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MUCOSAL-ASSOCIATED LYMPHOID TISSUE OF ESOPHAGUS AND STOMACH OF BACTRIAN CAMEL (*Camelus bactrianus*)

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ABSTRACT

The macroscopic and microscopic structure of the mucosal-associated lymphoid tissue of the oesophagus and stomach of bactrian camel were observed by histological and histochemical methods. The results showed that a plenty of lymphoid tissues were mainly distributed in the lamina propria of the glandular areas of stomach by diffuse lymphatic tissues, solitary lymphoid nodules and aggregated lymphoid nodules. Camel's stomach is not only a digestion organ but also an important organ of immunity with special structure, because there are a large number of lymphoid tissues in the stomach due to the existence of unique glandular sacs, developed mucosal duplicature, and especially the aggregated lymphoid nodules.

Key words: Bactrian camel, lymphoid tissue, oesophagus, stomach

There are lymphoid tissues in the mucosa of digestive tract, respiratory tract and eye conjunctiva in animals which are called mucosal-associated lymphoid tissue (MALT), and these are peripheral immune organs that belong to mucosal immune system (MIS) (Czyzewska and Majkowska-Skrobek, 2005). MIS is an independent immune system which is closely related to whole immune and also has important and unique function; it is regarded as another immune way except from cellular immunity and humoral immunity (Kohtaro *et al,* 1996; Didierlaurent and Simonet, 2005; Madhuri *et al,* 2008).

According to statistics, there is above 50 per cent lymphoid tissues that are located at mucosa, and lymphoid tissues of intestinal tract and respiratory tract, and these quantity are even more than that in spleen and lymph node, and the antibody generated by MALT is also more than that in humour. It is already identified that MALT is an important component and first line as defense of immune system, and also the morphological base of mucosal immune(David and Acheson, 2004;Wershil and Furuta, 2008). In recent years, studies on MALT has been not only progressed in theory, but also used in clinic, such as immunisation through the methods of dropping nose, pointing eye, drinking water and spraying, which can beforehand prevent human and animals (especially poultry) from some contagious disease and parasitosis. The oral immunisation has already been become the new thread of modern vaccinal development, and the exploration of immunologic mechanism of which also become the frontline discussion in immunology (Holmgren and Czerkinsky, 2005; Olga *et al,* 2010; Valeria *et al,* 2005). It is very important to reinforce such research for public health and protection from the animal contagious diseases.

In view of these, MALT of the esophagus and stomach was observed and studied carefully in bactrian camel.

Materials and Methods

The 20 healthy bactrian camels (3-15 years old) including 6 male, 11 female and 3 geld camels, after bloodletting from carotid arteries to death were quickly autopsied. The tissues were taken, such as the beginning end, midpiece, and posterior segment of esophagus; cardia; glandular areas in three glandular sac areas of first and second compartment of stomach, non-glandular areas and the junction of above too, the non-glandular sac areas in first and second compartment; oesophago-colpus; the left and right side walls, paries ventralis, the origin of front oncoides area of cardiac gland region at the stomach neck, ramified mucosa folds of beginning of curvatura ventriculi minor, dorsal of stomach neck and oncoides along front of which, vertical mucosa folds in different areas of stomach; different areas of

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fundus gland region; different areas of pyloric gland region.

Tissues were fixed in 10 per cent formalin. Paraffin sections, were stained with haematoxylin and eosin to observe the distribution and structure of lymphoid tissues in oesophagus and stomach under microscope.

Special staining by Masson-Fontana for determining the shapes and distribution of argentaffin cells was done.

The tissues fixed by Zenker were stained by Gordon-Sweet for observing the distribution of reticular fibres.

The tissues fixed by formaldehyde calcium were made to frozen sections and stained by six azo parafuchsin a-acetic acid naphthalinum ester for nonspecific esterase male cells.

