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HAEMATOPOIESIS IN THE HEAD KIDNEY OF FRESHWATER EEL, *ANGUILLA BICOLOR BICOLOR*(MCCLELLAND, 1844)

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Abstract: The present work focused on haematopoiesis in the headkidney of freshwater eel, *Anguilla bicolor bicolor*. The cells observed in the head kidney, includes the haemocytoblast- the stem cell and the developmental stages of different blood cell types: pro- erythroblasts, pro- erythrocyte and erythrocytes in the erythropoietic series, lymphoblasts and the lymphocytes (both large and small) in the lymphopoietic series, monoblasts, and monocyte in the monopoietic series and granuloblast, pro- neutrophils and neutrophils in the granulopoietic series. Only thrombocytes are observed in the thrombopoietic series. Macrophages and degenerating erythrocytes were also observed. The morphogenesis of developing blood cells and their lineages are similar to other teleosts. The morphological changes occur during maturation process are reduction in size and further increase at mature stage and gradual chromatin condensation of the nuclei. From the present study it can be concluded that the head kidney of *A. bicolor bicolor* is actively involved in the process of haematopoiesis and it could be the most important organ in the defence system.

Key words: Haemocytoblast, Morphogenesis, Defence system, Lymphomyeloid tissues, Pronephros

INTRODUCTION

Among vertebrates, fishes are the earliest group to possess a well-defined immune system with lymphomyeloid tissues consisting of mixed lymphoid and myeloid elements (Pica and Corte, 1987). Liu *et al*. (2004) and Patel *et al*. (2009) suggested that the lymphoid organs in fish include thymus, spleen and head kidney. The head kidney or pronephros in fish is the basic haematopoietic organ forming the blood elements (Rombout *et al*., 2005). The activity of the blood elements formation differs among teleost fish; it can be organ-forming erythroid lineages only in some fish, or all types of organ-forming blood cells in other fish (Esteban *et al*., 2000; Stephens *et al*., 2004). In some fishes, both haematopoietic organs function equally, whereas in others one is more active than the other. In *Salmotrutta*, only the spleen showed haematopoietic activity, but in *Rutilusrutilus* only the kidney, while in *Perca fluviatilis* both organs were active (Catton, 1951).

Previous histological studies have been concerned with determining the haematopoietic tissue and the series of haematopoiesis in different teleosts. However, little information is available on haematopoietic stem or progenitor cells in kidney haematopoietic tissue (Kobayashi *et al*. 2006) and very few quantitative data on the proportions among blood cell lineages in haematopoietic organs of teleosts (Fijan, 2002).

Anguilla bicolor bicolor, is a catadromous fish with a very complicated life history and during their migratory movements they have to survive in marine, estuarine and freshwater habitats. At this travel route they are exposed to attacks of pathogens such as bacteria, viruses and parasites and hence, they ought to have well-developed defence mechanisms.The blood cell plays a major role in providing immunity in all vertebrates including fishes and therefore the process of haematopoiesis is very crucial for the normal development of all the blood cell lineages. In the present study an attempt has been made in identifyingthe blood cell lineages in the head kidneyof freshwater eel, *A. bicolor bicolor* as a part of better understanding of their immune system.

MATERIALS AND METHODS

Live specimens of *A. bicolor bicolor* were collected from freshwater habitats of Alappuzha district, Kerala, India. A total of 20 young and adult healthy fish of both sexes were used for the study. The size of the fish varied from 35-76 cm in total length (TL) and 90-919 gm total weight (TW). The fish were killed by fast cutting the spinal cord just behind the head using sharp scissor as described by Kondera (2011). After opening the abdominal cavity, head kidney was collected. The impression smears were prepared on a clean micro slide from the cut surface of freshly dissected head kidney as described by Mahajan and Dheer (1980). The imprints were air dried for 24 h, fixed in methanol for 30 seconds and stained using Wright-Giemsa solutions. Morphology and staining characteristics of all developing blood cell types were studied using smears viewed with light microscope at varying magnifications. Photo-micrographs taken under research microscope supported with Q win software (Leica).

RESULTS AND DISCUSSION

The haematopoietic stem cells (HSCs), the most immature elements of the haematopoietic hierarchy, which are localized in lymphomyeloid or haematopoietic tissues, proliferate and differentiate into various classes of haematopoietic progenitor stem cells (HPSCs). The totipotent stem cell is the 'haemocytoblast' (Boomker, 1980) or 'haemoblast' (Romestand and Trilles, 1984). These progenitor cells have been differentiated into erythropoietic, granulopoietic, lymphopoietic, monopoietic or a combination, depending on the nature of progenitor cells comprising it.

