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Distribution of zinc iodide-osmium positive dendritic cells in the human appendix

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SUMMARY

Dendritic cells are antigen-presenting cells found in almost every type of tissue, including lymphatic tissue, blood and skin. In the intestinal tract, these cells are likely to play a pivotal role in the initiation and regulation of immune responses. Our earlier study of the human colon and ileum revealed the presence of zinc iodide-osmium-positive dendritic cells. In the present study we demonstrate the presence of ZIO-positive dendritic cells in the human appendix. ZIO-positive cells were seen in the region of crypts of Lieberkühn as well as in the surface epithelium. The cells showed a single long process directed towards the lumen. They were long, slender, and triangular. In the region of the lymphoid follicle, two different types of dendritic cells were noted. The follicular dendritic cells present in the germinal center were few in number, larger in size and with thick dendritic processes. However, in the mantle zone typical dendritic cells were seen. They were smaller in size and had many thin processes. The distribution of dendritic cells in the human appendix confirms the role of the appendix in the immune response.

Key words: Follicular dendritic cells – ZIO – Human appendix – Crypts of Lieberkühn

INTRODUCTION

Dendritic cells (DCs) are potent immunostimulatory cells (Steinman, 1991). They can take up and present both intestinally and orally administered antigen to naïve T cells (Liu et al., 1991; Liu and MacPherson, 1993). Dendritic cells are not only critical for the induction of primary immune responses, but may also be important for the induction of immunological tolerance as well as for the regulation of the type of T cell-mediated immune response (Banchereau et al., 2000).

DCs express several Ca^{2+}-dependent (C-type) endocytic lectins, exemplified by DEC 205 (Jiang et al., 1995), the mannose receptor (Sallusto et al., 1995) or Langerin (Valladeau et al., 2000). Although several markers have been used, Zinc-Iodide-Osmium (ZIO) has been extensively employed to identify the presence of dendritic cells in human and animal epithelia and subepithelial tissue (Crocker and Hopkins, 1984; Dagdeviren et al., 1994; Breathnach and Goodwin, 1965; Niebauer et al., 1969; Rodriguez and Caorsi, 1978; Hart and Fabre, 1981; Sertl et al., 1986; Prickett et al., 1988; Steinman, 1991).

Gut-associated lymphoid tissue (GALT) is the largest lymphoid organ in the body (Chandran et al., 2003). Dendritic cells are present in GALT, such as Peyer's patches, and are scat-

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tered through the lamina propria, and a dendritic cell population may also be located within the epithelium itself (Pavli et al., 1990; Maric et al., 1996). Lymphoid dendritic cells play an essential role in antigen presentation in primary immune responses and are believed to be important in normal healthy responses of the mucosal immune system. (Noble et al., 1996). Since the human appendix has not been studied in detail and since it contains lymphoid tissue in the lamina propria and in the submucosa, the present study was undertaken to demonstrate the distribution of ZIO-positive dendritic cells in the human appendix.

Materials and Methods

A normal human appendix was obtained from a 35-year-old woman who underwent right hemicolectomy for a pericaecal chronic abscess at the Christian Medical College, Vellore. The tissue was fixed in veronal-buffered zinc iodide-osmium tetroxide, pH 7.4 (Figueroa and Caorsi, 1980) for 48 hours at 4° C in the dark, washed in distilled water, dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin wax. Serial sections of 6 μm thickness were taken. The sections were transferred to glass slides, deparaffinised, mounted in Canada balsam without counterstain and viewed under microscope.

Results

ZIO-positive cells were located in the crypts of Lieberkuhn (Figs. 1, 2) and the surface epithelium (Fig. 2). These ZIO-positive cells in the lining epithelium and the crypts of Lieberkühn were few in number. They were long, slender, and triangular. They had a single long process directed towards the lumen (Fig. 3). In the subepithelial tissue, ZIO-positive cells were identified in a reticular formation (Fig. 1). In the region of the lymphoid follicle, two different types of dendritic cells were noted (Fig. 4). In the germinal center, the cells were few in number, larger in size, and had thick dendritic processes, which varied from one to three in number (Figs. 5, 6).

