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ELECTRON MICROSCOPIC STUDIES OF LUMBAR LYMPH NODES IN BACTRIAN CAMELS (*Camelus bactrianus*)

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ABSTRACT

The results show that the lumbar lymph nodes were located in the posteromedial aspect of the kidneys, and comprised of capsule and parenchyma. The trabeculas, which were slight, could not divide the parenchyma into obvious lobules. The parenchyma contained lymphatic nodules, dense anodular lymphoid tissue and diffuse lymphoid tissue. Numerous lymph nodules located among the parenchyma solitarily were slightly round and different in size. A layer of lymphatic endothelial cells lined marginal and intermediate sinuses, interestingly, numerous erythrocytes were observed in the periphery of the parenchyma and hemal sinuses inside the parenchyma. This study has shown that the lymph nodes of the camel are distinctively different from the other mammals.

Key words: Bactrian camels, lumbar lymph nodes, lymphatic nodules, morphology

Lymph nodes are not only important filters for lymphatic flow, but also important places where the body immune response too and are distributed throughout the body. They reflect the health status of the regions they drain and are thus routinely examined in abattoirs during meat inspection (Wilson, 1991). Camel meat is regularly consumed in many parts of the world and the major lymph nodes are regularly examined in slaughterhouses (Abdel-Magied *et al,* 2001). The lymph nodes have been reported in other animals, namely, in sheep (Yamashita *et al,* 1985) in rabbit (Compton and Raviola, 1985), in seal (Welsch *et al,* 1997) and in human (Tanegashima *et al,* 1999). About the camel lymph nodes, most of the studies focus on dromedary (Abdel-Magied, 1986; Osman, 1988; Abdel-Magied *et al,* 2001), however, many pertinent characteristics of the Bactrian camel lymph nodes currently lack description. In the present report lumbar lymph nodes from healthy adult bactrian camels were studied by anatomic and histological techniques and electron microscopy, was aimed to provide a basic knowledge for immune system of bactrian camel.

Materials and Methods

Fourteen specimens of the adult bactrian camels (7 male and 7 female) were obtained from the slaughterhouse of the Right Alasan Banner Food Company in Inner Mongolia Autonomous Region, China. The left and right lumbar lymph nodes were separated from fat and connective tissue rapidly, kept intact and weighted. The parameters of anatomical parts were measured by vernier caliper. Data were analysed using the SPSS version 11.5. Part of samples were dissected free and flushed with normal saline. Samples for light microscope (LM) were fixed in 10% formaldehyde for 72 h, dehydrated, cleared and embedded in paraffin. Embedded tissues were cut into 7-µm thick sections and stained with Hematoxylin and Eosin (HE). Pictures of all the sections taken and analysed by using MOTIC Images Advanced 3.0 software.

Small pieces of the tissue samples for Transmission Electron Microscope (TEM) were pre-fixed in 3% glutaraldehyde buffer (pH=7.2) for 1 week. The tissues were then washed thrice in 0.1 M phosphate buffer for 30 min before being cut into 1 mm^3 pieces and were post-fixed with osmium tetroxide for 1 h. The samples were washed thrice in 0.1 M phosphate buffer and then dehydrated in ascending grades of ethanol before being embedded in epon 812. Semi-thin sections of each tissue were collected and stained with toluidine blue. Ultra-thin sections were then collected on copper grids. The

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ultra-thin sections were stained with a saturated solution of uranyl acetate for 30min, followed by lead citrate for 7 min in a carbon dioxide-free environment. Sections were then washed in CO_2-free water, dried and examined under a Transmission Electron Microscope (JEOL, JEM-1230).

Results

Anatomic

Lumbar lymph nodes of bactrian camels were located in the posteromedial of the kidneys and were reddish, prolate and each one in the left and right. Greater differences in size and morphology, were seen in different individuals and also between the left and right in the same individuals.

Lumbar lymph node was about 13.44 ± 2.54 (g) in weight, the ratio of the lymph node weight / body weight (LyNW/BW) was 0.08 ± 0.01 (g/kg). It was 9.03 ± 1.87 (cm) in length, 2.41 ± 0.36 (cm) in breadth and 1.11 ± 0.45 (cm) in thickness (Table 1). On the weight the left lumbar lymph node was a little heavier than the right one but the difference was not significant (P<0.05). Similar observations were made for length, breadth and thickness too (Table 2). The node of female was heavier and the difference was non significant (P<0.05). Considering that male was much larger than female, in terms of the ratio (LyNW/BW), the ratio of female was 0.0857 ± 0.0104 (g/kg), and the ratio of male was 0.0669 ± 0.0030 (g/kg), the difference was found significant (P>0.05); in the length, the breadth, and the thickness, the volume of lumbar lymph node in female camel was larger (Table 3).