In the present study, thedeveloping blood cells in the head kidney were identified based on their morphological characteristics and staining properties as shown in table 1. The cells identified from the head kidney include, haemocytoblastact as the stem cell and the developmental stages of following series: erythropoietic, lymphopoietic, monopoietic and granulopoietic. Only thrombocytes are observed in the thrombopoietic series. Macrophages and degenerating erythrocytes were also observedin the head kidney of *A. bicolor bicolor*. In some teleosts similar observations were reported by Zuasti and Ferrer (1988) and Belosevic *et al*. (2006).

Haemocytoblast

They are round or oval cells with round or oval nucleus (Fig. 1 and 3). The cytoplasm stained blue and the nucleus stained pink as recorded in *Ictalurus punctatus* (Fijan, 2002), *Clarias batrachus* (Gangopadhyay and Home chaudhuri, 2011) and in *C. carpio* (Kondera, 2011). The nucleus is larger in size and over half of the cell, as recorded in rainbow trout by Peters and Schwarzer (1985).

Erythropoietic series

The erythropoietic series consisted of the pro-erythroblast (Fig. 1, 4 and 5), pro- erythrocyte (Fig. 6 and 4) and erythrocyte (young and mature) (Fig. 6). These developmental stages are similar to other teleosts which were clearly elucidated by Haider (1968). The shape of the pro-erythroblast was irregular or sub spherical cells. The cytoplasm stained blue and the irregular or subspherical nucleus stained pink. The pro- erythroblast typically showed round nucleus, more condensed than the haemocytoblast. The pro-erythrocyte was irregularly round to oval, larger than pro-erythroblast, with less condensed nucleus, and smaller than the mature erythrocytes. This result is in agreement with that obtained by Kondera (2011) in *C. carpio*. Erythrocytes are the commonest blood cells, elliptical or oval in outline. Mature erythrocytes were larger; more elongated and showed more intense light blue cytoplasm with deep blue nucleus than the immature one, the reticulocyte. This observation was similar to the mature erythrocytes of *Ictalurus punctatus* (Fijan, 2002) and common carp, *Cyprinus carpio* (Kondera, 2011). The cytological changes that observed during maturation in erythropoietic series are reduction of cell size, heterochromatinisation of the nucleus, and reduction of the cytoplasmic organelles. These changes were similar to those described in other species of teleosts (Zapata, 1980; Zuasti and Ferrer, 1989; Esteban *et al*., 1989) and maturation state terminate in the formation of oval nucleated erythrocytes in the blood vessels as reported by Romestand and Trilles (1984).

Table 1. Appearance (shape and staining property) of the developing blood cells in the head kidney of *A.bicolor bicolor* (Wrights –Giemsa stain)

CELL TYPE	SHAPE		STAINING PROPERTY	
	Cell	Nucleus	Cytoplasm	Nucleus
Haemocytoblast	Round/ oval	Round/ oval	Blue	Pink
Pro- erythroblast	Irregular/ Sub spherical	Irregular/ Sub spherical	Blue	Pink
Pro-erythrocyte	Oval	Oval	Blue	Pink
Erythrocyte	Oval	Oval	Light blue	Deep blue
Lymphoblast	Round/Sub spherical	Eccentric nucleus, sub spherical-round	Deep Blue	Pink
Lymphocyte	Round/Irregular	Round	Transparent	Pink
Monoblast	Spherical	Eccentric	Blue	Pink
Monocyte	Spherical	Eccentric	Transparent	Pink
Granuloblast	Round	Eccentric, spherical	Blue	Pink
Pro-neutrophil	Round/elongated	Eccentric, elongated/oval	Blue	Pink
Neutrophil	Round/Irregular	Eccentrically placed, bilobed, Kidney/bean shaped, horse-shoe shaped, dumb-bell shaped.	Light blue/ Transparent	Pink
Thrombocyte	Spherical/ oval, elongated/oblong, spindle shaped	Elongated/ oblong, round, spindle Shaped	Transparent	Pink

Lymphopoietic series

In the lymphopoietic series, two cells were observed, lymphoblast (Fig. 3,4 and 6) and lymphocyte (both small and large)(Fig. 1, 2, 3 and 5) as reported by Mulcahy *et al*. (1983) in *Esoxlucius*, while Zuasti and Ferrer (1988) found only two stages, immature and mature, in *Sparusaurata*, similar to those described in *Esoxlucius* (Savage, 1983). The lymphoblasts are round or subspherical cells with subspherical eccentric nucleus. The cytoplasm stains deep blue and the nucleus is pink in colour. The lymphocytes had a transparent, round or irregular cell with pink coloured round shaped nucleus. Similar results wererecorded in *C. batrachus* (Gangopadhyay and Home chaudhury, 2011).

