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Immunohistochemical Analysis of the Origin of Junctional Epithelium in Mice

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ABSTRACT

Junctional Epithelium (JE) is the front-line defense against bacterial infection by expressing cell adhesion molecule-1 (ICAM-1), which allows the emigration of immune cells into JE. The enamel organ has been considered the origin of JE; however, the actual source of JE is not clear. In this study, we examined the origin of JE during tooth development and eruption in ICR mice with special attention to ICAM-1 expression. During the pre-eruptive phase, ICAM-1 was expressed at the papillary layer of the enamel. During the eruptive phase, the reduced ameloblasts demonstrated no ICAM-1 expression, but still attached to the enamel surface. During the post-erupting phase, reduced ameloblasts were not detected and the ICAM-1-positive papillary layer directly attached to the enamel surface and formed JE. These results indicated that the origin of JE was cells of the papillary layer, which constitutively expressed ICAM-1 and helped, maintain a defense system against bacterial infection.

Keywords: Junctional epithelium, ICAM-, Enamel organ, Papillary layer

INTRODUCTION

Epithelial tissue covers the external and internal surfaces of the body and serves as the first line of host defense [1]. Rupture or disruption of the epithelial tissue permits the invasion of the foreign substances into the body, which may lead to the onset of disease. The continuity of the oral mucosa epithelium is naturally interrupted by tooth eruption. Therefore, analysis of the structure and function of the gingival epithelium provides important information regarding host defense mechanisms [2-4].

The gingival epithelium consists of the Oral Gingival Epithelium (OGE), Oral Sulcular Epithelium (OSE) and Junctional Epithelium (JE). The JE attaches to the tooth surface via hemidesmosomes and helps maintain the front line of defense against periodontal bacterial infection [5,6]. The JE is characterized as a non-keratinized squamous epithelium with high cell-proliferation activity [7-11]. To support the integrity of the periodontal tissue, neutrophils and lymphocytes emigrate into the JE [7-11].

It has been postulated that the JE is derived from cells of the enamel organ in tooth germ [12,13]; however, the actual cell source of JE is not clear. The enamel organ consists of inner enamel epithelium, outer enamel epithelium and stellate reticulum during the cap stage of tooth development. With tooth development, the inner enamel epithelium differentiates as secretory, transitional, mature and reduced stages of ameloblasts. The stratum intermedium, which is adjacent to the secretory ameloblasts, differentiates to the papillary layer. Mouse incisors are continuously erupting teeth and are easily examined for the differentiation of ameloblasts and odontoblasts at the growing end of teeth. We previously demonstrated in mice that the JE constitutively expresses intercellular adhesion molecule-1 (ICAM-1), which allows for the emigration of neutrophils and lymphocytes in the JE [11]. We also detected the expression of ICAM-1 in cells of the forming papillary layer [14], which suggests that the origin of JE may be cells in the papillary layer. In the current study, we examined the origin of JE during the eruption of molar tooth germs in ICR mice with special reference to the expression of ICAM-1.

MATERIALS AND METHODS

The experimental protocols used were reviewed and approved by the Animal Care Committee of Showa University (Shinagawa-ku, Tokyo, Japan).

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Animals

ICR mice were used throughout this study. 10 pregnant mice at 14 days gestation (E14) were purchased from Sankyo Laboratory Service Corporation (Tokyo, Japan) and maintained under routine conditions at the Laboratory Animal Center of Showa University. The mandibles from mice at 16 days gestation (E16) to 21 days postnatal (dPN) were used in the study.

Tissue preparation

For histological and immunohistochemical studies, the mandibles were dissected from the mice and fixed with 4% paraformaldehyde for 6 h at 4°C. After decalcification with 10% ethylene diamine tetraacetate (EDTA) for 2 weeks at 4°C, the specimens were embedded in OCT compound (Sakura, Torrance, CA, USA) and then fast-frozen in isopentane cooled in liquid nitrogen. Sagittal serial frozen sections parallel to the long axis of the first molars were cut (10 μm thick) and collected onto silan coated glass slides. Every third section was stained with hematoxylin and eosin (H-E) and evaluated by light microscopy to determine the area of JE. The remaining sections were processed for immunohistochemical evaluation.

