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Intestinal immune response of *Silurus glanis* and *Barbus barbus* naturally infected with *Pomphorhynchus laevis* (Acanthocephala)

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SUMMARY

Immunopathological and ultrastructural studies were conducted on the intestine of barbel Barbus barbus and sheatfish Silurus glanis that were naturally infected with the acanthocephalan Pomphorhynchus laevis. Enteric helminths often cause inflammation of the digestive tract, inducing the recruitment of different types of immune cells at the site of infection. The results of our study clearly demonstrated that mast cells (MC) were the dominant immune cells which occur at the site of inflammation in both hosts. MC were associated with fibroblasts and were found in close proximity to, and inside, the capillaries of the intestine, thus, migration of mast cells via the bloodstream was suggested. Significant degranulation of MC was present. Immunohistochemical staining revealed met-enkephalin and serotonin (5-HT) in intestinal MC of both uninfected and infected barbel and the absence of the antimicrobial peptides piscidin 3 and piscidin 4 in both species. Data are discussed with respect to host immune response to an intestinal helminth and compared with other host-parasite systems.

Keywords *fish, histopathology, immunohistochemistry, inflammation, mast cells, rodlet cells*

INTRODUCTION

Pomphorhynchus laevis (Acanthocephala) is a common endoparasite of several species of freshwater fish (1), but it matures in only a few hosts (2). Acanthocephala are parasites exclusively of the digestive tract of vertebrates and use arthropods as their intermediate host. In the life cycle of Acanthocephala, the arthropod intermediate host becomes infected by eating the mature egg, from which the acanthor larva is free into the lumen of the host's digestive tract. Later, this larva bores into the gut wall and enters the haemocoel. Here, the development of the parasite proceeds through the acanthella stage up to the cystacanth stage, which can infect the vertebrate host when the arthropod is ingested. Some species of fish-parasitic acanthocephalans use another vertebrate host, called a paratenic host, to bridge the trophic levels between predatory vertebrates and arthropods (3). In a paratenic host, the larvae of some acanthocephalans migrate from the gut lumen to encyst in the peritoneal cavity and viscera, where they remain as immature worms until ingested by the final host (3).

There are numerous records on histopathology of helminth infections in fish (4–9). Enteric helminth infections often incite inflammation of the digestive tract, and several studies suggest that the presence of a parasite within a host can induce the formation of various inflammatory cells, as well as a network of nervous fibres at the site of infection (6,7,10,11). Two of the most common cell types associated with enteric parasite infections in fish are mast cells (MC) and rodlet cells (RC). Mast cells, also known as eosinophilic granule cells (EGC), have cytochemical features and tissue locations that have led to the suggestion that they are analogous to mammalian mast cells (4). Indeed, it is widely accepted that mast cells have a role in fish immune responses (8,12–15). It was reported that mast cells are motile (16,17) and react to parasitic infections (8,9,15,18–20) with release of their contents by degranulation (20–22). Antimicrobial peptides (AMP), potent, antimicrobial host defence effector molecules that are present in virtually all life forms (23), include the piscidins, which are probably the most common AMP present in fish. Piscidins occur most commonly in mast cells, including those residing in the digestive tract (12,14,24). Recently, there is evidence for the presence of piscidin 3 in

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mast cells of gill of seabass infected with a monogenean ectoparasite, *Diplectanum aequans* (8).

Intestinal helminth infections of fish have also been associated with the presence of the neurotransmitters serotonin (5-HT) and met-enkephalin in the mast cells of brown trout *Salmo trutta* and powan *Coregonus lavaretus* (20,25). While serotonin is one of major mediators secreted by mammalian mast cells, little is known about its role in the immune system of fish. With regard to the met-enkephalin, its function in regulating gut contractile activity is well known in mammals (26), whereas in fish a great deal remains to be discovered.

Rodlet cells (RC) are found exclusively in fish, and the current view is that these cells are a component of a generalized host response to a variety of external stressors, including parasitic infections (4,8,19,20,27–29). Several records suggested that RC are closely linked to other piscine inflammatory cells such as mast cells (4,30–34).

