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Histopathological Study on the Distribution of Langerhans Cells in Oral Mucosa

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Background & Aims： Some oral mucosal diseases display clear predispositions toward certain sites of occurrence. Oral lichen planus is one such disease, and is thought to have some relationships with cell-mediated immunity leading to a tendency to develop on the buccal mucosa. The aim of this study was to investigate the distribution of Langerhans cells in various sites of oral mucosa. **Methods：** Immunohistochemical examination using an anti-S100 antibody was performed for 47 samples of healthy oral mucosa and skin. **Results：** S100-positive cells were more frequent in lining mucosa than in masticatory mucosa. Significantly more S100-positive cells were found in buccal mucosa than in other sites. **Conclusions：** Langerhans cells are positive for S100 and are thought to represent antigen-presenting cells. These cells were most frequent in buccal mucosa, a result that is in accordance with the predisposition of oral lichen planus. (Kitakanto Med J 2007；57：307～310)

Key Words： Langerhans cell, oral mucosa, oral lichen planus

Introduction

Many kinds of diseases appear on the oral mucosa, and some display clear predispositions toward certain sites. Oral lichen planus (OLP) is one such disease, tending to develop on the buccal mucosa.[1,2] Cell-mediated immunity is thought to bear some relationship to the disease. For cell-mediated immunity, T cells must be sensitized by information from an antigen-presenting cell (APC), such as a Langerhans cell (LC).[3,4] According to this theory, LCs might be closely involved in OLP.

LCs originate in bone marrow, and have antigen-presenting activity by the receptor for major histocompatibility complex (MHC) class II antigens and various complements.[5] LCs localize to stratified epithelium of the epidermis and mucosa, and present antigens to T cells after engulfing the antigen and migrating to the lymphoid system.[3,4]

LCs are characteristically large and dendritic, and display positive immunological reactions for anti-S100 protein, which is common to APCs.[3,4,6] LCs can thus be detected by immunohistochemical analysis. Based on this idea, we immunohistochemically studied the distribution of LCs on the oral mucosa in various sites.

Materials and Methods

A total of 47 samples of healthy oral mucosa and skin were obtained from surgical specimens of various oral diseases unrelated to inflammatory or immune diseases at Gunma University Hospital. Patients comprised 24 men and 23 women with a mean age of 46.9 years (range, 11-76 years). Oral mucosa sites comprised 5 cheek samples, 5 lip samples, 4 tongue samples and 4 floor of the mouth samples as movable lining mucosa, and 24 gingiva samples and 2 hard palate samples as immovable masticatory mucosa. In addition, 3 samples of healthy skin were stained as controls.

Formalin-fixed tissue sections of 3-μm thickness were sliced from each paraffin block of healthy oral mucosa or skin. These sections were reacted with anti-S100 antibody (Dako Cytomation A/S, Copenhagen, Denmark) diluted 1：500 at 4°C overnight. After treatment with 3％ H_2O_2 solution to reduce endogenous peroxidase activity, immunoreactive S100 protein was visualized using a Dako LSAB 2 kit (Dako Cytomation A/S) and diaminobenzidene/H_2O_2 according to the instructions of the manufacturer. Sections were then counterstained with hematoxylin.

Histomorphometric quantification was performed

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to count cells with S100-positive results in nucleic and cytoplasmic staining and dendritic shape. S100-positive cells in the basal cell layer were excluded as probable melanocytes. Numbers of cells were counted in 2 - 6 fields of 1 mm^2 in each section under ×200 magnification.

Differences in LC counts in healthy oral mucosa and skin were analyzed by analysis of variance (ANOVA) and the Tukey-Kramer honestly significant difference (HSD) test using JMP version 5.1 software (SAS Institute, Tokyo, Japan).

