Human Labial Gland Duct Epithelium and sIgA Secretion —An Ultrastructural and Immunohistochemical Study

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ABSTRACT. Objectives/Hypothesis : The aim of the present study was to clarify the variety of cell components, including acinous secretory cells and duct epithelial cells, of the labial glands, which participate in secretory IgA (sIgA) secretion.
Study Design : An ultrastructural and immunohistochemical investigation.
Methods : Using five autopsied cases, both secretory and duct portions of the human labial glands were examined ultrastructurally, and immunohistochemical observations of IgA, IgG, IgM, secretory component (SC) and lysozyme were performed using the avidin-biotin peroxidase complex (ABC) method.
Results : The secretory portions consisted of two types of secretory cells, seromucous cells and serous cells. The gland had long intralobular ducts with short intercalated ducts. The intralobular duct epithelium consisted of four types of epithelial cells ; D-1, D-2, D-3 and D-4 cells. Immunohistochemically, epithelial cells (especially D-1 cells) and intercalated duct cells were strongly stained not only by immunoglobulins but also by SC. D-3 cells were lysozyme positive.
Conclusion : In human labial glands, secretory cells and both D-1 epithelial cells of the intralobular duct and intercalated duct cells were considered to be responsible for sIgA secretion and lysozyme secretion.

Key words : human — labial gland — sIgA — SC — duct epithelium

SIgA is the predominant immunoglobulin in labial minor salivary gland secretions. Human saliva contains large amounts of immunoglobulin,[1,2] and compared with the major salivary glands, the labial minor salivary glands secret higher concentrations of sIgA.[3] Our previous histometrical observations confirmed that both the labial glands and sublingual glands, which are composed mostly of mucous acini, had much higher densities of plasma cells in the connective tissues around the glandular portion of these glands than did the parotid and submandibular glands.[4] IgA molecules were immunohistochemically identified in both the secretory cells of the labial gland acini and intercalated duct epithelium.[5] Specific receptor known as SC are also necessary for IgA secretion,[6] but these have been few reports on cellular involvement of the secretory cells and duct epithelium of the

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labial glands in sIgA secretion into the oral cavity. The aim of the present study was to clarify by electron microscopy and immunohistochemistry the variety of cell components, including acinous secretory cells and duct epithelial cells, participating in the sIgA secretion of the labial glands.

MATERIALS AND METHODS

Human labial mucosae from five autopsied cases were used in this study. None of the five cases, consisting of four males and one female aged 61-82 years old, had any diseases of the oral cavity, salivary glands or pharynx (Table 1). For ultrastructural and immunohistochemical observations, the labial mucosae were processed as follows.

TABLE 1. Cause of death of cases studied in this report

Cause of death	Number of Cadavers	Sex	Age (years)
Heart failure	4	♂3 ♀1	61-82
Lung Carcinoma	1	♂1	72

Electron Microscopy

Upper and lower labial mucosae were removed from the five autopsied cases in the Department of Pathology of Kawasaki Medical School, and cut into small blocks. The tissue blocks were immersed in 4% paraformaldehyde with 5% glutaraldehyde in 0.1 M cacodyrate buffer, pH 7.4, for 2 hr at 4°C. After being postfixed in 1% osmium tetroxide in 0.15 M cacodyrate buffer for 2 hr at 4°C, the tissues were dehydrated in a graded concentration of ethanol and embedded in Epon 812. Ultrathin sections were prepared on a Reichert-Jung Ultracut S microtome and stained with uranyl acetate and lead citrate for examination with a JEM-2000 EXII electron microscope at 80 kV.