The purpose of our investigation was to document the inflammatory response of *Barbus barbus* and *Silurus glanis* to the acanthocephalan, *Pomphorhynchus laevis,* with emphasis on the response of small size sheatfish (a paratenic host) to extraintestinal infection, and to probe for defensive chemicals that may be expressed in the predominant immune cell present in the response, the mast cell.

MATERIALS AND METHODS

Histology and electron microscopy

In September 2009, 26 specimens of *Silurus glanis*, with total length 55·09 ± 33·11 cm, (mean ± SD), were sampled by gill net in the terminal part of the River Po (Province of Ferrara, Italy). In October of the same year, 15 specimens of *Barbus barbus* were collected with gill net from the River Bormida (Province of Alessandria, Italy)(total length 34·07 ± 8·21 cm, mean ± SD). The fish were transported live to the laboratory where they were anesthetized using MS222 (Sandoz, Basel, Switzerland) and then weighed and measured before severing the spinal cord. The fish were then dissected ventrally, the whole digestive tract was removed from each fish, and pieces of intestinal tissue (mostly up to 12 mm in length) were fixed in chilled (4°C) 10% neutral-buffered formalin for 8 h. The samples were then paraffin-embedded following standard procedures, and 5-μm sections were stained with Giemsa, haematoxylin-eosin, or alcian blue/periodic acid Schiff (PAS) or used for immunohistochemical analysis. With regard to *S. glanis*, intestinal tissue (up to 10 mm in length), with encysted acanthocephalans on the serosal surface, were fixed with the same procedures. Light photomicrographs were taken using a Nikon Eclipse 80i microscope. Light photomicrographs were taken using a Nikon Eclipse 80i microscope.

For electron microscopy, pieces of infected and uninfected intestinal tissues of both species, measuring up to 7 mm in diameter, were fixed in 2·5% glutaraldehyde in 0·1 M cacodylate buffer for 3 h at 4°C before post-fixing them in 1% osmium tetroxide in the same buffer for 3 h. The specimens were dehydrated through a graded acetone series before being embedded in Epoxy resin (Durcupan ACM, Fluka, Buchs, Switzerland). Semi-thin sections (1·5 μm) were cut on a Reichert Om U 2 ultramicrotome using glass knives and then stained with toluidine blue. Ultra-thin sections (90 nm) were stained with a 4% uranyl acetate solution in 50% ethanol and Reynold's lead citrate and examined using a Hitachi H-800 electron microscope.

To quantify the differences in MC numbers in intestine in both healthy and fish infected with intestinal *P. laevis*, thick resin sections were analysed at 400× magnification using computerized image analysis software (Nis Elements AR 3.0; Nikon, Tokyo, Japan). One section was observed from each of 4 uninfected and 12 infected sheatfish as well as from each of 3 uninfected and 12 parasitized barbel. Cell counts were based on the same size tissue areas (50 000 μm^2) of the same region of the intestine. Results were analysed using Student's two tailed *t*-test, where significance was set at $P < 0{\cdot}05$.

Immunohistochemistry

Intestinal sections were processed using the indirect immunohistochemical method (peroxidase-anti-peroxidase immunocomplex). Briefly, sections (5 μm) were deparaffinized in xylene, rehydrated and then endogenous peroxidase activity and nonspecific staining were blocked in 3% H_2O_2 (10 min) and in normal goat serum (1 : 20) (30 min). After incubation with the rabbit primary antibody (see Table 1 for the working dilution and the incubation time of each antibody), sections were incubated for 30 min with goat anti-rabbit immunoglobulins 1 : 100 (DAKO, Milan, Italy) and then for 30 min with rabbit peroxidase-antiperoxidase 1 : 200 (DAKO). Then, the sections were developed using DAB (3,3′-diaminobenzidine), rinsed and counterstained with Alcian Blue and Harris's haematoxylin. Nonimmune rabbit serum and diluent only sections were used as negative controls.

