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RESEARCH ARTICLE

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Distribution of S-100 positive dendritic cells in bovine pharynx, tonsils, and retropharyngeal lymph nodes

Abstract Dendritic cells (DCs) are professional antigen-presenting cells. However, the distribution of bovine DCs in the pharynx, tonsil, and retropharyngeal lymph nodes has not yet been documented. To address this issue, immunohistochemistry was conducted using S-100 protein as a marker for DCs. It was observed that S-100 positive Langerhans cells (LCs) were primarily found in the basal layer of the pharyngeal epithelium. Some DCs were found in the outer layer of the epithelium and their dendrites extended out towards the epithelial surface. In the tonsil, S-100 positive DCs were found either in follicular germinal centers or in the T-cell areas. It is worth noting that the S-100 positive DCs were not only distributed in the cortex, but also in the medulla of bovine retropharyngeal lymph nodes. The distribution patterns of bovine DCs in the pharynx, tonsil, and retropharyngeal lymph nodes have an important implication for our understanding of the interaction between pathogens and host.

Keywords dendritic cells, bovine, pharynx, lymph node, tonsil, immunohistochemistry

1 Introduction

Dendritic cells (DCs) are professional antigen-presenting cells involved in the effective generation of antigen-specific T-cell responses (Perez-Torres and Ustarroz-Cano, 2001; Heath et al., 2004). It has been demonstrated that the DCs function as the first-line sentinels in immune surveillance of epithelia and peripheral tissues, such as pharyngeal epithelia, gut and airways, which are in direct contact with the external environment (Vermaelen et al., 2001; Garcia-Romo et al., 2004).

In the bovine pharynx, tonsils are well-developed and lie in a position critical to the immunological surveillance of airborne and orally presented antigens. The epithelium overlying the lymphoid follicles of the tonsils is closely adapted to this function as pathogens must traverse it before they can initiate immune responses to protect the body (Koshi et al., 2001).

Lymph nodes are the secondary immune organs that provide regional immunity. Recent studies show that the resident DCs within the T-cell area of lymph nodes take up soluble antigens that enter via the afferent lymphatics before the antigens carrying DCs arrive from the periphery (Sixt et al., 2005; Allan et al., 2006). The DCs immigrating from peripheral tissues enter the lymph node to initiate the primary immune response and trap immune complexes (Hoshi et al., 2001; Von Andrian and Mempel, 2003). However, the localization of bovine DCs in the pharynx, tonsils, and lymph nodes has not been fully characterized. Recently, the bovine dendritic cells in skin have been immunohistochemically stained with S-100 antibody (Oliveira-Sequeira et al., 2000). Also, it has been noted that the S-100 antibody is able to specifically stain only DCs rather than the macrophages in the lymph node and other tissues (Yamate et al., 2000; Madjid et al., 2007). These results indicate that the S-100 protein is one of the reliable markers for immunohistochemical detection of the bovine DCs in the lymphoid organs.

The pharynx, tonsil, and retropharyngeal lymph nodes comprise a local immune response unit in the mucosal immune system because the LCc in pharyngeal epithelium immigrate the draining lymph node and/or tonsil where to trigger the adaptive immune responses. To achieve a general knowledge of the DCs in such a functional unit, we collected bovine pharynxes, tonsils, and retropharyngeal lymph nodes for immunohistochemical investigation. It has long been documented that DCs are one of the most heterogenous populations in the

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body. There are many marker molecules, such as CD1, CD11c, CD205, and S-100 protein in the DCs, of which the CD1 and the S-100 protein have been widely utilized as specific markers for the DCs in the skin, intestine, liver, and respiratory tract in the paraffin-embedded tissues (Dziegiel et al., 2007; Nakahigashi et al., 2007). Double-staining for S-100 and CD68 confirmed that these two markers clearly distinguish between DCs and macrophages (Popov et al., 2006). Given that bovine DCs constitutively express the S-100 protein (Yamate et al., 2000; Dadabayev et al., 2004; Perez et al., 2005), the S-100 protein was used as a specific marker to examine the distribution of dendritic cells in the organs mentioned above.

2 Materials and methods

2.1 Preparation of tissue samples

Pharynxes ($n = 10$), tonsils ($n = 10$), and retropharyngeal lymph nodes ($n = 10$), were obtained from 10 healthy cattle at a slaughter house. The tissue samples were fixed in 10% buffered formalin (pH 7.2) for 48 hours and embedded with paraffin, followed by conventional practices.