Immunohistochemistry

Primary antibodies used in the study included rat anti-mouse ICAM-1 monoclonal antibody (BD Pharmingen, Tokyo, Japan). After serial sections of the tissue specimens were washed in PBS, they were immersed in PBS containing 1% H_2O_2 for 30 min to block endogenous peroxidase activity. The sections were then incubated with normal goat serum for 30 min at room temperature. The sections were incubated with the primary antibody for 24 h at 4°C and then thoroughly washed with PBS. The sections were incubated with biotin-conjugated goat anti-rat secondary antibody for 60 min and then incubated with avidin-biotin-peroxidase complex using Vectastain Elite ABC Kit (Vector Laboratories, Burlingame, CA, USA) for 30 min. The color was developed with 3, 3'-diaminobenzidine tetrahydrochloride in TRIS buffer plus hydrogen peroxide (DAB Reagent set, KPL, Gaithersburg, MD, USA). The sections were counterstained with hematoxylin. As negative controls, normal rat serum or normal goat serum was used instead of the primary and secondary antibodies.

RESULTS

ICAM-1 expression in tooth germ development

The tooth germ of the mandibular first molars of the mice differentiated to the cap stage at E16 of development, the bell stage at E17 and started dentin and enamel formation at parturition. Inner enamel epithelia differentiated into secretory ameloblasts around the time of birth and further differentiated to the maturation stage of ameloblasts around 7 dPN. At 7 dPN, the inner enamel epithelium differentiated to the secretory stage of ameloblasts and secreted enamel matrix on dentin **(Figure 1A)**. ICAM-1 expression was not detected in the stratum intermedium located at the apical side of the ameloblasts at 7 dPN **(Figure 1D)**. At 10 dPN, ameloblasts at the tip of the cusp differentiated to the transition stage and the stratum intermedium changed the form to the papillary layer **(Figure 1B)**. At 12 dPN, most ameloblasts had differentiated to the maturation stage and all the ameloblasts were covered by the papillary layer **(Figure 1C)**. ICAM-1 was expressed on the cells of the papillary layer starting at the transition stage of ameloblasts with strong reactions being detected on the cells of the papillary layer at 12 dPN **(Figure 1E)**.

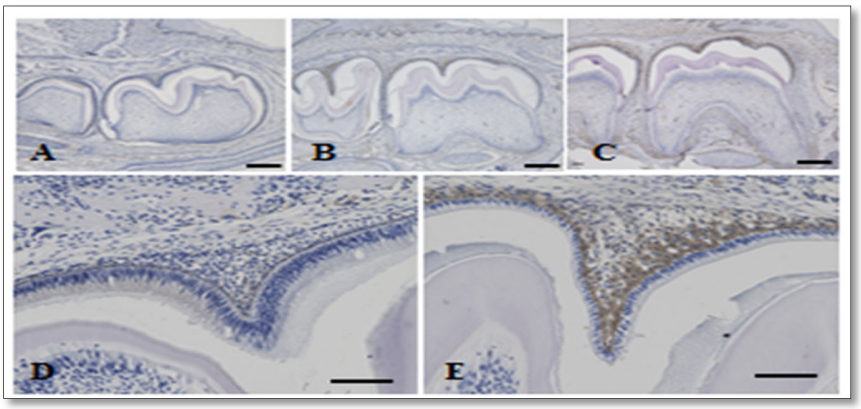

Figure 1. Immunohistochemical localization of ICAM-1 during development of the first mandibular enamel organs (A, D), 7 days postnatal (dPN) (B), 10 dPN and (C, E) 12 dPN. ICAM-1 expression in the papillary layer during tooth development. *Scale bar=200 μm (A, B, C) or 100 μm (D, E)*

