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The Diversity of Cells in the Thymic Microenvironment of *Aplocheilus panchax* (Hamilton, 1822)

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Abstract: *Thymus is a primary lymphoid organ in fish. This gland is situated at the upper most part of the mid-dorsolateral region of the opercular cavity, close to the base of 3rd and 4th gill arches. Aplocheilus panchax (Weight-2.5gm. Length-7.5cm.) a fresh water teleost belonging to the Order – Cyprinidontiformes, family – Aplocheilidae is examined under light and transmission electron microscopes (LM and TEM) to study the anatomical position and cellular composition of thymic environment. Histologically, the cortex and medulla is not well demarcated in this gland of A. panchax. The gland is packed with lymphoid cells and well vascularised. Under electron micrograph, the thymic tissue shows different lymphoid and non lymphoid elements. The thymic blood capillary is clearly marked. Apart from that, thymocytes, thymoblast, thymopharyngeal epithelial cell, reticular epithelial cell, rodlet cell, mast cell etc. are also characterized. The appearance of mast cell in thymic environment is also very significant for the assessment of thymic health.*

Keywords: *Aplocheilus panchax*, thymocytes, thymic blood capillary, rodlet cell, mast cell *etc*.

1. Introduction

The thymus is an 'old gland' [1]. Phylogenetically, the prototype thymus gland appears first in elasmobranches (*i.e.*, rays and sharks) which are located near the dorsal and medial part of the gill arches [2 and 3]. The fish thymus, which may be consider as a key organ of the immune system and seems to be the major location for T cells [4]. Stannius [5] was first described the gross anatomy of thymus gland in angler fish (*Lophius piscatorius*). This gland in angler fish is located at a distance from the branchial cavity [6]. The position of the thymus gland within the pharyngeal epithelium varies among the teleost. [7]. Like higher vertebrates, the thymus gland of elasmobranches is clearly divided into cortex and medulla [7]. Although, the cytological demarcation of cortex and medulla in teleosts is still a debatable part in thymic biology [8].The different type of lymphoid and nonlymphoid cellular structures has been identified within the thymic stroma of vertebrates. These cells are morphologically similar but vary greatly in occurrence and number among the vertebrate phyla [9]. Apart from that the cellular diversity and their true identification within the thymus gland of fish is also a contradictory part in thymic research. The present study emphasized on the microanatomy and the identification of different cell types within the thymic microenvironment in relation to their specific ecological habitat.

2. Materials and Methods

Aplocheilus panchax (Hamilton, 1822) is an indigenous larvivorous fish ('Least Concern'- IUCN) of South East Asia. This species is widely distributed in all over South-East Asia. For microanatomical studies, live and healthy *A. panchax* specimens were directly collected from the various ponds and sewage drain water of different area of village Rupasgori (Howrah districts of West Bengal, India, Latitude 22º46´N and Longitude 87º96´E). The collected specimens were brought to the laboratory and acclimatized with the laboratory conditions [temperature: 20˚ to 25˚C, humidity: >40%, *etc*.] for 24 hours. The thymus glands of *A. panchax* were dissected out from the opercular cavity and the procured thymus glands were separately fixed in aqueous Bouin's solution for (6-10) hours at room temperature (25˚C-30˚C). The tissues were then washed and subsequently dehydrated through graded ethanol. The dehydrated tissues were immediately transferred into Cedar Wood Oil for (24 – 48) hours. The tissues were then cleared in xylene and embedded in graded paraffin - xylene mixture under a thermostat vacuum paraffin-embedding bath [temperature 58°C–60°C] for a period of 60 minutes each respectively. The serial thin sections (about 5µm) of the thymus gland were cut by using rotary microtome and stretched on Mayer's albuminised glass slide. The sections were then stained with haematoxylin - eosin and examined under light microscope (LM - Model No micro imaging GmbH, serial no- 3109003139, Carl Zeiss-Germany). For transmission electron microscopical study, the thymus glands of *A. panchax* were separately dissected out and immediately fixed in 2.5% glutaraldehyde in 0.1 (M) phosphate buffer (pH.7.2–7.4) at 4°C for 1–2 hours. After completion of the primary fixation, the tissues were rinsed in the same buffer and further fixed in 1% osmium tetraoxide (OsO_4) in 0.1 (M) phosphate buffer (pH.7.2–7.4) for 1hour at 4° C. The thymus glands were then dehydrated in graded, chilled acetone (at 4°C). The dehydrated tissues were embedded in araldite mixture and incubated at 60°C for 48–72hours. Ultrathin sections (70nm–90nm) were cut with the help of ultramicrotome (Leica Ultracut: UC 6) and collected on copper grids; stained with uranyl acetate solution and lead citrate; examined under transmission electron microscope [TEM: MORGAGNI – 268D] operated at 40kV.

