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Gill Tissue Reactions to an Epitheliocystis Infection in Cultured Red Seabream, *Pagrus major*

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Tissue reactions in gills of cultured red seabream, *Pagrus major*, to an epitheliocystis infection are described. Basophilic intracellular inclusions in gills contained prokaryotes, most probably a *Chlamydia*-like organisms according to morphological characteristic. A few types of tissue reaction were found around the inclusions: encapsulation, epithelial hyperplasia, lamellar fusion, and inflammation. It was considered that eosinophilic granule cells and macrophages might take part in defense reactions against this prokaryotic organism.

Key words: Gill pathology, Red seabream, Epitheliocystis infection, Chlamydial organisms

Introduction

The family Chlamydiaceae contains two genera *Chlamydia* and *Chlamydophila* including a few species of gram-negative bacteria that cause a variety of diseases in humans and animals. Epitheliocystis is a well-known disease of fish, which is characterized by the presence of hypertrophied cells containing fine basophilic inclusions or cysts (Paperna, 1977). The causative agent is an intracellular prokaryote, named as a *Chlamydia*-like organism because ultrastructural characteristics and developmental cycles correspond to those of chlamydiae (Paperna, 1977; Desser *et al.*, 1988; Nylund *et al.*, 1998), but antigenic similarities with chlamydiaceae was shown for one infected fish species (Groff *et al.*, 1996) and not detected in other (Crespo *et al.*, 1999).

Epitheliocystis was first described as a benign, chronic, infectious gill and skin disease of the bluegill, *Lepomis macrochirus* (Hoffman *et al.*, 1969). Subsequently this disease has been observed in more than thirty-five fish species from both the freshwater and the marine environment (see review of Fryer and Lannan, 1994). It was reported in such marine species as basses, *Morone saxatilis* and *Morone americanus* (Wolke *et al.*, 1970; Paperna and Zwerner, 1976), mullet, *Liza ramada* (Paperna *et al.*, 1978), trout, *Oncorhynchus mykiss* (Rourke *et al.*, 1984) and *Salvelinus namaycush* (Bradley *et al.*, 1988), as well as American plaice, *Hippoglossoides platessoides* (Morrison and Shum, 1983). Among the *Sparidae* epitheliocystis was found in the gilthead sea bream, *Sparus aurata* from the Eastern Mediterranean and Red Sea (Israel) and Mediterranean coast of France (Paperna, 1977; Paperna *et al.*, 1978), the red seabream, *Pagrus major* transported to Japan from Hong Kong (Miyazaki *et al.*, 1986) and the black seabream, *Acanthopagrus schlegeli* from Japan (Egusa, 1987).

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Epitheliocystis has been registered by us in red seabream and hybrid fish cultured in Korea (Syasina *et al.*, in press).

Red seabream, *Pagrus major* Temminck et Schlegel, is widely distributed in coastal waters of Korea and is a valuable aquaculture species. A variety of parasites, bacteria and viruses are recognized as serious pathogens of this species. In addition, other diseases such as eye abnormality and cataracts were found recently in Korean red seabream and an induced hybrid (female red seabream, *Pagrus major* × male black seabream, *Acanthopagrus schlegeli*) (Syasina and Park, 2003). The purpose of the present paper is to describe the defense reactions in gills of the red seabream *Pagrus major*, cultured in Korea, to an epitheliocystis infection.

Materials and Methods

Fish

The description of epitheliocystis was based on observation in the group of captive-bred red seabreams (n = 100) over a 6-month period from November 2002 to April 2003. Fish were held in 2000 L tanks, each containing about 25 fish, and cultured in ambient water temperature conditions. The water exchange rate was 30 L min^{-1}, and tanks were illuminated for 10 h daily. Fish were fed with a commercial marine fish diet (Agiribrand Korea, Korea). Only sporadic mortalities were detected during the period of study. Before examination fish were anaesthetized and killed with an overdose of 100 ppm lidocaine-HCl/$NaHCO_3$ (Park *et al.*, 1998).

Histological and electron microscope techniques

The second left gill arch was collected for routine histological processing. Samples were fixed in Bouin's solution, processed, and embedded in paraffin. Sections of 5 ㎛ thickness were stained with Mayer's hematoxylin and eosin (HE) and examined under the light microscope equipped with digital camera Axio Vision (Zeiss, Germany). The occurrence of different types of tissue response (% of cysts) to the presence of cysts (n=60) of epitheliocystis was defined. Fresh gill samples from two fish sampled for histology were also fixed for transmission electron microscopy (TEM) in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, postfixed in 1% osmium tetroxide for 1 h, dehydrated and embedded in Epon. Semithin and ultrathin sections were cut in a LKB Nova ultramicrotome and stained with toluidine blue and lead citrate, respectively.

Results

The presence of basophilic intracellular inclusions that is characteristic feature of epitheliocystis was histologically revealed in gills of 100 % studied red seabream. All the cysts exhibited the same amorphous appearance under the light microscope and had a round shape predominantly. The localization of cysts in gills was different with 30% of cysts found in the interlamellar spaces of gills, and 70% in the middle part of the lamellae or at their tips. The type of infected cells was difficult to determine.