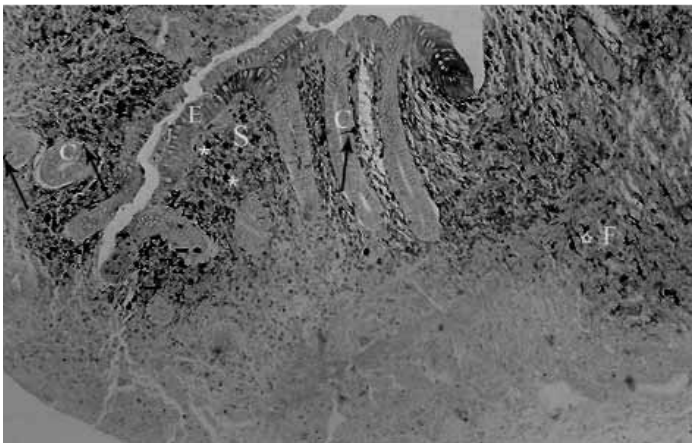

Fig. 1. The lining epithelium (E) and subepithelial tissue (S) of the human appendix. Arrows point to the ZIO-positive dendritic cell in the crypt of Lieberkühn (C); *: ZIO-positive dendritic cells in the subepithelial tissue. Open star: ZIO-positive dendritic cells in the lymphoid follicle (F). x 92.

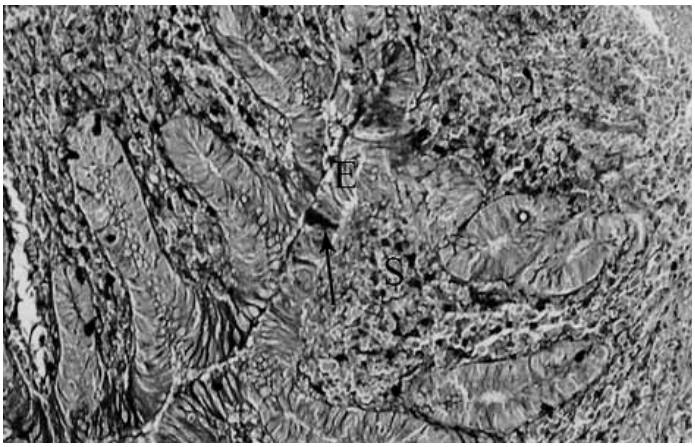

Fig. 2. High-power view of the lining epithelium (E) and subepithelial tissue (S) of the appendix. Arrow points to the ZIO-positive dendritic cell in the surface epithelium. x 212.

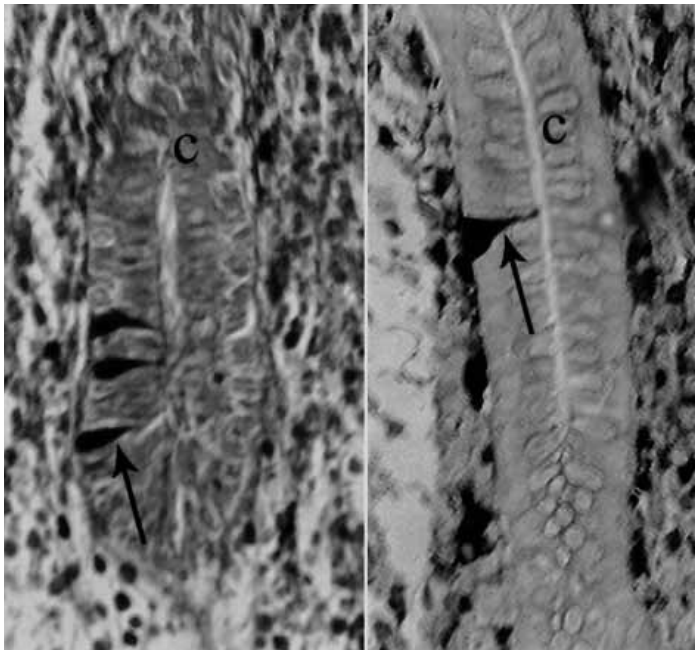

Fig. 3. High-power view of the crypt of Lieberkühn (c). Arrows point to the ZIO-positive dendritic cells. x 367.

However, in the mantle zone typical dendritic cells were seen. They were smaller in size and displayed many thin processes (Fig. 5).

Discussion

Dendritic cells are antigen-presenting cells (APC) that initiate several immune responses, such as the sensitization of MHC-restricted T cells, the rejection of organ transplants, and the formation of T-dependent antibodies and the presentation of antigen to B cells in the germinal centers of lymph nodes, tonsils and adenoids (Bernstein, 1992). DCs recognize and respond to microbial structures using pattern recognition receptors. In the intestine, DCs are pivotal in tolerance induction and they direct the differentiation of T cells (Hart et al., 2005).

