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Follicular dendritic cells in normal and infected human appendix

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SUMMARY

Follicular dendritic cells (FDCs) that reside within the lymphoid follicles play a central role in humoral immunity. They bind immune complexes and present antigen to follicular B cells and in the generation of B cell memory. This study aims to demonstrate the distribution of CD35 positive FDCs in normal and infected appendix by immunohistochemistry. Four normal and 5 infected appendix specimens were used for the study. Tissues collected were processed for immunohistochemistry, stained with mouse antihuman CD35 monoclonal antibody using the Polymer – HRP Detection System. Double immunostaining was done with mouse antihuman CD20 monoclonal antibody to find out the association CD35 positive Langerhans cells with B lymphocytes. Cells were viewed under the light microscope (Olympus DP21). In the normal appendix, CD35 positive FDCs were present in a reticular pattern in the germinal centre of the follicle. In acute appendicitis, the lymphatic follicles were not intact and FDCs were scattered in the mucosa of the appendix. Few discrete CD35 positive cells were seen surrounding the intestinal glands. CD20 positive B lymphocytes were noted in the lymphatic follicle, interfollicular areas, around the crypts and in the lamina propria. Apposition of CD35 and CD20 cells was noted. The dendrites of FDCs demonstrated in the follicles of appendix displayed an antigen-retaining reticulum which aids in trapping of immune complexes. Their association with CD20 positive B cells confirm the role of appendix in humoral immunity.

Key words: B-cells – Humoral immunity – Antigen trapping – Antigen retaining reticulum

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INTRODUCTION

Dendritic cells (DCs) are a heterogenous population of cells that exhibit great functional plasticity which is induced by different environment (Wu and Dakic, 2004). There are different types of DCs, including the Langerhans cells, interstitial DCs, veiled cells, interdigitating cells and follicular dendritic cells (FDCs). Follicular dendritic cells were first described by Maximow in 1927, as 'a nonlymphoid population of embryonic nonphagoytic reticulum cells'. They play a central role in humoral immunity in the lymphoid follicle (Nossal et al., 1964). They are specialized cells found only within lymphoid follicles. They are different from other types of DCs in that they are non-phagocytic cells, lacking phagosomes and lysozyme in their cytoplasm (Gerdes et al., 1983). In addition, they do not have typical Birbeck granules in their cytoplasm, which distinguishes them from Langerhans cells (Lindhout and De Groot, 1995). They can retain antigen on their surface for many months, and may have a role in maintaining the activity of memory cells, as well as stimulating a primary immune response. They reside in the microenvironment of secondary lymphoid tissue where antigen-activated B cells mature prior to becoming memory B cells (Lindhout and De Groot, 1995). FDCs express high levels of complement receptor 1 (CR-1, otherwise known as CD35) and complement receptor 2 (CR-2, otherwise known as CD21) (Heesters et al. 2014). The most widely used markers of FDC are CD21, CD23 and CD35 antibodies (Yamakawa and Imai, 1992; Han et al., 2000) . This study aims to demonstrate the distribution of FDCs in normal and infected appendix by immunohistochemistry.

MATERIALS AND METHODS

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Ethical clearance was obtained from the Institutional Review Board. Nine appendix specimens were used for the study, of which four normal appendix specimens were obtained from patients who underwent right hemicolectomy for carcinoma colon and seven from patients who underwent appendectomy for appendicitis after getting informed consent. Tissues collected were either embedded in paraffin wax or cryo-sections taken. The 4µm-thick sections were stained with mouse antihuman CD35 monoclonal antibody (Dako) using the Polymer - HRP Detection System. Double immunostaining was done with mouse antihuman CD20 monoclonal antibody (Dako) to find out the association CD35 positive Langerhans cells with B lymphocytes. Cells were viewed under the light microscope (Olympus DP21).

RESULTS

In the normal appendix, CD35 positive cells were present in a reticular pattern in the germinal centre of the follicle (Fig. 1). In acute appendicitis, FDCs were scattered in the mucosa of the appendix (Fig. 2a, b). The lymphatic follicles were not intact and few discrete CD35 positive cells were seen in the lamina propria surrounding the intestinal glands (Fig. 3a, b). CD35 positive FDCs, along with lymphocytes extruding into the lumen through the disrupted epithelium, were noted (Fig. 4). CD20 positive B lymphocytes were present in the lymphatic follicle, interfollicular areas, around the crypts and in the lamina propria. Apposition of CD35 and CD20 cells was noted (Fig. 5).