A few types of tissue reactions to the cysts of epitheliocystis were found in gills of red seabream: encapsulation, epithelial hyperplasia, lamellar fusion, and inflammation. One or two layers of squamous epithelial cells surrounded the majority of cysts and only 17% of cysts had a multilayer pseudocapsule. The variability in tissue response was present even within an individual fish (Fig. 1). Proliferation of epithelial cells leading to fusion of two or three lamellae was found in 83% of cases, and very strong hyperplasia (Fig. 2) leading to

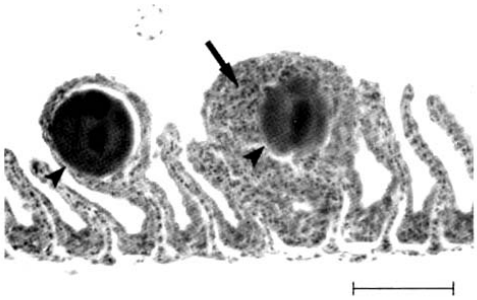

Fig. 1. The variability of the tissue response to the cysts (arrowheads) in the gill of cultured red seabream. Note the moderate hyperplasia of respiratory epithelium around one cyst (arrow) and the absence of proliferative reaction around the second cyst. Bar indicate 50 μm.

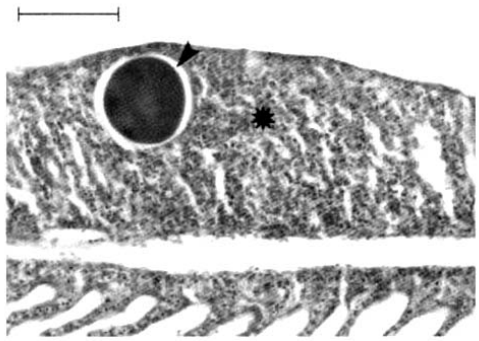

Fig. 2. Strong hyperplasia (asterisk) of epithelial cells around the amorphous cyst (arrowhead) in the gill of red seabream causing the fusion of lamellae. Bar indicate 50 μm.

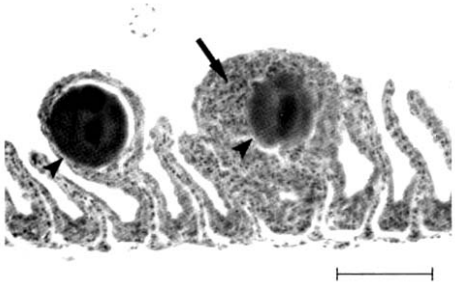

Fig. 3. Dystrophic changes of epithelial cells (arrow) and fusion of lamellae close to cysts (arrowheads). Bar indicate 50 μm.

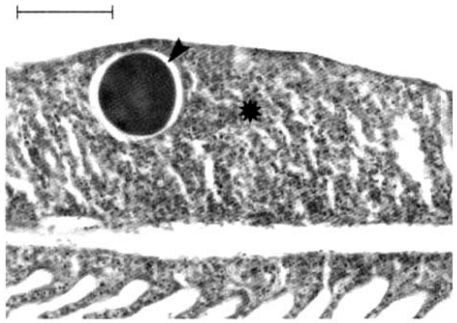

Fig. 4. Inflammatary reaction in the gill of red seabream with prominent morphological changes close to a cyst (asterisk): epithelial hyperplasia and fusion of lamellae. The arrow points to an eosinophilic granule cell. Bar indicate 50 μm. Inset: Higher magnification of eosinophilic granule cells (× 1000).

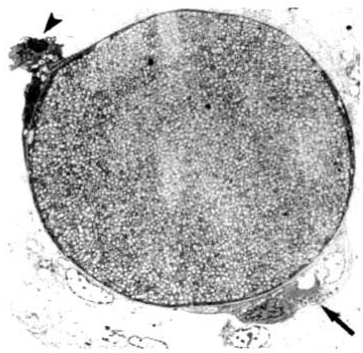

Fig. 5. TEM of cyst filled with prokaryotic organisms. Epithelial cells around the cyst have developed degenerative changes. The arrowhead points to a macrophage, whereas the arrow points to a granulocyte on the surface of cysts. × 1500.

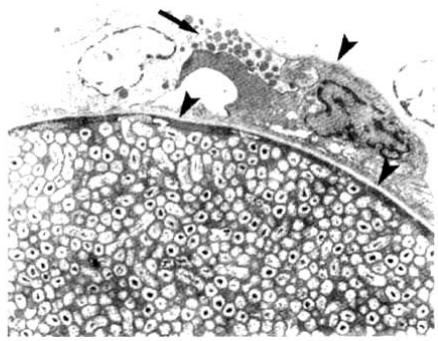

Fig. 6. Details of Fig. 5. A supplementary stratum composed of homogeneous material between the cyst and cells of the pseudocapsule (arrowheads). The cell with a large number of granules (arrow) is located on the surface of cyst. Primary and intermediate long cells with weakly condensed nucleoid, and small cells with highly condensed nucleoid and a few vacuoles around it are present inside the cyst. × 10000.

fusion of many lamellae was detected only in 17% of cysts. As shown in Fig. 3, dystrophic changes of epithelial cells were observed occasionally. Inflammation was registered around 20% of cysts. In the area of inflammation, round cells possessing numerous eosinophilic granules (Fig. 4) as well as lymphocytes were present.