2.2 Antibodies and immunohistochemical procedures

Polyclonal rabbit anti-cow S-100 protein (Dako Cytomation) was used as the primary antibody to detect the localization of DC in tissue sections, and goat anti-rabbit immunoglobulins (EnVision™ Detection Kit, Dako Cytomation) were used as the secondary antibody. A human melanoma biopsy was processed as a positive control, a rabbit anti-pseudorabies antibody (IgG) was used as an isotype control and phosphate-buffered saline (PBS) buffer was used as a negative control.

Paraffin sections, 8 μm in thickness, were mounted on aminopropylethoxysilane (APES) coated slides, and dried overnight at 37°C. Tissue sections were deparaffinized and rehydrated. After antigen retrieval in the citric acid buffer (pH 6.0) for 12 mins at 95°C, the sections were stained with a standard EnVision protocol. Briefly, an endogenous peroxidase activity was blocked with 3% hydrogen peroxide; then the sections were thoroughly rinsed three times in the PBS and incubated in goat serum albumin to block nonspecific binding of the antibodies. They were then incubated with the primary antibody (S-100, 1:400) at 4°C overnight. On the following day, they were washed three times in the PBS and incubated with the secondary antibody for 30 mins at room temperature. The sections were then washed three times in PBS and developed in 3, 3′-diaminobenzidine (DAB), following the manufacturer's instructions. The reaction was stopped by washing with PBS. The sections were dehydrated in gradient ethanol and cleared in xylene, then mounted with neutral balsam for light microscopic observation.

The stained sections were photographed on an Olympus microscope with 10 ×, 20 ×, and 40 × objectives connected to a digital camera. The images were processed using Adobe Photoshop 7.0.

3 Results

3.1 Distribution of S-100 positive DCs in bovine pharynx

In the pharynx, S-100 positive Langerhans cells (the dendritic cells in the epithelium known as LCs) were immunohistochemically detected in the mucosal epithelium, but found primarily in the basal layer of the epidermis (Fig. 1), whereas fewer LCs were detected in the outer layers of the epidermis. It is also clearly shown that there are some S-100 positive DCs in the periductular connective tissue beneath the basement membrane of the pharyngeal epithelium (Fig. 1, Fig. 2a).

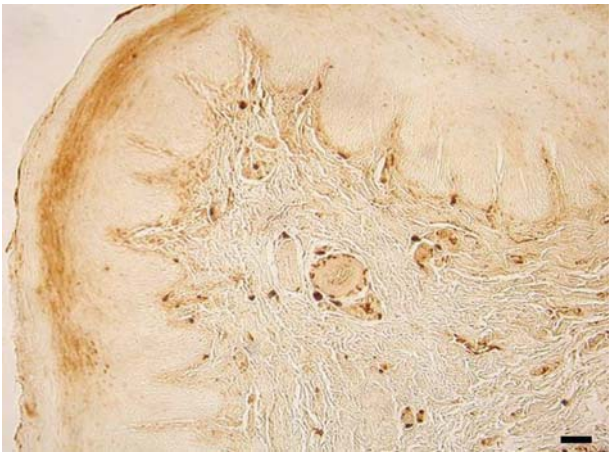

Fig. 1 Immunohistochemical reactivity of anti-S100 protein in paraffin sections of the bovine pharynx. (Scale bar, 50 μm)

3.2 Distribution of S-100 positive DCs in tonsils

It was clearly observed that the S-100 postive LCs were distributed in the basal layer of the tonsillar epithelium, especially in the heavily reticulated lining of the crypt (Fig. 2b), which is functionally referred to the follicle-associated epithelium. Interestingly, in the epithelium of the pharyngeal mucosa, LCs accumulated into a cluster (Fig. 2a). In the tonsillar parenchyma, the S-100 positive DCs were predominantly present in the germinal center of follicles (Fig. 2a), indicative of FDCs. Fig. 3b clearly showed that the FDCs appeared to be intensively nucleus-staining with the S-100 antibody, while few of those in the follicles showed cytoplasm-staining patterns. In the T-cell-dependent area of the tonsils, there were a

