Original Article

Functional Role of the Rodlet Cell and Macrophage in Neural Protection of the Olfactory Neuroepithelium in a Teleostean: Gobiid (*Pseudapocryptes lanceolatus* [Bloch and Schneider, 1801]): An Ultrastructural Study

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Abstract

Background: Neural protection of the olfactory epithelium against pathogenic invasion is still hardly addressed in fish chemosensory research. **Aims and Objectives:** The ultrastructural detail on the rodlet cell and macrophage has been studied within the olfactory neuroepithelium of *Pseudapocryptes lanceolatus* to correlate their role in the neural protection of the chemosensory system. **Materials and Methods:** The cellular structures were examined under light microscope (LM: Primo Star; Carl Zeiss Microscopy, GmbH, Germany) and transmission electron microscope (Morgagni 268D). **Results:** Three distinct stages of the rodlet cell (viz., immature, mature, and degenerative) and macrophages have been characterized at the various depths of the olfactory neuroepithelium in *P. lanceolatus*. The cytoarchitecture of degenerative rodlet cell indicates holocrine mode of secretion against pathogenic invasion into the nasal cavity. Macrophages possess prominent pseudopodia, extending toward invading pathogens. The interaction between macrophage and invading pathogens implicates the role of macrophage as a scavenger to eliminate the pathogens by phagocytosis from the neuroepithelial system. **Conclusion:** This study denotes a significant difference in the mode of action of rodlet cell and macrophages, but they are commonly involved in cell-mediated nonspecific immune response against the invading pathogens.

Keywords: Chemosensory cells, macrophage, phagocytosis, *Pseudapocryptes lanceolatus,* rodlet cells

Introduction

Olfaction is a primary chemosensory modality of fish. This sense is significantly involved in perceiving several chemical cues from the distant source of the external environment and elicits different behavioral responses in fish, viz., searching of food, recognition of sex, species identification, avoidance of predators, and orientation in migration.[1] The olfactory sensory receptor cells reside within the olfactory neuroepithelium and can recognize various classes of chemical cues.[2] Teleosts possess three different morphotype of olfactory sensory receptor neurons (i.e., ciliated sensory receptor cell, microvillous sensory receptor cell, and crypt cell) that has been identified at specific cellular layer of the olfactory neuroepithelium.[3-5] These cells can generate nerve impulse when interact with water-soluble odorants but experienced with vulnerable effect while exposed to various inorganic and organic pollutants and pathogenic invasion during water ventilation.[2,5] Therefore, neural protection of the olfactory neuroepithelium in fish is a great challenge as aquatic environments are severely affected by various anthropogenic activities. Although macrophage plays a pivotal role in cell-mediated neural protection, but it is still hardly detailed in fish

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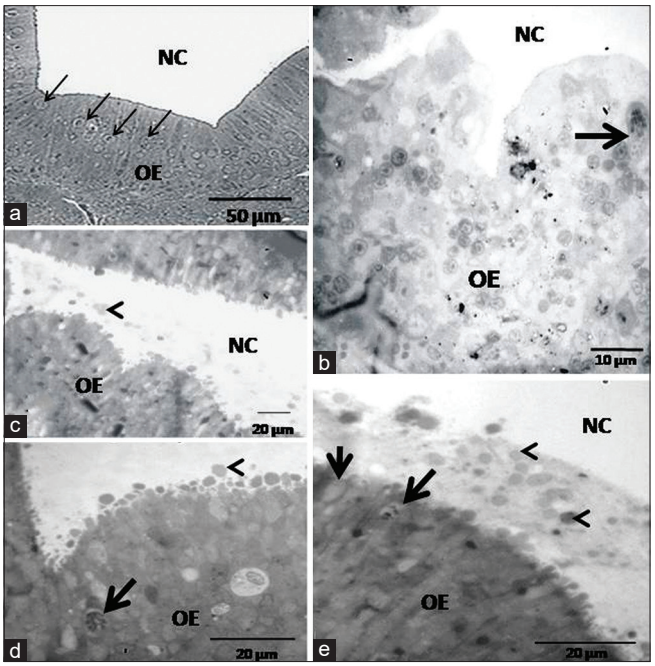
olfactory neuroepithelium.[6] Rodlet cell is also recognized as a special type of cell that is only found in various tissue systems of fish.[7] The functional importance of this cell is still a subject of variable interpretations.[8] It is hypothesized that the osmoregulation, ion transportation, and nonspecific immunity against invading pathogens are the most important functions of the rodlet cell.[9,10] The occurrence of the rodlet cell within the olfactory neuroepithelium of the zebrafish (*Danio rerio*) was first reported by Hansen and Zeiske, 1998,[11] but its functional correlation is still not addressed properly. The present study is aimed to unfold the role of rodlet cell and macrophage within the olfactory neuroepithelium of a teleost (*Pseudapocryptes lanceolatus* [Bloch and Schneider, 1801]) when exposed to pathogens into the nasal cavity.