Immunohistochemistry

The distributions of IgA, IgG, IgM, SC and lysozyme in the labial glands were studied using the avidin-biotin peroxidase complex (ABC) method. Labial mucosae removed from the female, were immersed in 5% formaldehyde in phosphate-buffered saline, pH 7.4, at approximately 4 hr after death. Tissues were immersed in 3.5% paraformaldehyde in phosphate buffer, pH 7.4, for 2 hr at 4°C and, after dehydration in a graded concentration of ethanol, embedded in paraffin. Sections, 4 μm in thickness, were placed on slides for staining. To block endogenous peroxidase, tissues on slides were incubated with 0.3% H_2O_2 in absolute methanol for 30 min. After washing in distilled water, the tissues were incubated in normal goat serum for 20 min to reduce non-specific reaction. Then, the tissues were incubated in primary antisera for 30 min at room temperature. Anti-human IgA-, IgG-, IgM-, SC- and lysozyme-rabbit polyclonal antibodies (Dako co., Denmark) diluted 1 : 150 to 1 : 200 with bovine serum albumin were used as antisera. Slides were placed in the biotin-labeled goat anti-rabbit IgG for 30 min, and then incubated in the

avidin biotinylated peroxidase complex (Vectastain Elite ABC-kit, Vector Lab., USA) for 30 min. Peroxidase was visualized by reaction with diamino-benzidine. For control staining, slides were placed in solution containing normal goat serum instead of primary antiserum. After the immunohistochemical procedure, slides were stained with hematoxylin.

RESULTS

A. Ultrastructure

The human labial glands consisted of a few lobes, with each lobe being composed of many lobules separated by intralobular connective tissue. The lobules were composed of glandular portions, connective tissue around those portions and short intralobular ducts. Intralobular and interlobular ducts drained into a single large main duct, which opened into the oral cavity.

a. Glandular portion

The glandular portion was lined with secretory cells, at the base of which myoepithelial cells were located. The secretory cells were columnar in shape and approximately 15 μm in height. Their apical cytoplasm was charged with a number of granules and, based on granular ultrastructure, the secretory cells could be divided into at least two groups; type 1 secretory cells (S-1) and type 2 secretory cells (S-2) (Fig 1). The majority of the secretory cells were S-1 cells with a flattened nucleus, containing large spherical granules, 1-2 μm in diameter, with electron-lucent content. The S-2 cells, on the other hand, contained a light content of small granules, and were 0.5-0.7 μm in diameter, with an electron-dense core. These cells contained numerous rough endoplasmic reticulum (RER), a number of microvilli on the apical surface and intercellular canaliculi on their lateral surface. Since the small number of electron-dense granules were observed in the S-1 cells, they were identified as seromucous cells, and the S-2 cells were considered to be serous secretory cells.

Intralobular connective tissue lay among the glandular portions, and, compared with the lamina propria mucosae of the lips and interlobular connective tissue, free cells appeared to be more numerous. These cells included plasma cells, mast cells and small lymphocytes, and the cells were found singly or in small cell clusters.

b. Intercalated and intralobular ducts

The glandular portions drained into intralobular ducts, then to interlobular ducts. Between the glandular portions and intralobular ducts, there were short intercalated ducts. These short intercalated ducts were narrow, 15-20 μm in diameter, and consisted of a single layer of cuboidal epithelial cells and myoepithelial cells situated outside the ducts. The epithelial cells, 6-7 μm in height, contained a few mitochondria and sparse cisterns of RER, but cytoplasmic organelles were generally scanty (Fig 2a).

The intralobular ducts measured 25-50 μm in inside diameter and a single layer of columnar epithelium lined the duct lumen (Fig 2b). The epithelial cells, 12-16 μm in height, had several ultrastructural diversities, with four types of epithelial cells; type 1 duct epithelial cells (D-1), type 2

duct epithelial cells (D-2), type 3 duct epithelial cells (D-3) and type 4 duct epithelial cells (D-4) (Fig 3). The D-1 cells were the most numerous cell type. They had shortmicrovilli on their apical surface, and both interdigitation and junctional complexes could be observed on their lateral surface. Their cytoplasm contained abundant polysomes and free ribosomes, and bundles of intermediate filaments were present in the apical and lateral cytoplasm. These D-1 cells often formed large blebs bulging and pinching off into the lumen like the secretory cells in the apocrine glands. The D-2