Mammalian (pig, rat) tissue sections were used as positive controls for met-enkephalin and serotonin, and the controls for the specificity of these immunohistochemical reactions were performed by the pre-absorption of each antiserum with the corresponding antigen. For piscidins, the positive control tissue was hybrid striped bass intestine.

Table 1 Primary antisera used in this study

Antisera raised in rabbit	Source	Dilution	Incubation
Met-enkephalin	Chemicon Int., Temecula, CA, USA	1 : 500	Overnight at 4°C
Serotonin	Chemicon Int., Temecula, CA, USA	1 : 1000	Overnight at 4°C
Piscidin 3[a] (anti-HAGR)	Bethyl Laboratories, Montgomery, TX, USA	1 : 400	3 h at RT
Piscidin 4[a] (anti-5.3)	Bethyl Laboratories, Montgomery, TX, USA	1 : 7000	3 h at RT

[a]The standard procedures used to produce anti-piscidin 3 and 4 antibodies were detailed in references 8 and 24, respectively.

All the positive control sections gave the expected immunoreactivities, and no immunoreactive signals were detected in the negative control sections.

RESULTS

Of 26 sheatfish examined, 12 (46%) harboured *P. laevis* in the intestine; infection intensity ranged from 1 to 12 worms per host (6·25 ± 3·39, mean ± SD). The parasites showed the aspect and sizes of young mature worms. At the site of attachment of *P. laevis*, the mucosa, submucosa and both muscle layers were completely disrupted to form a tunnel, which surrounded the long slender neck of the worm. The most pronounced reaction in the digestive tract was fibrosis (increased collagen fibres) and mast cell infiltration of the basal portion of the epithelium, adjacent to the basement membrane, and in the submucosa (Figure 4a). Comparison of the number of mast cells in uninfected (41·46 ± 8·68, mean ± SD, $n = 4$) and infected sheatfish (78·12 ± 25·17, mean ± SD, $n = 12$) in 16 microscopic fields showed that intestine of fish harbouring intestinal *P. laevis* had a significantly higher number of mast cells around the worms (t-test, $P < 0·05$).

In 10 small hosts (<40 cm total length), immature adult worms were encapsulated in the mesentery and peritoneum (Figure 1a). Host tissue encapsulated these extraintestinal worms, and two distinct layers were present within the capsule wall (Figure 1b). The outermost layer was comprised of collagen fibres infiltrated with MC (Figure 1c); MC were present inside the vessels and in the interstitium near the capillaries (Figure 1d). Mast cells were irregular in shape, with an eccentric, polar nucleus; their cytoplasm was filled with numerous large, electron-dense, membrane-bound granules (Figures 1d and 2a,d). The cytoplasm

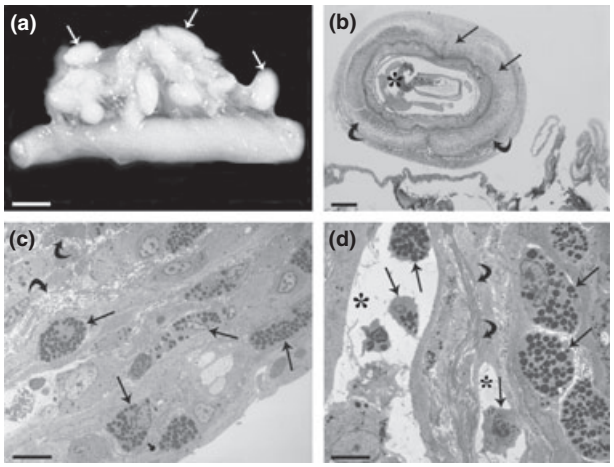

Figure 1 Mesentery of *Silurus glanis* infected with extraintestinal *Pomphorhynchus laevis*. (a) Gross lesions because of extraintestinal *P. laevis* specimens (arrows) within the mesenteries, bar = 2 mm. (b) Histological section of a worm (asterisk) that is surrounded by a host-produced capsule, including a cellular component (arrows) and collagen fibres (curved arrows), bar = 200 μm. (c) Transmission electron micrograph showing the outer part of the capsule which surrounds the extraintestinal *P. laevis* and has mast cells (thin arrows) and collagen fibres (curved arrows), bar = 6·0 μm. (d) Outer part of the capsule, showing capillaries (asterisks) and mast cells (arrows), both inside the vessels and in the interstitium; note the presence of numerous collagen fibres (curved arrows), bar = 3·7 μm.

contained a very few mitochondria, well-developed rough endoplasmic reticulum (RER) and an inconspicuous Golgi apparatus (not shown).