DISCUSSION

Follicular dendritic cells are long interdigitating cells of stromal origin found only within lymphatic follicles. While it was recognized that FDCs are a central component of B-cell follicles in spleen and in lymph nodes, their appearance was not limited to secondary lymphoid organs but also in non-encapsulated lymphoid structures, such as the isolated lymphoid follicles of the intestine. In addition to this, FDCs were frequently observed during certain chronic inflammations in non-lymphoid tissues (Kranich et al., 2016).

Follicular dendritic cells play a central role in humoral immunity in the lymphoid follicle (Tew et al., 1982; Yamada et al., 1997, Heesters et al., 2014). They bind immune complexes and present antigen to follicular B cells, and aid in the generation of B-cell memory (Heinen et al., 1984). The major constituents of germinal centres of the lymphoid follicles are activated B cells, tangible body macrophages, T lymphocytes and FDCs (Butcher et al., 1982; Stein et al., 1982). While primary follicles contain only a few FDCs, secondary follicles with well-developed germinal centres exhibit the dense three-dimensional network built up by the intercommunicating long, stretched cytoplasmic dendrites of FDCs (Heinen et al., 1991) Two types of FDC had been reported (Imai et al., 1986; Rabi et al., 2006). According to Imai et al., one cell type had cell processes with labyrinth-like structures, which are associated with the trapping of immune complexes, whereas the other cell type does not have labyrinth-like cell processes. Although the first cell type was only located in the light zone of the germinal centre, the latter was distributed throughout the follicle. Rabi et al. (2006) reported two types of dendritic cells in the lymphatic follicles of appendix using zinc iodide osmium as the marker. The cells in the germinal centre were larger in size with fewer thick dendritic process, while the ZIO positive cells in the mantle zone were smaller in size with many thin dendritic cells. In the present study, staining with anti-CD35 showed that FDCs formed an intricate network in germinal centres of lymph follicles of normal appendix which is in ac-

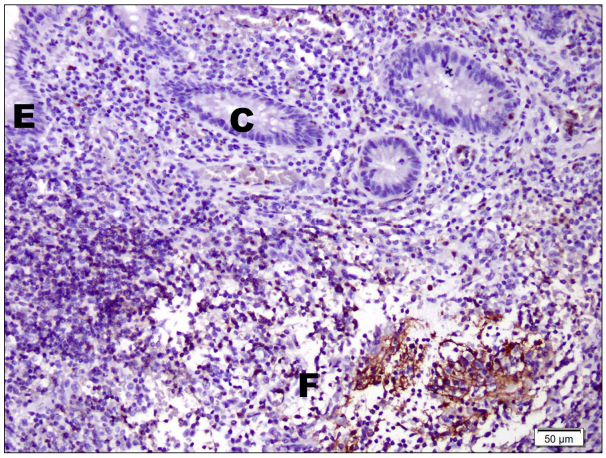

Fig. 1a. CD35 positive FDCs (brown cells) are seen in the follicle (F) of appendix. E – epithelium; C- intestinal crypt.

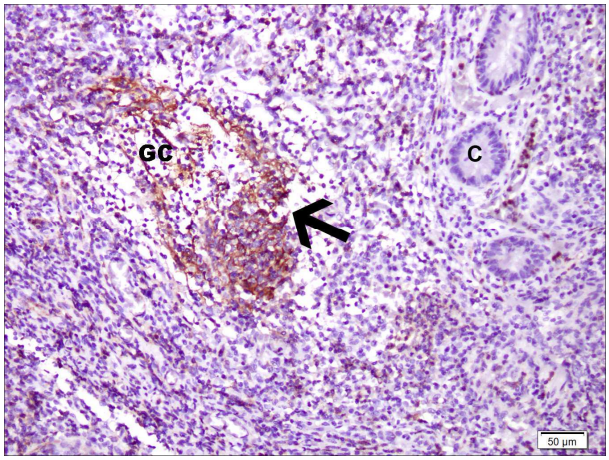

Fig. 1b. CD35 positive FDCs (brown cells) are seen in a reticular pattern in the germinal centre (GC) of a follicle of appendix. C- intestinal crypt.

