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The distribution of dendritic cells in healthy and inflamed human gingival tissue

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임상적으로 건강한 치은조직과 염증성 치은조직에서 수지상 세포의 분포에 관한 면역조직화학적 연구

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송광은의 박사학위논문을 인준함

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임상적으로 건강한 치은과 염증성 치은에서 수지상 세포의 분포에 관한 면역조직화학적 연구

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수지상세포는 치태관련 치주염에 대한 숙주반응에서 면역학적 역할을 하는 것으로 간주되고 있는 바, 이 연구는 임상적으로 건강한 치은과 염증성 치은조직에서 랑거한스세포의 분포를 면역조직화학적으로 확인하기 위해서 시행되었다.

치은조직은 14명의 환자에서 치주치료 (임상치관확장술, 치은절제술, 치은판막술, 발치 등) 도중에 침윤마취하에서 채취되었다. 채취된 조직은 10% 중성포르말린이 함유된 용액에 즉시 고정되었으며, 통상적인 방법으로 처리되었고 파라핀내에 포매되었다. 파라핀 플록은 6 ㎛ 두께의 박편들로 만들어졌으며, 조직학적으로 관찰하기 위하여 이 박편들은 hematoxylin-eosin으로 염색되었다. 치은조직내의 염증 정도에 따라 수지상세포들의 분포를 관찰하기 위하여, T-cell, 미성숙한 수지상세포 그리고 성숙한 수지상세포에 각각 특이한 항체 (CD4, CD1a, CD83)를 이용하여 박편을 면역조직화학적으로 염색하였다.

이 연구결과, 정상조직에 비하여 염증조직에서 수지상세포가 더 많이 발현되는 경향을 보였으나, 염증성 치은조직에서의 수지상세포는 염증의 형태와 심도에 따라 CD4+ T-cell, CD1a+ immature DCs, CD83+ mature DCs는 다르게 나타났다. 따라서 염증성 치은조직 뿐만아니라 임플란트 주위조직에서 DCs의 역할과 형태에 대한 보다 포괄적인 연구가 필요하리라 생각되었다.

I. Introduction

Dendritic cells (DCs) are very heterogeneous group of antigen-presenting cells. Most mucosal surfaces have at least two subsets of DCs. Langerhans cells (LCs) are dendritic, nonkeratinocytes which reside in the suprabasal layers of epithelial cells in the skin, oral, nasal, esophageal, pulmonary, virginal, and rectal mucosa.[1,2] Turville et al.[3] reported DCs subpopulation in the gingiva closely resembles a subpopulation of dermal DCs in the skin. Similar immature DCs populations have been reported in the lamina propria of other mucosal tissues like the rectum, uterus, and cervix and in lymphoid tissues such as lymph nodes, tonsils, and spleen.[4,5] Cirrincione et al.[6] reported that the contact occurs between the mature DCs and lymphocytes in inflamed gingiva.

In gingiva, LCs are found in oral epithelium of normal gingiva and in smaller amounts in the sulcular epithelium, but they are probably absent from the junctional epithelium of normal gingiva[7]. They belong to the mononuclear phagocytes system as modified monocytes derived from the bone marrow, contain elongated granules and are considered macrophages with possible antigenic properties, and play an important role in the immune reaction as antigen-presenting cells for lymphocytes[8,9].They are immature, 9 ~ 10 ㎛ diameter in blood. Their key receptors in interactions with antigens are IAM-1, LFA-1, MHC class Ⅱ, and CD 1. Important functions in inflammation are the processing and presentation of antigen[10]. They ingest antigen locally and transport the antigen to the lymph nodes through the afferent lymphatics.[8,11,12]

It is impossible to distinguish LCs from other dendritic cells such as melanocytes by routine hematoxylin and eosin stained material. They have an organelle (Birbeck granule) which appears to be unique to them. They were labeled with gold chloride and observed through a primitive light microscope.[13,14]

Immunohistological studies on human gingival LCs have been carried out using antibodies against HLA-DR, or Langerin, or CD1a molecule these days.[15-17]

CD1a is a marker of immature dendritic cells and LCs in the epidermis[18,19], and CD83 is a marker of mature dendritic cells[20].

Jotwani et al.[21] showed that in human chronic periodontitis, CD1a+ immature LCs predominantly infiltrate the gingival epithelium, whereas CD83+ mature mDCs specifically infiltrate the CD4+lymphoid-rich lamina propria.