Results

Immunohistochemically, all 47 samples of healthy oral mucosa and skin showed positive staining from the parabasal to upper stratum spinosum layers of the epithelium. Mean LC counts in lining mucosa were 35.0±2.1 cells in 24 fields from 5 cheek samples (Fig. 1A), 23.3±2.5 cells in 17 fields from 5 lip samples, 23.2±2.4 cells in 18 fields from 4 tongue samples and 17.5±2.6 cells in 8 fields from 4 floor of the mouth samples. Mean LC counts in masticatory mucosa were 16.9±1.4 cells in 53 fields from 24 gingiva samples (Fig. 1B) and 8.6±4.5 cells in 5 fields from 2 hard palate samples. Skin displayed 8.9±4.1 cells in 6 fields from 3 samples.

LCs showed significantly higher frequency in lining mucosa than in masticatory mucosa. In particular, significant differences were found between buccal mucosa and other sites of the oral mucosa and skin ($P<0.01$; Fig. 2, Table 1).

Discussion

Many reports regarding LC distribution in healthy and pathological oral mucosa have used immunohistochemical analysis, but inconsistent methods have led to discrepancies in findings.[3,4] The selection of LC marker significantly affects the outcome of quantitative LC analysis. LC is easily identified using an anti-S100 antibody in paraffin-embedded sections of oral epithelium, but the disadvantage of cross-reaction with melanocytes can lead to incorrect analysis.[6-8] Melanocytes are present in the basal cell layer of epithelium, whereas LC are found predominantly in suprabasal locations.[9] We considered LCs and melanocytes as distinguishable based on locations. CD1 and MHC class II molecules (HLA-DR, HLA-DP and HLA-DQ) are also sufficiently specific for use as LC markers, but antibodies to these molecules are restricted to use in frozen tissue sections.[3,9] In fact, our application for anti-CD1a monoclonal antibody to formalin-fixed and paraffin-embedded sections showed lower reaction of LCs than the present results (data not shown). For these reasons, an anti-S100 antibody was used in this study.

The present results show that the numbers of LC are higher in movable lining mucosa than in immovable masticatory mucosa. Lining mucosa consists of non-keratinized squamous epithelium at the lip, cheek, under surface of the tongue, floor of the mouth and soft palate. Conversely, masticatory mucosa consists of keratinized epithelium at the gingiva and hard palate. Another factor that might affect the number of LCs may be differences in origin of the mucosa. The mucosa of floor of the mouth and posterior parts of the oral cavity are thought to originate from the endoderm, whereas the remaining parts of the oral cavity mucosa originate from the ectoderm. The

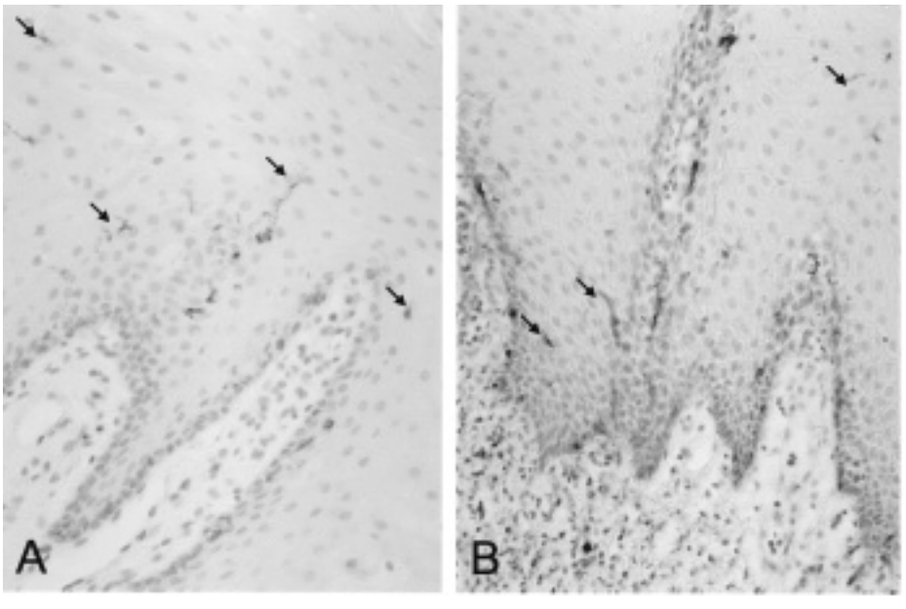

Fig. 1 S100-positive LC (arrows) in human buccal mucosa (A) and gingival mucosa (B). Original magnification ×200.