Results

Distribution and construction of MALT of glandular areas in stomach

Glandular sac areas

It was essentially identical about the distribution and construction of MALT in beginning of the first compartment, the areas of after glandular sac areas and the glandular sac areas of second compartment, which all concentrated in lamina propria and existed by solitary lymphoid nodules and diffuse lymphoid tissues (Fig 1). The solitary lymphoid nodules distributed in each three glandular sac area, but much more in the after and second compartment of glandular areas, and generally located in the superficial or deep layer, and sometimes it might be seen that the lymphatic nodules could breakthrough the mucosal muscle into or even occupy the lamina propria. The basic structure of lymphatic nodules was identical in solitary lymphoid tissues and lymph nodes, which had undeveloped germinal centre with light staining and mainly constructed by reticular cells, big or medium sized lymphocytes, and surrounded by small lymphocytes. The B cells were predominated in the whole lymphatic nodule.

There were also two patterns of manifestation on the diffuse lymphoid tissues. One was loose lymphoid tissue with rare lymphocytes that mainly distributed in superficial layer of lamina propria and scattered in glands. It was supported by reticular fibres, and there were unequal lymphocytes that were small lymphocytes at most more or less in reticulation. In addition, there were the classical plasma cells with various shapes and a few nonspecific esterase positive macrophages and reticular cells; Another was dense lymphoid tissues, the lymphoid cells collected together erratically and arranged densely but in irregular shape and without germinal centre. These were mainly constructed by small lymphocytes and had obvious boundary with peripheral tissues. The differences of the dense lymphoid tissues with solitary lymphoid nodules were not only in germinal centre, but also in the different distribution of reticular fibres. There were uniform reticular fibres in dense lymphoid tissues, but lymphoid nodule with little germinal centre had reticular fibres which were around. The diffuse lymphoid tissues were predominated by B cells, and often could see postcapillary venules. There were also few lymphoid cells in mucosal epithelium of three glandular sac areas.

Abomasums

There was same distribution and construction of lymphoid cells in every area of abomasums and glandular sac areas, but special in cardiac gland region.

Cardiac gland region: There were diffuse lymphoid tissues and solitary lymphoid nodules in reticulate and longitudinal mucosal folds except the neck paries dorsalis of stomach, and the band-like aggregated lymphoid nodule area (ALNA) of the cardiac gland region (Wang, 2003). There were more lymphoid nodules in the mucosal lamina propria of ALNA than other areas. In the lamina propria, there were some argentaffin cells which were ellipse, round or even fusiform in different size and distributed in clump. The nucleus was big and circular that located in the centre or side of cell. There were more argentaffin particles about 10-20 in the bigger volume cytoplasm than in smaller one which was only about 2-3, and the particles were in different size. The argentaffin cells were mainly located in deep layers of lamina propria (Fig 2), besides, there were also some single sporadic eosinophils in lamina propria.

Fundus gland region: Because the glandular areas and bottom of stomach ranked closely, therefore, the lymphoid tissues generally located under the mucosal epithelium and around the gland bucca areas. There were more dense lymphoid tissues which were often distributed near mucosal muscle of lamina propria bottom in the whole fundus gland. In addition, there were a few single sporadic argentaffin cells during the glands.

Pyloric gland region: It easily saw some lymphoid nodules and dense lymphoid tissues in this area. There

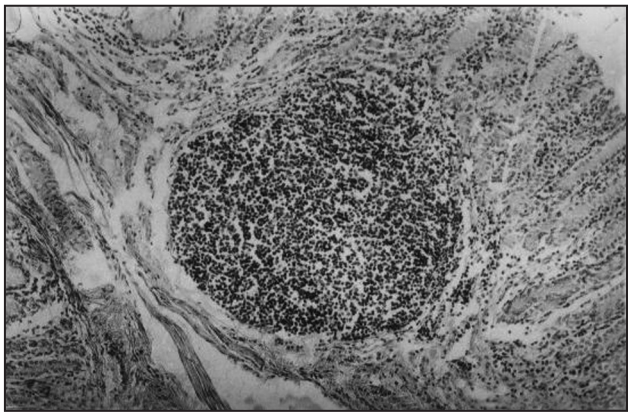

Fig 1. The solitary lymphoid nodule and diffuse lymphatic tissue in the second glandular sac area stained with haematoxylin-eosin, 100X.