LM and SEM

The overall organisation of the nodes mainly comprised of capsule and parenchyma. The parenchyma did not show the characteristic medulla, cortex and paracortex. Instead, it contained lymphatic nodules, dense anodular lymphoid tissue and diffuse lymphoid tissue. Numerous lymph nodules located among the parenchyma solitarily were round slightly and different in size (Fig 1).

Capsule and Trabecula: The surface of the lymph nodes was covered with a thin layer of dense connective tissue capsule. The capsule consisted mainly of densely packed bundles of collagen fibrils, which were tightly packed at the surface of the capsule. Not infrequently elastic fibres were interwoven between the collagen fibrils. Fibroblasts were the principal cellular components of the capsule, valve-containing afferent lymphatic vessels and blood vessels penetrate the capsule occasionally (Fig 2a). Outer layer of the capsule was the connective tissue containing more fat cells; the inner part of the capsule was a layer of lymphatic endothelial cells (En) that line the outer margin of the marginal sinus. Capsule penetrated the parenchyma forming trabeculas, which were slight and could not divide the parenchyma into obvious lobules. But trabeculas that originated in the hilus of the lymph node were distended, and larger blood vessels, lymphatic vessel were concentrated in them. The components of the trabeculas were similar to the capsule. The depression of the lymph node was hilus, which to not unique, and around the hilus there were more connective tissues and fat cells. Efferent lymphatic vessel, small arteries and nerves were consistently seen in or out the hilus (Fig 2b).

Parenchyma Trabeculars in which the branches of blood vessels and nerves were concentrated constituted a general stent of the lymph node, reticular tissues consisting of reticular cells and reticular fibres fill in between the trabeculaes. Reticular cells were characterised by their larger size, irregular shape, round and less heterochromatic nucleus. A number of spindly ramose cytoplasmic extensions from these cells branch out connected to each other to form a net of cells (Fig 3a). A variety of cells, i.e. lymphocytes, plasma cells, macrophages, interdigitating cells and mast cell filled in the mesh between the reticular cells, they consistently presented agglomerate or fascicular distribution forming no obvious lymphatic cords. Small lymphocytes were the principal lymphocytes of lumbar lymph node,

Table 1. Morphometrics analysis of the lumbar lymph nodes in bactrian camel.

Items	N	Mean	SD	Min	Max	Range
Gross weight (kg)	14	348.33	29.48	300	400	100
Weight (g)	14	13.44	2.540	10.1	18.2	8.1
Length (cm)	14	9.03	1.873	6.2	12.1	5.9
Breadth (cm)	14	2.41	0.356	1.9	3.2	1.3
Thickness (cm)	14	1.11	0.458	0.8	1.6	0.8
LyNW/BW (g/kg)	14	0.0773	0.0125	0.0631	0.0983	0.0352

Table 2. Morphologic variance analysis of left and right lumbar lymph nodes in bactrian camel.

Items	Mean ± SD		P
	Left (N=14)	Right (N=14)	
Weight (g)	13.87±2.805	13.01±2.352	<0.05
Length (cm)	8.73±2.248	9.33±1.487	<0.05
Breadth (cm)	2.33±0.413	2.49±0.291	<0.05
Thickness (cm)	1.08±0.268	1.14±0.61	<0.05
LyNW/BW (g/kg)	0.0400±0.0085	0.0373±0.0062	<0.05

Table 3. Morphologic variance analysis of the lumbar lymph nodes in female and male camels.

Items	Mean ± SD		P
	Female (N=7)	Male (N=7)	
Gross weight (kg)	332.0±23.61	368.8±23.94	<0.05
Weight (g)	14.32±3.079	12.34±1.050	<0.05
Length (cm)	9.05±1.372	9.02±2.47	<0.05
Breadth (cm)	2.50±0.301	2.30±0.405	<0.05
Thickness (cm)	1.22±0.589	0.97±0.161	<0.05
LyNW/BW (g/kg)	0.0857±0.0104	0.0669±0.0030	>0.05

