still to be done concerning the selection of resistant strains, as in most cases the genetic base is unknown. For *C. shasta*, multiple gene loci have been found associated to resistance in *O. mykiss* (Nichols *et al.*, 2003), for *M. cerebralis*, inheritance of resistance in disease resistant rainbow has been shown (Schisler *et al.*, 2006), and for PGD a genetic component for resistance in catfish has been suggested (Bosworth *et al.*, 2003).

ACKNOWLEDGEMENTS

Part of the information gathered in the present review has been generated in projects funded by EU and the Spanish *Ministerio de Educación y Ciencia* through research projects MyxFishControl (QLRT-2001-00722) and AGL2006-13158-C02-01.

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