ICAM-1 expression during tooth eruption

At 16 dPN, the tip of the cusp of the first mandibular molar was located just below the oral epithelium **(Figure 2A)**. Ameloblasts differentiated to reduced ameloblasts, which were not detected at the tip of the cusp **(Figure 2B)**. The reduced ameloblasts attached to the enamel at the cervical

side of the crown. Cells of the papillary layer expressed ICAM-1 as well as the blood vessels of the papillary layer **(Figure 2C)**.

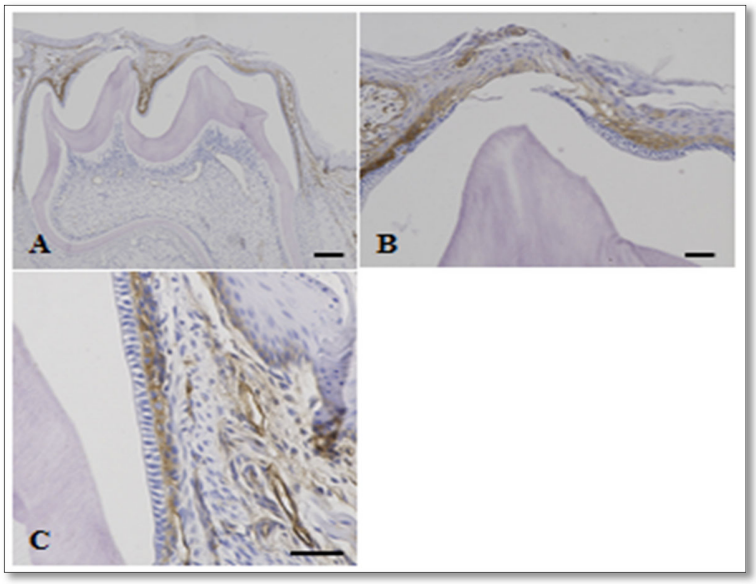

Figure 2. ICAM-1 expression in the first mandibular enamel organ at 16 days postnatal (dPN). (A) The tooth tip covered by oral mucosa. Ameloblasts at the tip area of the cusp disappeared and strong immunoreactive staining of ICAM-1 was detectable in the papillary layer. (C) Ameloblasts attached to the enamel at the marginal region, but ICAM-1 expression was not detected. Strong immunoreactive staining of ICAM-1 was detected in the papillary layer.
Scale bars=200 μm (A), 100 μm (B) or 50 μm (C)

At 19 dPN, the first mandibular molar erupted **(Figure 3A)**. Reduced ameloblasts still attached to the tooth surface at the medial side, but ICAM-1 expression was not detected in the interdental papilla between the first and the second molars. Cells of the papillary layer and the blood vessels beneath the papillary layer expressed ICAM-1 **(Figures 3B and 3C)**.