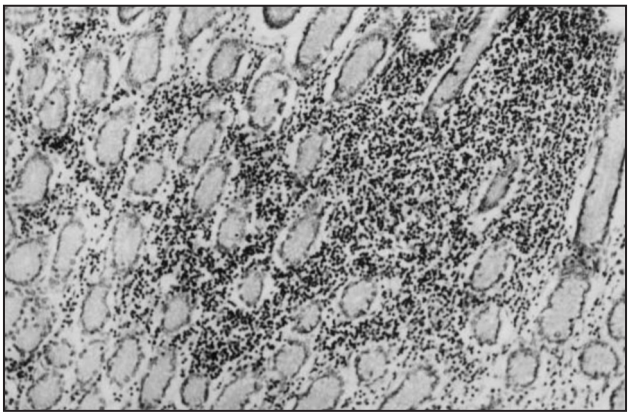

Fig 4. Dense and loose lymphatic tissue stained with haematoxylin-eosin, 100X.

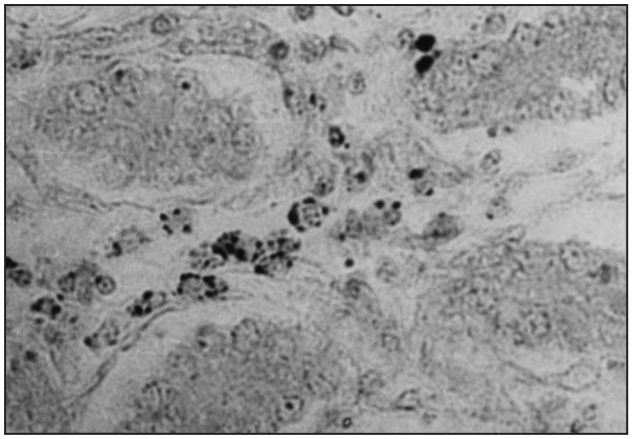

Fig 2. Argentaffin cells: Masson-Fontana's staining 400X.

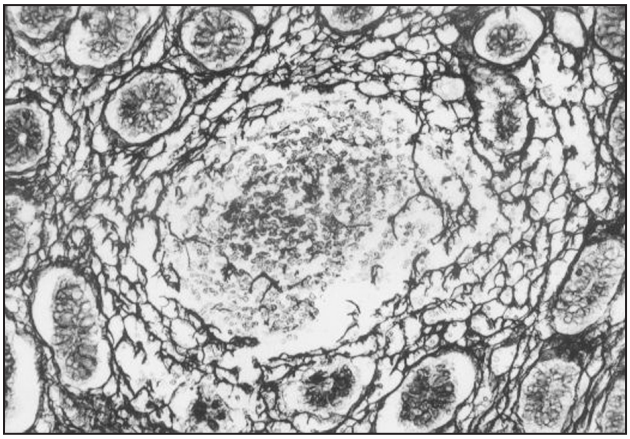

Fig 5. Reticular fibres distributed in lymphoid nodule Gordon-Sweet's staining, 200X.

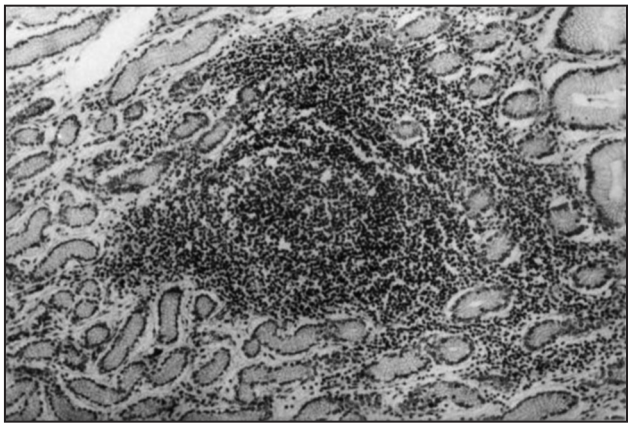

Fig 3. Dense lymphatic tissue near the solitary lymphoid nodule stained with haematoxylin-eosin, 100X.

