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Histological Aspects of Gut Associated Lymphoid Tissue in *Acanthopagrus latus*

[1]Negin Salamat, [1]Rahimeh Darbeh, [2]Naeem Erfani Majd and [1]Ali Reza Safahieh

[1]Department of Marine Biology, School of Marine Science,
Khorramshahr Marine Science and Technology University,
P.O. Box: 669, Khorramshahr, Iran
[2]Department of Histology, Faculty of Veterinary Medicine,
Shahid Chamran University of Ahvaz, Ahvaz, Iran

Abstract: The aim of the present study was to describe the histological and histochemical charachtristics of intestine and its associated lymphoid tissue (GALT) in marine water fish *Acanthopagrus latus*. 20 male *A. latus* (183.2±12.91g in weight) (17.65±0 .45 cm in length), were used for this study. The samples were taken from proximal, middle and posterior parts of intestine and were fixed by immersion in Bouin's solution. The samples were processed for histological examination and stained with hematoxylin and eosin, PAS, Alcian blue and AB-PAS. The mucosal surface of the intestine in *A. latus* had many folds lined by simple columnar cells and goblet cells, which later reacted positive to PAS, AB and AB-PAS. Then it became clear that goblet cells in the intestine of *A. latus* contain mix acidic and neutral glycoproteins. Eosinophilic granular cells were observed in lamina properia along the intestine. GALT existed in all over the intestine, as single cells or small aggregations in both the epithelium and lamina properia. Lymphocytes, plasma cells, granulocytes and macrophages were recognized in the intestinal mucosa, lamina properia and submucosa of *A. latus*. Significant increase in the number of lymphocytes toward posterior intestine, where some lymphocyte aggregations were seen.

Key words: Gut · GALT · Acanthopagrus Latus · Histology

INTRODUCTION

In mammals it has been proved that two sites can be recognized regarding to the gut mucosal immune system: the induction sites (gut-associated lymphoid tissue (GALT) and the effectors sites: lamina propria lymphocyte (LPL) and the intraepithelial lymphocyte (IEL) compartment [1, 2]. GALT, especially the Peyer's patches (PP), has a critical role in immune defense against antigens inserted to the alimentary tube. M-cells, existing in the epithelial layer above the induction sites, can vigorously transfer exogenous antigens to the underlying lymphoid tissue which results in the secretion of high amounts of IgA at the effector sites [1, 2]. Peyer's patches, M cells, IgA and also lymph nodes are not reported in teleost fish [2]. Thus, the existence of a common mucosal immune system is virtually rejected, but local mucosal defense system is reported repetitively [3]. Although GALT has lower organization in fish than mammals, but fish have more diffusely organized immune system in their gut, which is morphologically and functionally different from the mammalian GALT and containing all immune cells necessary for a local immune response include of many lymphoid cells, macrophages, eosinophilic and neutrophilic granulocytes [4].

Intraepithelial and lamina propria lymphocytes and T-cells catch the antigens and induce the suitable immune responses such as production of cytokines and specific antibodies [5] and antigen detection preservation in memory cells [6].

Although fish lack Peyer's patches and antigen-transporting M cells, the enterocytes in the hindgut of fish have an antigen-transporting capability and also many macrophages and lymphoid cells are distributed among the epithelial cells and in the lamina propria [7- 8].

The yellowfin seabream (*A.latus*) is one of the most important marine fish species with wild distribution. Due to lake of knowledge about the basic structure of intestine and gut associated lymphoid tissue in yellowfin seabream,

Corresponding Author: Negin Salamat, Department of Marine Biology, School of Marine Science,
Khorramshahr Marine Science and Technology University, P.O. Box: 669, Khorramshahr, Iran.

this research was carried out to describe the general histological structure of intestine and distribution of gut associated lymphoid tissue in this fish.