these cells (diameter 5-8μm) were characterised by their round nucleus, less cytoplasm, underdeveloped organelles and low electron density. Within the nucleus heterochromatin was massive and fenquently connected with the karyotheca. However, medium lymphocytes were less, these were characterised by their medium size, a deeply cleaved nucleus, and a cytoplasm poor in cellular organelles with mitochondria, rough endoplasmic reticulum and ribosome. The nucleus contains relatively loose heterochromatin (Fig 3b). Plasma cells were common, and were characterised by round to oval, excentrically located nucleus with thick chromatin and obvious karyotheca. Under the electron microscope, the large cytoplasm was filled with substantial forniciform dilated rough endoplasmic reticulum, and abundant polyribosome, large mitochondrial containing incomplete cristae. Partly cytoplasm appeared white vacuolation suggesting a self-dissolving occurrence (Fig 3c). The parenchyma possessed relative more macrophages, which exist in two ways. One kind of cells were fixed macrophages, that one side of the cell attached the fibre or the sinus wall, the other side was free; the other kind of cells were wandering macrophage, which are completely free, and their surface have many microvillus. The macrophage possessed large and flat nucleus, and in addition to rough endoplasmic reticulum and mitochondria etc, primary lysosome and secondary lysosome were also present in the cytoplasm (Fig 3d). Interdigitating cells were antigen-presenting cells and were occasionally found, these cells were characterised by many protrusions interleaving each other, an irregular deeply cleaved nucleus with a little heterochromatin. The cytoplasm was pale and on many sections was relatively poor in organelles. Irregular cytoplasmic finger like protrusions extended into the surrounding tissue. Numerous small lymphocytes were found adjacent to these cells (Fig 3e). Mast cells were also common, which were found grouped together or solitary nearby the blood vessels. These cells were round to oval, and the small round to oval nucleus was hypochromasia and centre-located. The cytoplasm of the mast cells was characterised by the presence of numerous fixed-size round granules, which were distributed around the nucleus (Fig 3f). The dense anodular lymphoid tissue formed patches of variable size that often contacted the lymphatic nodules. It contained mainly B lymphocytes, reticular cells, occasional plasma cells, mast cells and blood capillaries. This region was deep colour dyeing. However, the diffuse lymphoid tissue which appeared pale. It contains many branching sinuses and loosely arranged cells that included T and B lymphocytes, reticular cells, macrophages, and occasional plasma cells, interdigitating cells (Fig 4a). The whole parenchyma-mainly the diffuse lymphoid tissue was rich in lymph sinuses, blood vessels, particularly veins. Typically, numerous high endothelial venules (HEVs) were also present in the diffuse lymphoid tissue. These were the major channels for the lymphocytes in blood transferring into lymphoid tissues, and so cells in these regions were highly mobile. Some lymphocytes were seen passing through cubical endothelial cells into lymphoid tissues in the slices (Fig 4b).

Lymph sinus: The lymph sinuses comprised of subcapsular and intermediary sinuses. Subcapsular sinuses located below the capsule were narrow and had cord-like arrangement, and were thin, flat lymphatic endothelial cells line. Electron microscope showed that endothelial cells had both intracellular endocytic vesicles and secretory granules (Fig 5c). While the outer sinus endothelium next to the capsule was continuous and regular, some cells often interrupt the inner endothelium. Interstitium consisting of stroma and collagenous fibre bundles was the connection of the discontinuous endothelium and lymphatic tissue. Numbers of erythrocytes from the capsule crossed the subcapsular sinus and formed a loose interconnecting meshwork then go into the parenchyma (Fig 5a). The erythrocytes around

the sinuses were mixed up with macrophages, reticular cells, and rare veiled cells. Veiled cells were characterised by their surface with membrane protuberances, an irregular lobulate nucleus with less heterochromatic and a cytoplasm poor in cellular organelles where only a bit of mitochondrias and lysosomes were found (Fig 5b). Intermediary sinuses were internal of the parenchyma, making the round of the lymphatic nodules, and sinuses in the diffuse lymphoid tissue were distended and empty, forming networks of sinuses. Lymphatics in capsule and trabeculas often extended into parenchyma connecting sinuses. The endothelial lining of these sinuses were often discontinuous. Numerous erythrocytes were present within or around these sinuses. The ultrastructure of the intermediary sinuses closely resembled that of the subcapsular sinuses (Fig 5).

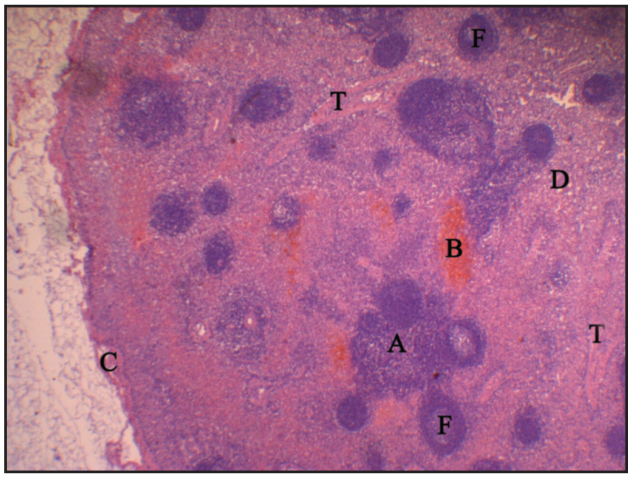

Fig 1. Light micrograph of the lumbar lymph nodes in the bactrian camel. Note the capsule (C), the scattered trabeculae (T), blood sinus (B), folliculi lymphaticus (F), dense anodular (A) and diffuse lymphoid tissue (D). H&E. Magnification: ×40.

