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INVESTIGATION ON DISTRIBUTION AND STRUCTURE OF MUCOSAL IMMUNOLOGICAL TISSUE AND CELL IN REPRODUCTIVE DUCT OF FEMALE TWO-HUMPED CAMEL

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ABSTRACT

Structure and distribution of the mucosal immunological tissue and cells in reproductive duct of female two-humped camels were observed with microscope and electro-microscope. Intraepithelial lymphocytes (IEL) were scattered extensively in the mucosal epithelium and gland epithelium of the oviduct, uterus and vagina, and lymphocytes infiltration in the mucous epithelium of the posterior segment of the cervix, uteri and the anterior segment of the vagina was found evenly. Variable numbers of lamina propria lymphocytes (LPL), plasma cells, macrophages and neutrophilic granulocytes were distributed in lamina propria along the duct from fimbriae tubea to the vagina. The special eosinophil cells appeared in groups in the lymph nodule arising in lamina propria of the uterus, which were found to be dendritic cells (DC). There were also some neutrophilic granulocytes in the epithelium of mucosa and gland and inside the glandular cavity of cervix uteri. Mast cells in groups were observed in deeper lamina propria and upper muscular layer of the uterus but they were not found in that of the pregnant camels. The multitudinous plica and glands of the mucosa in reproductive duct could enormously increase the surface area of the epithelium and then increased the number of mucosal immunological tissue and cell. The results suggested that the reproductive duct of the female camel may possess an active mucosal immune activity.

Key words: Female reproductive duct, mucosal immunological tissue, structure and distribution, two humped camel

Cavosurface of alimentary canal, respiratory passage, urinary tract and genital tract is covered with extensive areas of membrana mucosa and directly relate to the environment outside. Mucous membrane not only carries out special function in different systems, but also plays the first barrier role preventing infection. Considering the cell population, mucosal immunocyte takes 80% part of all the immunocytes *in vivo* (Wang, 2000), playing a very important role in immunity. In recent years, the investigation on cells and the molecular mechanism of mucosal immunological response and the mucosal lymphocytes homing mechanism mostly focuses on alimentary canal mucosal immunity, and the animals used for the experiment are always small ones such as mice and fowl (Camerini *et al,* 1998). Few reports relate to mucosal immunity in large livestocks, especially in camels, although there are some unique microanatomy structures (Wang, 2003 and Al-Zghoul *et al,* 2008). Recently Zidan and Pabst (2008) examined that the ileal payer's patches were still present with a comparable macroscopic and histological structure in one humped camels (*Camelus dromedarius*) in contrast to other species. Two humped camels live in the desert and aridity zone in northern and northwestern China year in year out, which adapt to extreme living environment better than other animals. As a key protected animals in the world, two humped camels have an important effect on maintaining fragile ecological environment in western China. Microscopy and electron microscopy of reproductive system of bactrian camels has been reported (Chen *et al,* 2002 and 2003). This paper explored the structure and distribution of the mucosal immunological tissue and cells in reproductive duct of female two-humped camels by using light microscope and electron microscopy technology in order to enrich the knowledge of mucosal immunology.

Materials and Methods

Twelve healthy adult female two-humped camels (3 of them got 10 to 12 months gestation)

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from Alashanyouqi of inner Mongolia in China were sacrificed by exsanguinating after intracarotid administration of sodium pentobarbital. Fimbria tubae, ampulla of uterine tube and tubal isthmus, uterine horn, uterine body, uterine cervix and vagina were sampled and fixed in 10% formalin solution. Paraffin embedding and H & E staining was used. Periodic acid-schiff's (PAS) staining was carried out. Samples were observed and photographed by Olympus microscope. Meanwhile electron microscope samples were prepared from these 12 camels. Every 1 mm^3 tissue blocks was fixed in 2.5% glutaraldehyde (4°C) and postfixed in 1% osmium tetraoxide. Double staining was done using heavy metal and these were finally observed and photographed by JEM-100CX II type TEM.

Results

Female two-humped camel's genital duct was made up with mucous layer, muscular layer and adventitia. But distribution of the tissue structure and immunological cells of each segment of genital duct wall was different.