Materials and Methods

P. lanceolatus is a teleostean: Gobiid of Gangetic Bengal. There are no known threats that have been recorded in IUCN red list category *P. lanceolatus* (i.e., "Least Concern") (Website: http://www.iucnredlist. org/details/169496/0). Adult specimens of *P. lanceolatus* were collected from the intertidal habitat of Hooghly River, Tribeni, West Bengal, India (influenced by a large amount of sewage and effluents, including inorganic and organic pollutants). The specimens were brought to the laboratory for acclimatization with the physical conditions (temperature: 20°C–25°C, humidity: >40%, and time: 24 h) and anesthetized by using MS– 222 (dose: 100–200 mg/L). The olfactory apparatus were dissected out from the dorsal side of the head and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) at 4°C for 2 h. After primary fixation, the olfactory tissues were rinsed in the same buffer and then fixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.3) for 1 h at 25°C. The olfactory tissues were then dehydrated through graded chilled acetone and embedded in Araldite CY212 (TAAB, UK) for 48 h at 60°C. Transverse sections of the olfactory lamella (thickness: 1 µm) were cut with an ultramicrotome (Leica ultracut UC6), stained with 1% toluidine blue and examined under a light microscope (LM: Primo Star; Carl Zeiss Microscopy, GmbH, Germany). For the transmission electron microscope (TEM) study, transverse ultrathin sections (70–80 nm) of the olfactory lamella were cut, collected on copper grids, and stained with uranyl acetate and lead citrate, respectively. The sections were observed under a Morgagni 268D TEM (Fei Electron Optics, Eindhoven, the Netherlands) operated at 80 kV. Digital images were captured at primary magnifications between ×4000 and ×9000 through the Mega view III camera, using iTEM software (soft imaging system, Münster, Germany) attached to the microscope. Diameter measurements of various organelles and cytoskeleton were performed on the acquired digital images.

Results

The pear-shaped rodlet cells frequently appear at different cellular layers (i.e., basal, middle, and superficial layers) of the olfactory neuroepithelium in *P. lanceolatus* in response to the



Figures 1: The microanatomical characterization of rodlet cells within the olfactory neuroepithelium (OE) of *Pseudapocryptes lanceolatus*. (a) The rodlet cells (arrows) with different morphometry identified at variable depths of OE. (NC: Nasal Cavity) (b) The matured rodlet cell (→) is marked at the middle part of OE. (c) During low tide, numerous unicellular pathogens (arrowheads) are marked. (d) The mature rodlet cell (→) is located at the upper middle part of OE in the presence of several pathogens (arrowhead). (e) The degenerating rodlet cells (arrows) are marked in the presence of different pathogens (arrowheads)