The TEM study showed the additional features of a cyst pseudocapsule structure. The pseudocapsule was actually formed by several layers of epithelial cells, which were densely connected to one another by numerous desmosomes. In some cases it was possible to observe the degenerative changes of epithelial cells constituting a pseudocapsule. Not only epithelial cells participate in the isolation of prokaryotic organisms, but also cells referring to the defensive system of fish. Macrophages were observed in cases where epithelial cells around the cyst were broken down. Macrophages were characterized by an irregular surface with protrusions and indentations and phagocytic vacuoles inside the cytoplasm (Fig. 5). Granular cells were found near to or on the surface of cysts (Fig. 6). These cells were observed both on the light microscope and electron microscope and characterized by the availability of numerous granules in the cytoplasm.

Discussion

Epitheliocystis is usually benign condition; nevertheless, a few types of tissue response to the presence of epitheliocystis inclusions in gills of different fish species have been described. The most typical reaction, the epithelial hyperplasia, have been seen in various fishes such as *Sparus aurata* (Paperna, 1977; Crespo *et al.*, 1999), *Salvelinus namaycush* (Bradley *et al.*, 1988), *Seriola dumerili* (Crespo *et al.*, 1990), *Bidyanus bidyanus* (Frances *et al.*, 1997) and *Dicentrarcus labrax* (Crespo *et al.*, 2001). Gill hyperplasia was found as well in *Pagrus major* (Miyazaki *et al.*, 1986) and present studies. The next most common reactions to epitheliocystis found in a few species are encapsulation of the cysts with a thin layer of squamous epithelial cells and lamellar fusion (Paperna, 1977; Miyazaki *et al.*, 1986; Frances *et al.*, 1997; Crespo *et al.*, 1999; 2001, Nowak and Clark, 1999). The more detailed histopathological investigation of epitheliocystis was performed in Atlantic salmon, *Salmo salar* L., and such types of tissue reactions as proliferation of mucous cells, epithelial lifting, necrosis and inflammation including infiltration with small mononuclear cells, macrophages and polymorphonuclear cells were observed additionally (Nowak and Clark, 1999). Nevertheless, inflammation with participation of eosinophilic granule cells induced by epitheliocystis pathogen was never described previously.

The immune system of fish is relatively well developed and a range of cellular and humoral mechanisms against parasites has been described (reviewed by Buchmann *et al.*, 2001). The cells participating in immune reactions are granulocytes, monocytes, macrophages, and also lymphoid cells - nonspecific cytotoxic cells, T- and B - lymphocytes. The present study detected not only epithelial cells, but also eosinophilic granule cells and macrophages in the structure of the pseudocapsule and in the injured tissues of red sea bream gills. It seems that these cells are involved in the immune defence of red sea bream against prokaryotic pathogens. Eosinophilic granular cells (EGCs) in teleost fishes have been proposed to be the equivalent of mast cells in mammals (reviewed by Reite, 1998). Little is known about the functional significance of EGCs in fish although they have been shown to participate in anti-parasitic responses to microsporidian (Reimschuessel *et al.*, 1987) and plerocercoid (Sharp *et al.*, 1989) infections.

There are data shown the epitheliocystis agent infects different types of gill cells. Paperna and Alves de Matos (1984) described epitheliocystis infection in the carp that occurred in the lining epithelial cells as well as mucous and chloride cells. In the amberjack, the target cell was the chloride cell since coccobacillary bodies were first found within the chloride cell and this type of cell underwent degeneration in the filament epithelium (Crespo *et al.*, 1990). In Atlantic salmon, *Salmo salar*, the appearance and position of the infected cells corresponded to the epithelial cells (Nowak and Clark, 1999). Desser *et al.* (1988) pointed out the possible involvement of a transformed macrophage in the earliest stages of these infectious organisms. In the present study only grown cysts were studied by TEM, and it was impossible to identify the nature of the host cells. Moreover, from our histological study it is apparent that cysts in red seabream are more frequently found in the interlamellar spaces as well as at the tips of lamellae. Miyazaki *et al.* (1986) found cysts on the capillary under respiratory epithelium of gill lamellae in the early stagein infected red seabream in Japan. Heavily infected gills showed many cysts among the hyperplastic epithelium of the interlamellar spaces.

Chlamydia-like organisms infected gills of cultured red seabream have induced a host protective response including epithelial hyperplasia, encapsulation, inflammation as well as dystrophy and necrosis of epithelial cells.

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