Uterine tube

Uterine tube was separated into 3 segments successively called tubal fimbria, ampulla of uterine tube and tubal isthmus. Its mucous layer formed much high and developed plica towards the lumens. Plica had complicated branches, which then formed reticular labyrinthus structure (Fig 1-1). Epithelium mucosae was simple columnar or pseudostratified columnar, more or less ciliated cells mingled with them. Sporadic lymphocytes could also be seen among these epithelial cells, showing an appearance of small round dark particle with a bright circle surrounding the cell (Figs 1-2). In the lumen formed by plica labyrinthus, there were usually a tuft of cells mainly including lymphocytes, macrophages, neutrophilic granulocytes and those similar to plasma cells. Lamina propria was thin, in which there were a few sporadic lymphocytes, plasma cells, neutrophilic granulocytes and macrophages, but mast cells and lymphatic nodules were not seen. Lamina propria of uterine tube was devoid of gland and conspicuous distribution of immunological cells was not seen in muscular layer.

Uterus

Two-humped camels had bicornuate uterus. The histological structure of left and right *cornu uteri* and uterine body was basically similar. The surface of *cornu uteri* and uterine body did not have plica, but their lamina propria contained developed ramous tubular gland. The mucosal epithelium and gland epithelium was mainly simple columnar and pseudostratified columnar epithelium was hardly seen. Some isolated lymphocytes were found in the mucosal epithelium and gland epithelium (Fig 1-3, 1-4), and most of them were distributed below the nucleus level in the epithelium, only a few inversely on top of the nucleus were seen. Neutrophilic granulocytes were inserted between the epithelial cells too. There were many lymphocytes between the gland epithelial cells. Group of lymphocytes was seldom observed in the lumen of gland. Uterus lamina propria was thick, constituted of embryonic connective tissues, containing rich of glands and blood vessels. Abundant lymphocytes, plasma cells, macrophages and leukocytes were distributed in lamina propria. Lymphocytes intensively formed well-demarcated lymphatic nodules near the uterine glands, sometimes with glandular tubes around or in them (Fig 1-3). In these lymphatic nodules there were some special kind of cells. Cell body was large, surrounded by many long branching processes which interlaced each other into a net. Cytoplasm had strong eosinophilia. Nucleus was ellipsoidal, clean and light stained, without conspicuous nucleolus. These cells usually stood in a group (Fig 1-3).

In the deep layer of the lamina propria and the superficial layer of the muscular part in *cornu uteri*, there were groups of mast cell. These cells were distributed along the blood vessel. Cell body was ellipsoidal and granas in the cytoplasm displayed positive reaction in PAS. The difference of mast cell quantity that was distributed in lamina propria and muscular layer was not significant. Under electron microscope, there were round or ellipsoidal granas which were close and uniform size in the mast cell substance. Some of granas had depression on the surface and unit membrane wrapped up around themselves. The inclusion appeared homogenous and the electro-dense was high (Fig 1-10). Some rough endoplasmic reticulum (RER), free ribosomes and mitochondrion were found dispersed among these granas. Nucleus had a shape of globe, inside massive trachychromatic heterochromatin were found near the inner nuclear membrane. Inversely in the body of uterus, few mast cells could be found and it hardly disappeared in the endometria the uterus having of pregnancy.

There were also some plasma cells in the lamina propria of *cornu uteri* and uterine body. Plasma cells were ellipsoidal or global. Cytoplasm was filled with rough endoplasmic reticulum, some of which

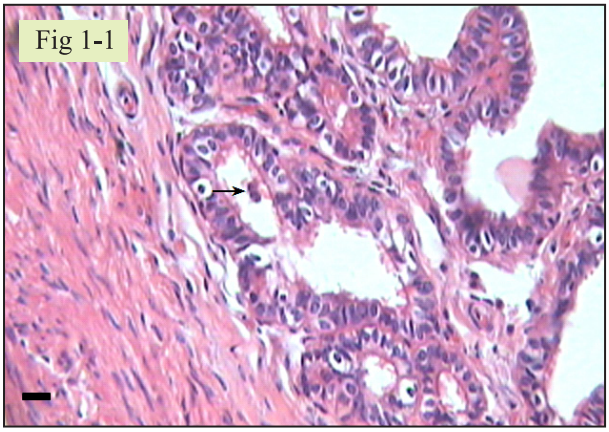
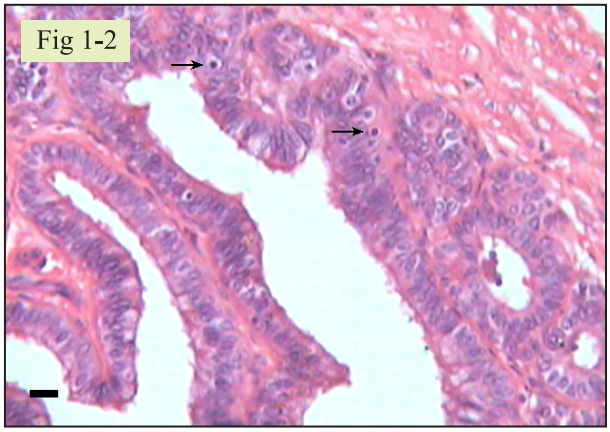