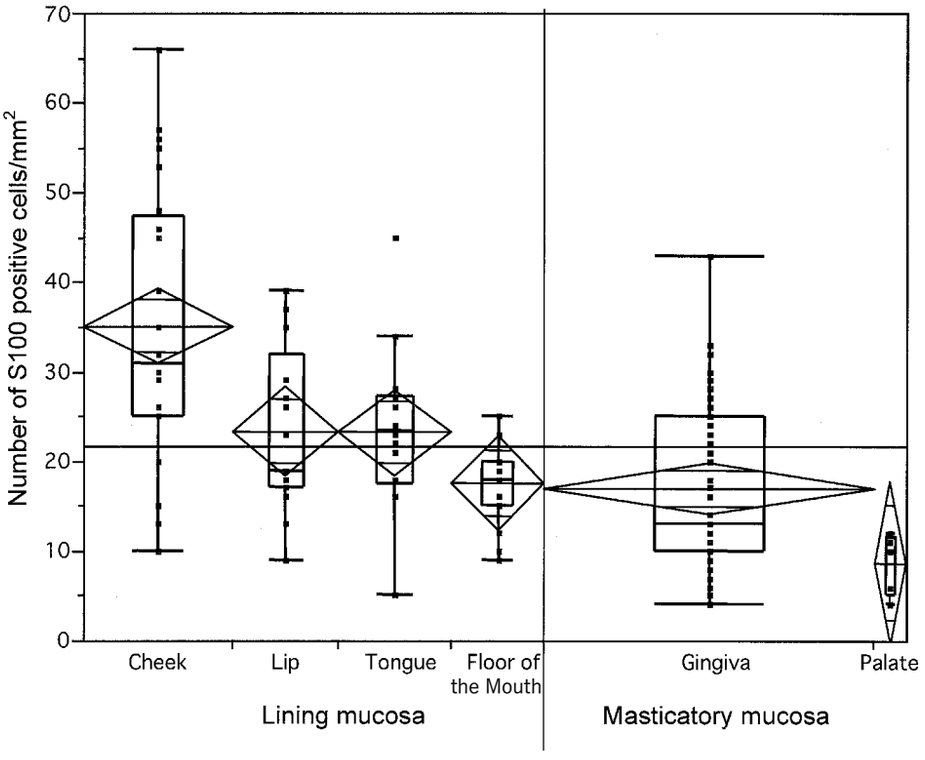

Fig. 2 S100-positive LC counts in oral mucosa.

Table 1 S100-positive LC counts in oral mucosa and skin.

		Mean±SE	Lip	Tongue	Floor of Mouth	Gingiva	Palate	Skin
Lining mucosa	Cheek	35.0±2.1	*	*	*	*	*	*
	Lip	23.3±2.5		N.S.	N.S.	N.S.	*	*
	Tongue	23.2±2.4			N.S.	N.S.	*	*
	Floor of the Mouth	17.5±2.6				N.S.	N.S.	N.S.
Masticatory mucosa	Gingiva	16.9±1.4					N.S.	N.S.
	Palate	8.6±4.5						N.S.
Skin		8.9±4.1						

N.S.: not significant * : $P<0.01$

posterior parts of the buccal mucosa, as the predisposed site of OLP, may originate from the endoderm. In a previous study using ATPase histochemistry on postmortem oral mucosa, non-keratinized mucosa displayed a higher LC density than keratinized mucosa.[10] The highest density of LCs was recognized in the lip and floor of the mouth and the lowest in the hard palate in that study.[10] In another study using monoclonal antibodies against CD1a and MHC class II molecules on postmortem oral mucosa, the dorsum of the tongue and buccal mucosa showed the highest density and the floor of the mouth the lowest.[9] The same author reported the highest density of CD1a -positive LC was in buccal mucosa in biopsy and autopsy samples.[11] The density of LCs in various oral mucosa in that report resembled our own results. There are minor differences between the present study and those studies. These discrepancies may be attributed to differences in methods used to detect LC and material sources.

