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Short Communication

Fine structure and cellular responses at the host–parasite interface in a range of fish–helminth systems

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ABSTRACT

A series of ultrastructural-based studies were conducted on the interface region in different fish–helminth systems: (a) an intestinal infection of the cestode *Monobothrium wageneri* in tench, *Tinca tinca*; (b) an extensive intestinal submucosa and mucosal infection in tench by metacercariae of an unidentified digenean trematode; (c) an intestinal infection in brown trout, *Salmo trutta*, by the acanthocephalan *Dentitruncus truttae*; (d) an extraintestinal infection by larvae of the acanthocephalan, *Pomphorhynchus laevis* in three-spined sticklebacks, *Gasterosteus aculeatus*; and (e) an infection in the livers of Eurasian minnow, *Phoxinus phoxinus*, by larvae of the nematode *Raphidascaris acus*. Endoparasitic helminths frequently cause inflammation of the digestive tract and associated organs, inducing the recruitment of various immune cells to the site of infection. In each of the fish–helminth systems that were studied, a massive hyperplastic granulocyte response involving mast cells (MCs) and neutrophils in close proximity to the helminths was documented. The current study presents data on the interface region in each fish–helminth system and documents the penetration of mast cells granules within the tegument of *P. laevis* larvae. No extracellular vesicles containing tegumental secretions from any of the four different taxa of endoparasitic helminths species at the host–parasite interface region were seen.

1. Introduction

Fish which include over 27,000 species are, phylogenetically, the oldest vertebrate group representing more than one-half of the vertebrates on the planet (Toledo-Ibarra et al., 2013). Understanding the immune systems of fish, therefore, is of great relevance as it provides information on the evolution of immunity in vertebrates (Rauta et al., 2012).

The innate immune system of fish comprises: (1) cytotoxic (i.e. natural killer) or phagocytic (i.e. macrophages, granulocytes) cells; (2) proteins that mediate the responses to helminth infection and (3) the use of physical (e.g. epithelial) and chemical (e.g. anti-microbial peptides) barriers to minimise the likelihood of parasitic infection (Dixon and Stet, 2001). In fish, neutrophils are the first cell type recruited to the site of an acute inflammatory response (Secombes, 1996; Katzenback and Belosevic, 2012) and their chemotaxis, phagocytosis and destruction of intracellular and extracellular pathogens demonstrate their important role in innate immunity (Secombes, 1996; Stakauskas et al., 2007; Katzenback and Belosevic, 2012).

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Mast cells (MCs), a type of granulocyte, are potent inflammatory cells that are present in most tissues and are commonly strategically positioned in close proximity to blood vessels (Reite and Evensen, 2006). In helminth-infected fish, MCs have been observed to migrate and accumulate in large numbers at the site of parasitic infection (Reite and Evensen, 2006; Dezfuli et al., 2008, 2011a, 2013a, 2014). In fish as in other vertebrates, MCs are very active and their role in the early orchestration of an immune response against a range of disease agents, including parasites, has been documented in several studies (Abraham and St John, 2010; Prykhozhi and Berman, 2014; Sfacteria et al., 2015). Mast cells in nonmammalian vertebrates contain a wide range of compounds (i.e. histamine, heparin, neuropeptides, proteases) and, in bony fishes, also antimicrobial peptides (AMPs) (Baccari et al., 2011; Masso-Silva and Diamond, 2014).

Recently the investigation of host–parasite interactions has increased considerably, numerous studies focusing on the identification of mammalian helminth excretory/secretory (ES) proteins (Marcilla et al., 2012; Smith and Maizels, 2014). Knowledge on the occurrence and effects of helminth ES proteins on the immune systems of fish, however, is still limited (Buchmann, 2012; Bahlool et al., 2013).

For the current study, transmission electron microscopy is used to study and comment on the interface region in four different taxa of endoparasitic helminths and their hosts.