Fig 1-1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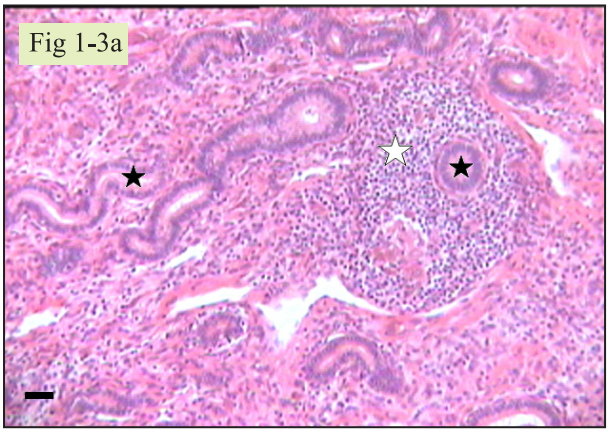
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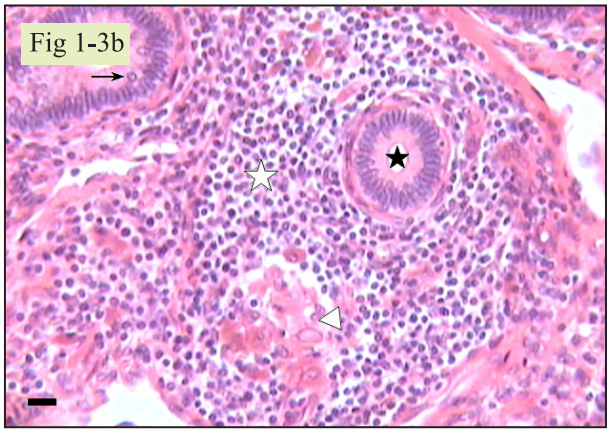
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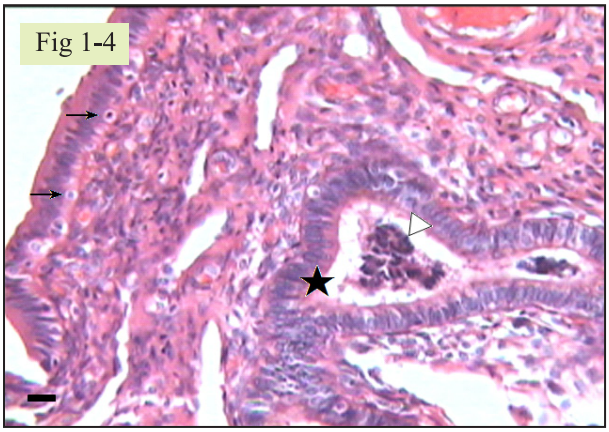
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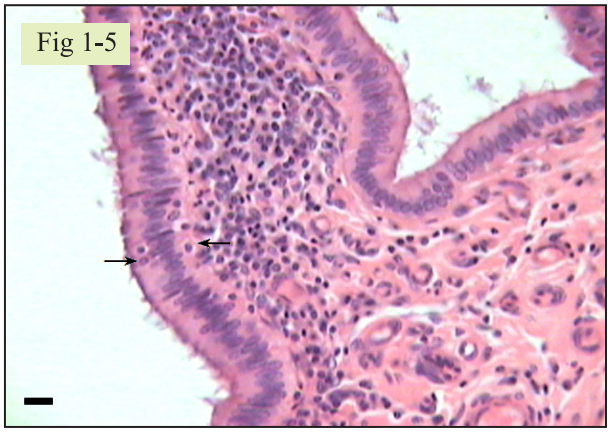
Fig 1-5