3. RESULTS

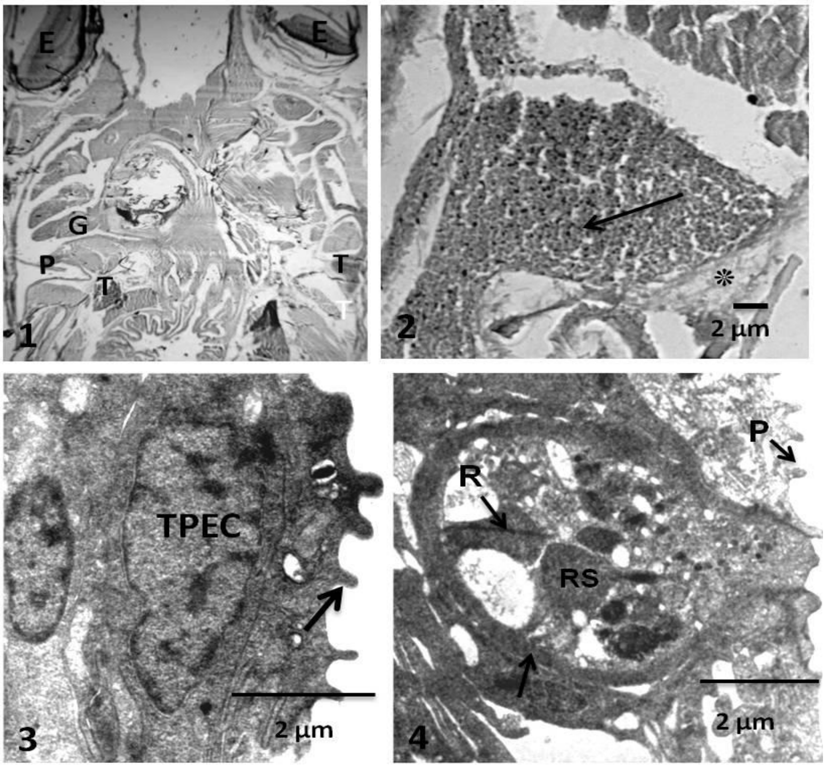

Fig1. *The microanatomy of thymus gland shows the bilaterally position of thymus gland (T) within the opercular cavity of A. panchax (from paraffin section). [Eye (E), gill (G), pharyngeal wall (P), etc.]. [Mag. x100 (approx.)]*

Fig2. *The histological section of the thymus gland shows thymocytes (arrow heads) and muscular sheath (star).*

Fig3. *The electron micrograph shows thymopharyngeal epithelial cells (TPEC), pharyngeal wall (P) etc., within thymic tissue of A. panchax.*

Fig4. *The thymic rodlet cell with several rodlet sac (RS), rodlet (R), fibrous exoplasm (arrow), etc. are identified within the thymus gland of A. panchax. Pharyngeal wall (P) is also observed.*