Lymphatic nodules Lymphoid nodules were also known as lymphoid follicles, which were round to oval, variable in the size, larger in quantities and distributing throughout the whole lymph nodes dispersedly. The median section of the well-developed secondary lymphoid nodules usually showed large pale germinal centre and surrounded by a darker stained mantle zone containing tightly packed monomorphic intensely stained small lymphocytes called nodule hat. The germinal centre were divided into two parts (Figs 6a and b). First is a darker stained and consisted of centroblasts which can be recognised by their basophilic cytoplasm with cisternae of rough endoplasmic reticulum (RER), mitochondrias and a large round nucleus containing several marginally located nucleoli. These cells were tightly packed in groups with close apposition of the neighbouring cell membranes (Fig 6c). The second zone was stained lighter consisting predominantly of centrocytes, which were produced by centroblasts proliferating continuously, and can be recognised by their smaller size, a cytoplasm poor in cellular organelles, and a deeply cleaved nucleus. The nuclei contain relatively little heterochromatin and only very occasionally have more than two nucleoli (Fig 6d). This zone is oriented towards the marginal and radial sinus. Primary lymphoid nodules were smaller, in there centre contains a small quantity of being transformed B cells, and so the light zone and dark zone were blurring the lines. However, the dark coronae of secondary lymphoid nodules is fuscous circularity and also impossible to separate

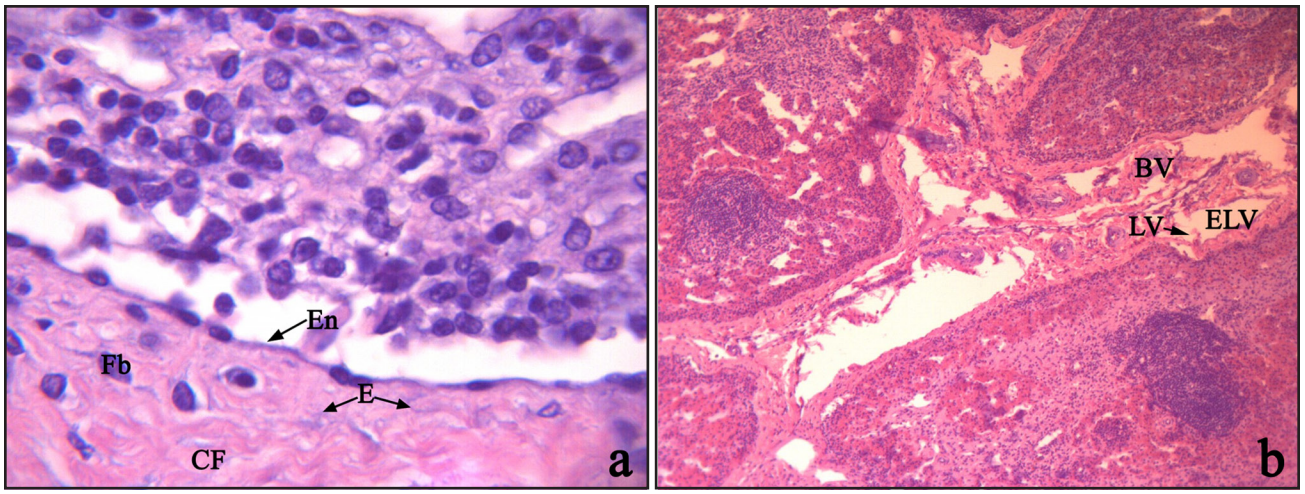

Fig 2. Capsule and Hilus of the lumbar lymph node. a. Capsule: note fibroblast (Fb), collagen fibrils (CF), elastic fibres (E). And a layer of Lymphatic Endothelial Cells (En) is next to the capsule. H&E. Magnification: ×1000. b. Hilus: note the small blood vessel (BV), efferent lymphatic vessel (ELV), lymphatic valve (LV). H&E. Magnification: ×100.