MATERIALS AND METHODS

A. latus (183.2 ± 12.91g in weight) (17.65 ± 0 .45 cm in length), were collected from North of Persian Gulf (Khure Mousa). The samples were taken from proximal, middle and posterior parts of intestine and were fixed in Bouin´s solution. The samples were dehydrated in graded ethanol solution, cleared in xylene and embedded in paraffin. Five-micrometer-thick sections were obtained and stained with hematoxylin and eosin (H&E), Periodic Acid Schiff (PAS), Alcian Blue (AB) (PH=2.5) and PAS-AB [9].

Histological sections of intestine were submitted to counting of intraepithelial lymphoid cells. Six observations per section and five sections per fish were used for histometric evaluation. Counting was performed with light microscope using Dino lit lens (with Dino capture software).

RESULTS

Results showed that the basic structure of intestinal wall in *A. latus,* was as same as other vertebrates: tunica mucosa (simple epithelium lining lamina propria/submucosa of loose connective tissue), tunica muscularis with inner circular and outer longitudinal layers of smooth muscles and tunica serosa (Fig. 1). There were many nerve plexuses (Auerbach´s plexuses) between inner circular and outer longitudinal muscle layers.

In *A. latus*, the intestine had a short length and thicken wall. The mucosal surface of intestine had numerous long folds, the length of which didn't have significant differences among different parts of intestine. The folds covered with simple tall columnar epithelial cells containing a basal nucleus and an apical brush border with goblet cells (mucus secreting cells) and intra-epithelial lymphocytes (IELs) (Fig. 2).

The goblet cells were characterized by a swollen supranuclear region containing cytoplasm stained purple with PAS, blue with AB and dark blue with AB-PAS. Findings showed that the intestine of *A. latus* contained one type of goblet cells which secrete mixed mucus consist of neutral and acid glycoprotein. The number of goblet cells significantly ($p<0.05$) increased toward posterior intestine (Fig. 3) and their concentration was more at the base of folds. Extensive capillary network was observed under epithelium and within lamina properia.

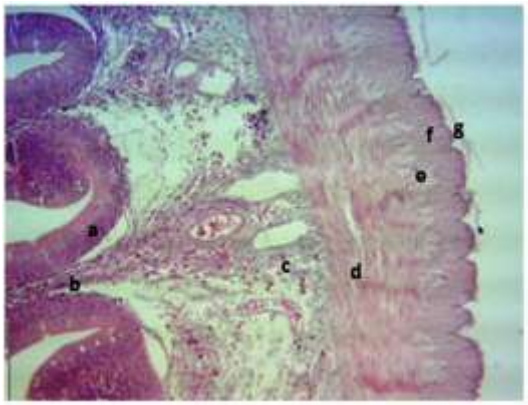

Fig. 1: Intestinal wall in *A. latus*: a. epithelium, b. lamina propria, c. submucosa, d. circular muscle layer, e. Auerbach´s plexuses, f. longitudinal muscle layer, g. serosa, (H&E; ×290).

Fig.2: Intestinal epithelium: 1. Eosinophilic granular cell, 2. goblet cell, 3. columnar epithelial cells,4. intraepithelial lymphocyte (IEL) (H&E;×2900).

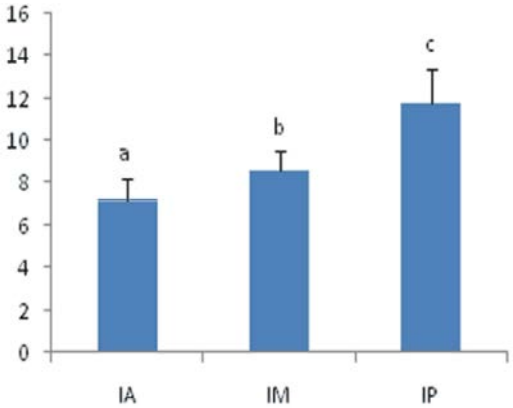

Fig.3: Goblet cells increase toward posterior intestine ($p<0.05$),AI: anterior intestine, MI: middle intestine, PI: posterior intestine

