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Original Article

# Ultrastructural demonstration of antigen presenting cells in appendix

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ABSTRACT

*Introduction:* The human Appendix is a component of gut associated lymphoid tissue (GALT). Lymphoid denritic cells play a pivotal role in antigen presentation in mucosal immune system. A very few studies have demonstrated antigen presenting cells present in it either by cytochemistry or by immunohistochemistry. The aim of the present study is to demonstrate the antigen presenting cells present in normal and infected Appendix by electron microscopy.
*Materials and methods:* Tissues obtained from two normal and five infected appendices were processed for electron microscopy and viewed under Philips EM 201C electron microscope.
*Results:* In addition to the enterocytes, goblet cells and enteroendocrine cells lining the appendix, M cells were present in the lining epithelium of both normal and infected appendix. Intraepithelial lymphocytes were present in relation to the base and basolateral surface of the M cells. In addition to these cells, dendritic cells (DCs) were present in the epithelium of infected appendix. DCs were present in the subepithelium of normal and infected appendices. In the region of lymphatic follicles, follicular dendritic cells (FDCs) were present which showed ultrastructural heterogeneity. In the interfollicular area, high endothelial venules (HEVs) were present. Transendothelial migration of lymphocytes through the HEVs could be demonstrated.
*Discussion:* The ultrastructural demonstration of DCs, FDCs, M cells and the transendothelial migration of lymphocytes through HEVs in Appendix confirms that Appendix is truly a secondary lymphoid organ.


## 1. Introduction

Gut associated lymphoid tissue, a component of mucosa associated lymphoid tissue (MALT) is the largest lymphoid organ in the body.[1] Antigen presenting cells like dendritic cells (DCs), B-lymphocytes and M-cells are present in the epithelium, lamina propria and lymphatic aggregation of GALT.[2,3] In the intestine, DCs are pivotal in tolerance induction and they direct the differentiation of T cells.[4] Lymphoid DCs play an essential role in antigen presentation in primary immune responses and are considered to be important in normal healthy responses of the mucosal immune system.[5] Though the human Appendix is thought to be a vestigial organ, it is a component of GALT. It is considered as a secondary lymphoid organ as it contains lymphoid tissue in the lamina propria and in the submucosa. Though various antigen presenting cells like Langerhans cells and follicular dendritic cells (FDCs) have been demonstrated in the human Appendix by immunohistochemistry, ultrastructural demonstration of antigen presenting cells has not been done earlier. The present study was undertaken to demonstrate the presence of antigen presenting cells in Appendix by electron microscopy.

## 2. Materials and methods

After getting the ethical clearance from the Institutional Review Board, two normal Appendix specimens were obtained from patients who underwent right hemicolectomy for carcinoma colon and five infected Appendix specimens were obtained from patients who underwent appendectomy for acute appendicitis. Tissue were fixed in 3% gluteraldehyde, post-fixed in osmium tetraoxide and embedded in resin. Ultrathin sections were taken, stained with freshly prepared saturated aqueous uranyl acetate and counterstained with Reynolds lead citrate and viewed under Philips EM 201C electron microscope at 40 KV.

## 3. Results

The Appendix lined by simple columnar epithelium had enterocytes, goblet cells and enteroendocrine cells. In the epithelium, intraepithelial lymphocytes (Fig. 1) and M cells were

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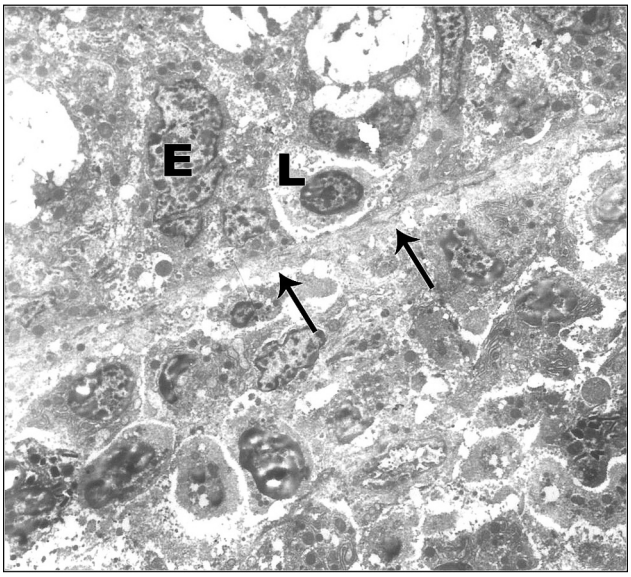