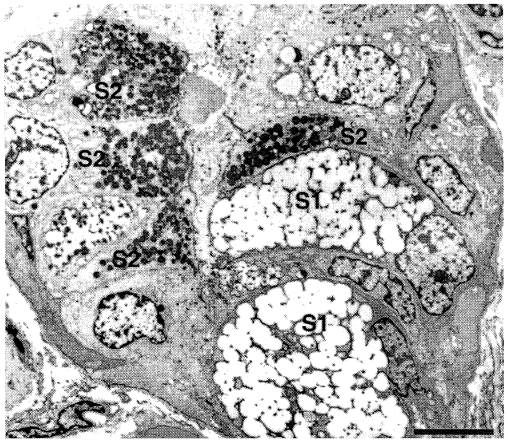

Fig 1. Glandular portion of a labial gland.
The S-1 cells (S1), which contain electron-lucent granules, are seromucous cells, and they form a majority of the glandular portion of the labial gland. S-2 cell (S2) are serous cells, containing electron-dense granules. Bar : 5 μm

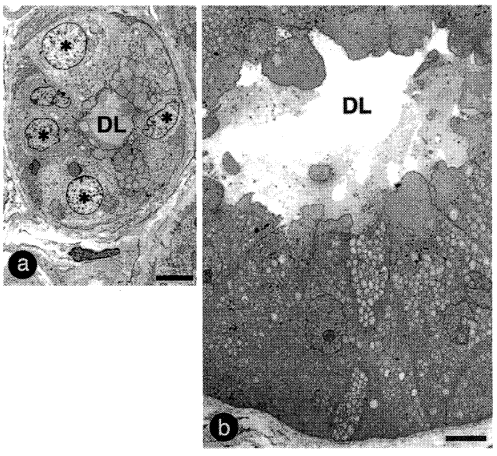

Fig 2. Intercalated and intralobular ducts.
a) An intercalated duct consisting of a single layer of cuboidal epithelial cells. The duct lumen (DL) is narrow and round, and the epithelium is composed of simple cuboidal cells (asterisks). Bar : 5 μm
b) Intralobular duct.
High columnar epithelial cells line the duct lumen (DL). The profile of the duct lumen is irregularly wide. Bar : 5 μm

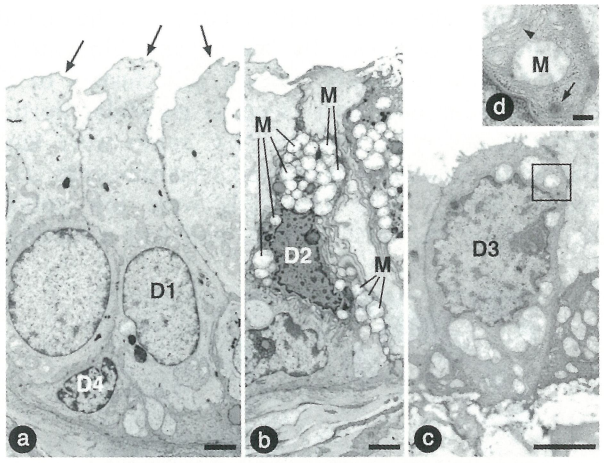

Fig 3. Duct epithelial cells of intercalated and intralobular ducts.
a) The D-1 cells often formed large blebs bulging and pinching off into the lumen (arrows). The D-4 cells were low basal cells (D4). Bar : 2 μm
b) The D-2 cells were charged with abundant mitochondria (M), and other cell organelles were scanty. Bar : 2 μm
c) D-3 cells.
The cytoplasm is narrow, and contains a few granules (arrow). Bar : 2 μm
d) The cytoplasm in the rectangle in c).
The solid arrow indicates a small granule, and the arrowhead shows RER.
M : mitochondria, Bar : 0.2 μm