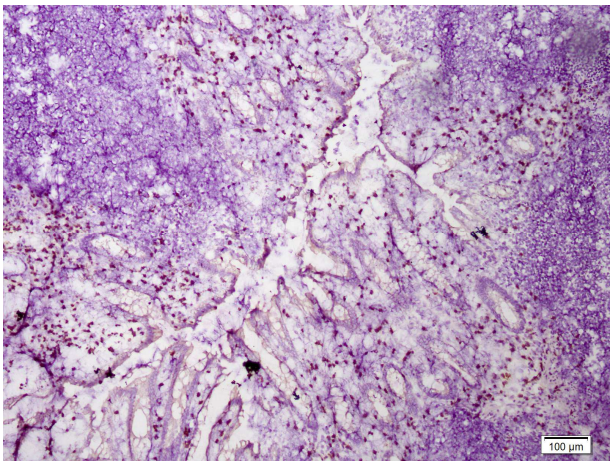

Fig. 2a. Panoromic view of appendix in a case of acute appendicitis.

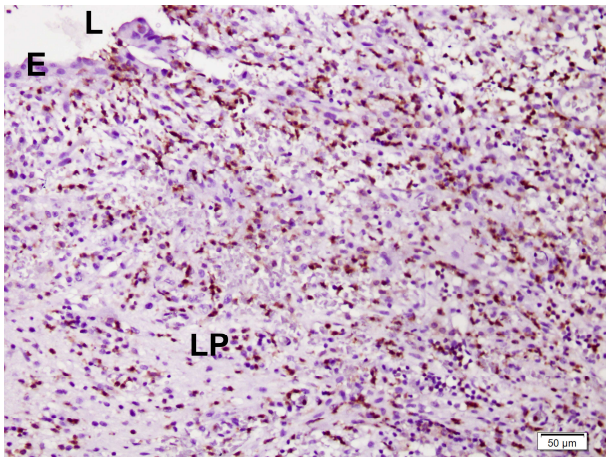

Fig. 2b. The mucosa in a case of acute appendicitis. CD35 positive FDCs (brown cells) are scattered in the lamina propria. L – lumen; LP – lamina propria.

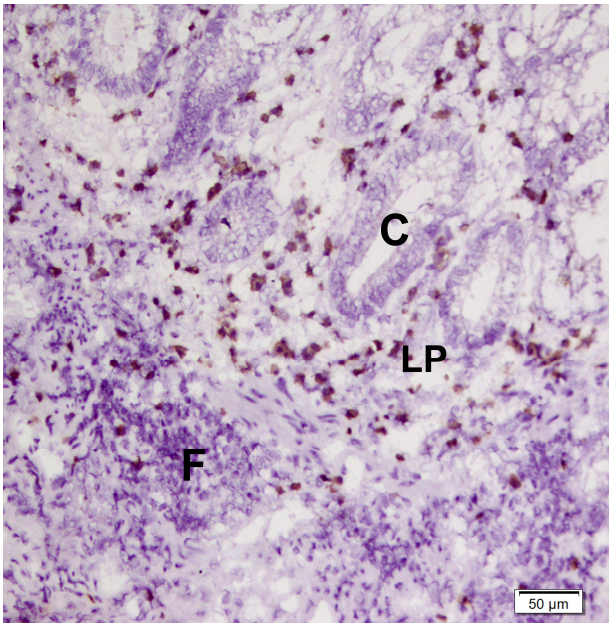

Fig. 3. The mucosa in a case of appendicitis. Note there is no well-defined follicle (F). Discrete CD35 positive FDCs are seen in the lamina propria (LP) surrounding the intestinal crypts (LC).

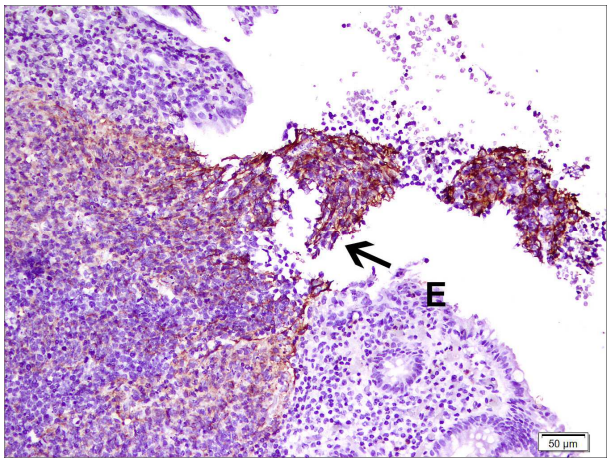

Fig. 4. Arrow indicates CD35 positive FDCs along with the lymphocytes extruding into the lumen through the disrupted epithelium (E).