The thymus gland of *Aplocheilus panchax* is paired in nature and bilaterally placed (Figure-1). The gland is located at the uppermost part of the mid-dorsolateral region on the either side of opercular cavity, close to the base of 3rd and 4th gill arches (Figure-1). The thymus gland is covered by a fibro muscular sheath (Figure-2). Micro anatomically, thymus is hardly differentiated into cortex and medulla (Figure-2). Thymocytes are not uniformly distributed in thymic environment. The thymus gland is compact with varieties of lymphoid (thymocytes, thymoblast *etc.*) and non lymphoid elements (thymopharyngeal epithelial cell, reticular epithelial cell, endothelial cell, thymic blebs etc.). Thymocytes are the one of the chief cellular components of the thymic tissue in *A. panchax* and possess prominent chromatinized nucleus with nucleolus (Figures-1,5,6). The ovoid shaped thymoblasts are also observed within the thymic tissue. This cell shows large and round shaped nucleus. Elongated mitochondria are noted within the cytoplasm of thymoblast cell (Figure-6). Thymic blebs are also observed and it is closely attached with thymoblast cells (Figure-6). The thymopharyngeal epithelial cells with extended nucleus and very closely associated with pharyngeal epithelium (Figure-3). Heterochromatine materials are observed towards the inner part of the nuclear membrane. Reticular epithelial cells are identified within the thymic tissue (Figure-7). These cells possess large nucleus with characteristic extended cytoplasmic processes. The vascular drainage through thymic blood capillary is also examined (Figure-7). The endothelial cell lining of the blood capillary is separated from the epithelial cell of the thymic tissue. Apart from that, special type of cell *i.e.*, rodlet cell are also evident within the thymic tissue of concerned species (Figure-4). The rodlet cell are located various depth of the thymopharyengeal region of the thymic tissue. The elongated membrane bounded sacs like structure is also noted. Rodlet sacs are extended towards the periphery of the cell (Figure-4). Rodlet are also observed within the rodlet sac. Mast cell is well noted in the thymic environment of *A. panchax* (Figure-8). It is pentagonal in shape which contained variety of cytogranules. The granules are round or elongated in shaped. The heretochromatin materials are very prominent and distributed towards the peripheral part of nucleoplasm. Nucleus is basally placed with very prominent nucleolus.

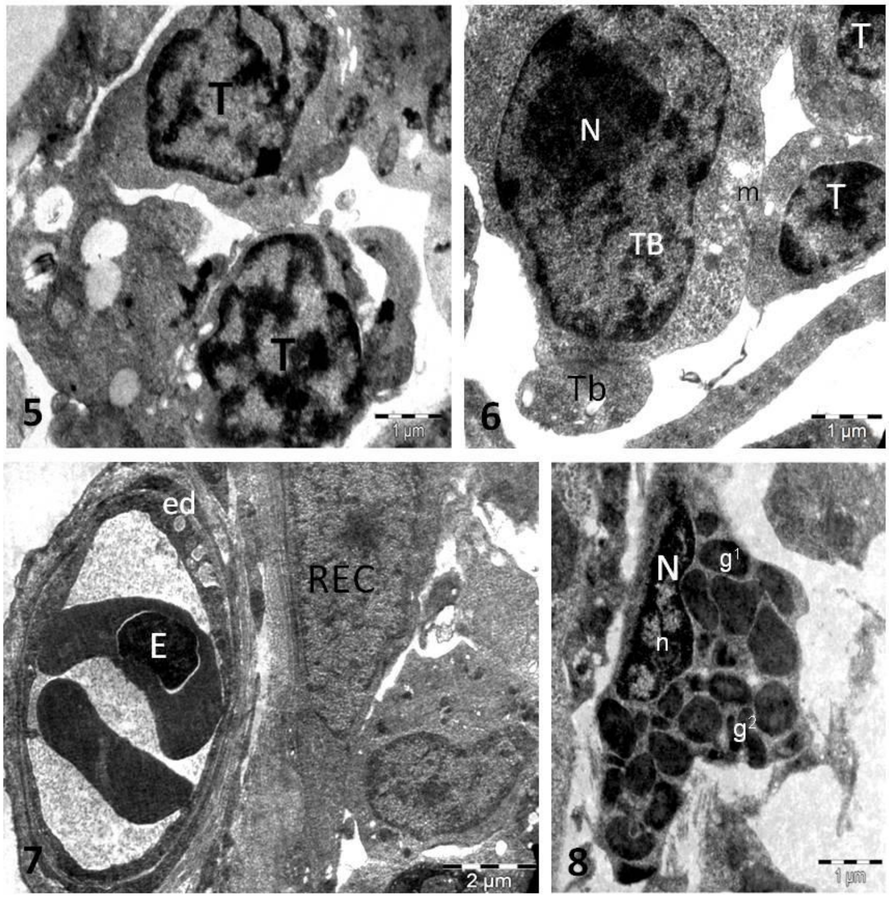

Fig5. *The electron micrograph shows prominent thymocytes (T) within the thymic microenvironment.*