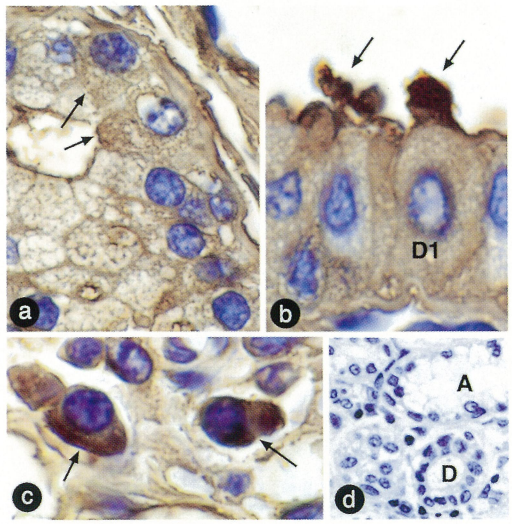

Fig 4. IgA staining.
a) Glandular portion. ×1,100
Some secretory cells (arrows) were positive.
b) Intralobular duct. ×1,600
The D-1 cells (D1) were positive, and the cell blebs (arrows) appeared strongly positive.
c) Connective tissue around secretory portion. ×1,500
Many plasma cells (arrows) were strongly positive.
d) Control secretions of labial gland. Any positive cells cannot be observed in duct epithelium (D), acinus (A) and intralobular connective tissue. ×300

cells were charged with abundant mitochondria. The basal, lateral and perinuclear cytoplasm was occupied by round and elongated mitochondria, and other cell organelles were scanty. Small cell clusters of D-2 cells could be found in the intralobular duct very close to glandular portions. D-3 cells were the least frequent cell type, and the N/C ratios were higher than those of D-1 cells. The apical cytoplasm contained electron-dense secretory granules as well as abundant RER. The D-4 cells were low cuboidal cells with scanty cytoplasm, lying on the basal lamina, and had a tapering upper end.

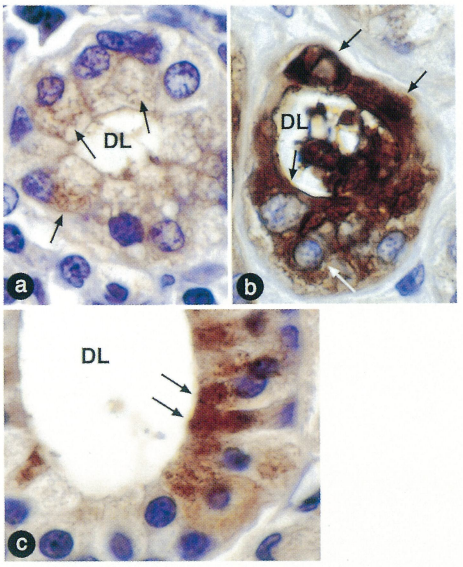

Fig 5. SC staining. DL : Duct lumen
a) Secretory cells in the acinus.
Arrows show weakly positive secretory cells. ×1,000
b) Intercalated duct cells.
Duct epithelia appear strongly positive (arrows). ×1,100
c) Duct epithelial cells.
Many columnar cells appear positive (arrows). ×1,000

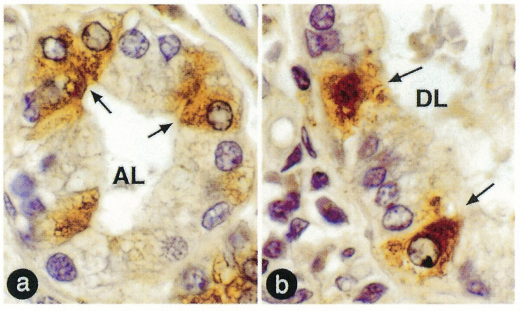

Fig 6. Lysozyme staining. ×730
a) Secretory cells.
Arrows show strongly positive cells (arrows). AL : Acinus lumen
b) Intralobular duct.
Arrows show positive duct epithelial cells. DL : Duct lumen