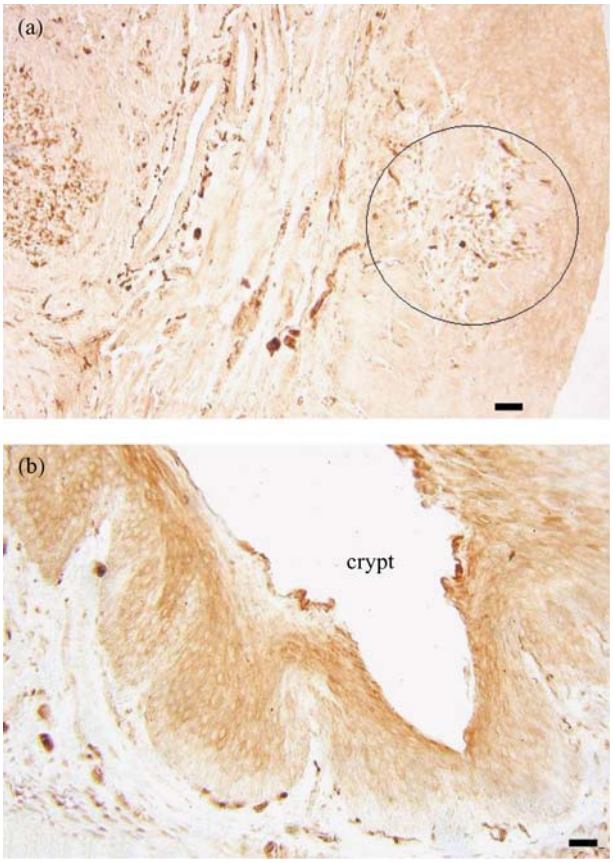

Fig. 2 A number of S-100 positive LCs seen within both types of tonsillar epithelia
Note: (a) In the epithelium of pharyngeal mucosa, LCs accumulated in a cluster to form an island-like appearance (as shown in the circle). Scale bar, 50 μm; (b) LCs are in the basal layer of the heavily reticulated lining of the crypt. (Scale bar, 25 μm)

few S-100 positive DCs that were usually known as interdigitated dendritic cells (IDCs) (Fig. 3a).

3.3 Distribution of S-100 positive DCs in retropharyngeal lymph nodes

In this study, the S-100 protein produced immunolabeling of the cytoplasm of DCs located in both the cortex and the medulla of retropharyngeal lymph nodes in cattle, but predominantly in the germinal center of lymphoid follicles (Figs. 4–6). In subcapsular and peritrabecular sinuses, some migrating DCs were revealed by S-100 immunostaining (Fig. 4). In the outer layer of the cortex, there were many S-100 positive DCs which we prefer to call antigen transmitting cells (ATC) (Fig. 4). FDCs in the germinal centers of the follicles were intensively stained with the S-100 protein. Between the follicles, the S-100 staining DCs were scattered in the T-cell-dependent area, which could have been interdigitated dendritic cells (IDCs) in the paracortex (Fig. 5).

In the medulla, the S-100 positive DCs were densely-populated. Morphologically, these DCs were quite different