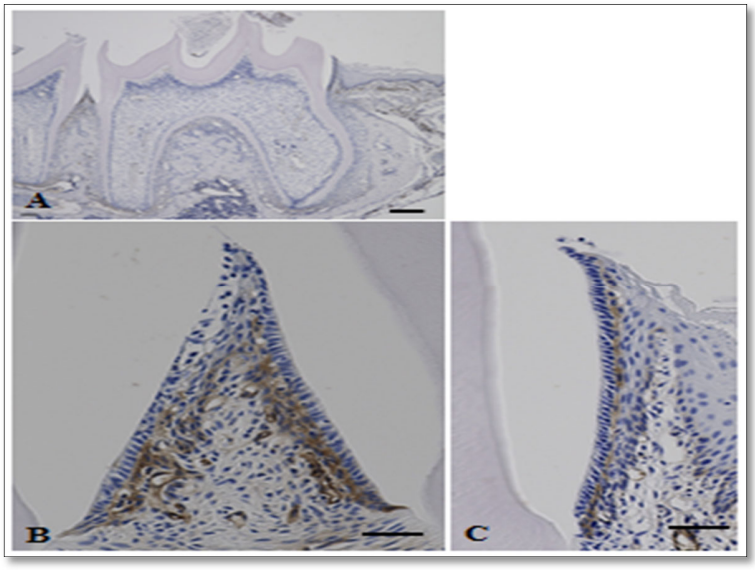

Figure 3. ICAM-1 expression in the first mandibular enamel organ at 19 days postnatal (dPN). (A) Tooth post eruption. (B) Interdental papilla between the first and second molars. (C) Cervical region of the gingiva. Ameloblasts attached to the enamel even in the absence of ICAM-1 expression. The strong immunoreactive staining of ICAM-1 was detectable in the papillary layer.
Scale bars=200 μm (A) or 50 μm (B, C)

At 21 dPN, reduced ameloblasts were not observed and cells of the papillary layer expressing ICAM-1 directly attached to the tooth surface of both the mesial and interdental papillary layers **(Figures 4A-4C)**. The epithelial cells of the OSE showed no ICAM-1 expression **(Figure 4C)**, but the blood vessels located adjacent to the ICAM-1-positive epithelium did express ICAM-1 **(Figure 4C)**.

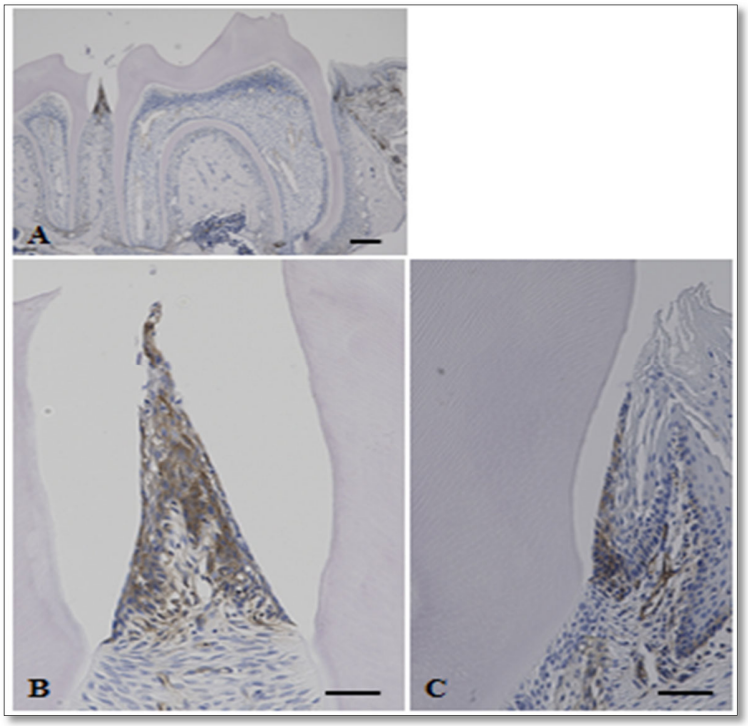

Figure 4. ICAM-1 expression in the first mandibular enamel organ at 21 days postnatal (dPN). (A) Overview of the first mandibular molar. (B) Interdental papilla between the first and second molars. (C) Cervical region of the gingiva. Ameloblasts were no longer detected and ICAM-1-positive epithelial cells attached directly to the enamel.
Scale bars=200 μm (A) or 50 μm (B, C)