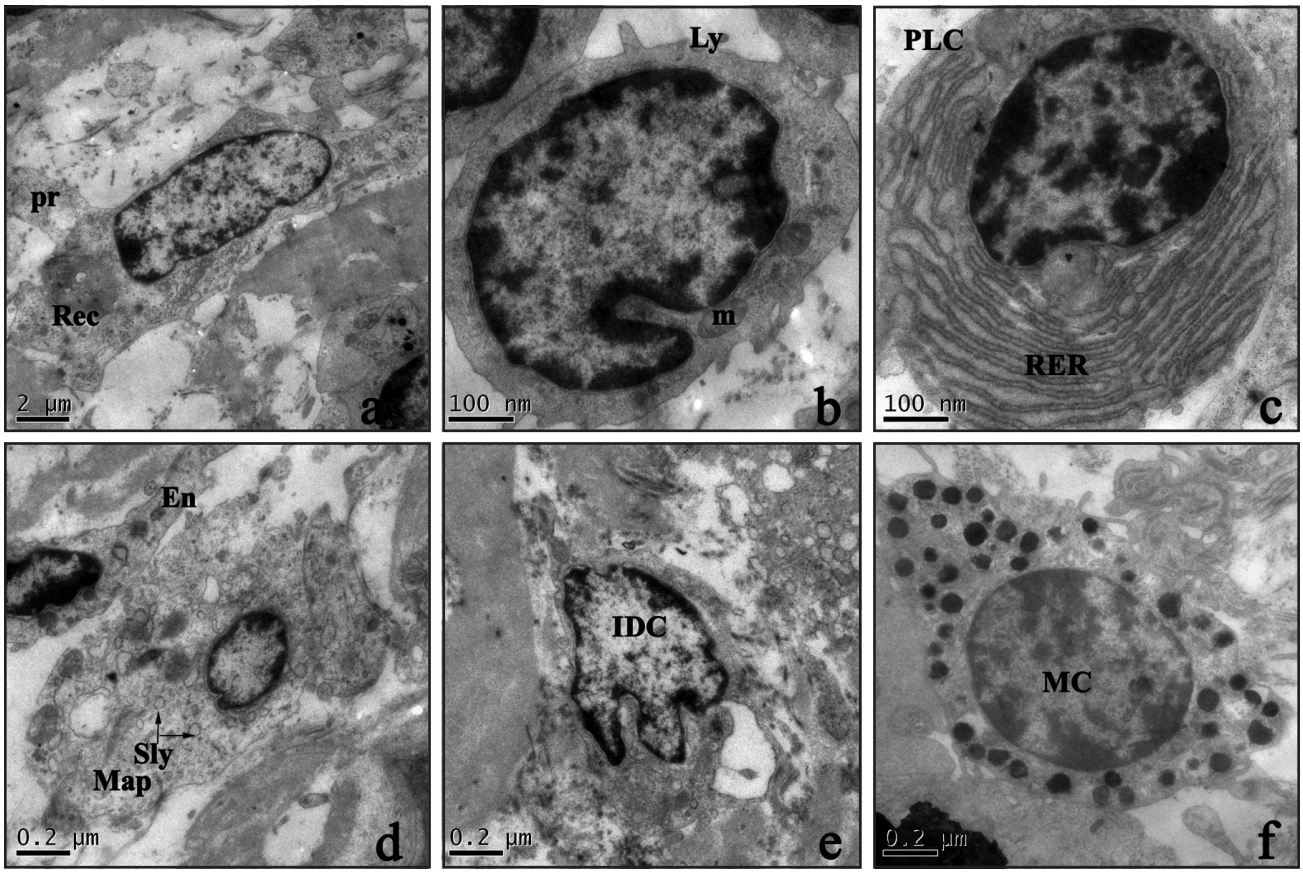

Fig 3. Ultrastructure of cells in the parenchyma. a. reticular cells (ReC), protrusion (pr), Scale bar = 2 µm; b. lymphocyte (Ly), mitochondrial (m), Scale bar = 100 nm; c. plasma cell (PLC), rough surfaced endoplasmic reticulum (RER), Scale bar = 100 nm; d. macrophagocyte (Map), secondary lysosome (sly), Endothelial Cell (En), Scale bar = 0.2 µm; e. Interdigitating cells (IDC), Scale bar = 0.2 µm; f. mast cell (MC), Scale bar = 0.2 µm.