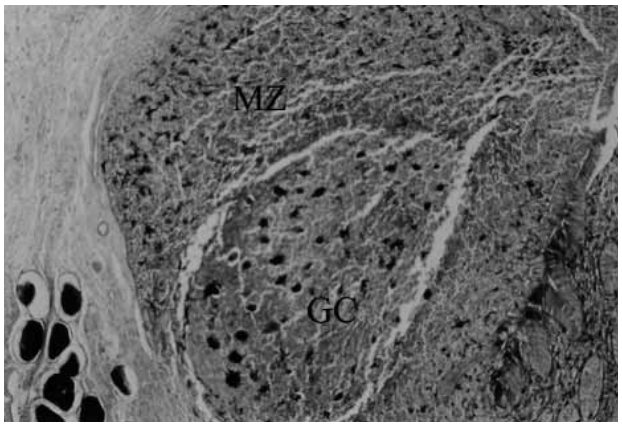

Fig. 4. ZIO-positive cells in the lymphoid follicle. GC: germinal center; MZ: marginal zone. x 106.

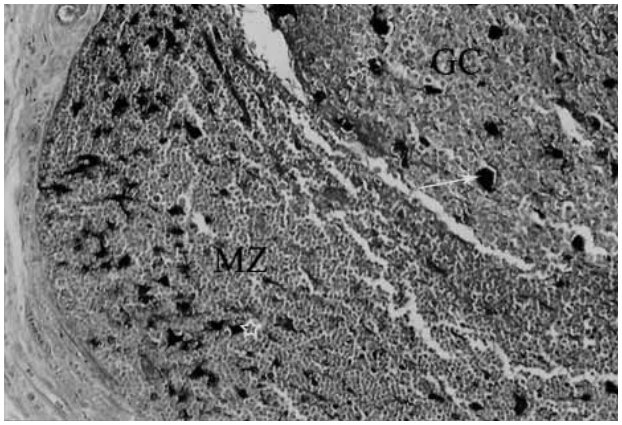

Fig. 5. High-power view of lymphoid follicle. The arrow points to a ZIO-positive cell in the germinal center (GC). Open star: ZIO-positive dendritic cells in the marginal zone (MZ). x 212.

Here, the DCs in the lining epithelium and the crypts of Lieberkuhn of the appendix were long, slender and triangular. They showed a single, long process directed towards the lumen. These DCs resemble the crypt DCs reported for the human colon by Indrasingh et al. (2003) and in human ileum by Koshy et al. (2003). The lining and the crypt epithelium of human intestines share a common morphological feature.

The lamina propria of the intestine contains many DCs, which are likely to be in close contact with luminal antigens. The DCs of the lamina propria play a central and unique role in immune homeostatsis in the gut (Chirdo et al., 2005). The human lamina propria is an effector site of intestinal secretory IgA responses that originate from the organized gut-associated lymphoid tissues (Boursier et al., 2005). In the appendix, the DCs form a reticular framework throughout the lamina propria. This type of reticular framework is also identified in skin (Hume et al., 1983) and in the large airways of the lungs (Holt et al., 1989).

Follicular dendritic cells (FDCs) play a central role in events related to humoral immunity in the lymphoid follicle (Tew et al., 1982; Yamada et al., 1997). They are specialized cells found only within lymphoid follicles.

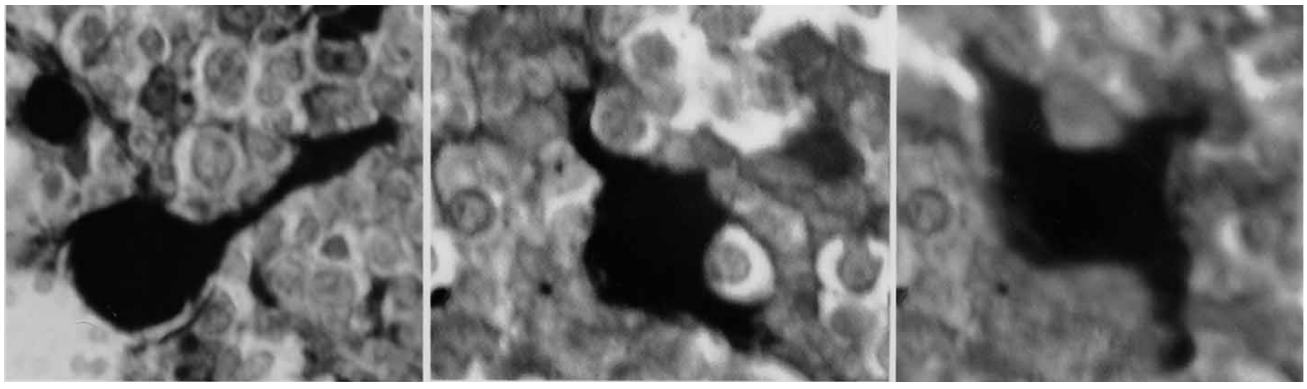

Fig. 6. High-power view of ZIO-positive dendritic cells in the germinal center. x 875.