**Fig. 1.** Electron micrograph of the epithelium of Appendix showing an intraepithelial lymphocyte (L). Arrows indicate the basement membrane. E- enterocyte. x6,357.

identified (Fig. 2). Apically, the cell membrane for M cell was thrown into irregular, short microfolds. Desmosomes and tight junctions were present between M cells and the adjacent enterocytes. Cellular organelles such as Golgi bodies and mitochondria were in the basal part of M cells. Lymphocytes were present in relation to the base and basolateral surface of the M cells. In addition to these cells, in a case of acute appendicitis in a child, the epithelium showed a DC with indented nucleus (Fig. 3). DCs were present in the subepithelium (Fig. 4) of normal and infected appendices. In the region of lymphatic follicles, FDCs were present (Fig. 5). FDCs showed ultrastructural heterogeneity. In the interfollicular area, HEVs were present (Figs. 6). They were lined by cuboidal epithelium. Transendothelial migration of lymphocytes could be found. Fig. 7 shows a pseudopodium of the migrating of lymphocytes attached to the luminal surface of the endothelium.

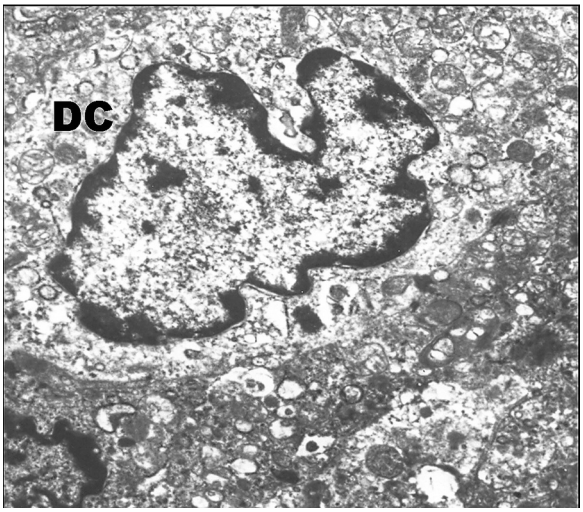


**Fig. 3.** Electron micrograph, showing a dendritic cell (DC) in the surface epithelium of a child with acute appendicitis. DC is pale, with indented nucleus. Abundant cytoplasmic organelles were seen. X31,785.

## 4. Discussion

The human Appendix is a component of MALT, which is one of the largest lymphoid organs. It contains upto 70% of all the body's immune cells.[6] The antigen presenting cells present here include DCs, macrophages, B lymphocytes and M cells.

Dendritic cells are professional antigen presenting cells, located at various surveillance sites where they capture the antigens, process and present them to the T lymphocytes. When they come across danger signals, they undergo a differentiation and maturation process. Maturing DCs usually migrate to lymphoid tissues where they present antigens to T lymphocyte to commence a primary immune response.[7,8] Recent evidence suggests that DCs play a crucial role in mediating tolerance against self-antigens, commensals and dietary antigens while mounting inflammatory responses against harmful pathogens.[9] Our previous study had demonstrated a few Zinc-iodide osmium (ZIO) positive DCs in the

