SHORT COMMUNICATION

Langerhans cells in rat junctional epithelium after the application of hyaluronidase as revealed by electron microscopy

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Introduction

Langerhans cells are bone marrow derived, dendritic clear cells found in stratified squamous epithelia including oral epithelium1-3). Ultrastructurally, Langerhans cells contain specific cytoplasmic granules called Birbeck granules3,4). Langerhans cells are known to have antigen-presenting functions and play an important role in the local immune system of the oral mucosa5). Although many investigators have observed Langerhans cells located in the oral gingival epithelium and oral sulcular epithelium2,5-15), few reports on Langerhans cells in the junctional epithelium are found in the literature16,17).

In these reports Langerhans cells in the junctional epithelium were identified immunohistochemically by anti-CD1 (previously called T6) monoclonal antibody. With an electron microscope, on the other hand, no Langerhans cells have been found in the junctional epithelium12). Therefore, the existence of Langerhans cells with definite Birbeck granules is not yet evident in the junctional epithelium.

In this study we examined rat molar gingiva by electron microscopy to confirm the existence of Langerhans cells in the junctional epithelium, under normal state and experimental condition where the permeability of the junctional epithelium was enhanced to make a passage of antigenic exogenous substances from the gingival sulcus to the gingival connective tissue easier.

Materials and methods

Fifteen 8 weeks-old male Wistar rats were used. Three untreated animals were used for the normal condition group. After sacrificing by an overdose of diethyl ether, the upper molars were removed en block with the attached gingival tissue. Immediately, tissue blocks were fixed in a 0.1 M cacodylate-buffered 4% paraformaldehyde and 5% glutaraldehyde solution at 4°C for 2 hours. After decalcification of hard tissue in a 10% EDTA solution (pH 7.4, 4°C) for 2 weeks, from each tissue block, palatal dento-gingival tissue of the first molar and that of the second molar were cut buccopalatally into about 0.5 mm thick slices in a longitudinal direction along the tooth axis under a dissecting microscope. These slices were then post-fixed in cacodylate-buffered 1% osmium tetroxide (pH 7.4, 4°C), stained en block with 1% uranyl acetate,
dehydrated in a graded series of ethanol and then embedded in epoxy resin. Thick sections cut from each block were stained by toluidine blue and lead citrate. Junctional epithelium of these sections were buccopalatally examined by a JEOL-100S transmission electron microscope at 60 or 80 kV.

For enhancement of the permeability of the junctional epithelium, bovine testicular hyaluronidase (Type 1-S, Sigma Chemical Co., St. Louis, 12 mg/ml in physiological saline) was topically applied to the gingival sulcus of the palatal side of the upper molars for 90 minutes using a small cotton applicator containing the solution under intraperitoneal anaesthesia with ethyl carbamate (1 mg/g body weight). At 12 hours, 1, 2 and 3 days after the application, the specimens from each of the three animals were taken, processed for electron microscopy and observed as described above.

Results

In the untreated rats, the junctional epithelium was observed as described before and in that epithelium no apparent Langerhans cells with their characteristic morphologic features were revealed. Non-epithelial mononuclear cells such as lymphocytes or macrophages were only occasionally seen between the junctional epithelial cells.

In the experimental group, the junctional epithelium exhibited dilatation of the intercellular spaces, infiltration of many polymorphonuclear leukocytes, and shedding of the cells consisting the most coronal portion of the junctional epithelium. In addition, in the specimen at 1 to 3 days after the application, two or three non-epithelial clear cells resembling Langerhans cells were found between the junctional epithelial cells. Some of these clear cells had Birbeck granules with a central striation. Such an apparent Langerhans cell located in the innermost layer of the junctional epithelium, facing the enamel space, is illustrated in Figure 1. Langerhans cells or Langerhans cell-like clear cells without Birbeck granules were also located in the basal or suprabasal layer of the junctional epithelium.

Discussion

Birbeck et al. first defined the ultrastructural features of Langerhans cells: a lobulated or indented nucleus, clear cytoplasm with a developed rough endoplasmic reticulum and Golgi apparatus, an absence of tonofilaments, desmosomes and melanosomes, and a presence of unique cytoplasmic granules, now termed as Birbeck granules. In thin sections, Birbeck granules are rod-shaped with a central striation. Breathnach regarded the presence of these characteristic granules as an essential criterion for the identification of Langerhans cells at the ultrastructural level. In contrast to no Langerhans cells in the junctional epithelium of untreated animals, our electron microscopic observation revealed several non-epithelial clear cells reminiscent of Langerhans cells in the junctional epithelium in the specimens at 1 to 3 days after the hyaluronidase application. Because of the detection of specific cytoplasmic granules, some of these clear cells could be confirmed to be Langerhans cells.

This appearance of Langerhans cells in the junctional epithelium is probably due to the immune response of the host to hyaluronidase or the external antigens that could get access into the intercellular spaces of the junctional epithelium expanded by the hyaluronidase treatment. Dilatation of the intercellular spaces of the junctional epithelium following application of hyaluronidase to the gingiva was observed by Ijuhin et al. and Schultz-Haudt et al. The increase of Langerhans cells related to antigen stimulation was shown in the oral epithelium of mice when they were conventionalized from germ-free condition by Bos and Burkhardt. They interpreted the increase of Langerhans cell number as a response to external antigens.

The morphological changes following the hyaluronidase application observed in the present study such as increased migration of polymorphonuclear leukocytes into the junctional epithelium and shedding of the coronal portion of the junctional epithelium are probably identical with the changes as seen in the initial lesion during the periodontal inflammatory process as
Fig. 1 A Langerhans cell located in the innermost layer of rat junctional epithelium (JE) at 2 days after the hyaluronidase application (at arrow in (a)) is shown at higher magnification in (b). Note the presence of specific granules (an example in a square) and large mitochondria (M).

a: ×2,850, b: ×17,000 Insert: higher magnification of a Birbeck granule shown in the square in (b). ×69,200 ES = decalcified enamel space; OSE = oral sulcular epithelium; PMN = polymorphonuclear leukocyte.

described by Page and Schroeder\textsuperscript{22}. In addition, Juhl \textit{et al.}\textsuperscript{16} suggested that clusters of Langerhans cell in junctional epithelium in their observation might represent reactions to initial/early plaque formation. Thus, in the beginning stage of periodontitis, Langerhans cells are likely to immigrate into the junctional epithelium and play some immunological functions. However, further experimental studies is thought to be needed to examine the possible role of Langerhans cells in the process of periodontitis.

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