B. Immunohistochemistry

In the glandular portion, some of secretory cells were positively stained for IgA (Fig 4a) and IgG, but no IgM positive cells could be detected. Almost all epithelial cells of the intralobular and the intercalated ducts were stained for IgA, and especially the blebs of the D-1 cells were intensely IgA-positive (Fig 4b). In both the intralobular and intercalated ducts, IgG-positive cells were rare, and no IgM-positive cells could be found. Secretory cells and duct epithelial cells appeared weakly positive (Fig 5a, c), but the cuboidal epithelium of the intercalated ducts was strongly positive for SC (Fig 5b). Both secretory cells and few duct cells (D-3 cells) in the intralobular ducts were positive for lysozyme (Fig 6a, b).

Immunoglobulin staining on plasma cells showed that the most common cell type were IgA plasma cells (Fig 4c), and IgG plasma cells were the second common cell type. Russell body in plasma cells were only IgM-positive. All plasma cells were negative for both SC and lysozyme. Control slides had no positive cells for IgA (Fig 4d).

DISCUSSION

Our present results showed that ductal elements rather than acinus cells play an important role in the secretion of sIgA and lysozyme in the human labial glands.

When compared with the major salivary glands, secretions from the minor salivary glands were smaller in amount and lower in the concentration of digestive enzymes.[7] However, the concentration of sIgA has been known to be 4.4 times greater in secretions from the labial glands than in those from the parotid gland.[3] Tissues from the minor salivary glands have been known to contain various kinds of immunoglobulin, and more than 70% of the immunoglobulin in secretions from the labial glands has been IgA.[8,9] As reported previously,[4] the densities of plasma cells in the connective tissue surrounding the secretory acini of the human labial glands were significantly higher than those of parotid and submandibular glands. The intralobular connective tissue of the labial glands also contained higher numbers of IgA positive plasma cells than IgG and IgM positive plasma cells. Therefore, in IgA secretion into the oral cavity, special attention should be paid to the labial glands and sIgA production in situ. In addition to IgA positive plasma cells in the connective tissue surrounding secretory cells of the labial glands, present observations revealed that IgA could also be recongnized not only in secretory cells but also in the duct epithelium. SIgA composed of monomeric IgA molecules and SC is known to be essential in protection of mucosal surfaces, and SC is directly involved in sIgA function in vivo.[10-12] Both secretory cells and duct epithelial cells were positive for IgA and SC, but the intensity of the staining proved to be much stronger in intralobular duct epithelial cells than in secretory cells. Therefore, the intralobular duct elements rather than the acinar secretory elements should be considered to have specialized roles in transepithelial secretion of IgA originally produced by plasma cells in connective tissues around the glandular portion. Our present observation revealed that the most common type of duct epithelial cells, D-1 cells, could be responsible

for sIgA synthesis and secretion through an apocrine-like mode of secretion.

D-2 cells were ultrastructurally distinguished from other duct epithelial cells by the presence of numerous mitochondria. As is well known, in the major salivary glands, striated duct cells are highly folded, and numerous elongated mitochondria occupy the basal cytoplasm for ion-pumping energy supply.[13] D-2 cells could be considered to correspond to these striated duct cells in the major salivary glands, although the basal foldings were underdeveloped. Duct cells having extremely numerous mitochondria have also been reported in human laryngeal glands and anterior lingual glands.[14-16]

Lysozyme has been known to have a direct bacteriolytic action by hydrolyzing glycosidic bonds in the bacterial cell wall,[17] and a high concentration of lysozyme was found in serous acinus cells in the parotid glands. However, the enzyme has not been found in the duct epithelium. In the labial glands, as shown in our observations, lysozyme was clearly positive in both the duct epithelium and secretory cells. Thus labial gland duct epithelium could play an important role not only in IgA reaction but also in the lysozyme reaction of the oral mucosal immune defense system.