Fig6. *The thymic stroma shows thymocytes (T), thymoblast (TB) with prominent nucleous (N), mitochondria (m) and thymic blebs (Tb) etc.*

Fig7. *The developing erythrocyte (E), endothelial cell (ed) etc. are marked within the thymic vessels. Reticular epithelial cell (REC) is also observed at the peripheral part of thymic vessels.*

Fig8. *The mast cell in the thymic tissue of A. panchax shows nucleus (N) with prominent nucleolus (n) mitochondria (m), granule (g^1 and g^2) are clearly demonstrated.*

4. DISCUSSION

The thymus is an important lymphohaemopoietic organ in fish [10]. Morphoanatomy of thymus in some fishes is already known [11]. The position of thymus gland within the pharyngeal epithelium varies slightly in different fish species [7]. The different positions of the thymus observed in different species of fish may represent the character of a particular species [12]. It is reported that the position of thymus is located over the first and second gill arches in *Oncoryhnchus mykiss* [13], in *Cyprinus carpio* the second and third [14], in *Harpagifer antarcticus* the second, third and fourth [15] and in cichlid fishes the third and fourth gill arches [16]. The association of paired thymus gland within the pharyngeal epithelium is very common in *Aplocheilus panchax* and situated upper most part within the opercular cavity, close to 3rd and 4th gill arches. The gland shows a wide range of cellular diversity [17]. The occurrence of the various types of cells within the thymic microenvironment is also very unique and plays various functional activities. The demarcation of the true cotico-medullary junction is not properly known in *A. panchax*. The cortex and medulla in fish thymus is still a contradictory part in thymic research [8]. In many higher vertebrate, the thymus is hardly differentiated into cortex and medulla [18]. During the development of thymocytes, the non-lymphoid thymic epithelial cells are significantly involved in differentiation as well as maturation of T-lymphocytes within the thymic tissue [19]. The interaction between thymocytes and epithelial cells are required for development of functional cellular elements within the thymus gland [20]. Several environmental conditions modify the structural as well as cellular diversity of thymus gland in *A. panchax* [21] and also exposed the similarities with other telelostean species. Thymopharyngeal epithelial cell is close to pharyngeal structure may perform as a blood thymic barrier, as reported in other vertebrate species [22]. The functional significance of rodlet cell in thymic stroma of teleosts is not well detailed but the presence of rodlet cell within the thymopharyngeal region in the thymus gland of *A. panchax* might have some advantages from the respective aquatic ecosystem. Because, the recruitment of rodlet cells at the site of infection is very important for tissue concern [23]. Apart from that, the increased numbers of rodlet cells in fish exposed to adverse environmental conditions and toxic substances [24 and 25]. The blood vascular channels are also reported by Zapata [10] in fish. Chilmonczyk [26] reported on fenestrated endothelial cell and this type of cellular structure is quite similar in thymic blood vessels of *A. panchax*. The appearance of mast cell in thymic environment is also very significant for the assessment of thymic health. The present study emphasized on the cellular diversity within the thymic microenvironment of *A. panchax* in relation to their specific ecological habitat and also raised a frame work for further investigation.

5. CONCLUSION

Aplocheilus panchax is a hardy larvivorous fish in fresh water, polluted water respectively. This paper emphasizes on the microanatomical detail on the thymus gland as well as the identification of its cellular diversity *i.e*., thymocytes, thymopharyngeal epithelial cells, reticular epithelial cells, thymic blebs, rodlet cells, mast cells, *etc.* to know their specific functions in their respective ecological habitat. The appearance of rodlet cell, mast cell *etc*., in thymic microenvironment is significant in relation to assess the specific ecological state for that unique gland.

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