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SHORT COMMUNICATION

Epithelial and PGP9.5-immunoreactive cells of Malassez epithelium in the periodontal ligament of cats: a transmission electron microscopic study

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Abstract

Objective. The aim of the study was to investigate the ultrastructural features of Malassez epithelium (ME) containing protein gene product 9.5 (PGP9.5)-immunoreactive (IR) cells in the cat periodontal ligament (PDL). ***Material and methods.*** Specimens from the teeth and tooth-supporting tissues of four mature cats of both sexes, 18 to 24 months of age, were used. The fixed jaws were decalcified in EDTA. Frozen sagittal sections 20 μm thick were immunostained for PGP9.5, and the ME, containing IR cells in the PDL, were evaluated under a transmission electron microscope. ***Results.*** Several epithelial cells and PGP9.5 IR cells formed clusters and were enveloped by a basal lamina and separated from the surrounding connective tissue. A large nucleus and scanty cytoplasm were observed in most of the ME cells, which contained abundant keratin filaments and mitochondria. Caveolae-like structures and vesicles were found in the periphery of the ME. The small cytoplasmic processes of some of the epithelial cells extended toward the surrounding connective tissues. The cytoplasmic matrix of one type of cell comprising the ME showed immunoreactivity for anti-PGP9.5 antibody. The IR cell in the cell clusters was connected to adjacent epithelial cells and extended cytoplasmic processes toward the adjacent epithelial cells. The IR cell contained keratin filaments and abundant densely cored vesicles approximately 100–250 nm in diameter. ***Conclusions.*** The findings of the study suggest endocytotic capabilities of the epithelial cells and neuroendocrine functions of the IR cells. It is possible that the two different cell types react to extrinsic stimuli and interact with cells comprising the clusters and cords in the PDL. These ultrastructural evidences may imply functional heterogeneity of the ME in the PDL.

Key Words: *Feline, functional heterogeneity, Malassez epithelium, periodontal ligament, ultrastructure*

Introduction

Periodontal epithelial cells, known as epithelial cell rests of Malassez (ERM) or Malassez epithelium (ME), are distributed along the tooth root in a network. The ME are frequently observed as clusters of epithelial cells on the cementum surface. In sections cut tangentially to the tooth root surface, epithelial cells can be observed arranged in a meshwork encircling the tooth root [1]. Ultrastructurally, a continuous basal lamina separates the epithelial cell clusters, which are composed of several epithelial cells joined by desmosomes and gap junctions, each with a large nucleus (sometimes deeply constricted), scanty cytoplasm, containing relatively abundant keratin filaments, mitochondria, and Golgi apparatus [2,3].

A study of human ME has reported a close relationship between periodontal neural endings and the cell clusters [4]. Simultaneously, the presence of protein gene product 9.5 (PGP9.5) and calcitonin gene-related peptide (CGRP) immunopositive reactions in the ME was shown in the periodontal ligament (PDL) of cat canines [5]. Some cells of the ME have been shown to express immunoreactivity for neuropeptides such as vasoactive intestinal peptide (VIP) and substance P (SP) [6,7]. The ME is composed of different cell types and the presence of neuroendocrine cells in the cell populations strongly suggests a biological function of this tissue, and the neuropeptide content may indicate endocrine function of the cells [6]. Antibodies to PGP9.5 and cytokeratin have been used and proved the presence of single intensely labeled cells and some nerve fibers invested between the ME cells [7]. These cells contain cored vesicles that

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are similar to neuroendocrine cells, possibly Merkel cells [8]. However, the focus of these previous studies has been cells immunoreactive to neuropeptide antibodies, but there have been no reports dealing with the ultrastructurural features of ME cells expressing neuropeptide PGP9.5 immunoreactivity in the PDL.

The aim of the present study was to investigate the ultrastructure of ME containing PGP9.5-IR cells in the feline periodontal ligament by transmission electron microscopy.

Material and methods

The four mature cats of both sexes used in this study (18 to 24 months of age) were housed in polycarbonate cages in a specific pathogen-free environment. The Institutional Committee for Ethics and Animal Use and Care of Matsumoto Dental University approved all protocols for the animal experiments.