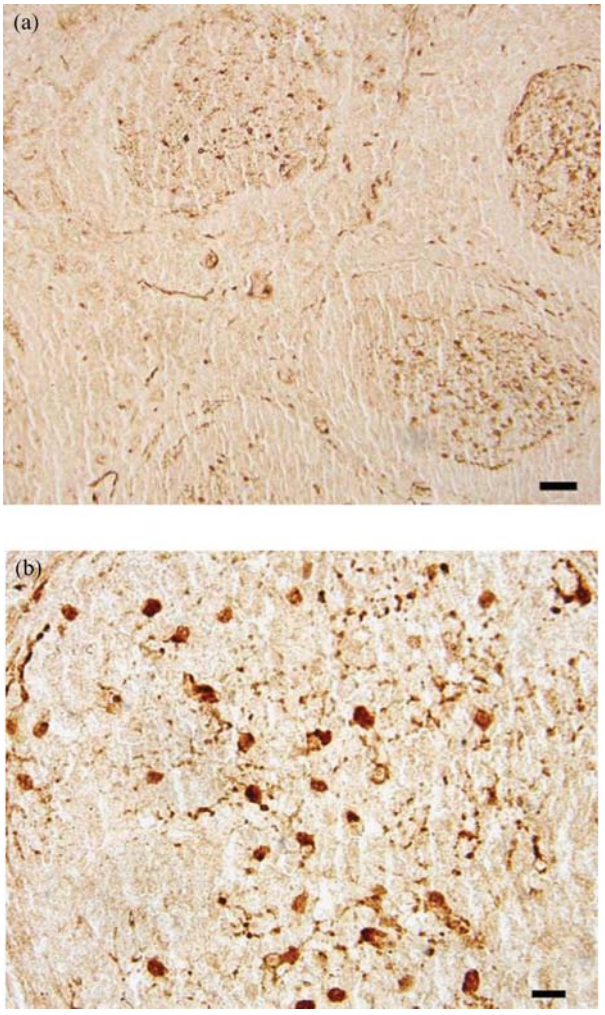

Fig. 3 DCs in the tonsillar parenchyma
Note: (a) In the T-cell-dependent area of tonsils, there are a few S-100 positive DCs. (Scale bar 50 μm); (b) DCs in follicles show intensively S-100 stained. (Scale bar, 12.5 μm)

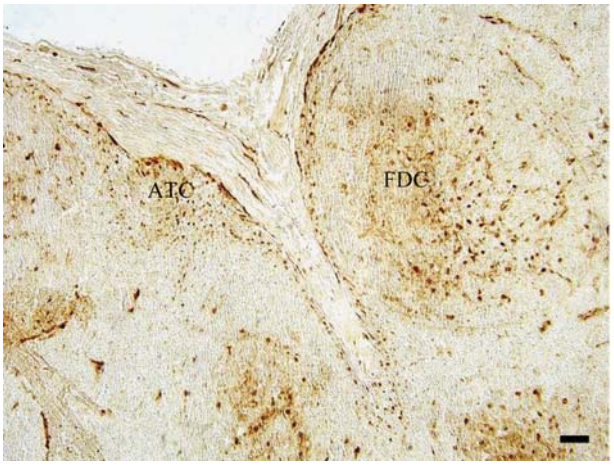

Fig. 4 Distribution of DCs in the draining lymph node
Note: The S-100 positive DCs are predominantly seen in the germinal center of follicles, known as FDC. Many S-100 positive DCs in the outer layer of cortex are called ATC. (Scale bar, 50 μm)

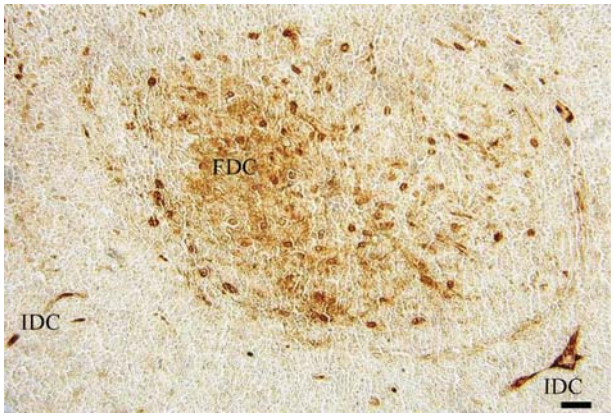

Fig. 5 Higher magnification of Figure 4.
Note: S-100 proteins expressed in the cytoplasm of FDCs in the germinal center of a lymphoid follicle and IDCs in the paracortex of the lymph node. (Scale bar, 25 μm)

from macrophages, which are predominantly located in the sinuses. In contrast, most medullary S-100 positive DCs were attached to the lining of medullary sinuses, while a few dendritic cells were seen in the sinuses lumen (Fig. 6).

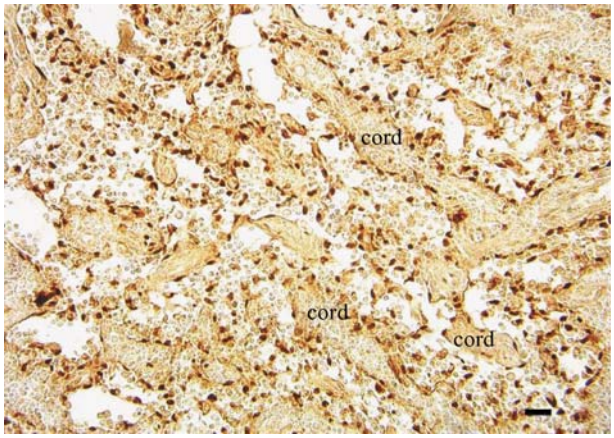

Fig. 6 Distribution of DCs in the medulla of the lymph node.
Note: S-100 positive DCs are densely-populated in sinuses. Most DCs are attached to the sinus lining. A few DCs could be found in the sinus (Scale bar, 50 μm). "Cord" refers to medullar cord.

