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Histological Structure of the Colonic Lymphoglandular Complex in the Angora Goat

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Abstract: In this study, the histological structure of the colonic mucosal lymphoid tissues was investigated in the Angora goat. Tissue specimens taken from the proximal and distal colon of 5 healthy adult Angora goats, which were slaughtered at slaughterhouses in the vicinity of the Ankara province, were used as the material for histological examination. Histological examination demonstrated the presence of the colonic lymphoglandular complex (LGC), which had a dome-like structure and was composed of solitary lymphoid follicles. It was observed that the follicle-associated epithelium of the LGC was composed of prismatic enterocytes and M cells, the latter of which were ascertained to have taken up intraepithelial immune cells. M cells were distinguished from enterocytes by the presence of irregular microvilli, referred to as microfolds and also pinocytic vesicles on their apical surface. M cells had oval nuclei, which were localised to the basal region of the cell and in this respect, showed histomorphological similarity to the M cells of the Peyer's patches. High endothelial venules (HEVs) were observed at the base of the lymphoid follicles. In result, it was determined that, solitary mucosal lymphoid follicles displayed a dome-type lymphoglandular complex configuration in the colon of the Angora goat.

Key words: Colon · Lymphoglandular Complex · Angora Goat · M Cells

INTRODUCTION

In mammals, the digestive system mucosa is the main site, where the organism is exposed to antigens. Both solitary and aggregated lymphoid follicles play an important role in the immune protection of the digestive system. In several animal species, gut-associated lymphoid tissue (GALT) has been reported to exist in the form of the ileocecal tonsil in the lower part of the small intestine [1-3]. The mucosal lymphoid tissue shows two different localisation patterns in the terminal area of distribution of the GALT in the gastrointestinal tract. The first localisation pattern is characterized by the presence of solitary and isolated lymphoid follicles restricted to the lamina propria of the colonic mucosa, the luminal surface of which is lined by follicle-associated epithelium (FAE). These structures are referred to as the "propria nodules (PN)" [1,4-6]. In the other immunological tissues of the colon, the lymphoid follicles are, to a large extent, localised to the submucosa beneath the lamina muscularis. Differently from the PN, on the luminal surface of these follicles, the crypts closely associated with the colonic lumen give off many branches into the inner regions of the lymphoid tissue, in the form of smaller diverticula. In several species, these structures are referred to as the "lymphoglandular complex (LGC)" [1,7,8]. The LGC, which is a structure specific to the colon, is suggested to be analogous to the Peyer's patches and appendix [1].

O'leary and Sweeney [7] described two different types of LGC in the human colon, namely, the non-dome and dome configurations. These researchers also reported the presence of M cells, similar in structure to those of the Peyer's patches, in the follicle-associated epithelium of only the dome-type lymphoid follicles. Later, Fujimura *et al.* [2] reported to have observed features typical of colonic M cells upon the transmission electron microscopic (TEM) examination of biopsy samples taken from the human colon. Accordingly, the M cells were observed to have taken up many mature and immature lymphocytes and plasmocytes into their cytoplasm. Their apical cytoplasm contained microfolds and a well-developed tubulo-alveolar system [2].

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On the basis of their investigation on the human colon, Jacob *et al*., (1987) reported that the columnar cells lining the follicles of the LGC possessed a centrally or basally located nucleus and multiple mitochondria. These researchers also ascertained that the supranuclear area of these cells contained membrane-bound structures, which resembled lysosomes. Although not as frequent as in crypts, goblet cells generally exist in follicle-associated epithelium [3].

Lymphocytes are continuously circulated from the bloodstream into secondary lymphoid organs and from secondary lymphoid organs into the bloodstream. In secondary lymphoid organs, this continuous circulation is regulated by specialized post-capillary venules, referred to as the "high endothelial venules (HEVs)" and by a series of adhesive interactions which occurs between lymphocytes [9].

To date, research on the GALT has mostly focussed on the investigation of the Peyer's patches, appendix and the avian bursa of Fabricius. To the authors' knowledge, the colonic lymphoid tissues of the Angora goat, which is a local breed native to Anatolia, has not been investigated before. This study was aimed at the investigation of the histological structure of the LGC in the Angora goat.

MATERIALS AND METHODS

In this study, tissue specimens taken from the proximal and distal colon of 5 healthy adult Angora goats, slaughtered at slaughterhouses in the vicinity of the Ankara province, were used for histological examination. After being prefixed in glutaraldehyde and paraformaldehyde and then fixed in osmic acid, the tissue samples were passed through a series of graded alcohols and propylene oxide and finally, were embedded in Araldite M. One-micron-thick semi-thin serial sections were cut and stained with toluidine blue. After the targeted areas were marked in these sections, thin sections of a thickness of 300-400 Angstrom were cut. These thin sections were stained with uranyl acetate and lead citrate [10] and examined using a Carl Zeiss EM 9S2 model electron microscope.