Fig 1-6

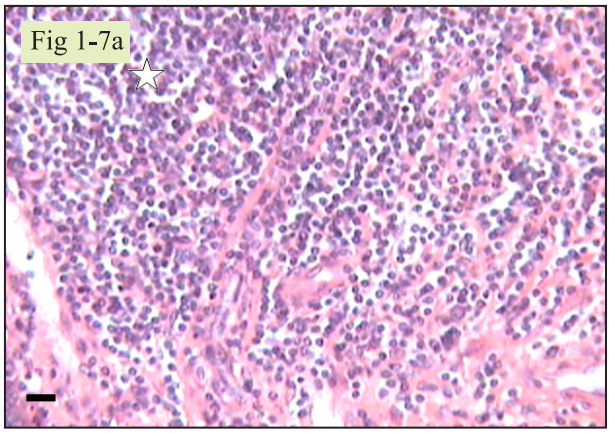
Fig 1-7a

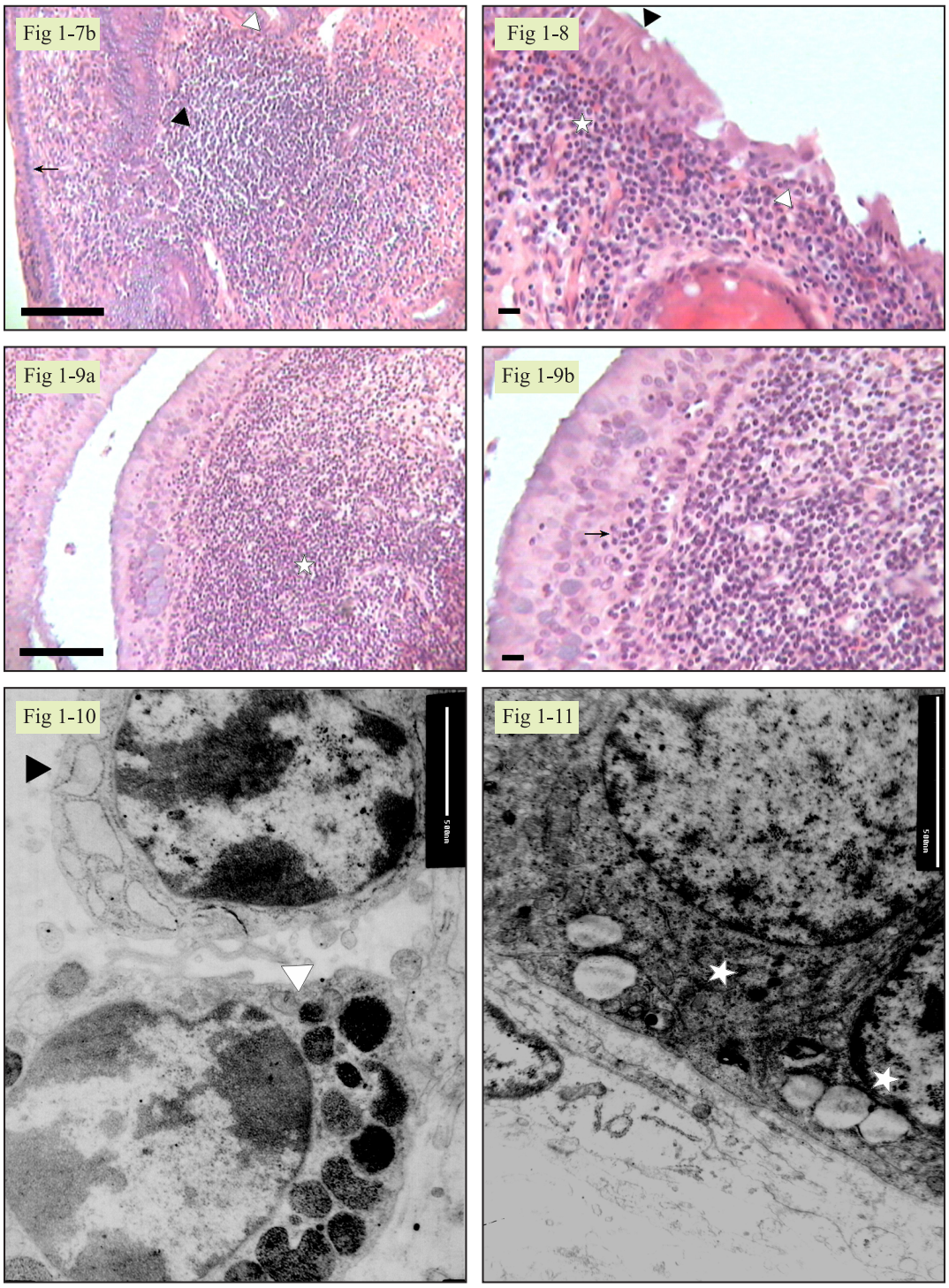

Fig 1. Structure of mucosal immunological tissue and cell in reproductive duct of female two-humped camel 1. The fimbriae tubae of the oviduct: Group of immunocytes (↑)×100; 2. The isthmus of the oviduct: IEL (↑)×400; 3. Lamina propria of uterine horn: Lymphatic nodule (☆), uterine gland (★), a group of special cells (△), IEL in gland epithelium (↑)×400; 4. Mucous layer of uteria corpus: IEL (↑), uterine gland (★), IEL in gland epithelium (△)×400; 5. Uterocervical mucous: IEL (↑)×400; 6. Cervix (posterior segment): Neutrophil leucocytes (▲) in the gland cavity×100; 7. Cervix: Mucous epithelium (↑), immunocytes in upper lamina propria (☆), uterocervical gland (△), lymphatic nodule (▲)×100; 8. Junctional part of cervix and vagina: The immunocytes (☆) are densily distributed beneath the lining epithelium, lymphocytes infiltrated (△) in some segments of the epithelium (▲)×400; 9. Vagina (anterior segment): Accumulated immunocytes (☆) in upper layer of lamina propria, lymphocytes infiltration (↑) in the epithelium×100; 10. Electronograph of mast cell (△) and plasma cell (▲) in lamina propria of uterine horn×13 000; 11. Electronograph of the gland epithelium (☆) in uterine horn, the basement membrane of the epithelium is not clear×8 300 (1-9 HE staining)

were lamellar flat capsule, as well as other RERs showed an obvious extended state. The chromatin was often arranged in peripherally located clumps or in centrally converging strands that gave it a "cartwheel" appearance (Fig 1-10).

Cervix of uterus

Lamina propria of cervix not only contained glands but also formed branching plica, which was different from that in *cornu uteri* and body of uterus. Cervial epithelial mucosae was simple high columnar or pseudostratified columnar, which had a few cilia on it. Epithelial cell nucleus was ellipsoidal and cytoplasm upward side was compact