4 Discussion

The pharynx is a passageway shared by both the digestive and respiratory systems. It has been demonstrated that in most other species, such as mice and humans, the DCs reside in the pharyngeal mucosa as sentinel cells which provide a link between the innate and adaptive immune responses (Maeda et al., 2002). However, in cattle, the distribution of DCs in the pharynx has not yet been immunohistochemically determined. In our present study, the S-100 positive DCs were found to be widely distributed in the bovine pharynx, tonsils, and retropharyngeal lymph nodes (Figs. 1–6). Interestingly, it was observed that many LCs in the epithelium accumulated in a cluster, and their dendrites extended out towards the epithelial surface (Fig. 2a). This distribution pattern provides a great possibility for the LCs capturing the luminal antigens because the morphology of bovine DCs is quite similar to that of the murine DCs in the small intestine (Niess et al., 2005). Recently, it has been indicated that the propagation of the foot-and-mouth disease virus (FMDV) takes place in the bovine pharynx in both vaccinated and unvaccinated cattle (Zhang and Alexandersen, 2004; Zhang et al., 2006). This unexpected outcome is presumably consistent with the finding that non-replicating FMDV induced the apoptosis of DCs[1]. Thus, our data may pave the way to understanding the pathological mechanism of FMDV which persistently invades the body through the pharynx.

In the tonsil, the S-100 positive DCs are predominantly located in the mucosa layer and lymphoid germinal centers. This distribution pattern is quite similar to that of DCs in human tonsils (Koshi et al., 2001; Summers et al., 2001), despite the LCs being only detected in the basal layer of the epidermis in the bovine tonsil. In the tonsillar parenchyma, the S-100 positive FDCs are predominantly present in the germinal center of follicles (Fig. 2a). In the T-cell-dependent area of tonsils (Fig. 3a), there are a few S-100 positive DCs that are usually known as interdigitated dendritic cells (IDCs) (de Baey et al., 2003).

More importantly, not only are FDCs and IDCs well-recognized in lymph nodes with the S-100 staining protocol, but some S-100 positive DCs are also found in the outer layer of the cortex (Fig. 4), which could be known as antigen transmitting cells (ATCs) on the way to the T-cell area where they initiate cellular immune responses.

It is widely known that DCs are usually distributed in the cortex of the lymph nodes. However, in our present study, the S-100 positive DCs were also detected in the medulla of lymph nodes at a great density (Fig. 6). To our knowledge, this is the first study to clearly reveal the medullar DCs in cattle. The function of medullar DCs remains to be investigated.

The DCs are thought to provide potential mechanisms for the transmission of prion diseases, such as Creutzfeldt-Jakob disease (CJD) in humans, scrapie in sheep, and bovine spongiform encephalopathy (BSE) in cattle (Rybner-Barnier et al., 2006; Huang et al., 2002; Aucouturier et al., 2001). Recently, the DCs in the connective tissue have been addressed to be involved in

[1] Jin H, Xiao C, Zhao G, Du X, Yu Y, Kang Y, Wang B (2007). Induction of immature dendritic cell apoptosis by foot and mouth disease virus is an integrin receptor mediated event before viral infection. Journal of Cellular Biochemistry (in press).

the spread of disease-associated prion protein (Koperek et al., 2002). It has been assumed that the gastrointestinal tract is the leading site of entry for the pathological isoform of the prion protein (Miyazawa et al., 2007). However, the general morphology of bovine DCs in the digestive system has not yet been reported. Our data that describe the distribution pattern of DCs in the bovine pharynx, tonsils, and retropharyngeal lymph nodes for the first time have an important implication for the understanding of early events in the development of an robust immune response against BSE, whose DCs are suspected to carry prions through the lymphoid system and to transfer them towards the peripheral nervous system (Dorban et al., 2007).

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