Jotwani and Cutler[1] reported that the lamina propria in chronic periodontitis contains CD83+mature dendritic cells (mDCs) and CD4+T-cells. They show that the gingiva contains DDCs in the lamina propria, which increase in number during chronic periodontitis. DDCs, LCs and B-cells co express CD83 in chronic periodontitis and contribute to the mDCs pool.

Recently, Bodineau et al.[22] demonstrated that the tissue inhibitors of matrix metralloproteinase-positive LCs were mainly observed in the upper epithelial layers, the other hand, marix metralloproteinase 9-positive LCs were observed especially during periodontitis and ine the basal epithelial layer or crossing the basement membrane.

An immunologic role in the host response to plaque-associated periodontitis is postulated for LCs. CD1a+ LCs appear to be principal leukocytes involved in the response of the oral mucosal epithelium to infection[23]. The specific role of LCs is unclear at present. These days, research about the role of LC in the pathogenesis of chronic periodontitis is in progress. The purpose of this in situ study was to immunohistochemically confirm the distribution of LCs in epithelium and lamina propria of patients with plaque-associated periodontitis.

Ⅱ. *Materials and Methods*

1) Selection of patients

Twenty sites from 14 patients, with a mean age of 47 years (range 11 to 68) were evaluated. All the patients were medically healthy. Informed consent was obtained form all subjects before commencement of the study.

Gingival tissue specimens from 14 subjects were excised under infiltrative anesthesia by sharp dissection during the periodontal therapy (clinical crown lengthening, gingivectomy, flap operation, extraction, etc.).

2) Methods

The specimen was immediately fixed in a solution containing 10% neutral formalin. They were processed routinely and were embedded in paraffin, The paraffin blocks were sectioned in 6 ㎛ thin slices and the sections were stained with hematoxylin-eosin for general histological observations. The sections were evaluated under a light microscope for detection of normal epithelium, inflammatory epithelium (gingivitis and chronic periodontitis).

For the immunohistochemistry, monoclonal mouse anti-human antibodies were purchased from DakoCytomation Inc. (Glostrup, Denmark). The primary Abs used were CD4, CD83, and CD1a. Sections were stained by the biotin-streptavidin-peroxidase method (Vectastain® ABC-AP kit, Burlingame, CA, USA.). The paraffin sections were pre-incubated with 1.5 % blocking normal serum diluted in 1X PBS for 20 min, and incubated for overnight with primary antibody (anti-CD4, CD83, and CD1a) diluted (1:200) in the diluted normal serum. After washing in 1X PBS, the sections were incubated for 30 min at room temperature with diluted biotinylated universal sencondary antibody. The sections were then rinsed briefly with 1X PBS, reacted with Vectastain® ABC-AP Reagent for 30 min. After color development with 0.05% DAB (diaminobenzidine tetrahydrochloride, 나라이름), the sections were washed and counterstained with hematoxylin. The number of positively stained cells in the normal epithelium and inflammatory epithelium were compared with each other.

III. Results

We have evaluated immature CD1a+ LCs and mature CD83+ DCs by using immunohistochemistry within the epithelium and lamina propria of the gingiva in healthy and inflammatory gingival tissues (Table 1-2).

Microscopic analysis of healthy epithelium revealed the presence of large numbers of CD1a+ LCs, but only a few CD83+ mature DCs.

Transition from health to chronic inflammatory tissue was marked by increase in numbers of CD4+ T cells and DCs (Fig. 1-3), both of which were predominantly present in lamina propria.

In inflammatory gingival tissues, there was a slight increase in the numbers of CD1a+ - labeled cells in the epithelium. However, the transition from health to chronic periodontitis was associated with a increase in numbers of LCs in the epithelium, and was associated with a increase in numbers of mature CD83+ DCs in the lamina propria.

Table 1. The histological findings of the Langerhans cells in normal gingiva and inflammatory tissue.

No	Age	Sex	Dx	Primary Antibody		
				CD4	CD83	CD1a
1	68	F	normal gingiva	−	−	+
2	25	F	normal gingiva	+	−	−
3	25	F	inflammatory gingiva	++	−	−
4	51	F	inflammatory gingiva	−	+	−
5	66	F	inflammatory gingiva	+	+	−
6	46	F	inflammatory gingiva	+	−	++
7	57	F	normal gingiva	+	−	−
8	50	F	inflammatory connective tissue	+	−	−
9	50	F	inflammatory gingiva	++	−	+
10	50	F	inflammatory gingiva	−	+	+
11	11	F	inflammatory gingiva	−	−	+
12	55	F	normal gingiva	−	+	+
13	51	F	inflammatory gingiva	+	+	+
14	62	F	inflammatory gingiva	+	+	−
15	62	F	inflammatory gingiva	+	−	+
16	52	F	normal gingiva	−	+	+
17	39	F	inflammatory gingiva	+	−	−

