Demonstration and Organization of Duct-Associated Lymphoid Tissue (DALT) of the Main Excretory Duct in the Monkey Parotid Gland

Midori MATSUDA1, Keisuke INA1, Hirokazu KITAMURA1, Yoshihisa FUJIKURA1 and Tatsuo SHIMADA2

Department of Anatomy1 and Department of Fundamental Nursing2, Oita Medical University, Oita, Japan

Received September 11, 1997

Summary. Duct-associated lymphoid tissue (DALT) of the main excretory duct in the monkey parotid gland was first demonstrated by light microscopy and by transmission and scanning electron microscopy. The DALT included a follicular area, a parafollicular area and a specialized overlying epithelium with distinct fine-structural elements. There was usually a solitary lymphoid follicle located in the subepithelial area near the orifice of the parotid duct. The lymphoid follicles typically had a distinct germinal center. Numerous immune cells often infiltrated into the epithelium overlying the lymphoid follicle. The superficial epithelial cells of the DALT were larger and flatter than the ordinary duct epithelial cells, and had short irregular microvilli on their luminal surface. They were also in close contact with immune cells such as dendritic cells and lymphocytes. Goblet cells were rare in this area. In addition, bacteria, seen at the duct orifice, were sometimes taken up by the flattened epithelial cells near the orifice. Latex microspheres administrated as particulate antigens at the duct orifice were selectively taken up by the flattened epithelial cells and also by the intraepithelial dendritic cells of the DALT.

These morphological findings suggest that the epithelial cells of the DALT in parotid glands take up antigens from the duct lumen and transport them to adjacent immune cells, and that the DALT in parotid glands may serve as one of the inductive sites in the common mucosal immune system.

Mucosa-associated lymphoid tissue (MALT) like gut-associated lymphoid tissue (GALT) exhibits specific anatomical features so as to be distinguishable from other secondary lymphoid tissues. The most obvious of these are the lack of a defined capsule and of afferent lymphatics, and the presence of a specialized follicle-associated epithelium (FAE). Morphologically, GALT represented by the Peyer's patches is separated into three major domains: a follicular area, a parafollicular area, and an FAE. The FAE includes specially differentiated microfold (M) cells which take up antigens and transport them from the lumen to the adjacent antigen presenting cells and lymphocytes (Owen and Nemanic, 1978; Kuhn and Kaup, 1996).

The palatine tonsil and the nasopharyngeal tonsil showing characteristic lymphoid architecture (nasopharyngeal-associated lymphoid tissue: NALT) are also present in the oral pharynx and the nasopharynx, respectively. Additionally, these tonsils represent a defense line against antigens such as bacteria and viruses as known in GALT. It is well established that these lymphoid tissues, including bronchus-associated lymphoid tissue (BALT), are a part of the inductive sites in the common mucosal immune system (Croitoru and Bienenstock, 1994).

Tonsil-like lymphoid follicles have been found in various sites of the oral mucosa in human and nonhuman primates (Gorlin, 1957; Knapp, 1970; Scott, 1980). Around 1980, a close association between these oral mucosal lymphoid tissues and the ducts of minor salivary glands was elucidated (Klein et al., 1979; Schroeder and Dörg-Schwarzenbach, 1982). Both gram-negative bacteria attached to the duct epithelium of minor salivary glands in healthy monkeys (Schroeder et al., 1983) and organized lymphoid tissues were demonstrated in juxtaposition to their ducts (Schroeder et al., 1983; Nair and Schroeder, 1985). The lymphoid tissue of minor salivary glands was designated as duct-associated lymphoid tissue (DALT) which was comparable to GALT in the gastrointestinal tract in origin, tissue organization, and function (Nair and Schroeder, 1986; Challacombe and Shirlaw, 1994).