invasion of various pathogens in the nasal cavity [Figure 1a-e]. This cell is characterized under the LM by the presence of outer dense exoplasm, faintly stained endoplasm with rodlet sacs, and basally located nucleus [Figure 1b]. Three distinct stages of rodlet cell, namely, immature, mature, and degenerative stages have been identified under TEM within the olfactory neuroepithelium of *P. lanceolatus* [Figures 2-4]. The immature, oval-shaped rodlet cells with intact fibrous capsule (thickness: 200–400 nm) are located near the basal lamina of the olfactory neuroepithelium [Figure 2]. The euchromatinized nucleus is located at the basal part of the cell [Figure 2]. Heterochromatin materials are scattered at the peripheral part of the nucleoplasm. Polygonal rodlet sacs vary in diameter (diameter: 0.3–1 µm). Rodlet sacs are single-membrane-bound structure and contain numerous granulated substances [Figure 2]. The cytoplasm of immature rodlet cell is less compact in nature and shows vesicular dilatation of rough endoplasmic reticulum (rER) with scattered, free ribosomes at the perinuclear cytoplasm [Figure 2]. The elongated, mature rodlet cell is located at the middle part of the olfactory neuroepithelium of *P. lanceolatus* [Figures 3a and 5]. The thickness of the fibrous capsule-like exoplasm is greater than immature stage of the rodlet cell (300–500 nm) [Figures 3d and 5]. At the apical part of this rodlet cell shows cytoplasmic protrusion, i.e., microvillar appearance [Figure 5]. The lateral part of the plasma membrane shows distinct glycocalyx and few

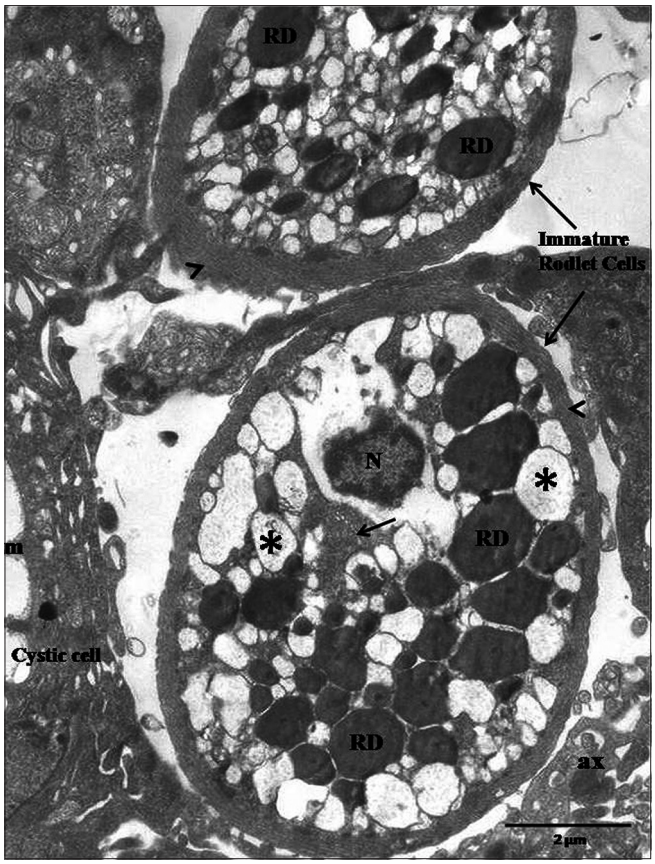
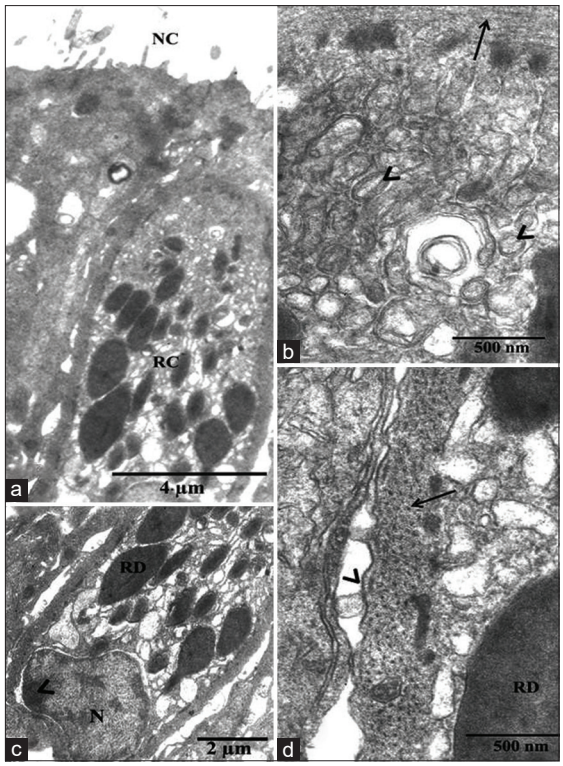