The innermost layer of the capsule was adjacent to the *P. laevis* tegument and also had numerous MC (Figure 2a,b). Often it seemed that mast cell granules were present extracellularly on the acanthocephalan tegument (Figure 2b). In some mast cells, presumable formation of electron-dense granules was observed. Initially, well-developed RER in close contact with electron-dense granules start to be formed and surrounded by membrane. Between the membrane and the electron-dense contents of the granule, there was an electron-lucid space (Figure 2d); this space appeared to be gradually filled with a fine amorphous substance and later in mature secretory granules, the granule membrane surrounds the dense material very closely (Figure 2c,d).

Of the 15 barbel examined, 80% harboured *P. laevis* in the intestine, with an infection intensity ranging from 2 to 25 worms per host (10·13 ± 7·63, mean ± SD). In barbel, *P. laevis* was only found in the intestinal lumen, where it reached the largest size recorded for the species. At the penetration site, the mucosal folds were absent (Figure 4d), with the acanthocephalan penetrating deeply through all the layers of intestine by means of its slender

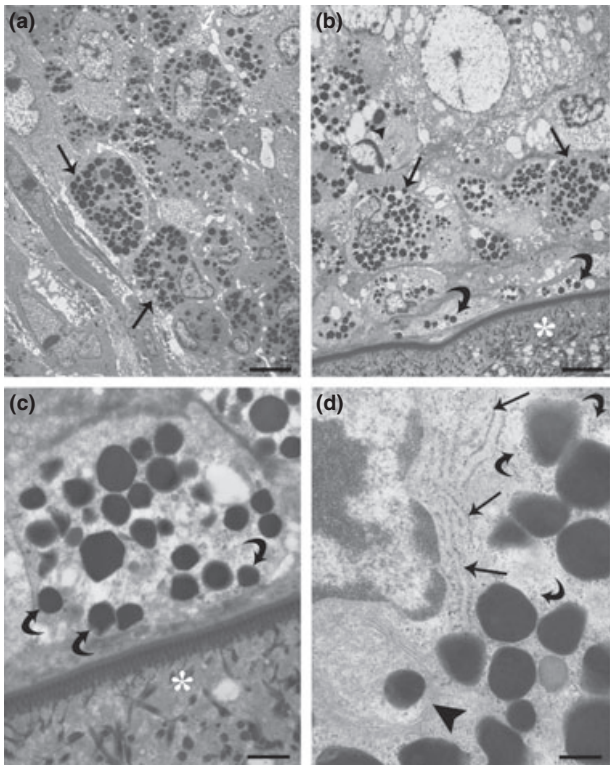

Figure 2 Infected mesentery of *Silurus glanis*. (a) Numerous mast cells (thin arrows) within the capsule and in proximity to the parasite; note the pleiomorphic shape of the mast cells (MC) with their eccentric nuclei, bar = 4·5 μm. (b). Interface between the parasite tegument (white asterisk) and the mast cells (arrows); MC near the parasite have lost their membrane integrity, and some granules (curved arrows) are near the parasite's body surface, bar = 4·5 μm. (c) High magnification of the interface region, with the mast cells appearing to release granules (curved arrows) adjacent to the acanthocephalan tegument (white asterisk), bar = 0·9 μm. (d) Well-developed rough endoplasmic reticulum (arrows) and free ribosomes (curved arrows) among the MC granules are visible; note circumferential presence of membranes (arrowhead) around a small electron-dense granule, bar = 0·4 μm.

neck, bulb and proboscis; consequently, the bulb and proboscis were encapsulated beneath the serosa and mesentery (Figure 4d). As a result, a capsule was usually present on the external surface of the intestine. The capsule was composed of two fibroblast layers: some were fibroblasts of the tunica propria, while others belonged to the muscularis. The above-mentioned fibroblasts extended into the capsule form a tunnel; thus, two distinct layers were recognized in the capsular wall (not shown). Infiltration of MC, RC and collagen fibres was great within the capsule around *P. laevis* bulb (e.g., Figure 4d).