With respect to the ducts of major salivary glands, Gorlin (1957) previously suggested the presence of
lymphoid masses with germinal centers as a "hetero-
topic lymphoid tissue" near the duct orifices of hu-
man parotid glands. However, the organization of
these lymphoid masses near the duct orifices has re-
mained obscure. The present study, which is believed
to be the first to mention the presence of an organized
lymphoid tissue of the main duct in the parotid gland,
discusses its ultrastructure and functional significance.

MATERIALS AND METHODS

A total of 15 adult monkeys, *Macaca fuscatus* (8 males
and 7 females) weighing 6.0-8.0 kg were used for this
study. The animals were fed in accordance with our
university's guidelines for the care of experimental
animals. Eleven animals were deeply anesthetized
with ketamine HCl (20 mg/kg) followed by sodium
pentobarbital (25 mg/kg). Each side of the main ex-
cretory duct, which extended from the gland hilus of
the parotid gland to the oral cavity, was excised and
processed for light microscopy (LM), transmission
electron microscopy (TEM), and scanning electron
microscopy (SEM). Four animals were used for an
experiment to take up latex microspheres.

Light and electron microscopy

For LM, eight animals were used. Ten parotid ducts
(from five animals) were fixed in 2% paraformalde-
hyde in 0.1 M phosphate buffer (pH 7.4) for 3 h at 4°C,
then cut into small blocks, dehydrated in ascending
grades of ethanol and embedded in paraffin. Speci-
mens were cut serially at a 5 μm thickness. Sections
were stained with hematoxylin-eosin and an immuno-
histochemical method using immunogold-silver tech-
niques (TAKITA, 1990). Rabbit polyclonal anti-S100
protein (DAKO, Glostrup, Denmark) was used as the
primary antibody to identify dendritic cells (WOOD et
al., 1985; OKATO et al., 1989). Goat anti-rabbit IgG
conjugated to gold particles 5 nm in diameter (Amer-
sham, Buckinghamshire, UK) was used as the secon-
dary antibody. For negative controls, sections were
prepared as above, using normal rabbit serum as the
first antibody. Blood
ultramicrotome and stained with 1% toluidine blue.

For TEM, the specimens were derived from the six
Epon-embedded tissue blocks used for semithin sec-
tions as described above. The ultrathin sections were
cut on an LKB ultramicrotome with a diamond knife,
double-contrasted with methanolic uranyl acetate
and lead citrate, and then examined in a JOEL-
1200EX transmission electron microscope.

For SEM, six parotid ducts from three animals
were excised, immersed in Karnovsky's fixative for
2 h at 4°C, then cut longitudinally with razor blades.
After rinsing in 0.1 M cacodylate buffer (pH 7.4), they
were postfixed with cacodylate buffered 1% osmium
tetroxide for 2 h, dehydrated in graded series of
ethanol, and were freeze-dried by the t-butyl alcohol
drying method (INOUE and OSATAKE, 1988). The speci-
mens were sputter-coated with gold, and examined in
a Hitachi-S 800 scanning electron microscope.

Uptake of latex microspheres

An experiment was carried out on four animals. One
ml of 5% aqueous suspension of 0.3 μm latex micro-
spheres [Sigma, St. Louis, USA (3.05±0.0084 μm)]
was administrated topically at each side of the duct
orifices of the parotid glands under deep anesthesia.
Under the anesthesia, the main excretory ducts of the
parotid glands were excised from two animals at 15
min after the labeling period, and from the other two
animals at 60 min after the labeling. The tissues were
washed in 0.1 M cacodylate buffer, placed in Karnov-
sky's fixative and cut into small blocks. They were
processed according to the same procedure men-
tioned above, and investigated by TEM and SEM.