The animals were deeply anesthetized with sodium pentobarbital and transcardially perfused with 4% paraform aldehyde plus 0.01% glutaraldehyde in phosphate buffer (pH 7.4). The upper and lower

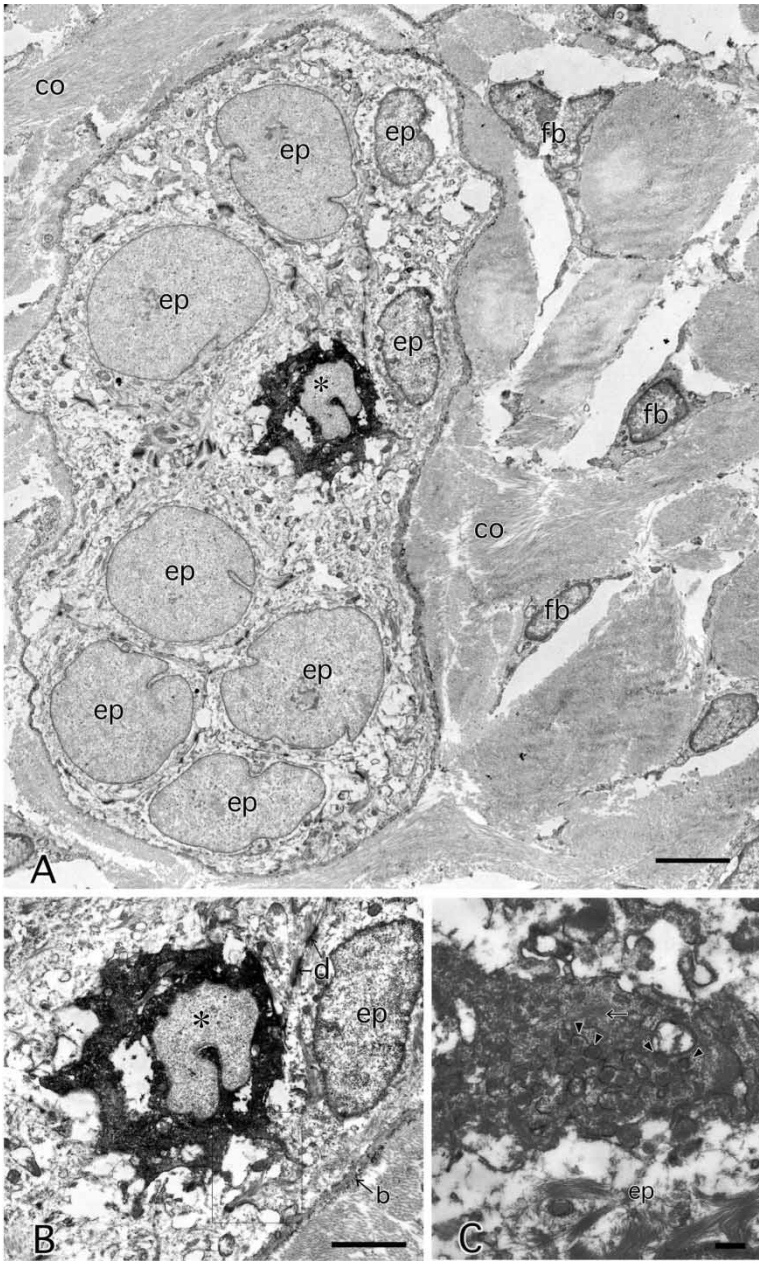

Figure 1. A. ME containing a PGP9.5-IR cell in the cat PDL. The ME is covered with a basal lamina and composed of cells containing a large nucleus and scanty cytoplasm. PGP9.5 immunoreactivity is noted in the cytoplasmic matrix of one type of cell (*) comprising the ME (co = collagen; ep = epithelial cell; fb = fibroblasts). Scale bar = 2 μm. B. A higher magnification of the PGP9.5-IR cell (*) shown in A. Immunoreactivity is observed in the cytoplasmic matrix of the cell in ME (b = basal lamina; d = desmosome-like structures; ep = epithelial cell). Scale bar = 1 μm. C. A serial section of the specimen shown in the boxed region in B. The IR cell extended cytoplasmic processes containing abundant cored vesicles (*arrowheads*) and keratin filaments (*arrows*) in between the adjacent epithelial cells (ep). Scale bar = 500 nm.

jaws were excised and post-fixed in the same fixative for 8 h. The specimens were decalcified in 10% EDTA for approximately 6 weeks. After demineralization and rinsing with phosphate-buffered saline (PBS), the specimens were cut sagittally into about 20 μm thick sections on a freezing microtome for the following immunocytochemical analysis.