Figure 2: Immature rodlet cells are characterized under the transmission electron microscope , located at the lower part of the olfactory neuroepithelium. Axons (ax) of different sensory receptor cells are noted in association with immature rodlet cell. The fibrous exoplasm (arrowheads) is marked at the outer region of the rodlet cell. The cytoplasm of immature rodlet cell shows the prominent presence of polygonal rodlet sacs (RD), basally located euchromatinized nucleus (N), vesicular structures, electron-lucent vesicles (stars), and dilated cytoplasm with Golgi complex (→)



Figures 3: The photomicrographs show mature rodlet cell. (a) The mature elongated rodlet cells (RC) are present at the middle part of the olfactory neuroepithelium. (b) The anterior part of matured rodlet cell shows fibrous exoplasm (→) and double-membrane vesicles (>). (c) The cytoplasm of the rodlet cells also shows club-shaped rodlets (RD) with electron-dense core material. Nucleus (N) with distinct nucleolus (>) is located at the basal part of the cell. (d) The outer membrane of the rodlet cell shows prominent glycocalyx (>). The exoplasm possesses numerous microfibril-like structures (→)

cellular junctions with the adjacent epithelial cells of the olfactory neuroepithelium [Figures 3d and 5]. The rodlet sacs are apparently large in number, and the diameter ranges from 0.3 to 2 µm [Figures 3c and 5]. Dense, granular substances are very much prominent within the rodlet sacs of mature stage. Electron-lucent vesicles are observed within the cytoplasm [Figures 3b and 5]. These vesicles are different in morphometry, and sometimes, it shows a double membrane-bound structure. Nucleus is round in shape and present at the basal portion of the mature rodlet cell [Figures 3c and 5]. Nucleolus is prominent and located near the nuclear membrane. An accumulation of chromatin granules is also observed within the nucleoplasm of the mature stage of the rodlet cell [Figures 3c and 5]. Ribosomes are prominent on the outer nuclear membrane. Few ribosomes are also scattered within the cytoplasm. Endoplasmic reticulum (ER) is present in vesicular form, but other subcellular organelles are not very prominent [Figure 6]. A large number of degenerative rodlet cells are clearly noticeable at the superficial layer of the olfactory neuroepithelium in the presence of unicellular pathogens within the nasal cavity of *P. lanceolatus* [Figure 4]. The existence of fibrous exoplasm is not clearly identified under the TEM study. Mitochondria with dilated cristae, degenerating rodlet sac, and granulated secretory materials are marked within the cytoplasm [Figure 4]. This cell contains several vesicular structures with electron-dense materials. The dilated rodlet sacs and its secretion toward the nasal cavity are also observed [Figure 4]. Apart from the various stages of rodlet cells, macrophages are also present within the olfactory neuroepithelium of *P. lanceolatus*. Macrophages are roughly round or oval in shape with irregular outlines and distinct pseudopodia [Figure 7a and b]. This cell is located within the middle and lower part of the olfactory neuroepithelium of *P. lanceolatus* and shows phagocytic activity by extending pseudopodia when contact with invading pathogens [Figures 7a-c]. Macrophage possesses large, eccentric, and kidney-shaped nucleus [Figure 7b]. The heterochromatin materials are distributed at the peripheral region of the nucleus and closely associated with the nuclear membrane [Figure 7b]. Nucleolus is prominent. The cytoplasm of the macrophage cell shows primary and secondary lysosomes, rER, well-developed Golgi complex, and other cellular debris [Figures 7b and c]. Moderate amount of free

Figure 4: The photomicrograph of degenerating rodlet cell shows the absence of fibrous exoplasm at the outer cytoplasm. The dilated rodlet sacs (→), mitochondria with degenerating Cristea (mt) and other vesicular contents (*) have been identifiable