RESULTS

Light microscopy

The main excretory duct was principally lined with
the pseudostratified columnar epithelium, but gradu-
ally changed near the duct orifice into the stratified
squamous epithelium that was identical to the lining
of the oral mucosa. Near the duct orifice, an organ-
zied lymphoid follicle was found in the subepithelial
area of the parotid duct in all monkeys used (Fig. 1a,
c). This was always located just beneath the bound-
ary between the stratified squamous epithelium and
the pseudostratified columnar epithelium. Most of the
lymphoid follicles included a distinct germinal center
(81%, 13/16) (Fig. 1a). This was always located just beneath the boundary between the stratified squamous epithelium and the pseudostratified columnar epithelium. Most of the lymphoid follicles included a distinct germinal center (81%, 13/16) (Fig. 1a). The duct epithelium overlying the lymphoid follicles often contained a large number of mononuclear cells (81%, 13/16). The infiltrated duct epithelium, appearing to be pushed by the mononu-
clear cells, had few goblet cells (Fig. 1b). Blood
Fig. 1. Light micrographs of 1 μm thick Epon sections from the main excretory ducts belonging to the parotid glands. 

a. A longitudinal section of the parotid duct. A lymphoid follicle (LF) with a germinal center can be seen near the duct orifice (O). L, duct lumen. 

b. Higher magnification of a part of a. The specialized epithelium overlying the lymphoid follicle (SE) considerably differs in appearance from the ordinary duct epithelium (DE) which contains many goblet cells. 

c. A cross section of the parotid duct near the orifice. A lymphoid follicle (LF) can be seen in the subepithelial area. a: ×125, b: ×260, c: ×84
Fig. 2. Immunoreaction to anti-S100 protein antibody. S100 protein positive cells with dendritic shapes can be seen not only within the epithelium but also in the subepithelial region overlying the lymphoid follicle. ×350

Fig. 3. Low-power TEM image of the infiltrated epithelium overlying the lymphoid follicle. Note the flattened superficial epithelial cells (double arrow), numerous infiltrating lymphocytes (arrows) and dendritic cells (arrowheads). Other epithelial cells are also deformed and show an electron dense configuration. ×900
capillaries existed throughout the lymphoid follicles. High endothelial venules (HEV) existed in the parafollicular area, and lymphatic capillaries lay beneath the follicles. Immunohistochemical study demonstrated that S100 protein positive cells with dendritic shapes were distributed within the epithelium overlying the lymphoid follicles in addition to the subepithelial and parafollicular areas (Fig. 2).

Transmission electron microscopy

The epithelial cells of the ordinary main duct consisted of principal cells, goblet cells, and basal cells. Immune cells such as lymphocytes and dendritic cells were rarely scattered within the duct epithelium (data not shown).

With regard to the epithelium overlying the lymphoid follicle, the infiltrated duct epithelium showed a characteristic morphology in comparison with the ordinary duct epithelium (Fig. 3). The superficial epithelial cells were often flattened, tightly connected to each other by the junctional complex containing desmosomes, and had short irregular microvilli. Their cytoplasm was of increased density, having a few vesicles and lysosomes, numerous mitochondria, moderately developed Golgi apparatus, and rough endoplasmic reticulum (Figs. 4a, 7b). Especially, two types of intraepithelial immune cells could be predominantly identified: lymphocytes and dendritic cells (Fig. 3). The dendritic cells were characterized by lobulated nuclei and cytoplasmic processes. Their cytoplasm was less dense, having mitochondria, Golgi apparatus, rough endoplasmic reticulum and few lysosomes (Fig. 4b). Birbeck granules as demonstrated in the Langerhans cells of the skin were not found. The dendritic cells were very close to the intraepithelial lymphocytes, epithelial cells, or each other without a junctional complex (Fig. 4b). Plasma cells and macrophages were rarely encountered within the duct epithelium (data not shown).

The follicular and parafollicular compartments of the lymphoid follicle showed particularly fine structures. The follicular area or the germinal center contained numerous small and large lymphocytes, macrophages, reticular cells, and follicular dendritic cells (data not shown). The parafollicular area contained numerous lymphocytes, macrophages, plasma cells, dendritic cells, fibroblasts, and HEV (data not shown).