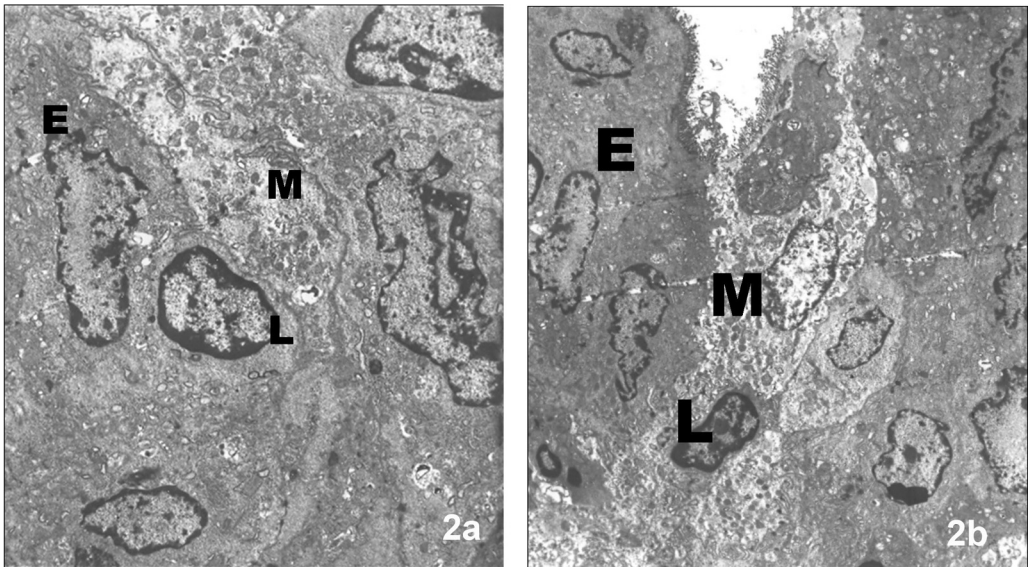


**Fig. 2.** (a) An M cell (M) in the epithelium of Appendix showing abundant cell organelles and vesicles. L-lymphocyte in close association with M cells. E-enterocyte. x14,303. (b) Another M cell (M) in the epithelium of appendix. The basolateral surface is invaginated by a lymphocyte (L). E-enterocyte. x9,535.

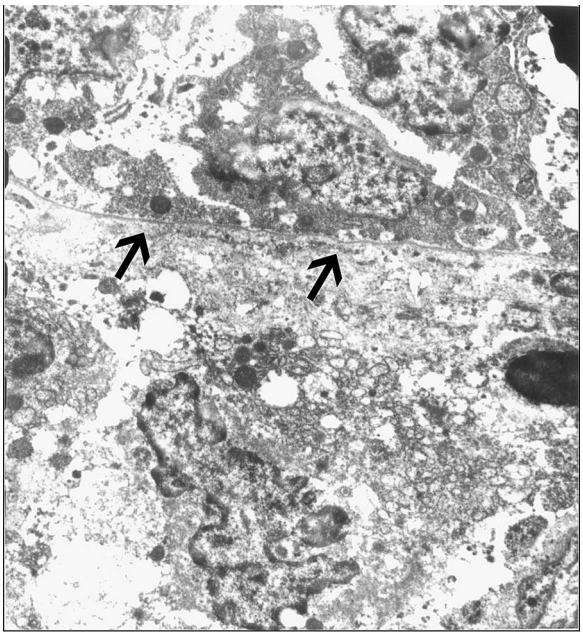

**Fig. 4.** Electron micrograph showing a dendritic cell (DC) with indented nucleus in the subepithelium. Arrows indicate the basement membrane. X14,303.

lining epithelium and the intestinal crypts of the appendix, which had a single, long process directed towards the lumen.[10] The present study demonstrates the presence of DCs in the surface epithelium of the infected appendix. In addition, DCs are present in the lamina propria of both normal and infected appendix. The DCs present in the mucosa are likely to be in close contact with luminal antigens and play an inimitable role in immune homeostasis in the gut.[11] They respond quickly to environmental changes and differentiate to become either mature or immunogenic accessory cells.[12] In the lamina propria, DCs provide a special microenvironment for the extensive traffic of lymphocytes into these organs during antigenic challenge.[13]