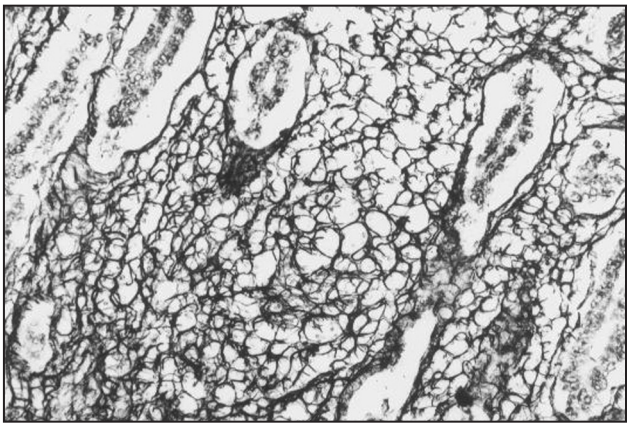

Fig 6. Reticular fibres distributed in dense lymphatic tissue Gordon-Sweet's staining, 200X.

were more diffuse lymphoid tissues surrounded some lymphoid nodules, the dense lymphoid tissues were seen both in superficial and deep layer of lamina propria (Fig 3 and Fig 4). Near the pylorus, there were more lymphoid tissues in superficial layer of lamina propria. The reticular fibres distributed uneven in lymphoid nodules that were few in centre, but was normal in dense lymphoid tissues (Fig 5 and Fig 6). Otherwise, sometimes, it could see some bigger argentaffin cells in pyloric gland region.

There were intraepithelial lymphocytes which were easily seen in pyloric and fundus gland region

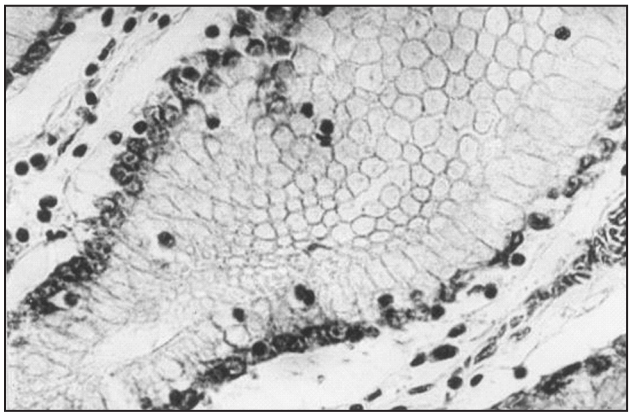

Fig 7. Intra-epithelial lymphocytes in the pyloric glandular region stained with haematoxylin-eosin, 400X.

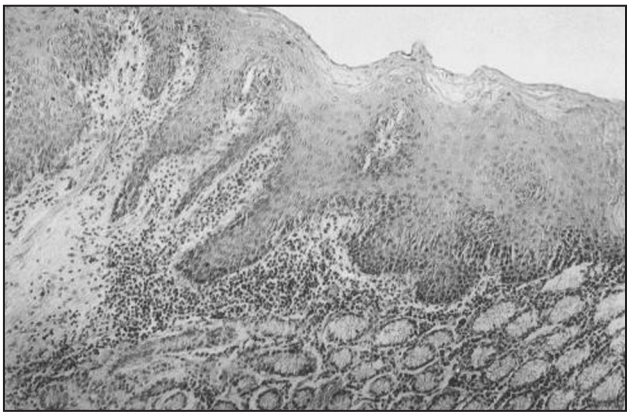

Fig 9. Diffuse lymphatic tissue under the stratified squamous epithelium stained with haematoxylin-eosin, 100X.