2. Materials and methods

In 2013, a total of 28 specimens of tench, *Tinca tinca* (L.) (47.36 ± 4.55 cm, mean total length TL ± standard deviation [SD]) and 40 specimens of brown trout, *Salmo trutta* (L.) (28.9 ± 7.48 cm, mean TL ± SD) were processed from Lake Piediluco situated in the Province of Terni, Central Italy (42°31′01″ N; 12°45′00″ E). The fish were caught by gill net that was deployed on three occasions by professional fishermen operating within the lake. Twenty-five specimens of Eurasian minnow, *Phoxinus phoxinus* (L.), (60.96 ± 3.73 mm, mean ± SD), and 39 three-spined sticklebacks, *Gasterosteus aculeatus* (L.) (47.80 ± 4.62 mm, mean ± SD), were sampled by electrofishing a tributary of the River Brenta, North Italy.

After capture, the fish were transported live to the laboratory, euthansed using an overdose of 125 mg L^{-1} MS222 (tricaine methanesulfonate, Sandoz, Basel, Switzerland) and thereafter, the spinal cord was severed. The fish were lengthed and weighed and a complete necropsy was performed, with particular interest to gills, gonads, liver, kidney, spleen and the alimentary canal which was completely dissected and opened.

For light and electron microscopy, small pieces (i.e. 7 mm × 7 mm) of the following tissues were excised and fixed in chilled (4 °C) 2.5% glutaraldehyde solution in 0.1 M sodium cacodylate buffer, pH 7.3 for 3 h: parasite-infected intestines from brown trout and tench, parasite-infected liver from minnows, encysted larval acanthocephalans on the outer surface of the intestine of three-spined sticklebacks. Thereafter the fixed tissues were post-fixed in 1% osmium tetroxide for 2 h and then rinsed and stored in 0.1 M sodium cacodylate buffer containing 6% sucrose for 12 h. Then, the samples were dehydrated through a graded acetone series and then embedded in epoxy resin (Durcupan ACM, Fluka, Buchs, Switzerland). Semi-thin sections (i.e. 1.5 μm) were cut on a Reichert Om U 2 ultra microtome (Reichert-Jung, Austria) and stained with toluidine blue. Ultra-thin sections (i.e. 90 nm) were stained with a 4% uranyl acetate solution in 50% ethanol and Reynold's lead citrate and observed using a Hitachi H-800 transmission electron microscope (Hitachi High-Technologies Europe GmbH, Krefeld, Germany).

Corresponding pieces of intestine and liver were prepared from uninfected fish for comparison with parasite-infected tissues. The absence of parasites in uninfected fish was established by the necropsy and trough fresh microscopic smears which were performed on all the examined organs to rule out microparasites and related lesions. Histological sections confirmed that tissues of these control fish were parasites-free.

3. Results

Table 1 summarises the main information on host–parasite systems including fish and helminth species, prevalence and intensity of infection, parasite tissue location, host cell types and pathology.

3.1. *T. tinca* and the cestode *M. wageneri* (Table 1)

The attachment of the *M. wageneri*, typically in tight clusters of variable number, resulted in the formation of a raised, surrounding, inflammatory swelling. Cestode attachment to its host was effected by means of a simple, rounded scolex inserted deep into the intestinal wall, extending into the *mucosa* and *submucosa* as far as the *muscularis* layer. While these inflammatory swellings consist primarily of fibroblasts, there are also a large number of two different granulocytes, i.e. neutrophils and MCs. Interestingly, rodlet cells (RCs) were also found to co-occur with these granulocytes within the *submucosa* of the resultant nodule. Neutrophils and MCs were also recorded within the connective tissue surrounding capillaries and within the blood vessels within the *submucosa* and *muscularis* layer. MCs were observed to be irregular in shape with an eccentric, polar nucleus, and a cytoplasm characterised by numerous large, electron-dense, membrane-bounded granules (Fig. 1a). The cytoplasm typically contained two to three mitochondria and an inconspicuous Golgi apparatus. MCs were frequently surrounded by collagen fibres of the *submucosa* or by fibroblast-like unsheathing cells. Within the nodule, there were numerous neutrophils which appeared round to oval in shape though their outline was commonly irregular. These cells also contained a round nucleus and a cytoplasm with dark, elongated granules which were fibrous in appearance (Fig. 1b). Only a small number of mitochondria and some fragments of rough endoplasmic reticulum were seen within the cytoplasm.

Degranulation of the MCs, which was common in the *submucosa*, was characterised by the conspicuous swelling of granules, with free granules frequently seen in close proximity to the capilliform filitriches or adjacent to or between the coniform spinitriches of the