Scanning electron microscopy

The luminal surface of the parotid duct was observed by SEM (Figs. 5, 6). Three different regions in the
Fig. 5. SEM images of the luminal surface of the parotid duct near the orifice. 

a. Low magnification shows three different regions of the duct (1-3). 
b-d. High magnification of the three different epithelial surfaces of the duct (a, 1-3).

b. The orifice (a, 1) is composed of stratified squamous epithelial cells with microridges, which are identical with those of the oral mucosa. Note the many bacteria attached to the surface.
c. Epithelial cells of the parotid duct near the orifice (a, 2) are large in size as compared with the ordinary duct cells. They have numerous short microvilli.
d. The ordinary duct surface (a, 3) is composed of principal cells (P) and goblet cells (G).

a: \( \times 70 \), b-d: \( \times 3,200 \)
surface configuration were clearly identified near the duct orifice (Fig. 5a). The orifice was covered with stratified squamous epithelial cells. These cells were flattened and polyhedral in shape, and their surface was covered with twisted ridges or microplicae. Bacteria were attached to these cells (Fig. 5b). The epithelial cells, which existed at the transitional region between the squamous epithelium and the ordinary duct epithelium, were larger in size than those of the principal cells and the goblet cells, ranging from 10 to 15 μm in diameter and varied in surface appearance. Most of them had numerous short microvilli (Fig. 5c). Bacteria were sometimes attached to the cell surface or taken up by the cells in this transitional region (Fig. 6). The ordinary luminal surface of the main duct was lined with principal cells and goblet cells. Bacteria were not detected in this region (Fig. 5d).

**Uptake of latex microspheres**

To confirm morphologically whether the epithelial cells overlying the lymphoid follicle in the parotid duct may be one of the points of entrance for oral antigens, a latex suspension was administrated topically at the duct orifices of the parotid glands. SEM observation revealed that latex microspheres were picked up only by the large superficial epithelial cells near the duct orifice within 15 min after the administration (Fig. 7a), but not by other epithelial cells. TEM observation demonstrated that only the flattened epithelial cells overlying the lymphoid follicle took up these latex microspheres into their cytoplasm within 60 min following the administration (Fig. 7b). In addition, latex microspheres were found between the cytoplasmic processes of intraepithelial dendritic cells as well as within their cytoplasm (Fig. 7c). In the subepithelial and parafollicular areas, they were also taken up by macrophages and dendritic cells within 60 min. No latex microspheres were found in the follicular area or in the remainder of the duct epithelium during these periods (data not shown).

**DISCUSSION**

Organized lymphoid tissues in oral mucosa have been known in juxtaposition to ducts of the minor salivary glands in primates, and termed duct-associated
Fig. 7. SEM and TEM images of the epithelium overlying the lymphoid follicle after the administration of latex microspheres from the duct orifice. a. SEM observation demonstrates that latex microspheres (arrows) are picked up by the surface of the epithelial cell 15 min after administration. b. The superficial epithelial cells take up latex microspheres (arrows) 60 min after administration. c. Latex microspheres are detected between the cytoplasmic processes of intraepithelial dendritic cells (arrows) as well as within their cytoplasm (arrowhead) 60 min after administration. a: ×16,000, b: ×9,500, c: ×5,800
lymphoid tissue (DALT) by NAIR and SCHROEDER (1986). Most of them are preferably located not only at the confluence of the interlobular ducts, but also in the lamina propria mucosae along the course of the excretory ducts. Despite these findings, the presence of the follicle-associated epithelium (FAE) including M cells has not been described at all with regard to DALT in the minor salivary glands. Additionally, the organization of DALT in the major salivary glands has remained unclear. In the present study, DALT consisting of a follicular area, a parafollicular area, and a specialized overlying epithelium, was initially demonstrated in the main excretory duct of the monkey parotid gland by LM, TEM, and SEM. Our attention was focused on the morphology near the duct orifice, especially the transitional region between the stratified squamous epithelium and the pseudo-stratified columnar epithelium, as the lymphoid follicle is located just beneath the epithelium in this region.