Assessments of LC in OLP have been reported. Considerably more LCs were found in buccal mucosa with OLP than in normal tissue using an OKT6 (CD1) antibody.[12] In another study, more S100-positive LCs were found in buccal mucosa with OLP than in normal mucosa adjacent to the disease, but the difference in numbers of HLA-DR-positive LCs between OLP and normal tissue was minimal.[13] In the examination of MHC class II molecules in buccal mucosal with OLP, more HLA-DP- and HLA-DQ-positive

LCs were found than in normal mucosa, although equal numbers of CD1- and HLA-DR-positive LCs were seen between OLP and normal mucosa.[14] These results suggest that numbers of LCs rise in OLP due to increases in alternative phenotypes such as HLA-DP- and HLA-DQ-positive LCs activated by T cells.

OLP is a chronic mucocutaneous inflammatory disease with a clear predisposition toward certain sites in oral mucosa. As inappropriate T-cell mediated immune response is observed in OLP, APCs are thought to play a central role. The present results show LCs as APCs distribute in buccal mucosa at high density, in accordance with the predisposed site of OLP.

References

1. Mescon H, Grots IA, Gorlin RJ. Mucocutaneous disorders. In : Gorlin RJ, Goldman HM (eds). Thoma's Oral Pathology. St. Louis : Mosby, 1970 : 681-683.
2. Bhaskar SN. Surface lesions of oral mucosa. In : Bhaskar SN (eds). Synopsis of Oral Pathology. St. Louis : Mosby, 1986 : 417-422.
3. Barrett AW, Cruchley AT, Williams DM. Oral mucosal Langerhans' cells. Crit Rev Oral Biol Med 1996 ; 7 : 36-58.
4. Lombardi T, Hauser C, Buudtz-Jörgensen E. Langerhans cells : structure, function and role in oral pathological conditions. J Oral Pathol Med 1993 ; 22 : 193-202.
5. Katz SI, Tamaki K, Sachs DH. Epidermal Langerhans cells are derived from cells originating in bone marrow. Nature 1979 ; 282 : 324-326.
6. Nakajima T, Watanabe S, Sato Y, et al. S-100 protein in Lamgerhans cells, interdigitating reticulum cells and histiocytosis X cells. Gann 1982 ; 73 : 429-432.
7. Kurihara K, Hashimoto N. The pathological significance of Langerhans cells in oral cancer. J Oral Pathol 1985 ; 14 : 289-298.
8. Charbit Y, Monteil RA, Hitzig C, et al. S-100 immunolabelling of Langerhans cells in oral epithelium. J Oral Pathol 1986 ; 15 : 419-422.
9. Cruchley AT, Williams DM, Farthing PM, et al. Regional variation in Langerhans cell distribution and density in normal human oral mucosa determined using monoclonal antibodies against CD1, HLADR, HLADQ and HLADP. J Oral Pathol Med 1989 ; 18 : 510-516.
10. Daniels TE. Human mucosal Langerhans cells : Poatmortem identification of regional variations in oral mucosa. J Invest Dermatol 1984 ; 82 : 21-24.
11. Cruchley AT, Williams DM, Farthing PM, et al. Langerhans cell density in normal human oral mucosa and skin : relationship to age, smoking and alcohol consumption. J Oral Pathol Med 1994 ; 23 : 55-59.
12. Rich AM, Reade PC. A quantitative assessment of Langerhans cells in oral mucosal lichen planus and leukoplakia. Brit J Dermatol 1989 ; 120 : 223-228.
13. Regezi JA, Stewart JCB, Lloyd RV, et al. Immunohistochemical staining of Langerhans cells and macrophages in oral lichen planus. Oral Surg Oral Med Oral Pathol 1985 ; 60 : 396-402.
14. Farthing PM, Matear P, Cruchley AT. The activation of Langerhans cells in oral lichen planus. J Oral Pathol Med 1990 ; 19 : 81-85.