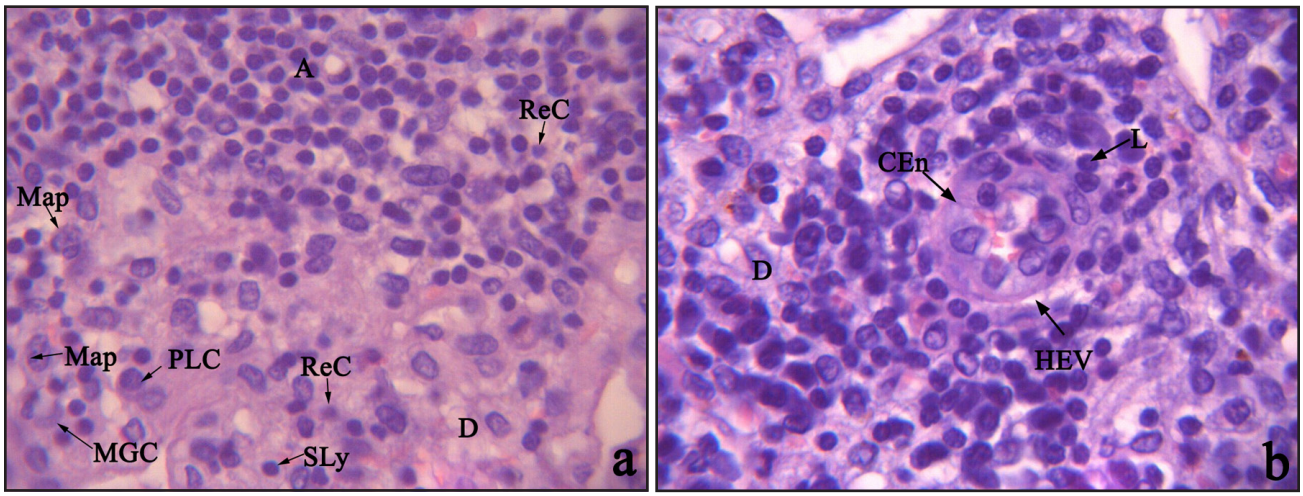

Fig 4. Parenchyma of the lumbar lymph node. a: note dense anodular (A), diffuse lymphoid tissue (D), reticular cells (ReC), macrophagocyte (Map), plasma cell (PLC), multinucleated giant cell (MGC), small lymphocyte (SLy). H&E. Magnification: ×1000. b: high endothelial venule (HEV), cuboidal endothelium (CEn), lymphocyte in passage (L). H&E. Magnification: ×1000.

the light zone and dark zone, if the cross-section were pericentric. Reticular fibres and capilliares were sparsely distributed and without a particular orientation within the lymphatic nodules, but collagen fibres and elastic fibres were almost completely absent in the germinal centre. However, substantial collagen fibres and elastic fibres were present in the region between lymphatic nodules. In addition to B

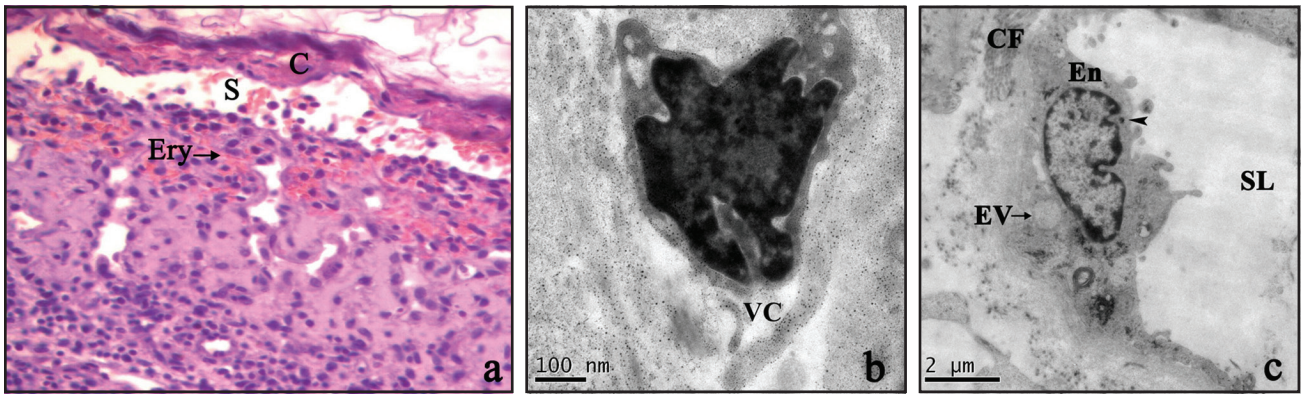

Fig 5. Lymph sinuses of the lumbar lymph node. a. subcapsular sinus: capsule (C), subcapsular sinus (S), erythrocytes pass through the sinus (Ery). H&E. Magnification: ×400; b. A veiled cell (VC) in the lymph sinus. Scale bar = 100 nm; c. Electron micrograph showing an intermediate sinus: the lumen of the sinus (SL), Endothelial Cell (En), secretory granules in the endothelial cell (arrowhead), endocytic vesicles (EV), collagen fibrils (CF), Scale bar = 2 µm.