The avidin biotin complex (ABC) method was applied to the sections, which were incubated with polyclonal rabbit anti-PGP9.5 antiserum (1:20,000; Ultraclone, Yarmouth, UK) overnight at 4°C. After rinsing in PBS, the sections were incubated with biotinylated goat anti-rabbit IgG (1:1,000; Vector Laboratories, Burlingame, Calif., USA) for 2 h at room temperature and subsequently with ABC complex (Vector Laboratories) for 90 min, and then visualized by incubation with 0.05 mol/l TRIS-HCl buffer (pH 7.6) containing 0.04% 3′3-diaminobenzine and 0.01% H_2O_2. Controls for immunoreaction specificity were made by omitting/substituting the primary antibody with PBS. After confirmation of the DAB reaction in the cat ME under a light microscope, the sections were post-fixed in 1% OsO_4 in 0.1 mol/l cacodylate buffer for 2 h at room temperature. The sections were dehydrated with a graded series of ethanol and embedded in EPON 812 resin (TAAB Laboratories Equipment, Aldermaston, UK). Ultra-thin sections were prepared with a diamond knife and examined with a JOEL 1200 EX-2 (Nihon Denshi, Tokyo, Japan) transmission electron microscope under an accelerating voltage of 80 kV, after staining with uranyl acetate and lead citrate.

Results

The ME-containing PGP9.5-IR cells were mainly scattered on the surface of the cervical cementum covering the tooth root, appearing as clusters of elliptical or club-shaped cells (Figure 1A). The cell clusters were enveloped by a basal lamina separating them from the surrounding connective tissue. The surface of ME showed an irregular border (Figure 1A, B, 2A, 2B). The epithelial cells were joined to each other by desmosomes. A high nucleus/cytoplasm (N/C) ratio was observed in most of the ME cells. The nuclei were round or oval, with heterochromatin scattered around the nuclear membrane (Figure 1A). In most of the cells, the nuclear membrane was partly deeply invaginated. Keratin filaments and mitochondria were observed in the ground substance of the ME cells. Vesicles were also noted in the peripheral zones of each cell (Figure 2A, B). Small cytoplasmic processes and caveolae-like structures were identified in some of the ME cells (Figure 2A). These cell surfaces were surrounded by a discontinuous basal lamina. Blood vessels were sometimes found close to the ME (Figure 2B). Caveolae-like structures were seen in the periphery