and darkly while down the nucleus it was stained light and clear. Intraepithelial lymphocytes (IEL) were distributed in the cells down the nucleus (Fig 1-5). In the epithelium mucosae of posterior segment of cervix, besides lymphocytes there were many neutrophilic granulocytes which could be seen in basilar part, middle part and upper part. Some of them and their lobed nuclei were squeezed into sliver shape (Fig 1-5). Cervical glandular epithelium also had IELs and neutrophilic granulocytes on it. The glandular cavity of posterior part of cervix often contained neutrophilic granulocytes, sometimes full of them (Fig 1-6). In the superficial layer of cervix lamina propria, a great number of lymphocytes and neutrophilic granulocytes were intensively ranked among glandular tubes, with lots of capillaries. However, the deep layer of lamina propria had few immunocytes but sometimes they formed lymphatic nodule (Fig 1-7) around or near the gland. Cervix of uterus contained few mast cells but many plasma cells and macrophages.

Vagina

Vaginal epithelium mucosae was stratified columnar epithelium or stratified cuboidal, the anterior segment of which had many clumped goblet cells in it. A great quantity of lymphocytes and neutrophilic granulocytes were distributed in the epithelium mucosae of junction between cervix and vagina and anterior segment of vagina. These cells could be found not only in the basal part of epithelium but also in the middle and upper part, even conspicuous lymphocytes infiltrating phenomenon was seen (Fig 1-8). Lymphocytes in the lamina propria under epithelium were intensive and turned out to be obvious diffuse lymphoid tissue (Fig 1-9) while immunocytes in deep layer of lamina propria were rare.

Under electron microscope, the basement membrane of genital duct epithelium mucosae and the glandular epithelium (GE) was not obvious (Fig 1-11). There were cell junction complex between cells of epithelial free surface.