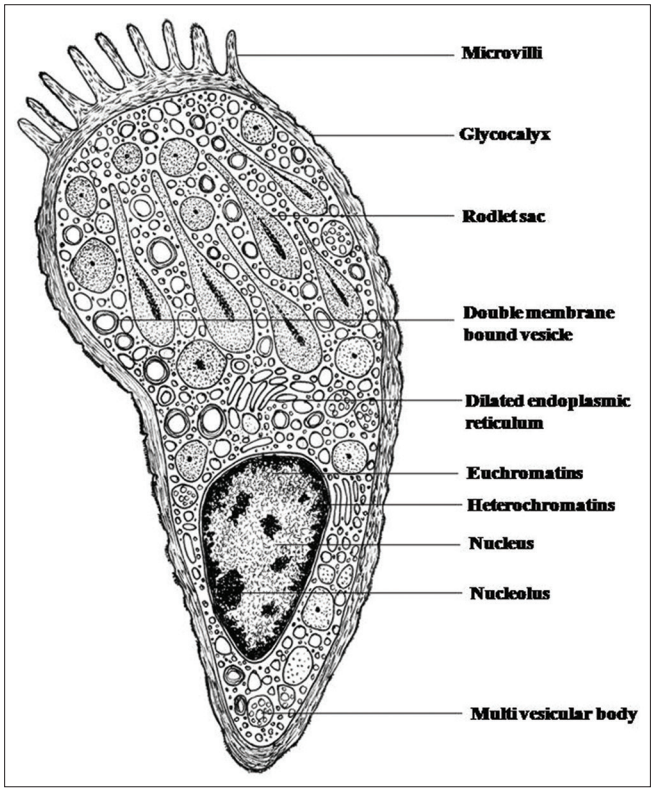


Figure 5: The diagram represents the mature rodlet cell with distinct cellular organelles (Microvilli, rodlet sacs, double-membrane-bound vesicles, multivesicular body, glycocalyx, nucleus, euchromatin and heterochromatin materials, nucleolus, and dilated endoplasmic reticulum) within the olfactory neuroepithelium of *Pseudapocryptes lanceolatus*. (Not to scale)

ribosomes are also marked within the cytoplasm [Figure 7b]. Some electron-dense vesicles are also found at the peripheral region of the cell [Figure 7d]. The distinct cellular debris is marked within the electron micrograph of the macrophage cell of the olfactory neuroepithelium [Figures 7b-d].

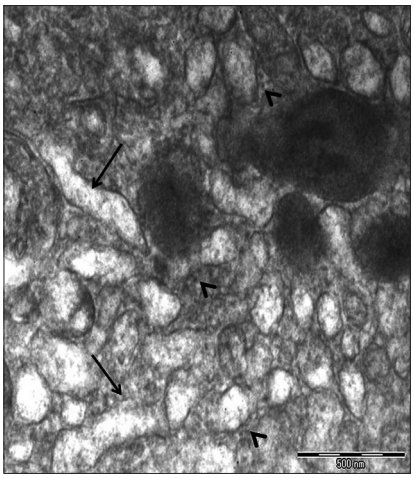
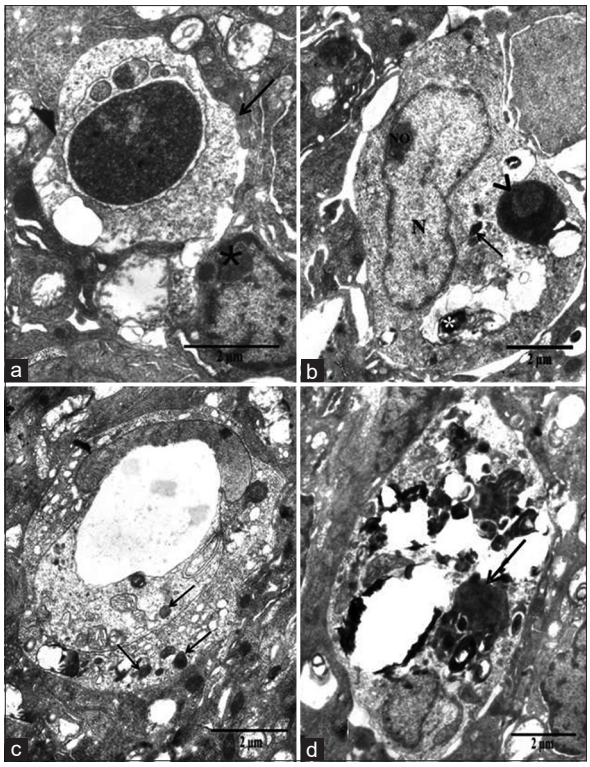