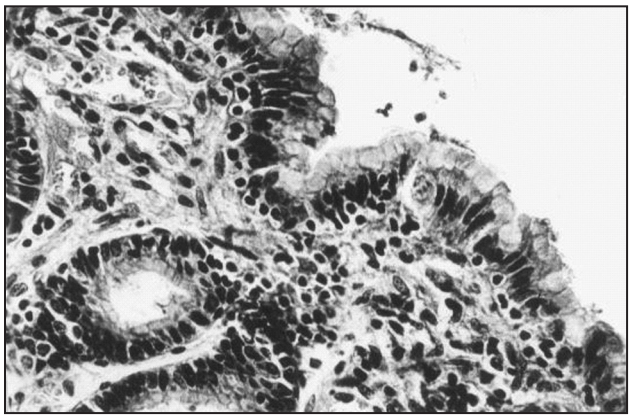

Fig 8. Intra-epithelial lymphocytes in the fundic glandular region stained with haematoxylin-eosin, 400X.

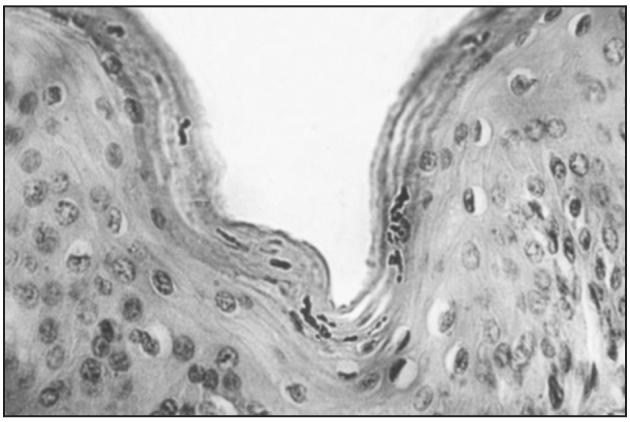

Fig 10. Langerhans' cells stained with haematoxylin-eosin, 400X.

that distributed in each area of abomasums, and the cell was small lymphocyte which was round in deep staining, and some nucleolus ambient was transparent. These cells generally located up, under and around the nuclear of epithelium (Fig 7 and Fig 8).

Distribution and construction of lymphoid tissues in oesophagus and non-glandular regions of the stomach

Oesophagus

There were few lymphoid tissues in the anterior segment, midpiece and posterior segment of oesophagus, which were some sporadic lymphoid cells, plasma cells, macrophages and dense lymphoid tissues. The dense lymphoid tissues mainly distributed around bleeder lines of oesophageal glands. There were more capillaries near the epithelium and high endothelial venules, and in the mesh constructed by reticular fibres, there were many small lymphocytes, few plasma cells and macrophages. The cells which constructed dense lymphoid tissues were in different number.

In addition, there was round, oval or ellipse Langerhans' cells in the granular layers and prickle cell layers of oesophageal epithelium. Sometimes, there was hollow in the side of these cells that liked half-moon, and sometimes, the cytoplasm that was transparent and bubble-like in the centre of cells. The number of these cells gradually increased from the anterior segment to posterior segment of oesophagus.

The non-glandular regions of stomach

The results of cardia, the first and second compartment of the non-glandular regions, the non-glandular areas of three glandular sac areas, sulcus oesophagus and the nape mucosa of the stomach indicated that there were few lymphoid tissues, such as more sporadic lymphocytes, plasma cells and very few abnormal dense lymphoid tissues in different size in mucosa which covered by stratified squamous epithelium. There were more diffuse lymphoid tissues distributed in the junction of the glandular and non-glandular areas (Fig 9).

The Langerhans' cells could be seen in each area of the non-glandular region of stomach (Fig 10).

Discussion

Gastric mucosal-associated lymphoid tissues of bactrian camels compared with other animals

The results indicated that the MALT intensively located in the gastric glandular areas in bactrian camels, therefore, the lymphoid tissues mainly distributed in the gastric glandular areas. It was suggested that there were more lymphoid tissues stored in bigger glandular areas.

There were three glandular sac areas in the stomach of camels (first and second compartment of stomach), and each glandular sac area were constructed by many glandular sacs which was capsular. According to the observation and study of stomach of bactrian camel, the glandular sac often had small mouth but big bottom, and the mouth area was non-glandular, but the bottom area was glandular. The mucosal appearance of bigger bottom areas was often enlarged by abundant mucosal folds and deeply dense gastric groove. In addition, there were well developed mucosal folds in cardiac gland region and fundus gland region of camels which could also make the mucosal appearance of abomasums enlarged (Wang and Chen, 2003a, b). These structures could let camels have glandular areas with sizable mucosal appearance and distributed a lot of lymphoid tissues.