Discussion

Veazey's work on monkey by using flow cytometry (FCM) and immunohistochemistry indicated that above 80% IEL cells were CD3+ and CD103+T cells and NK cells, only 6% of them were CD20+B cells. So the main function of IEL is cell-killing effect that defends enteropathogen invading the body such as bacteria or epithelial cells infected by virus (Xueyuan, 1999). But in LPL, T and B cells are both quite rich. B cells are mainly made up of IgA secretory cells, and very small percentage of B cells are IgG and IgM secretory cells. Otherwise mast cells, granulocytes and macrophages are plentiful too in the lamina propria. Consequently lamina propria is the chief place of mucous membrane immunological reaction (Husband *et al,* 1996). Our study showed that there were lymphocytes in epithelium mucosae distributed from two-humped camel's *fimbriae tubae* to vagina. These lymphocytes belonged to IEL. Even lymphocytes infiltration phenomenon could be seen in epithelium of the posterior part of cervix and anterior part of vagina. That situation was more obviously in lamina propria where the lymphocytes were called LPL. In addition, lamina propria also contained plasma cells, neutrophilic granulocytes, macrophages and so on. IEL and LPL were part of dispersed immunocytes. Besides epithelium mucosae, two-humped camel's uterine glandular epithelium had a copious distribution of IEL too. Developed plica and rich glands of genital duct greatly increased the surface area of epithelium mucosae and glandular epithelium to make larger number of lymphocytes. Lymphatic nodules were usually found in lamina propria of two-humped camel's *cornu uteri*, body of uterus and cervix, which indicated that two-humped camel's female genital duct immune system not only had an enormous number of dispersed immunocytes but also formed mucosa associated lymphoid tissue.

In lymphatic nodules of the lamina propria, there are considerable antigen presenting cells (APC), such as macrophage and dendritic cells (DC). Processed antigens are transmitted to B— lymphocytes to produce antigen specific B— lymphoblasts. After proliferating in germinal centre, these B— lymphocytes migrate through the blood circulation to mucous membrane and gland tissue far away, where they further mature and differentiate into plasma cells secreting IgA. When the IgA formed amphiploid or haploid, they selectly combine to

the polyIgA receptor on the epithelial cell and then stepped over the epithelial cell so as to be released to the excretion of mucous membrane or gland (Kagnoff, 1996). Our experiment had observed that lymphatic nodules appeared in lamina propria of two-humped camel's uterus. Inside the nodule there was a kind of special eosinophil cell which was inferred to be follicular dendritic cell according to the characteristic of appearance, structure and distribution. There were also many macrophage in lamina propria. All the facts comfirmed morphologically that the mucous membrane of two-humped camel's female genital duct contained 2 kinds of APC, called follicular dendritic cell and macrophage morphologically. They can deal with the transmitted antigen and participate in induction of B cell differentiation, maturation of plasma cell and secreting IgA in the lymphatic nodule of lamina propria. Different from other animals, the lamina propria lymphatic nodules were not located below the epithelium mucosae but near the uterine gland, very close to or surrounding the gland, in addition to the IEL in gland epithelium. All the facts above suggested that uterine gland of two-humped camel was an important part of mucous membrane immunity.

It is generally considered that distribution of mast cell in animal's uterus is different according to the species. Rat and Hamster mast cells appear in lamina propria as well as in myometrium, but the quantity in lamina propria is less than that in muscular layer. Quantity of Cricetulus griseus' mast cells has no differences between muscular layer and lamina propria. Mouse mast cells are mainly distributied in muscular layer, but few in tunica intima (Zhang Wenxue *et al*, 2000). Two-humped camel's uterus has generous clumped mast cells and their distribution in lamina propria and muscular layer is similar with Cricetulus griseus's. But these mast cells are mainly distributed in deep layer of lamina propria and surperficial muscular layer in the camel, and the quantity is far more in *cornu uteri* than in body of uterus. When investigating the distribution of mast cells in mouse's uterus, Zhou Zhanxiang (1998) suggested that as a half-allotransplant the embryo could set up a relationship with parent to develop normally and succeeded in avoiding rejection from parent, the most important reason was the local regulation of cell immunity level in the uterus Zidan and Pabst (2008). As a kind of constant immunocyte in uterus, mast cells decreased in number during the gestation in order to depress the immunity level in uterus. Thus the embryo having the father's antigenic character was able to avoid rejection. The phenomenon that mast cells seldom be seen in pregnant camel's uterus supported the above-mentioned view morphologically.

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