Figure 6: Several stages of the dilated rough endoplasmic reticulum (rER) devoid of ribosomes (arrows) have been identifiable. Free ribosomes are also (arrowheads) marked within the cytoplasm of mature rodlet cell

Discussion

The neural protection of the peripheral olfactory system against invading pathogens is one of the important aspects of fish chemosensory research. This study highlights on the occurrence and subcellular details of the rodlet cell and macrophages that are frequently observed within the olfactory neuroepithelium in response to various pathogens within the nasal cavity of *P. lanceolatus*. The rodlet cell is considered to be a migratory blood cell derived from the circulating stem cell.[12] This cell shows a common cytological architecture (i.e., outer fibrous cortex or exoplasm and inner cytoplasm with numerous rodlet sacs). The fibrous exoplasm is largely aggregated at the peripheral part of the cytoplasm to maintain the structural integrity of the rodlet cell (both in immature and mature stages). The presence of glycocalyx at the surface region of mature rodlet cell may protect the cell from mechanical or chemical damage and also keeps various other cells at a distance to prevent unwanted protein–protein interactions.[13] Apart from that, the granulated meshwork of chromatin material is largely noted at the inner nuclear membrane that may help to provide the structural stability of the nuclear envelope.[14] The irregular shape of the outer nuclear membrane in the rodlet cell may an indicative of degenerating nature. The features of dilatation in ER, ribosomes, mitochondria, and Golgi complex are also denoted in various stages of rodlet cell within the olfactory neuroepithelium of *P. lanceolatus*. The cytological integrity of fibrous exoplasm in degenerating rodlet cell is not well demarcated. The disintegration of cytoplasmic and subcellular components may be indicative of a holocrine mode of secretory nature of degenerating rodlet cell. The lack of fibrous exoplasm in degenerative rodlet cell probably promotes the secretion of cellular component toward the nasal cavity of *P. lanceolatus*. Although the biochemical nature of secretory materials of degenerative rodlet cells is still not examined, it assumed that the rodlet cell is involved in "nonspecific immune response" through the holocrine mode of secretion to protect the olfactory neuroepithelium from invading pathogens present within the nasal cavity. Macrophages are also



Figures 7: The electron micrograph showing different characters of macrophage within the olfactory neuroepithelium. (a) The cellular structure of macrophage (*) with distinct pseudopodia (→) are indicated. (b) Nucleus (N), nucleolus (NO), cellular debris (>), and secondary lysosome (→) are also indicated within the macrophage. (c) A degenerating cell is identified within the cytoplasmic part of a macrophage. Different phases of lysosomes (arrows) are observed within the cytoplasmic matrix. (d) The cellular debris (→) is prominently noted in neuroepithelial macrophage of *Pseudapocryptes lanceolatus*

well known for their phagocytic capacity and general defense mechanism according to their respective site of origin.[15,16] Activated macrophages are heterogeneous group of cells that perform distinct immunological functions.[17] This cell possesses a variety of surface receptors specialized for the recognition of nonself pathogen or pathogen-derived structures.[18] Macrophage migrates toward the intercellular pathogens, encounters them and ultimately degrades with the help of lysosomal enzymes.[18] The notable diversities of primary and secondary lysosomal structures are studied within the olfactory neuroepithelium of *P. lanceolatus*.

Conclusion

This research contribution indicates a dynamic interaction between macrophage and invading pathogens within the olfactory neuroepithelium which is implicating the role of macrophage as a scavenger. In comparison to macrophage, the rodlet cell is also involved in "nonspecific immune response" through the holocrine mode of secretion for protecting the olfactory neuroepithelium from invading pathogens during water ventilation. Therefore, this study concludes that rodlet cell and macrophages are significantly different in their mode of action but play significant roles in cell-mediated nonspecific immune response against invading pathogens.

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Conflicts of interest

There are no conflicts of interest.

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