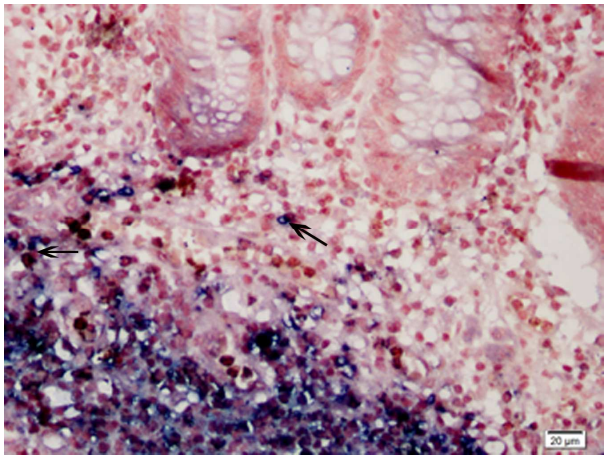

Fig. 5. Double immunostaining shows the presence of CD20 postie B cells (dark blue cells) and CD35 positive FDCs (brown cells) in the lamina propria (LP)and follicles of appendix. Arrow indicates apposition of CD35 positive FDCs and CD20 positive B lymphocytes both in the lamina propria and follicular region.

cordance with Heinen et al. (1991).

Trapping of antigen by FDCs as immune complexes is thought to play a crucial role in the generation of memory B lymphocytes during germinal centre reactions (Thorbecke et al., 1974; Klaus et al., 1980). The dendrites of FDCs intertwined to form a network called the antigen-retaining or FDC -reticulum (Imai et al., 1986). Antigens are trapped on the surface of the FDCs as immune complexes and are retained for very long periods (Nossal et al., 1964; Klaus et al., 1980; Tew et al., 1980) allowing B cells to endocytose, process and present antigens to CD4 T lymphocytes (Dubois et al., 1999). Binding of immune complexes to FDCs is complement-mediated (Klaus and Humphrey, 1977; Joling et al., 1993). FDCs control humoral immunity directly by interacting with B cells and indirectly by inducing the expansion and differentiation of $CD4^+$ helper T cells (Palucka and Banchereau, 2012). Double immunostaining with CD20 revealed that CD20 positive B cells were seen within the follicles, around the follicles, in the interfollicular area and in the lamina propria of appendix. They express abundant co-stimulatory and adhesion molecules needed for T cell interactions. They also express HLA-DM and HLA-DO, which contribute to peptide loading onto MHC-II molecules (Trombetta and Mellman, 2005). It had been shown in the ageing mouse, that in the absence of antigen presenting cells, immunological recognition and appropriate maturation of B cells into both memory as well as into immunoglobulin secreting cells do not occur (Tew et al., 1989).

FDCs promote the formation and maintenance of germinal centres where B cells differentiate into memory cells or antibody-producing plasma cells. It has been demonstrated that interaction of B cells and FDC is needed to generate the follicular structures, with lymphotoxin acting as crucial mediator. In mice lacking B cells, mature FDC are not formed and secondary lymphoid organs are highly disorganized (Aguzzi and Krautler, 2010). In selective ablation of FDCs, the lymphatic follicles lose their round shape and become disorganized bands of cells (Wang et al., 2011; Heester et al., 2014) The present study indicated that in acute appendicitis, the lymphatic follicles were not intact and the lymphocytes were dispersed along with the CD35 positive FDCs in the lamina propria and also extruding into the lumen where there was disruption of epithelium. It could be possible that the invading microbes in acute appendicitis destroy the FDCs that they lose their defensive capacity. Recently it has been demonstrated that infection of arbovirus like blue tongue virus induces an acute immunosuppression by rapidly infecting and destroying FDC network and hampering the capacity of germinal centres to produce antibodies (Melzi et al., 2016).

In conclusion, FDCs are demonstrated in the follicles of normal appendix. The dendrites of FDCs form an antigen-retaining reticulum and are associated with trapping of immune complexes. Their association with CD20 positive B cells confirm the role of appendix in humoral immunity.

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