The response of barbel at the attachment site of the worm was hyperplasia of host connective tissue, with cellular infiltration. Penetration of the neck through the intestinal wall resulted in a dense accumulation of fibroblasts in the tunica

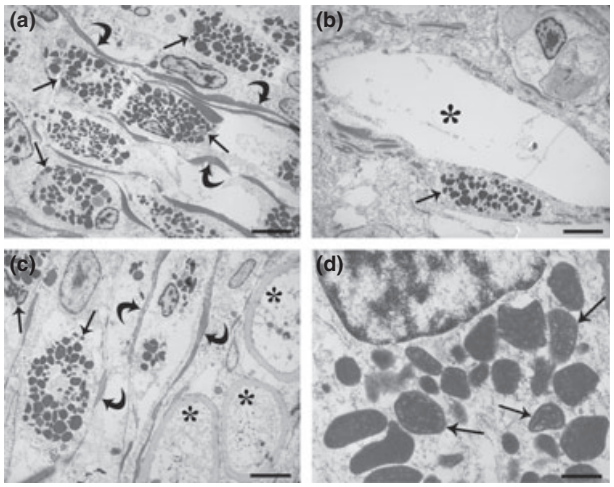

Figure 3 Intestine of *Barbus barbus* infected with adult *Pomphorhynchus laevis*. (a) Electron micrograph showing a portion of the host-produced capsule around the bulb and proboscis of the parasite; several collagen fibres (curved arrows) are scattered among the mast cells (arrows), bar = 3·4 μm. (b) Close contact of a mast cell (arrow) with a capillary (asterisk) within the capsule, bar = 4·2 μm. (c) Co-occurrence of mast cells (arrows), collagen fibres (curved arrows) and rodlet cells (asterisks) inside the capsule, bar = 3·3 μm. (d) High magnification of a mast cell showing some electron-dense granules (arrows) which appear to be degranulating, bar = 0·6 μm.

propria around the tunnel, and this layer appeared to be thicker (Figure 4d). Within the capsule around the bulb and proboscis, numerous MC and collagen fibres were encountered (Figure 3a). Mast cells counted in 15 microscopic fields showed that the intestine of parasitized barbel had a significantly higher number of MC (125·13 ± 53·92, mean ± SD, $n = 12$) than those of uninfected ones (54·01 ± 23·72, mean ± SD, $n = 3$) (*t*-test, $P < 0·05$).

Often, MC were in close contact with capillaries present in the capsule (Figure 3b). Moreover, within the capsule, some RC co-occurred with MC and collagen fibres (Figure 3c). Degranulation of the mast cells was frequent; where this was observed, the granules appeared less homogenous, and less electron-dense with white-empty spaces inside them (Figure 3d).

The comparison of damage in histological sections of both hosts showed that the damage was greater in the intestine of barbel, followed by intestine of *S. glanis* and then by sheatfish with extraintestinal worms. Barbel also had a higher intensity of infection and harboured the largest worms, which destroyed more mucosal folds and deeply penetrated all layers of the intestinal wall. The general host response in the intestine of both fish species were similar, but in barbel, mast cells and collagen fibres were more numerous and a second type of immune cell (rodlet cell) was present.