RESULTS

The light microscopic examination of the semi-thin sections of the proximal and distal colon revealed the presence of the dome-type LGC in the lymphoid tissue (Fig. 1). It was observed that the LGC was composed of solitary lymphoid follicles. The secondary lymphoid follicles, which contained a germinal centre, penetrated

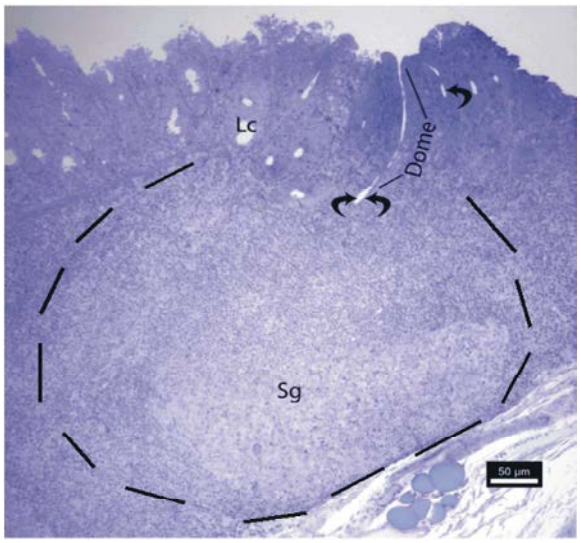

Fig. 1: The structure of the colonic lymphoglandular complex. A secondary follicle with the germinal centre (Sg) (the inside of the circle drawn with the dashed line). The epithelial diverticula of the dome-type LGC (curved arrows), Lieberkühn's crypts (Lc) (Toluidine Blue Bar:50 µm)

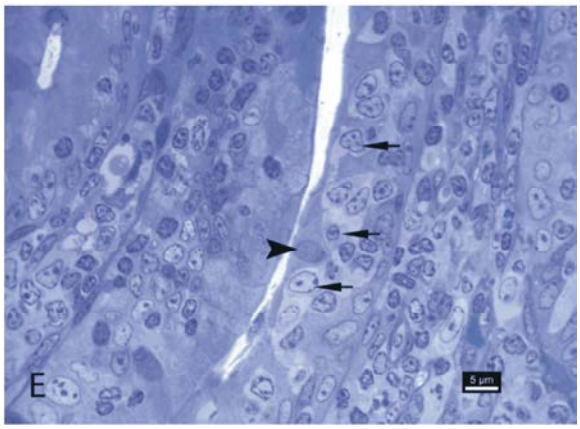

Fig. 2: The FAE of the dome region of the LGC. M cell (arrowhead), lymphocytes(arrows), enterocyte (E). (Toluidine Blue Bar:5 µm)

the lamina muscularis and extended from the lamina propria to the submucosa. In this region, the lamina muscularis was observed to be disrupted. A distinct dome region was distinguished on the luminal surface of the follicles. The dome region harboured high prismatic enterocytes and M cells (Fig. 2), but lacked goblet cells. The cells in the dome region were predominated by columnar cells. Lymphocytes were observed in the proximity of the cytoplasm of the M cells. High endothelial venules, of typical cubic shape, were present at the base of the lymphoid follicles (Fig. 3).

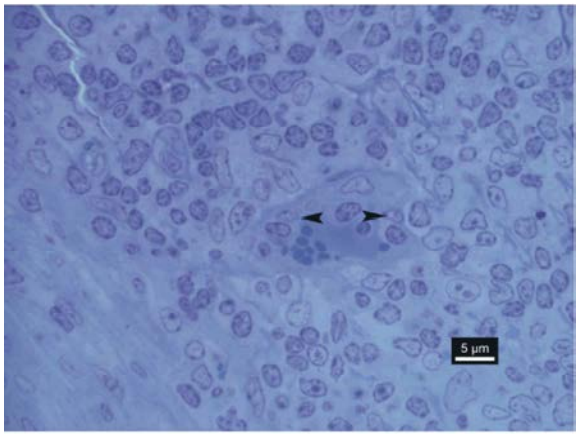

Fig. 3: The HEVs at the base of the follicles. Cubic endothelial cells (arrowheads). (Toluidine Blue Bar:5 µm)

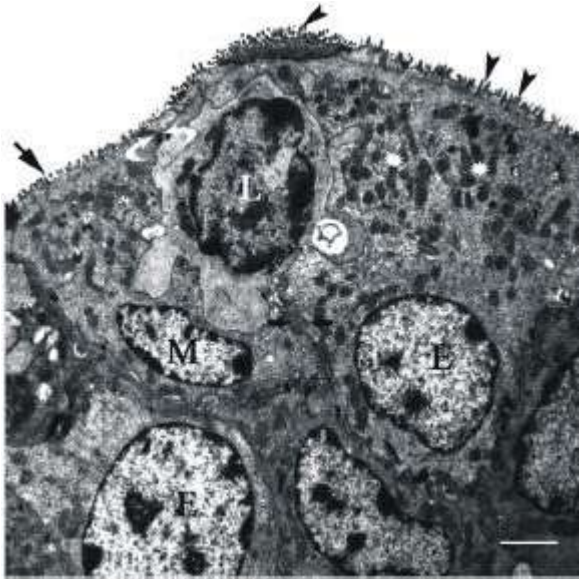

Fig. 4: The ultrastructure of the FAE of the dome region of the LGC.Microfold (arrows) and an M cell (M) displaying a vesicle (V) in its apical cytoplasm. The intercellular connections of the M cells, namely, the tight junctions (curved arrows) and desmosomes (bold arrows). Intraepithelial lymphocyte (L). Enterocytes (E) with dense mitochondria (stars), regular microvilli on their apical surface (arrowheads) and a euchromatic nucleus. Bar=3 µm.