The present LM observations of serial sections demonstrated an organized lymphoid follicle located near the duct orifice in all the animals used. Most of the lymphoid follicles possessed a germinal center, and numerous immune cells often infiltrated into the duct epithelium. Histological findings of the DALT in the parotid gland were fundamentally similar to those of GALT (CROITORU and BIELENSTOCK, 1994). SEM observation revealed bacteria lying on the epithelial cells at the duct orifices, and sometimes being taken up by the duct epithelial cells near the orifices. These findings suggest that the duct orifice may be an easy position for such oral antigens to invade along the entire extent of the main duct of the parotid gland, and that the location of DALT may be highly concerned with these oral circumstances.

The ultrastructure of the epithelial cells of the main duct was similar to the previous findings in primates and humans (SATOH et al., 1993; TANDLER, 1993; TESTA-RIVA et al., 1995). However, the ultrastructure of the infiltrated epithelium of the DALT was apparently different from the ordinary part of the duct epithelium. This epithelium had few goblet cells. Most of the superficial epithelial cells of the DALT were larger and flatter than the ordinary duct epithelial cells and had short irregular microvilli. They were also in close contact with immune cells. The ultrastructural appearances of the epithelial cells of the DALT were similar to those of M cells in NALT and GALT in human and non-human primates (OWEN and JONES, 1974; CLAEYS et al., 1996). However, it is not clear whether these cells are really identical with M cells functionally. Recent TEM studies have demonstrated that particulate antigens such as latex microspheres selectively bound to and were taken up by M cells of Peyer's patches (LEFEVRE et al., 1978; SASS et al., 1990). Moreover, JEPSON et al. (1993) reported that selective binding and transcytosis of latex microspheres might be utilized as a functional marker in the identification and isolation of M cells. In our experiment, latex microspheres were selectively incorporated by the superficial epithelial cells overlying the lymphoid follicle, and then transported to the immune cells beneath the epithelium. These findings strongly indicate the presence of M cells at the site in question.

A point of further interest in the present results is the presence of numerous intraepithelial dendritic cells as well as lymphocytes in DALT of the parotid gland. It would seem that these dendritic cells correspond to interdigitating cells (IDC) on the basis of the presence of lobulated nuclei, cytoplasmic processes, clear cytoplasm with few remarkable organelles, and the absence of Birbeck granules (TEW et al., 1982). These structural appearances, except for Birbeck granules, are similar to Langerhans cells of skin. Anti-S100 protein antibody was shown to direct itself to the cytoplasmic antigens of dendritic cells, including both IDC and Langerhans cells (WOOD et al., 1985; OKATO et al., 1989). S100 protein positive cells with dendritic shapes were also demonstrated to distribute themselves into the dome epithelium of the Peyer's patches and the crypt epithelium of palatine tonsils (NAGURA et al., 1991; PERRY, 1994). According to the present immunohistochemistry, S100 protein positive cells with dendritic shapes appear to be identical to IDC. In addition, intraepithelial dendritic cells were sometimes in close contact with lymphocytes. These findings may support the idea that the intraepithelial dendritic cells play a key role in inducting T cells in the DALT of parotid glands, as indicated in MALT (NAGURA et al., 1991; PERRY, 1994).

Taken together with these observation, it can be concluded that the organized duct-associated lymphoid tissue (DALT) which exists at the orifice of the main duct of the parotid gland may indeed serve as a part of the inductive sites in the common mucosal immune system.

Acknowledgements. The authors thank Prof. Toshie SAKATA, Department of Internal Medicine I, Oita Medical University for valuable advice and help in preparation of the manuscript, Prof. Masakazu HORITA, Department of Gerontological Nursing, Oita Medical University for critical advice, and Mr. Shuji TATSUKAWA and Miss Yukari GOTO for experimental and secretarial assistance.
REFERENCES