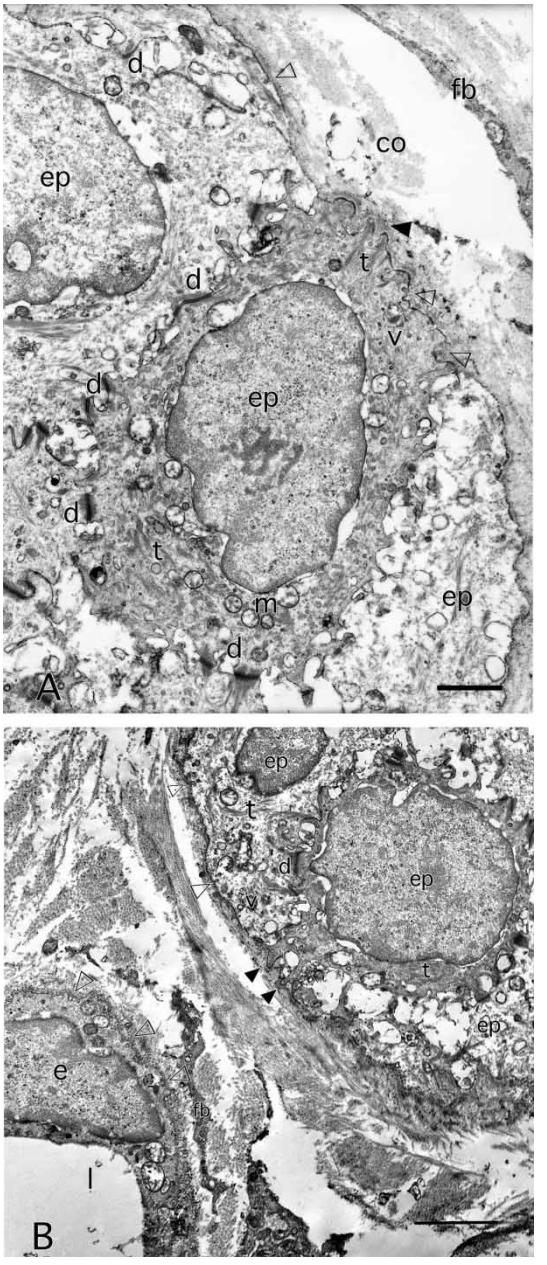

Figure 2. A. Epithelial cells (ep) immunonegative for PGP9.5 in the ME. Small processes (*black arrowhead*) and caveolae-like structures (*white arrowheads*) can be seen on the outer surface of the cells. These structures are covered by a discontinuous basal lamina. Mitochondria (m) were found, but were sometimes swollen (co = collagen; d = desmosomes; fb = fibroblasts; t = tono filaments). Scale bar = 1 μm. B. Epithelial cells (ep) immunonegative for PGP9.5 in the ME. Cytoplasmic processes (*black arrowheads*) of the cell extended toward periodontal ligament fibers. Endothelial cells and fibroblasts (fb) were found close to the ME cell. Caveolae-like structures (*white arrowheads*) and vesicles (v) can be seen in the ME, as well as endothelial cells (co = collagen; d = desmosomes; e = endothelial cells; l = lumen; t = tonofilaments). Scale bar = 2 μm.

of the endothelial cells. The distance between the tip of the processes and the vessel was approximately 3 μm. Cored vesicles were not found in the ground substances of these cells.

The cytoplasmic matrix of a cell comprising the ME was immunoreactive for PGP9.5 (Figure 1A–C).

The IR cell had a nucleus with a deeply invaginated nuclear membrane and was joined to adjacent epithelial cells (Figure 1B). Cell junctions could not be observed. The IR cell had spiny cytoplasmic projections that extended toward the adjacent epithelial cells in the ME. In the ground substance of the cell, cored vesicles approximately 100–250 nm in diameter and fine filaments were also identified (Figure 1C). The structure of ME in the cat PDL mentioned above is summarized in Figure 3.

Discussion

This present study reports the ultrastructure of ME containing PGP9.5-IR cells in the cat PDL. To my knowledge, only two previous studies have been reported dealing with PGP9.5-IR cells [7,8]. The PGP9.5-IR cell shown in this study had cored vesicles and keratin filaments in the cell body characteristic of Merkel cells [9,10]. However, the overall features of the ME have not been fully elucidated. This study reveals that two cell types, i.e. PGP9.5-IR and epithelial cells immunonegative for PGP9.5, form epithelial clusters. Caveolae-like structures and vesicles were seen in the epithelial cells. Similar findings have been reported in human and rat ME [4,11]. Caveolae are plasmalemmal invaginations that are considered subtypes of lipid rafts enriched with cholesterol, glycosphingolipids and signaling molecules, and indicate vesicular trafficking [12,13]. Studies *in vitro* and *vivo* have reported the expression of extracellular matrix-degrading proteinases and also lysosomes in the ME cells [13–18]. In addition, ME has been reported to exhibit potential bone-resorbing activity [19]. These reports, together with the findings of the present study, suggest endocytotic capabilities of epithelial cells as well as endothelial cells.