DISCUSSION

ICAM-1 is a critical adhesion molecule for the migration of neutrophils and lymphocytes. Expression of ICAM-1 is regulated by proinflammatory cytokines, such as interleukin (IL)-1β and tumor necrosis factor alpha (TNFα) [15,16]. The expression of ICAM-1 concurrently decreases with recovery from inflammation. In humans, JE constitutively expresses ICAM-1 following bacterial stimulation by dental plaque [17,18]. Our previous studies demonstrated constitutive ICAM-1 expression in the JE of mice, even under germ-free condition [10]. In addition, Heymann et al. [19] reported the expression of carcinoembryonic antigen-related cell adhesion molecule 1, a transmembrane cell-adhesion molecule, within the JE of germ-free mice. The JE is the only place in which the continuity of the epithelial tissue is naturally disrupted *in vivo*. Therefore, the constitutive expression of ICAM-1 and the emigration of neutrophils and lymphocytes into the JE may be key to maintaining the JE structure and the defense against bacterial infection. We also previously reported the constitutive expression of IL-1 and TNF in the JE [11], which may regulate the expression of ICAM-1 in JE.

ICAM-1 was detected at the papillary layer of the mouse molar enamel organ, consistent with previous reports for ICAM-1 expression in continuously erupting incisors [14]. Tooth eruption is divided into three stages, the pre-eruption stage, the erupting stage, and the functional stage. Our study indicated that the first molar began to erupt at 16 dPN and reached the functional stage at 21 dPN. During tooth development, ameloblasts differentiated from the inner enamel epithelium and functionally differentiated through the secretory, transitional and maturation stages to form enamel. After the formation of enamel, ameloblasts reduced their height and were no longer detected after eruption. At the cervical region of the tooth crown, ameloblasts were no longer detected after the functional stage. Nasmyth's membrane, the primary dental cuticle, is the product of ameloblasts, which quickly disappear after eruption [20,21]. In the current study, ameloblasts disappeared by 21 dPN and the JE consisted of ICAM-1-positive cells. These results indicated that the functional JE was derived from the papillary layer of the enamel organ.

With the development of amelogenesis, the stratum intermedium is differentiated at the apical side of secretory

ameloblasts. The stratum intermedium differentiates into the papillary layer with differentiation of the ameloblasts during progression from the secretory stage to the maturation stage. During tooth development, the expression of ICAM-1 is detected at the papillary layer. However, the mechanism by which the papillary layer develops during the successive changes of amelogenesis remains unclear. Recently, Liu et al. [20] indicated that the cells in the stellate reticulum adjacent to the stratum intermedium differentiate and form the papillary layer. The major function of the papillary layer has been suggested to be the transport of mineral ions to ameloblasts for enamel mineralization [21,22].

Expression of ICAM-1 in the papillary layer may be not related to the function of the papillary layer for the enamel formation. During eruption, the tissues overlying the enamel organ must be degraded. Immunohistochemical evaluation has indicated that the pericoronal connective tissue expresses IL-1β [23]. To induce a mild inflammation during tooth eruption, which may be associated with the degradation of connective tissue and the securing of the space needed for eruption. Expression of ICAM-1 is induced by proinflammatory cytokines such as IL-1β and TNFα [14,15]. The expression of IL-1β during eruption might initiate the expression of ICAM-1 in the papillary layer cells. In the current study, blood vessels adjacent to the enamel organ expressed ICAM-1, which may also be induced by the expression of IL-1β from the pericoronal connective tissue [24].

CONCLUSION

Our study indicated that JE was derived from the papillary layer of the enamel organ. However, it remains controversial whether JE might be replaced from the enamel organ derived cells to the oral epithelial cells with aging. Yagima-Himuro et al. [13] suggested the replacement of JE by oral epithelial cells, while Soda et al. [25] suggested the existence of an enamel organ-derived cell niche in JE. Further studies are needed to analyze newly formed regenerative JE after periodontal treatments such as gingivectomy. Regardless the origin of the regenerated JE, our previous studies [10,11] indicate that the constitutive expression of ICAM-1, the expression of proinflammatory cytokines and chemokines, and the emigration of lymphocytes and neutrophils are important for the self-defense function of JE.

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CONFLICTS OF INTEREST

The authors declare that no competing financial interests exist.

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