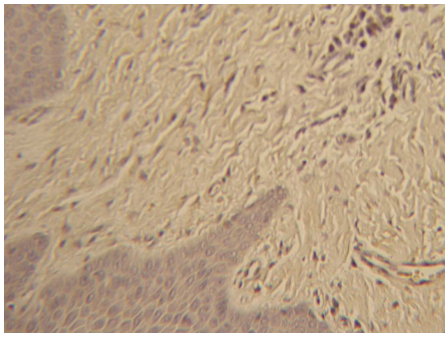
Table 2. The distribution percentage of the Dendritic cells in normal gingiva.

	Primary Antibody			Total cases
	CD4	CD83	CD1a	
Cases(%)	2 (40%)	2(40%)	3(60%)	5

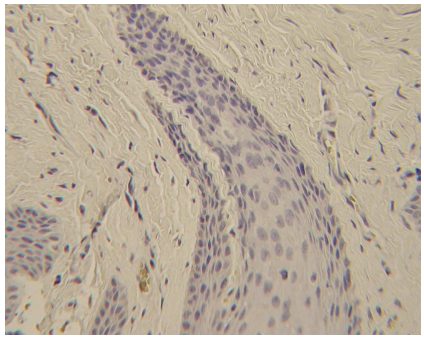
Table 3. The distribution percentage of the Dendritic cells in inflammatory gingiva.

	Primary Antibody			Total cases
	CD4	CD83	CD1a	
Cases(%)	9 (75%)	5(42%)	6(50%)	12

a.



b.



c.

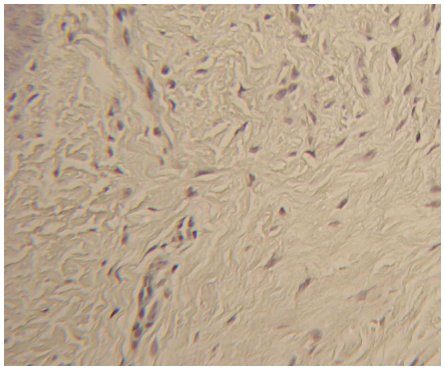
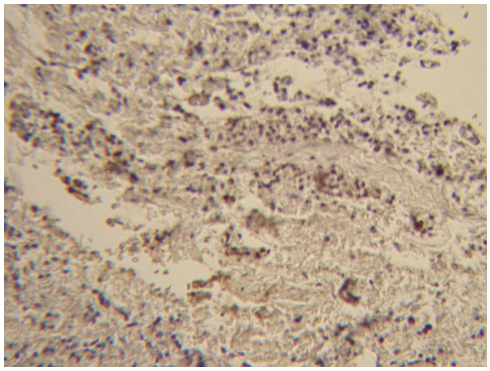
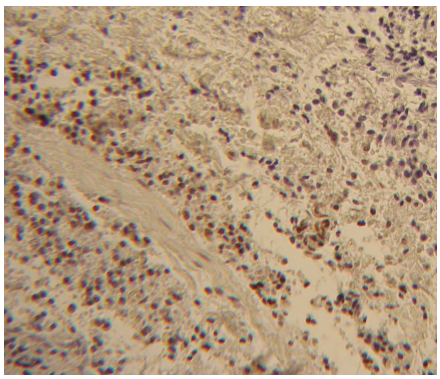


Fig 1. CD4+, CD1a+, and CD83+ cells was weakly shown in normal gingiva. a. CD4+ cells, b. CD1a+ cells, c. CD83+ cells, Immunohistochemical staining ×200.

a.



b.



c.

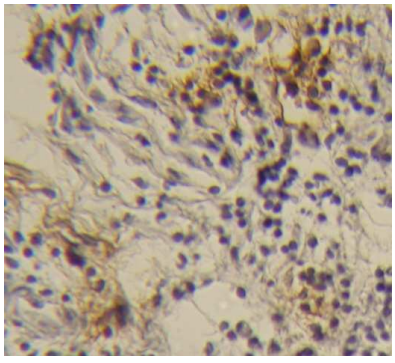


Fig 2. CD1a+, CD4+, and CD83+ cells are strongly observed in inflammatory gingiva. a. CD4+ cells, b. CD1a+ cells, c. CD83+ cells, Immunohistochemical staining ×200.