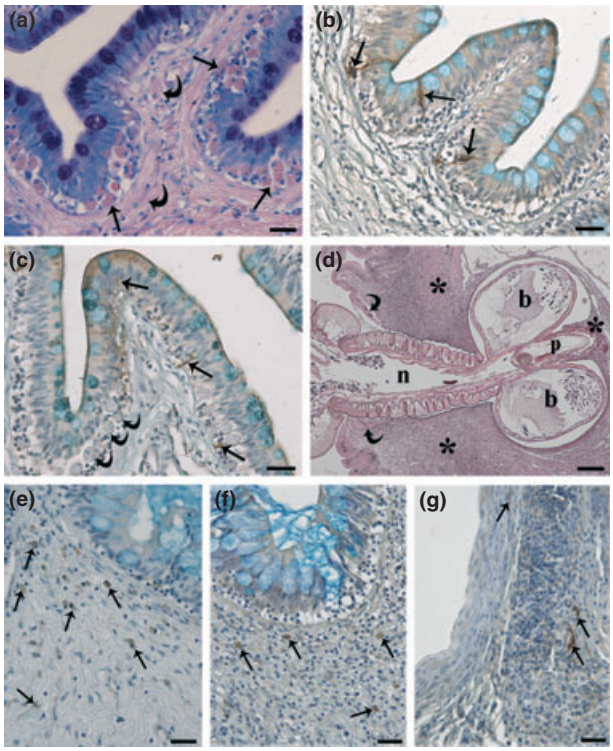

Figure 4 Histological sections of infected intestine of sheatfish and barbel. (a) In intestinal folds of sheatfish with numerous mast cells (thin arrows) at the base of the epithelium and a few in the submucosa (thick arrows), bar = 20 μm. (b) Cells (arrows) immunoreactive to serotonin in the intestinal epithelium of *S. glanis*, bar = 20 μm. (c) Cells positive for met-enkephalin (arrows) among the intestinal epithelial cells of parasitized intestine of sheatfish; curved arrows show the mast cells, bar = 20 μm. (d) In barbel, adult worm has deeply penetrated through all intestinal layers, causing tissue compression and atrophy of the intestinal folds (curved arrows) at the attachment site. There is intense host reaction (asterisks) around the neck (n), bulb (b) and proboscis (p), bar = 200 μm. (e) Intestinal folds of parasitized barbel with mast cells (arrows) in the submucosa that are immunoreactive for met-enkephalin, bar = 20 μm. (f) Mast cells (arrows) immunopositive for anti-serotonin in the submucosa of parasitized barbel intestine, bar = 20 μm. (g) Nerve fibres (arrows) of infected barbel intestine immunoreactive for serotonin in the connective tissue capsule surrounding the bulb of *P. laevis*, bar = 20 μm.

In sheatfish, MC were immuno-negative for both serotonin and met-enkephalin, but several positive endocrine cells were seen within the intestinal epithelium of both infected and uninfected fish (Figure 4b,c). In barbel, several MC in the submucosa were positive for met-enkephalin and serotonin (Figure 4e,f). In infected barbel intestine, serotonin-positive nerve fibres were observed in inflamed tissue around the bulb and proboscis of *P. laevis* (Figure 4g).

In parasitized and uninfected intestine of both fish species, histochemical staining with anti-piscidin 3 or anti-piscidin 4 antibodies did not reveal any positive mast cells and/or other positive cells.

DISCUSSION

Pomphorhynchus spp. and other acanthocephalans can use several fish species as a paratenic host (5,35,36). In most northern Italian rivers, barbel and chub, *Leuciscus cephalus* are among the few species which act as the preferred definitive hosts for *P. laevis*; in these species, the parasite reaches its largest size. In these same rivers, sheatfish appears to be unsuitable as a preferred definitive host and the small size conspecifics act as paratenic host harbouring *P. laevis* encysted in extraintestinal sites.

In fish, as in other vertebrates, the digestive tract is the main route of infection for many intestinal worms (37), and this is likely due to several factors, including the relative ease of pathogen access via the oral route, ready availability of attachment sites and nutrients, and a relatively nonaggressive immune response (38). Often, infection of the gastrointestinal tract by protozoa or helminths has detrimental effects on digestive function (26,39). Accordingly, the attachment organ of endoparasitic helminths frequently induces inflammation of the alimentary tract (5,17,20). Members of the genus *Pomphorhynchus* are among a few acanthocephalan species which, as a result of their morphological adaptation, often penetrate deeply through the host alimentary canal and inflict extensive damage.