The aggregated lymphoid nodules areas which were band-like in cardiac gland region of camels could further increase the storage of lymphoid tissues. The aggregated lymphoid nodules are the important place for the lymphoid tissues of digestive tract to participate in immune response, and the aggregated lymphoid nodules of other animals and human all reside in intestinal tract, but in camels, it was present in the stomach and intestinal tract. It suggested that the position of camels digestive tract to immune response is much more than other animals and human, which will profit camels to develop oral vaccine, and can also effectively prevent contagious disease and parasitosis.

Distribution and construction of lymphoid tissues of gastric glandular areas in bactrian camel

The basic structures which were mainly constructed by diffuse lymphoid tissues and solitary lymphoid nodules in lamina propria of MALT in gastric glandular areas were same except aggregate lymphatic nodule in cardiac gland region. There were two kinds of shapes on diffuse lymphoid tissues, one was loose lymphoid tissue which scattered in all glands of stomach and located near the epithelium with loose structure, and was supported by reticular fibres. The lymphoid cells, plasma cells in different number and few macrophages were in mesh, and the lymphocytes were main small lymphocytes; the other was dense lymphoid tissue which was collected by small lymphocytes irregularly in different shapes and size. It was different with solitary lymphoid nodule because it had no germinal centre, but was supported by abundant reticular fibres, and there were few germinal centres in lymphoid nodules which were surrounded by reticular fibres. The dense lymphoid tissues were easily seen in fundus glands and pyloric gland region. The solitary lymphoid nodules were distributed in all glands, but were more in the mucosal lamina propria of aggregate lymphatic nodule area in cardiac gland region and pyloric gland region. Otherwise, there were a few intraepithelial lymphocytes in glandular areas. Sometimes, there were clumped argentaffin cells in cardiac gland region, but few sporadic argentaffin cells in fundus gland region and pyloric gland region were also present. The argentaffin cell is a cell which can mainly secrete endocrine cell of 5-HTA and also reside in gut associated lymphatic tissue of other animals.

Distribution of lymphoid tissues of the oesophagogastric non-glandular areas

In the oesophagogastric non-glandular areas which were covered by stratified squamous epithelium, there were few lymphoid tissues, only could see some sporadic lymphocytes and plasma cells, and sometimes dense lymphoid tissues. It might be the result of the thickest epithelium of stratified squamous epithelium which is a native barricade and has strong mechanical protective effect. It is hard for general antigen to invade in the mucosal layer from this epithelium. Otherwise, the process of producing and secreting antibody from mucosa of digestive tract was participated by lymphocytes, plasma cells, macrophages and epithelial cells (non-stratified epithelium) in common, therefore, the lymphoid tissues mainly concentrated in the areas which were covered by columnar epithelium of stomach.

Distribution of Langerhans'cell of oesophagus and stomach in bactrian camel

The Langerhans'cell (LC) is an antigen presenting cell which can swallow foreign bodies, present and transfer antigen. It is closely related to lymphoid cell and join in the immune function of digestive tube. The appearance of this cell distribute the abundant MHC-I, II antigen and FcgR, C3bR. LC mainly locates at the epidermis, but some scholars

find this cell from the human oesophageal epithelium which concentrate at the epithelial intercellular layer and with the same shape of human epidermis. The study of morphometry indicates that the number of this cell will obviously increase with the development of body, and the number of this cell in inferior segment is more than superior segment of oesophagus. There is no report on this cell in animals until now, but from the result of stratified squamous epithelium of oesophagogastric non-glandular areas in bactrian camel, this cell was found in this study which had the same shape with human, and the distribution of which in oesophagus was also identical with human that mainly located at the granular layer and prickle cell layer. But the sub-microcosmic characteristics and functions on this cell need more studies to prove.

Acknowledgement

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