The examination of the ultrastructure of the follicle-associated epithelium (Fig. 4) demonstrated that the enterocytes making up the epithelium had euchromatic nuclei and contained multiple mitochondria in their cytoplasm. The luminal surface of these cells was covered by regular microvilli and beneath these microvilli a terminal web existed. It was determined that, while the M cells formed junctional complexes with tight junctions and desmosomes, they did not have any connection with the intraepithelial immune cells. M cells were distinguished from enterocytes by the irregular microvilli and pinocytic vesicles on their apical surface. M cells were observed to have a basally situated oval nucleus. Furthermore, it was observed that the intraepithelial lymphocytes with heterochromatic nuclei surrounded the M cells.

DISCUSSION

The presence of GALT in the large intestine has been reported in cattle, sheep, pigs, horses, dogs, cats, rats, primates and humans [1,3,11-16]. In the large intestine, the lymphoid follicles of the GALT display an aggregated distribution in only rats and a solitary distribution in humans, pigs and horses [4,5,12-15].

Histologically, the colonic lymphoid follicles exist either in the form of a lymphoglandular complex (LGC) or in the form of propria nodules (PN). The LGC is composed of submucosal lymphoid follicles, which are distributed throughout the mucosa and contain epithelial diverticula [4]. The histomorphology of the lymphoid tissue of the large intestine varies among animal species. While both the PN and LGC exist in the bovine large intestine, in other animal species either the PN or the LGC exists in the large intestine Liebler *et al*. [4]. suggested that while the propria nodules existed in greater numbers in one-week-old calves, the LGC predominated in older calves. In the present study, the dome-type LGC was detected in the colon of the Angora goat.

Histomorphologically, the colonic LGC displays similarity to the Peyer's patches. These two structures are differentiated by the Peyer's patches containing aggregated lymphoid follicles and the colonic lymphoid follicles having a solitary distribution. The lymphoid follicles of the LGC and the germinal centre of these follicles are smaller than those of the Peyer's patches [8]. In their research on the human colon, O'leary and Sweeney (1985) determined that in normal individuals, approximately 1% of the LGC possessed a germinal centre. In agreement with the findings reported by Owen *et al*., (1997), in the present study, it was observed that the LGC contained solitary secondary lymphoid follicles with a germinal centre.

The LGC contains all types of cells found in the lymphoepithelial structure of the small intestine. In healthy individuals, in the LGC of the proximal colon, each 20 colonic epithelial cells contain 1 or 2 M cells and it has

been indicated that this number is four-fold in the rectum [7]. In the distal colon of mice, in each centimetre square, on average 1.4 LGC is randomly distributed. On the other hand, in the proximal colon of mice and rats, the LGC shows an antimesenteric distribution [5,17,18]. In the present study, in agreement with the findings reported by O'leary and Sweeney (1985), it was ascertained that the FAE of the LGC was predominated by prismatic enterocytes and contained only a few M cells.

In calves, the FAE lining the PN and LGC contains cells, which resemble the M cells localised to the dome region of the Peyer's patches in the small intestine. These cells display similarity to the M cells of the Peyer's patches in that they have an electron-lucent cytoplasm and harbour short and thin microfolds and numerous apical vesicles. Furthermore, these cells are associated with intraepithelial immune cells [4]. The ultrastructure of the lymphoid tissue in the large intestine has been described in rodents. In their research on rats, Bland and Britton [1] reported that colonic M cells took up ferritin and antigens, such as bacteria, into their cytoplasm and transferred them to intraepithelial lymphocytes. In the present study, it was determined that M cells displayed these features previously reported by the indicated researchers.

Although the localisation of HEVs in lymphoid organs concentrates in T lymphocyte regions, these specialised blood vessels serve for the entry of not only T lymphocytes but also B lymphocytes into lymphoid organs [19] When examined by light microscopy, it is observed that the endothelial cells of the HEVs are typically of cubic shape and contain abundant lymphocytes in their lumen [9]. In humans, HEVs are found in all secondary lymphoid organs, excluding the spleen [19]. The development of the organised mucosal lymphoid response in all mucosae is made possible through the transfer of memory cells to other mucosal lymphoid tissues by circulation [4]. In the present study, the observation of HEVs at the base of lymphoid follicles suggests that this structure is essential to the mucosal defence of the colon.

In conclusion, the determination of the presence of the dome-type LGC in the colon of the Angora goat and the presence of M cells in the FAE of the LGC suggests that the colon is involved in the mucosal defence system.

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