The functional significance of the remaining duct epithelial cells, the D-4 cells, is unclear at present, but, on the basis of their localization within the duct epithelium, they may correspond to basal cells in the excretory duct of the glandular portion of the glands.

CONCLUSION

Ultrastructurally, the acinous portion of the labial glands consisted of two types of secretory cells, seromucous cells and serous cells, and the lobules consisted of long intralobular ducts and short intercalated ducts. The epithelium of intralobular ducts was composed of four cell types ; D-1, D-2, D-3, D-4 cells. Immunohistochemical studies showed that not only duct epithelial cells, especially D-1 cells, but also intercalated duct cells were strongly stained by both IgA and SC. Both D-3 cells and secretory cells were positively stained by anti-lysozyme antibody.

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REFERENCES

1) Carpenter GH, Proctor GB, Garrett JR : Preganglionic parasympathectomy decreases salivary SIgA secretion rates from the rat submandibular gland. J Neuroimmunol **160** : 4-11, 2005
2) Proctor GB, Carpenter GH : Chewing stimulates secretion of human salivary secretory immunoglobulin A. J Dent Res **80** : 909-913, 2001
3) Crawford JM, Taubman MA, Smith DJ : Minor salivary glands as a major source of secretory immunoglobulin A in the human oral cavity. Science **190** : 1206-1209, 1975
4) Takemoto T : Human labial salivary glands and plasma cells - a histological and histometrical study. Kawasaki Ikaishi **22**(1) : 19-28, 1996 (in Japanese with English summary)
5) Takemoto T : Ultrastructural and immunohistochemical observation of plasma cells of the human labial glands. Kawasaki Ikaishi **23**(2) : 87-97, 1997 (in Japanese with English summary)

6) Tomasi TB Jr. : Secretory immunoglobulins. New Eng J Med **287**(10) : 500-506, 1972
7) Ten Cate AR : Oral Histology. 4th ed, St. Louis, Mosby. 1994, pp356-388
8) Smith DJ, Taubman MA, King-WF : Immunological features of minor salivary gland saliva. J Clin Immunol **7**(6) : 449-455, 1987
9) Smith DJ, Taubman MA, Ali-Salaam P : Immunoglobulin isotypes in human minor gland saliva. J Dent Res **70**(3) Mar : 167-170, 1991
10) Mostov KE, Kraehenbuhl JP, Blobel G : Receptor-mediated transcellular transport of immunoglobulin : synthesis of secretory component as multiple and larger transmembrane forms. Proc Natl Acad Sci USA **77**(12) : 7257-7261, 1980
11) Corthesy B, Spertini F : Secretory immunoglobulin A : form mucosal protection to vaccine development. Biol Chem **380** : 1251-1262, 1999
12) Phalipon A, Cardona A, Kraehenbuhl JP, Edelman L, Sansonetti PJ, Corthesy B : Secretory component : a new role in secretory IgA-mediated immune exclusion in vivo. Immunity **17**(1) : 107-115, 2002
13) Cook DI, van Lennep EW, Roberts ML, Young JA : Secretion by the Major Salivary Glands. In Physiology of the Gastrointestinal Tract, 3rd, edited by Johnson LR. New York, Raven Press. 1994, pp1061-1117
14) Pastor LM, Ferran A, Calvo A, Sprekelsen C, Horn R, Marin JA : Morphological and histochemical study of human submucosal laryngeal glands. Anat Rec **239** : 453-467, 1994
15) Tandler B, Denning CR, Mandel ID, Kutscher AH : Ultrastructure of human labial salivary glands. III. Myoepithelium and ducts. J Morph **130** : 227-246, 1970
16) Tandler B, Pinkstaff CA, Riva A : Ultrastructure and histochemistry of human anterior lingual salivary glands (glands of blandin and nuhn). Ant Rec **240** : 167-177, 1994
17) Dobrovski LS : Lysozyme expression in the rat parotid gland : light and electron microscopic immunogold studies. Histochem Cell Biol **107**(5) : 371-381, 1997