It is generally accepted that the pathogenecity of acanthocephalans is attributed to two factors: density of the parasite burden and depth of worm penetration (5,40). As we reported in the previous section, the intensity of *P. laevis* in barbel was higher than that in sheatfish. Furthermore, in barbel (which acts as preferred definitive host), worms reached the largest size recorded for the species. It also penetrated deeper into the intestinal tissue, presumably to allow firmer attachment to prevent its expulsion. Consequently, worms induced intense inflammation; within the capsule, there was abundant infiltration of mast cells and rodlet cells, as well as greater collagen deposition. Large sheatfish appeared to be less preferred as a host for *P. laevis* in our study area, as suggested by the parasites being small, young adults. In sheatfish intestine, the worms penetrated less deeply and thus provoked less immune reaction than in barbel. In small sheatfish (<40 cm total length), which act as a paratenic host for *P. laevis*, inflammation was less severe than in either barbel or large sheatfish and was limited to the mesentery and peritoneum, where the immature worms were encysted.

In the great majority of previous studies of host-parasite interactions in fish, there has only been a description of

the physical damage caused by the parasites. Frequently, information is lacking about the inflammatory response. Inflammation is a protective reaction of the host in response to injury, resulting in specific chemical and morphological alterations to the cells and tissues (41). Inflammation consists of a complex series of homeostatic mechanisms involving the circulatory, immune and nervous systems in response to tissue injury or infection (42). Cellular events in the inflammatory response in teleostean fish could be biphasic, beginning with an influx of neutrophils, followed by the subsequent arrival of monocytes/macrophages (28). In fish, rodlet cells and mast cells are considered to be components of the teleost innate immune system and closely linked to other piscine inflammatory cells (4,19,33). For instance, in *B. barbus*, RC and MC co-occurred in the capsule around the bulb and proboscis of the acanthocephalan. With regard to RC, there is agreement on their development, stages, location and migration, whereas the origin and role of RC are still under debate (20,29,32). The possible function of RC as immune cells was suggested from studies that reported an increase in the number of these cells in fish infected with protozoan (27) and metazoan parasites (9,28,29,34,43,44).

As we reported in the previous section, barbel had both MC and RC in the capsule around the parasite's bulb and proboscis, while sheatfish only had a MC infiltrate. Helminth parasites of fish elicit different immune/inflammatory reactions, depending upon the tissue, type of parasite and stage of infection. For instance, in brain of fathead minnows and minnows infected with trematode larvae, only RC were observed (29,43). Conversely, in the liver and pancreas of minnow infected with larvae of the nematode *Raphidascaris acus*, many immune cells (i.e., MC, RC, neutrophils and macrophages) appeared to participate (31). Both MC and RC are especially common when gill or intestine is the target (8,15,17).

Most teleosts appear to have within their alimentary canal, gills and other major organs a cell type that possesses structural and functional properties similar to those of mammalian mast cells (16,28). In all vertebrates, mast cells may be strategically positioned at perivascular sites to regulate inflammatory responses and this places them in a unique position to encounter invading organisms and orchestrate a response (45). In fish infected with helminths, mast cells tend to migrate and accumulate in large numbers at the site of infection (11,17,20,28). Our present study demonstrates the occurrence of numerous mast cells in connective tissue and blood vessels of *S. glanis* and *B. barbus* intestine infected with *P. laevis*. The same phenomenon was observed in intestine of brown trout infected with another acanthocephalan, *Echinorhynchus truttae* (17). Moreover, the number of mast cells was high at parasite attachment sites. Based on a considerable amount of published data, it is reasonable to assume that fish may have two populations of mast cells, resident and circulating (45) and the presence of parasites induces recruitment of mast cells to the site of infection (8,17,20,25). Accordingly, acute mast cell activation is a feature of many types of tissue injury and it has been demonstrated that pathogen products can also activate mast cells (46).