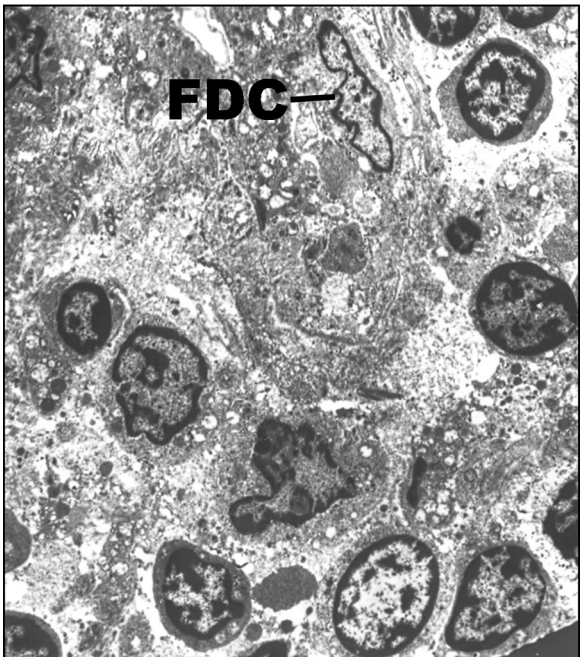


**Fig. 5.** Electron micrograph showing a follicular dendritic cell (FDC) within the lymphatic follicle.

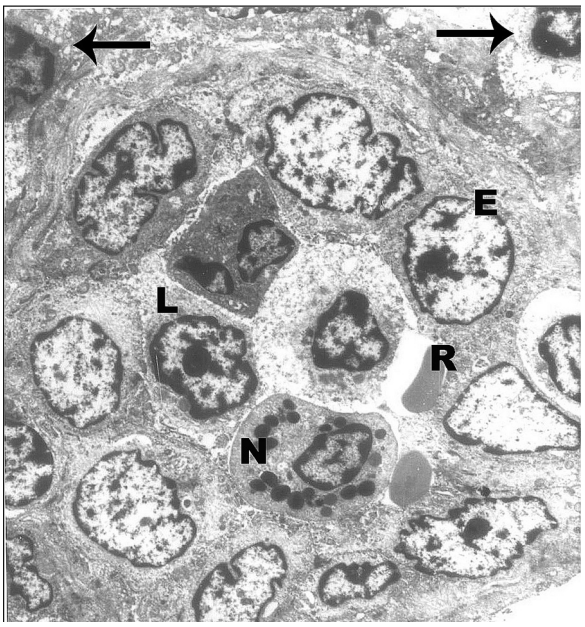


**Fig. 6.** HEV in the interfollicular region. E-endothelial cells lining the HEV. Red blood cells ®, neutrophil (N) and lymphocytes (L) are seen within the lumen. Arrows indicate lymphocytes in the wall of the HEV. x9,535.

Follicular dendritic cells are stromal cells that reside within the primary follicles, in the germinal centres of secondary lymphoid organs and in non-capsulated lymphoid structures such as the isolated lymphoid follicles of the intestine. They play a central role in B-cell activation.[14] They are different from other types of DCs in that they are non-phagocytic cells, lacking phagosomes and lysozyme in their cytoplasm.[15] In addition, they do not have typical Birbeck granules in their cytoplasm which distinguish them from Langerhans cells.[16] The intertwined dendritic processes of FDCS form a three-dimensional network, which trap antigens. Then they present the antigen to follicular B cells and aid in the generation of B cell memory.[17] They control humoral immunity directly by interacting with B cells and indirectly by inducing the expansion and differentiation of $CD4^+$ helper T cells.[18] Previous