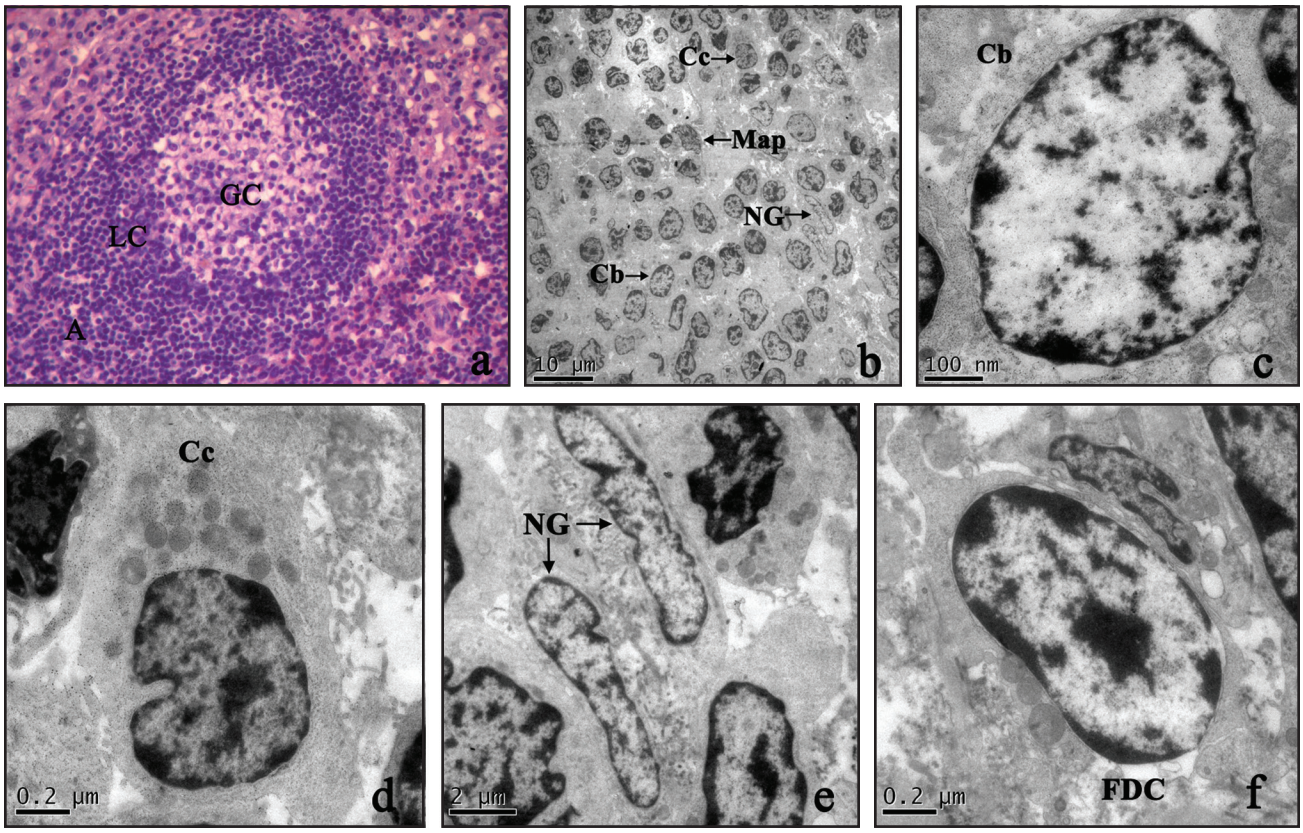

Fig 6. Lymphatic nodule of the lumbar lymph node. a. Light micrograph showing the germinal centre (GC), lymphocyte cap (LC), light zone (L), dark zone (D), dense anodular (A). H&E. Magnification: ×400; b. Electron micrograph showing cells in the lymphatic nodule: centroblasts (Cb), centrocytes (Cc), macrophagocyte (Map), neutronphile granulocyte (NG). Scale bar = 10 µm; c. centroblasts (Cb), Scale bar = 100 nm; d. centrocytes (Cc), Scale bar = 0.2 µm; e. neutronphile granulocyte (NG), Scale bar = 0.2 µm; f. follicular dendritic cell (FDC), Scale bar = 0.2 µm.

lymphocytes, macrophages were common, follicular dendritic cells with irregular shape and multiple processes were occasionally found in lymphatic nodules. The processes were various in shapes showing pseudopodium-like or buninoid processes extend into the surrounding tissue, numerous small lymphocytes were found adjacent to these cells. The follicular dendritic cells had a pale, round to oval, deeply cleaved nucleus, and only very occasionally cells had two irregularity nucleuses (Fig 6e and f).