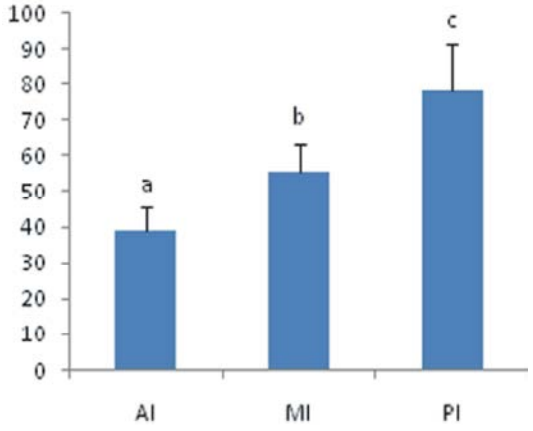

Fig. 4: Significant difference between IELs along the length of the intestine ($p<0.05$), AI: anterior intestine, MI: middle intestine, PI: posterior intestine

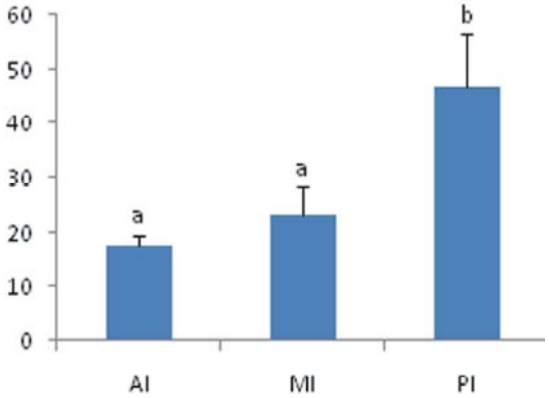

Fig. 5: Increase of apical IELs toward the posterior intestine AI: anterior intestine, MI: middle intestine, PI: posterior intestine

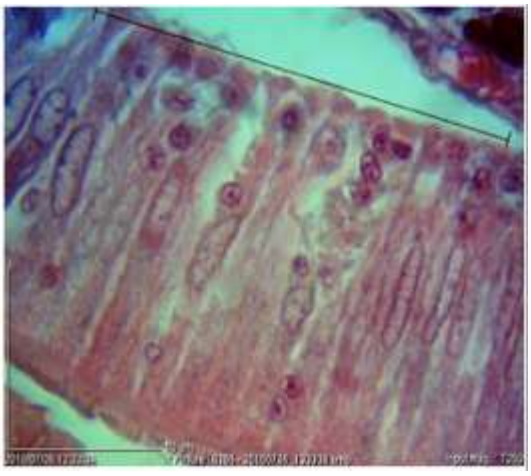

Fig. 6: Lymphocyte, arrow demonstrate IELs(×7250)

Gut Associated Lymphoid Tissue (GALT): GALT was demonstrated as single cells or as small aggregations in both the epithelium and the lamina propria in all over the intestine (anterior, mid and posterior intestine). However, a few accumulations were detected. IELs were found in apical and basolateral regions of entrocytes, but the number of the later was more than those detected in apical region.

Figure (4) illustrates the mean number of IEL detected in the mucosa of the intestine. There was significant ($p<0.05$) difference in the number of these cells along the length of the intestine. Apical IELs significantly ($p<0.05$) increased toward posterior intestine (Fig. 5).

There were numerous lymphocyte, macrophage, plasma cell and eosinophilic granular cells/mast cell in lamina properia and submucosa. Lymphocytes marked with nucleus contained condensed chromatin enclosed with a thin margin of basophilic cytoplasm (Fig. 6). Significant increase was observed in the number of LPLs toward posterior intestine, where some lymphocyte aggregations were seen. Plasma cells were found in both lamina propria and epithelium and its number in lamina properia was significantly more than it in epithelium. Eosinophilic granular cells were detected in stratum granulosum layer.

DISCUSSION

The histological study of fish gut is becoming more precious as the interest in fish culture increases and therefore, more information is necessitated with regard to feeding and nutrition. The intestinal mucosa acts as selective barrier to nutrients and also prevents many toxins and pathogens [10].