Monopoietic series

In the development of monocytes two stages have been identified: monoblast (Fig. 3) and monocyte (Fig. 2 and 4). Monoblasts are large, slightly irregular but roughly subspherical cells, usually larger than the granuloblatsts and light blue cytoplasm with pink coloured eccentric nucleus. The nucleus occupied over half of the cell as in common carp, *Cyprinus carpio* (Kondera, 2011). Monocytes were spherical cells with eccentric nucleus. Jordan and Speidel (1931) described monocytes in the blood and haemopoietic sites of lungfish, *Protopterus aethiopicus*. They recognized the precursor of monocyte as monoblast dividing from the lymphoid haemoblast. The mature monocyte had a more abundant transparent cytoplasm and eccentric, more compact reniform or irregularly

Developing blood cells in the head kidney of *A.bicolor bicolor* (Wright-Giemsa stain)

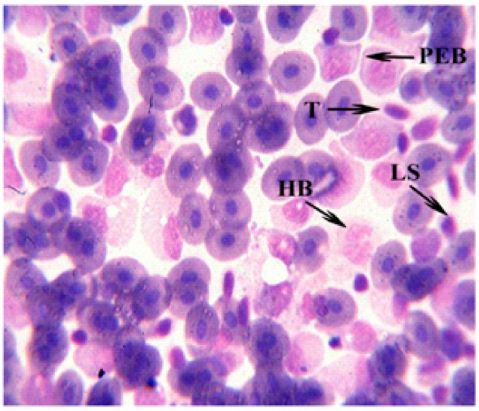

Fig : 1

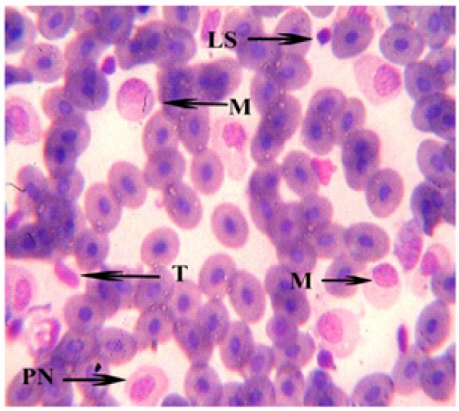

Fig : 2

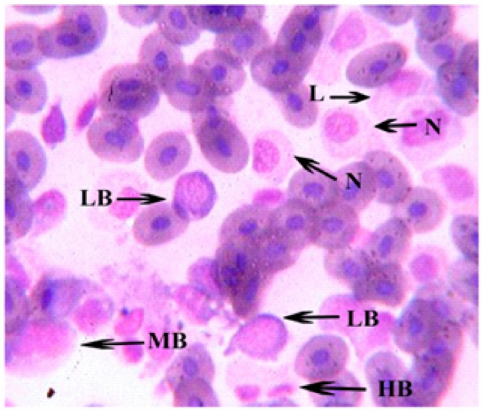

Fig : 3

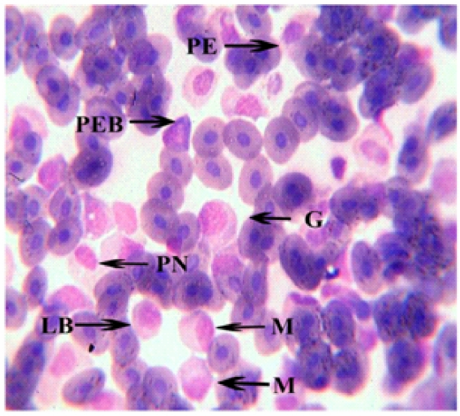

Fig : 4

G- Granuloblast; HB- Haemocytoblast; L- Lymphocyte; LB- Lymphoblast; LS- Small lymphocyte; M- Monocyte;MB- Monoblast; N- Neutrophil; PE- Pro-erythrocyte; PEB- Pro-erythroblast; PN- Pro-neutrophil; T- Thrombocyte.