Mucosal mast cells are known to play a role in the expulsion of nematode *Trichinella spiralis* in mice (47,48). Within the intestinal epithelium of *S. glanis*, high numbers of mast cells were observed; thus, mucosal mast cells in sheatfish might have the same function (47,48), but our knowledge is too limited to make such a generalization with confidence. In *S. glanis*, there was evidence for extensive mast cell degranulation within the capsule surrounding the extraintestinal worms, especially those near the parasite's tegument. Descriptive data exist detailing how mast cells degranulate in response to their interaction with parasites (17,20). Mast cell secretions may have a role in attracting other types of cells (i.e. neutrophils) involved in the inflammatory process, especially during initial pathogen challenge (28). Recently, it was reported that the mast cells of certain perciform fish possess histamine, which can regulate fish inflammatory responses (49). Murray *et al.* (50) suggested that mast cells could also be directly involved in the destruction of pathogens based on the evidence of the multifunctional role of these cells in teleosts.

In barbel and sheatfish parasitized with *P. laevis*, mast cells were the most common immune cells at the site of infection; thus, determining the immune chemicals they expressed was one purpose of this paper. Our immunohistochemical analysis revealed that intestinal mast cells of both species did not contain piscidin 3 or piscidin 4; moreover, only some barbel mast cells expressed serotonin and met-enkephalin. The same finding was reported in brown trout and powan infected with the acanthocephalan *Dentitruncus truttae* (20,25). Endocrine cells immunopositive for serotonin and met-enkephalin were present in the intestinal epithelium of *S. glanis* and *B. barbus* and in barbel, immunopositive fibres were also in the serosal capsule around the parasite's bulb and proboscis. Information on these neuromodulators and their role in the piscine immune system is scarce (51,52); nonetheless, their presence has been documented in other fish-parasite systems (see 7,53-55). It has been suggested that in rat serotonin affects vascular permeability, lymphocyte activity (56) and plays an important role in regulating intestinal function (57). A significant increase in this biogenic amine in the intestine and muscle of rats infected with nematodes has been reported in the study by Terenina et al. (58), and

serotonin exerts a variety of effects that may favourably affect the survival of the parasite (26). If parallels between these host-parasite systems can be drawn, this suggests that *P. laevis* infection incites the recruitment of cells to secrete serotonin at the site of infection to ensure their survival. Enkephalins belong to the endogenous opiate system and in mammals they play an important role in modulating inflammation (52), but the same function has yet to be established in fish. With the presence of cells positive for met-enkephalin in the epithelium of infected *S. glanis* and *B. barbus*, we speculate that by increasing the amount of the met-enkephalin-like peptide secreted, inflammation caused by the acanthocephalan may be modulated.

Several studies in fish have shown that mast cells produce antimicrobial peptides (AMPs) and therefore are presumed to be directly involved in killing microbes (12,15,24,33,50). Piscidin 3 was identified in mast cells of different organs, including intestine and stomach, of uninfected fish (12,14,33) and in MC of gill of fish infected with ectoparasites (the monogenean *Diplectanum aequans* and the copepod *Chondracanthus goldsmidi*)(8,15). Furthermore, piscidin 4 was present in mast cells of uninfected gills stomach and intestine of several fish species; nevertheless, not all MC were piscidin-positive (24). In the present study, no MCs of uninfected or parasitized intestine of *S. glanis* and *B. barbus* were positive for piscidin 3 or 4. Immunohistochemistry on a wide taxonomic range of teleosts showed that these two piscidins were encountered only in MC of fish belonging to the orders Perciformes (14,24) and Gadiformes (59). Their absence in barbel (Order Cypriniformes) and sheatfish (Order Siluriformes) may provide additional evidence in favour of the taxonomic distribution of piscidins previously suggested by Silphaduang *et al.* (33), Mulero *et al.* (14) and Corrales *et al.* (24). Alternatively, because the piscidin 3 and piscidin 4 antibodies do not recognize the highly conserved N-terminus of the piscidin family, other piscidins might not have been detected with our assays.

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