Although there are critical dissimilarities in the microscopic structure of intestinal tract among different fish species, the wall of the intestinal tract of *A. latus*, as also occurs in other fish and is consisted of the four layers described for vertebrates [11]. The present study revealed that in this fish, the intestinal wall had mucosal folds lined by columnar epithelium with goblet cells scattered between the epithelial cells. The results of other investigations on other species are in agreement with this study [12-14]. The increased crowdedness of goblet cells toward the posterior intestine observed in the present study, has also reported in fish such as rice field eel and may relate to the need for increased mucosal protection and lubrication for faecal exclusion [15].

The mucus-secreting cells are a general characteristic of teleosts, while muco substance composition varies between species and even different parts of digestive

tract [16]. The mucus-secreting cells react positively to PAS, representing that they contain neutral glycoproteins and those react positively to alcian blue, are containing acidic glycoproteins [17]. Histochemical results of the present investigation showed that the goblet cells of intestine in *A. latus* have compound acidic and neutral glycoproteins, due to the positive reaction of these cells to PAS and alcian blue at the same time. Similar findings have been reported in northern pike and European catfish [9]. Structure of gut mucosubstances is directly correlated to environmental conditions which in turn may involve the function of the digestive tract [9]. Neutral mucosubstances in combination with alkaline phosphatase involve in emulsification of food into chyme in vertebrates. Acidic mucins act in protection of the intestinal epithelium against the glycosidase enzymes [18].

In this research mucosal tubular glands were not seen in the intestine of *A.latus*, though codfish has been reported to have deep glands in intestinal mucosa [19].

The pattern of muscularis layers followed evenly the pattern of intestine mucosal folds. The tunica muscularis consist of an outer longitudinal and a thicker inner circular layer of smooth muscle in *A. latus*, the same results described for *Ambassis sp*. Tilapia and *Rhamdia quelen* [14-20].

In the last decades mucosal immunology of more developed vertebrates is extremely attracted so much attention. Though, persistent of mucosal immune system with effective function in fish would be very valuable for living in a pathogen rich aquatic environment, not many details are known about this system in fish [4].

GALT has not been widely studied in fish and in those that have been studied, no accumulations of leucocytes have been reported [7]. In *A. latus* although a few accumulations have been observed, but they have been detected in just a few individuals. Large accumulation were located in the lamina propria and consisted of aggregations of Lymphocytes together with granulocytes, macrophages and plasma cells. Large accumulations also have been reported in *Oreochromis* mossambicus [21].

The GALT of *A. latus* mainly consisted of diffuse populations of leucocytes, both in the epithelium and lamina propria and the number located in the intestinal epithelium was increased. There was a significant difference in the number of IEL along the length of the intestine and IELs showed significantly increased toward posterior intestine. The same result was also reported in *Barbus sharpeyi* [22] and in sea bass *Dicentrarchus labrax* (L.) [23]. Nevertheless, Rombout *et al*. (1993b) did not observe significant difference in density of IELs along the length of intestine [24].

Intraepithelial lymphocytes present in the gut epithelium probably play important roles in protection of the epithelium from infection [25]. The greater part of the IEL was including of lymphocytes that were normally placed in the basal portion of the epithelium. A similar feature was identified in the intestine of the sea bass *Dicentrarchus labrax* [25].

However, plasma cells, not abundant in number, were recognized as similar as those in mammalian and other teleosts [24]. In most teleost species studied, a layer called stratum granulosum consisting of one or a few rows of mast cells/ eosinophilic granule cell is located in lamina properia submucosa [26], as observed in the present study. As far as present knowledge goes, the main characteristics of mast cells/ eosinophilic granule cell in teleosts are quite identical to those of mast cells in mammals, except that some of the chemical materials of the mammalian mast cell, such as histamine, are replaced with others that have effects in teleosts same as those of mast cell in mammals [27].

In conclusion, all cells necessary for a local or mucosal immune response appear to be present in the intestinal mucosa of *A. latus*. In addition, high amounts of mucus-secreting cells demonstrated in gut of *A. latus*, possibly having a significant defense function.

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