Small cytoplasmic processes that extended toward the surrounding tissues were sometimes found in some of the ME cells. Similar findings of cytoplasmic processes extending towards the outer surface of the ME and periodontal immunocompetent cells have been reported [17,18]. This morphology resembles that of sensory epithelial cells in glandular tissue, or the intestinal lumen. On the other hand, a close apposition has been shown between Ruffini-like and free nerve endings and the basal lamina of the ME [4]. Considering that the ME surrounds the tooth root in a network-like manner, along with nerve endings in the cat PDL [20,21], it is possible that the epithelial cells with PGP9.5-IR cells react on extrinsic stimuli and interact with cells comprising the ME.

In the PDL, immunocompetent cells are known to be present widely and abundantly [18,22,23]. A close relationship between ME and immunocompetent

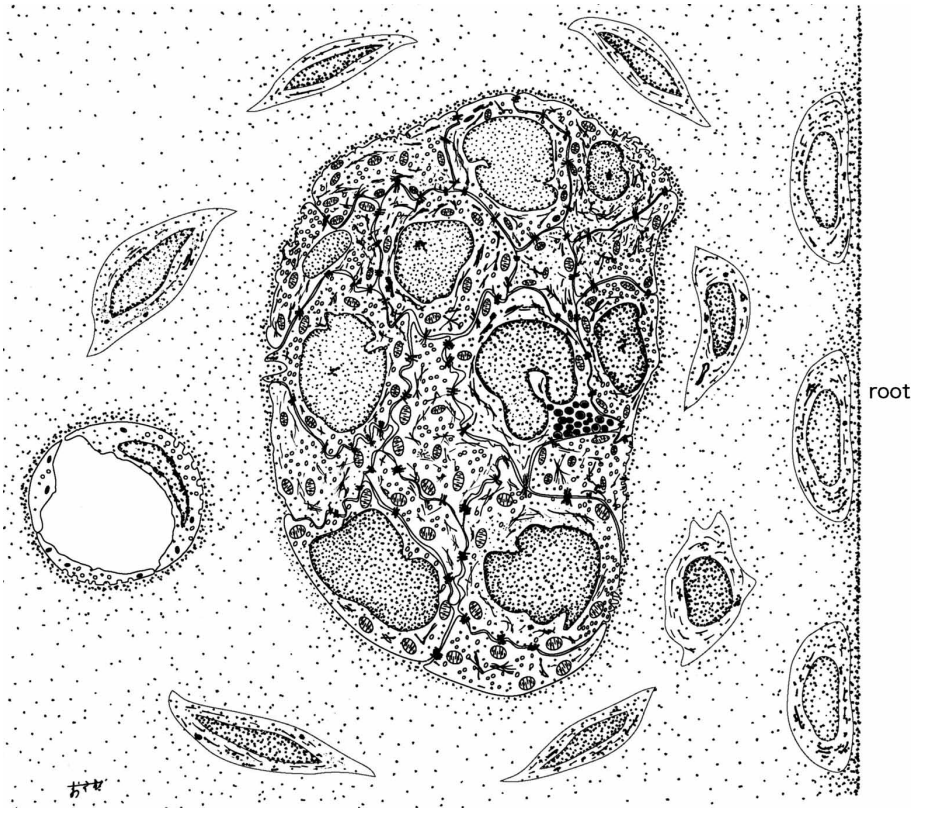

Figure 3. Diagram summarizing the ME in cat PDL. Cellular components of ME and surrounding cells are illustrated. Two different cell types, epithelial and cored vesicles contained cells, are composed of the ME in the PDL.

cells has been reported in the rat PDL [17,18]. In the present study, blood vessels were sometimes observed close to the ME cells. Similar findings have been reported in previous studies [18,24]. In such areas, immunocompetent cells are close to or in contact with the ME cells [18]. However, the present study did not focus on the relationship between them. Further investigations are needed to clarify their relationship in the cat PDL.

In conclusion: (1) the feline ME is composed of epithelial and PGP9.5-IR cells, (2) caveolae and vesicles are found at the periphery of epithelial cells, (3) keratin filaments and cored vesicles are contained in PGP9.5-IR cells, in agreement with previous reports, (4) some ME cells extend their cytoplasmic processes toward PDL fibers, and (5) it is possible that epithelial and PGP9.5-IR cells respond to extrinsic stimuli and interact with cells comprising the clusters and cords in the PDL.

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