Developing blood cells in the head kidney of *A.bicolor bicolor* (Wright-Giemsa stain)

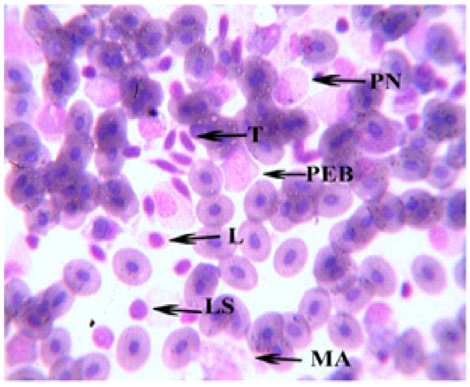

Fig : 5

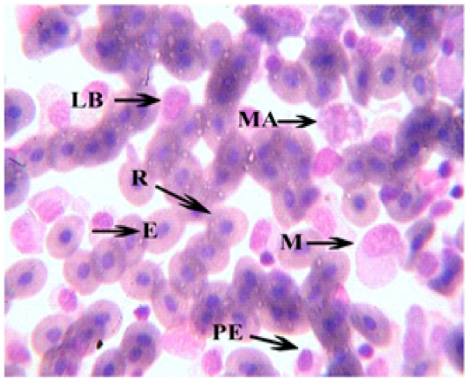

Fig : 6

E- Erythrocyte; L- Lymphocyte; LB- Lymphoblast; LS- Small lymphocyte; M- Monocyte;MA- Macrophage; PE- Pro-erythrocyte; PEB- Pro-erythroblast; PN- Pro-neutrophil; R- Reticulocyte; T- Thrombocyte

oval nucleus as in *C. carpio* (Kondera, 2011). Some monocytes showed different sizes and contained many vacuoles in the cytoplasm, which confirms the findings by Kondera (2011) and Abdel- Aziz *et al.* (2010).

Granulopoietic series

The granulopoiesis in the head kidney of *A. bicolor bicolor* includes neutrophilic series, which consists of granuloblast (Fig. 4), pro-neutrophil (Fig. 2, 4 and 5) and neutrophil (Fig.3). More than three granulopoietic types have been described in other teleosts (Morrow and Pulsford, 1980; Parish *et al*., 1986) while one or two granulopoietic series are frequently been found (Bayne, 1986). Romst and Trilles (1984) recognized the granuloblast as the earliest cell recognizable in the granulocytic line of *D. labrax*. The granuloblasts were round cells with eccentric spherical nucleus. It showed light blue cytoplasm with spherical and mostly eccentric nucleus, which occupied about half of the cell. These morphological characters were similar to the granuloblast observed in *C. carpio* (Kondera, 2011). The pro-neutrophil is a round cell in *A. bicolor bicolor*, with light blue cytoplasm and appears finely granular. The large, elongated or oval eccentric nucleus stained pink. It occupies nearly half of the cell volume. Similar observations were made by Kondera (2011) in *C. carpio*. The granuloblasts with neutrophilic granules, arbitrarily referred to as pro- neutrophils (Mc Arthur, 1977) have a relatively large nucleus with an undulating rough surface tincture which occupies approximately one third of the cell. In *A. bicolor bicolor* the neutrophils observed in the haematopoietic sites, the head kidney and the cells are round or irregular in shape. Bi- lobed, kidney or bean shaped, horse- shoe shaped and dumb-bell shaped eccentric nuclei are observed. The cytoplasm stained transparent and nucleus stained pink. Mc Arthur (1977) recorded same observations in New Zealand eels.

Thrombopoietic series

In *A. bicolor bicolor* thrombocytes (Fig. 1, 2 and 5) alone is identified in the thrombopoietic series. The thrombocytes were oval or spherical, elongated or oblong and spindle shaped with transparent cytoplasm. They are predominantly smaller than the lymphocytes with a central compact nucleus and a minimum or no cytoplasm. Similar observations were made by Fange (1994) in some fishes. As in common carp, *C. carpio* the developmental stages of thrombocytes were not identified in the present study (Kondera, 2011), while in New Zealand eels, *A. australisschimidii* and *A. dieffenbachii*, the thromboblast and thrombocytes were observed in the thrombopoietic series. Recently spindle shaped thrombocytes were observed in *T. niloticus* (Abdel-Aziz *et al*., 2010).

Macrophages (Fig. 5 and 6) were also more abundant in the head kidney of *A. bicolor bicolor* and it also showed variations in size and morphology. The cytoplasm was very faintly stained and appeared non- granular and the nucleus stained deep purple. These cells were early recognized by vacuole in the cytoplasm. Similar cell types were also observedin the head kidney of *C. batrachus* (Gangopadhyay and Homechaudhury, 2011).

CONCLUSIONS

The present study confirms the role of head kidney as a major haematopoietic organ. It contains all the blood cell lineages except for eosinophils, basophils and thrombocytes. The structure and the lineages of developing blood cells reflect the physiological condition of the fish. A combination of light and transmission electron microscopic characterization makes it possible to recognize different blood cell lineages present in the headkidney with a high degree of certainty.

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