They are unrelated to other types of DCs, including interdigitating cells found in T cell areas, Langerhans cells, dermal dendritic cells and dendritic cells isolated from periperhal blood (Lindhout and de Groot, 1995). They are non-phagocytic cells, lacking phagosomes and the typical phagocytic enzyme, lysozyme, in their cytoplasm (Gerdes et al., 1983). They reside in the microenvironment of secondary lymphoid tissue, where antigen-activated B cells mature prior to becoming memory B cells (Lindstedt et al., 2003). They prevent apoptosis of germinal center B cells and stimulate cellular interaction and proliferation (Park and Choi, 2005).

Secondary germinal centers consist of a basal dark zone, mainly composed of immature blast cells showing extensive mitotic activity, and an apical lightly stained zone containing a heterogenous population of lymphoid cells. FDCs are present in the dark- and light-stained zones of the germinal center. The FDCs of germinal centers are probably the most antigen-presenting cells for B cells (Yamakawa and Imai, 1992). These authors suggested that there is a functional difference between the light and dark zone and that complete activation of the complement system occurs only in the light zone. Marginal zone B cells play important roles in the early phases of humoral immune responses. In addition to having an inherent capacity to rapidly differentiate into antibody-secreting cells, marginal zone B cells also help to regulate the fate of both T-independent and T-dependent blood-borne antigens in the spleen (Ferguson and Corley, 2005). In the present study, FDCs were present in the germinal center. They were few in number, large in size and had thick dendritic process, the number of these latter varying from one to three. However, in the mantle zone typical dendritic cells were seen. They were smaller in size and had many thin processes. This morphological difference of the dendritic cells in the lymphoid follicle of the human appendix correlates the functional difference of FDCs reported by Yamakawa and Imai (1992). The presence of FDCs in the germinal center of the human appendix suggests that this organ plays a central role in the humoral immune response.

Follicular dendritic cells also serve as a reservoir for HIV infection. These cells trap HIV on their surfaces because they possess surface lectin. Weissman et al. (1995) found that when HIV encounters a dendritic cell, some of the HIV is taken up and processed, while some of it remains stuck to the surface as an infectious virion. Bhardwaj (1997) calls dendritic cells a 'double edge sword' because they are so efficient a taking up and presenting antigen to T cells; however, they also bring the HIV to the target of its destruction-T cells.

Dendritic cells are under investigation as immunotherapeutic agents in the treatment of cancer and infectious diseases. It has been reported that peptide-pulsed DC therapy is effective against melanoma (Nakamura et al., 2005). Hsu et al. (1995) found that dendritic cells could successfully stimulate T cells to attack B cell lymphomas. Furihata et al. (2005) reported that the presence of CD83(+) DCs are a useful prognostic factor for patients with gall bladder carcinoma and Tsukayama et al. (2005) reported that the maintenance of mature dendritic cell density could prolong the survival of patients with advanced gastric cancer.

Ulcerative colitis and Crohn's disease, collectively termed inflammatory bowel diseases (IBD), are chronic inflammatory diseases of the intestine that afflict more than four million people worldwide. Intestinal inflammation is characterized by an abnormal mucosal immune response to normally harmless antigens in the gut flora (Karlis et al., 2004). Clinical and experimental studies suggest that appendectomy can protect against development of ulcerative colitis and Crohn's disease (Farkas et al., 2005). Several studies have been performed to uncover the role of dendritic cells in early immunologic events leading to T cell activation and chronic intestinal inflammation. Mature intestinal dendritic cells may form a nucleation site for a local T cell response and may play an important role in the pathogenesis of inflammatory bowel disease (Karlis et al., 2004). But how T cells in the appendix can affect the development of colitis is not known. The studies by Farkas (2005) demonstrate the preferential migration of CD62L+CD4+ cells into the appendix as compared to the colon. This migration pattern suggests an important role of the appendix in the pathogenesis of colitis. In the present study, the distribution of dendritic cells in the human appendix confirms the migratory nature of DCs as well as their role in immunity.

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