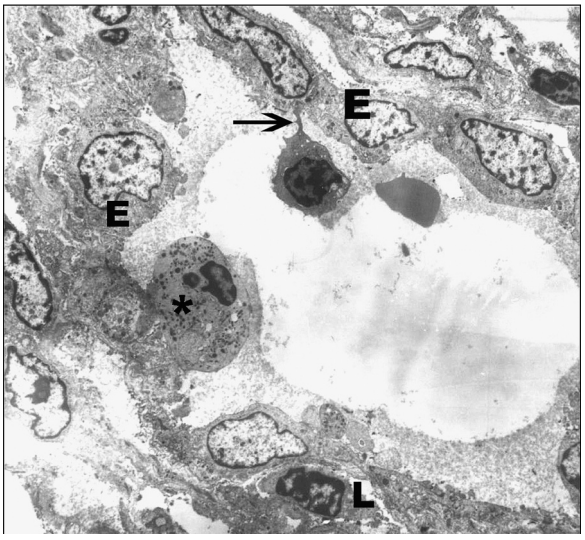


**Fig. 7.** Arrow indicates the pseudopodium of a migrating lymphocyte attached to the luminal surface of endothelium (E). another lymphocyte (*) is seen adherent to the luminal surface of endothelium. Another lymphocyte (L) is seen in the subendothelial layer of the wall of HEV. x6,357.

studies have demonstrated ZIO positive FDCs[10] and CD35 positive FDCs and their interaction with the CD20 positive cells in the human appendix.[19] The lymphoid follicle of normal Appendix displayed two different types of ZIO positive DCs. In the germinal center, the FDCs were few in number and larger in size while smaller DCs with many thin processes were present in the mantle zone. The present electron microscopical study showed that FDCs present in the lymphatic follicles of Appendix displayed ultrastructural heterogeneity.

In addition to the DCs and FDCS, M cells were identified electron microscopically in the epithelium of appendix. M cells are highly specialized epithelial cells, exclusively found in the epithelia that cover mucosa-associated lymphoid tissues.[20] They differ from enterocytes in shape and function. The apical surface is characterized by the fewer, shorter and wider microfolds.[21] The basolateral surface is invaginated by the intraepithelial lymphocytes or other antigen presenting cells. M cells lack lysosomes. The main function of M cells is to transport antigens from the intestinal lumen into the underlying lymphoid tissue.[21] M cells facilitate transcytotic traffic of vesicles. The interactions of antigens with the apical surface of M cells play an important role in the initial step of intestinal and systemic immune responses or tolerance.[20] It had been hypothesized that the M cells in the dome epithelium of Peyer's patches are a primary entry site for many pathogens. In the present study, often they were in relation to the intraepithelial lymphocytes at the base or basolateral position. Intraepithelial lymphocytes are important in cell mediated immune responses, in the regulation of secretory immunity and in the mediation of systemic tolerance.[20] M cells express MHC class II molecules and are capable of presenting antigens to lymphoid cells.[22] Therefore it can be concluded that the antigens acquired by M cells through their apical surface are rapidly shuttled via vesicular transport to the basolateral membrane and are presented to the adjacent intraepithelial lymphocytes.

Migration of lymphocytes is essential for specific immune reactions in the lymphatic lymphoid organs. HEVs are the sites of migration of lymphocytes from blood vascular system to lymph nodal parenchyma. In a multistep fashion, adhesion molecules on lymphocytes and endothelial cells of HEV interact, resulting in rolling, adhesion activation and transmigration of lymphocytes.[23] In the present study, the migration of lymphocytes through HEV could be seen in appendix. Though the entire process of migration of lymphocyte could not be demonstrated in the present study, a pseudopodial process of a lymphocyte adhered to the luminal surface of endothelial cell of HEV could be demonstrated. Other lymphocytes in the subendothelial spaces between the endothelial cells and basement membrane were also seen. Some lymphocytes could be seen just outside the wall of HEV, after migrating out through HEV.

## 5. Conclusion

In conclusion, the ultrastructural demonstration of DCs, FDCs, M cells and the transendothelial migration of lymphocytes through HEVs in Appendix confirms that Appendix is truly a secondary lymphoid organ.

## Conflict of interest

The authors have none to declare.

## Acknowledgement

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