Discussion and Conclusions

All the lymph nodes of the camel were located in their typical anatomical sites and were possessed of

unique morphological characteristics (Saar and Getty, 1975; Schummer *et al,* 1981; Jackson and Morris, 1984; Barrell and Simpson-Morgan, 1990). Unlike other ruminants, in the camel the lumbar lymph nodes were not present in groups but as solitary nodes, and each one in the left and right. The comparisons of male and female in this study indicated that female lumbar lymph node were larger than the male on the length, the breadth, the thickness, and these differences were significant, which was the same as other mammals. The histological observations of this study had shown that the lumbar lymph nodes of the Bactrian camel lack characteristic medulla, cortex and paracortex. Instead they showed lymphatic nodules, dense anodular lymphoid tissue and diffuse lymphoid tissue, and lymph nodules located among the parenchyma solitarily were round slightly and different in size. In this respect they resembled the lymph nodes of the dromedary, but were distinctively different from the lymph nodes of other domestic ruminants (Abdel-Magied *et al,* 2001; Harris and Templeton, 1966; Blau and Gaugas, 1968). Available information indicates that the capsule of seal lymph nodes is thick, and a well-developed system of trabeculae penetrated the parenchyma of the lymph node in which clear subdivision (Welsch *et al,* 1997), in dromedary each lymph node was also lobulated. However, the present study has shown that the capsule was thin and trabeculas were slight which can not divide the parenchyma into obvious lobules. In addition, the lumar lymph nodes of bactrian camel examined in this study showed lymph nodes were differed from those of the pig and other mammals in containing blood sinuses. The erythrocytes were seen consistently in lymph nodes of healthy camels in this study and by Osman (1988), Abdel-Magied *et al* (2001). Lymph nodes do not contain extravascular blood and blood-filled sinuses except in rare lymphadenopathies characterised by capillary proliferation and lymphoid atrophy (Lucke *et al,* 1987; Welsh *et al,* 1999). However, blood sinuses were normally present in the haemal and haemolymph nodes of other mammals (Gargiulo *et al,* 1987; Abu-Hijleh and Scothorne, 1996; Marniok *et al,* 1997; Sakita *et al,* 1997). Available information about other animals indicates that the haemal nodes have blood sinuses but no afferent lymphatics, lymph sinuses and no clear line between cortex and medulla, whereas the haemolymph nodes resemble lymph nodes in having blood sinuses, lymph sinuses, lymphatics, and a clear boundary between cortex and medulla. The bactrian camel lymph nodes under consideration were neither typical lymph nodes nor typical haemolymph nodes. The presence of afferent lymphatics and blood-filled sinuses in camel lymph nodes suggest that they were functionally similar to the haemolymph nodes of other mammals, which have the ability to cope with both lymph-borne and blood-borne antigens (Abbas *et al,* 1994).

Osman (1988) hinted that afferent lymphatics could be a possible route of entry of erythrocytes into the nodal sinuses. Abdel-Magied *et al* (2001) held that septal blood vessels might be possible sources for erythrocytes in camel lymph nodes. The results of the present study suggest that part of erythrocytes from the capsule gain access to the parenchyma via the subcapsular sinus, and other large numbers of erythrocytes originate from trabecula blood vessels via peritrabecular sinus into intermediate sinus. Extravasation of large numbers of erythrocytes from the nodal sinuses into the surrounding diffuse lymphatic tissue was seen in this study. This may indicate that the lymph nodes of the camel may participate in clearing the body of ailing erythrocytes. The presence of macrophages within the diffuse lymphatic tissue around nodal sinuses was seen in this study, and Abdel-Magied (1986), Abdel-Magied *et al* (2001) and Osman (1988) supports this suggestion.

However, during the long process of evolution, the camel had long been renowned for its ability to withstand disease and these unique lymph nodes may play a significant role in it. The capsule and trabeculaes of bactrian camel lymph node were undeveloped compared with the developed parenchyma. Therefore lymphoid nodules and lymphocytes were relatively more, which can effectively enhance their resistance to diseases. In a similar manner both the haemolymph nodes and haemal nodes of other mammals, the lymph nodes of the bactrian camel were apparently able to cope with antigens carried in either blood or lymph. Moreover, their ability to produce antibodies was probably higher than that of ordinary lymph nodes. It is concluded that the basic histology of the lymph node in the camel is different from that of other mammalian species. Bactrian camels have a strong immunity and adapt well to the harsh climatic conditions.

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