IV. Discussion

DCs are antigen-presenting cells that capture antigens in their immature state, process them into peptides, and present them to T-cells in their mature CD83+ state. The immune function of DCs depends upon their state in maturation. In their immature phase, DCs encounter and capture in infectious agents like bacteria and bacterial products, resulting in the release of inflammatory cytokines such as TNF-α and IL-1β. These cytokines activate and mobilize DCs and initiate the process leading to DC maturation. In adaptive phase, mature DCs prime native helper/cytotoxic T-cells to undergo clonal expansion in the lymph nodes.[24)]

Jotwani and Cutler[1)] reported first demonstration of the presence of an additional subset of immature DCs, besides LCs, in the inflamed gingiva. Most LCs remain isolated anatomically from CD4+ T-cells in the healthy gingiva, and in disease, CD83+ cells are associated with large clusters of CD4+ T-cells, suggestive of local antigen presentation.

To determine whether human gingiva harbors a separate subpopulation of DCs according to the inflammation, we performed immunohistochemical staining of paraffin sections using antibodies (CD4, CD1a, and CD83) specific for T-cell, in epithelium and lamina propria of patients with plaque-associated periodontitis. respectively.

We used the anti-CD1a immunolabelling for identifying LCs in the human epithelia. Hutchens et al[25)] demonstrated histologic structure and quantitative distribution of oral epithelial dendritic cells of the Rhesus monkey. The number of LCs in heavily keratinized areas was significantly greater than in unkerainized areas. They suggested a direct relationship between LCs and degree of keratinization. And DiFranco et al[26)] demonstrated the number of LCs in the biopsy specimens of clinically inflamed human gingiva was 2 to 10 times greater than in clinically normal gingiva of the same patient. But, the speculation about the number of LCs in the gingival epithelium is controversy, with increased numbers, decreased numbers, and no quantitative change, being reported during inflammation[27-29)]. Séguier et al.[30)] evaluated the number of CD1a+ LCs in healthy and diseased human gingival tissues. They demonstrated that a significant decrease in the number of CD1a+ LCs in gingivitis and periodontitis groups when compared with clinically healthy group. And, they concluded that the decrease of such cells could represent a way to avoid

immune overstimulation.

Katou et al.[31] reported that formation of mature DCs and CD4+ T-cell clusters has been demonstrated in chronically inflamed skin infected with Candida albicans.

We used the anti-CD83 immunolabelling for identifying LCs in the human lamina propria. Jotwani et al[21] reported that in chronic periodontitis, immature LCs predominantly infiltrate the gingival epithelium, whereas CD83+ mature DCs specifically infiltrate the CD4+ lymphoid-rich lamina propria.

Gustafson et al.[32] examined expression of Langerin and CD83 molcules on LCs in patients with oral lichen planus. Frequency of CD83+ cell in oral lichen planus epithelium didn't differ compared with healthy oral epithelium, but that in oral lichen planus connective tissue showed significantly higher of CD83+cells compared with healthy connective tissue.

Jotwani et al.[21] believe that as periodontitis progress, LCs install in the lamina propria and become CD83+ mDCs, and mDCs are precursors of CD83+ mDCs. Jotwani and Cutler[1] suggest that multiple DC subsets mature in the gingiva and that mature DCs engage in antigen presentation with T-cells in chronic periodontitis. Both DC subpopulations (LC and dermal DC) as well as B-cells have been shown to contribute to the CD83+ population observed in the inflamed gingiva.

Recently, Bodineau A. et al.[33] compared the number, the distribution and the expression of markers of maturation of LCs in elderly and younger patients with chronic periodontitis to evaluate the effect of aging on LC in inflammatory gingival tissue. In elderly group, the number of CD1a+ LC was significantly decreased in the epithelium and significantly increased in the upper connective tissue, and the expression and the numbers of CD83+ cells were significantly increased compared with younger patients. We could find the CD83+ mDCs in the normal gingival tissues samples obtained, but could not confirm difference between the aged and the younger.

On the basis of our results, data demonstrated that DCs (immature and mature DCs) remain isolated anatomically from CD4+ T-cells in the healthy gingiva, and also showed that the immature DCs and mature DCs (specially mature DCs) were increased in the inflamed gingiva. Expecially, our data indicated that T-cell rich severe and chronic inflammation is associated with a significant increase in the number of mature DCs in the lamina propria, relative to gingival health. And CD4, CD1a, and CD83 were not detected in acute inflammation. In other words, we think that in inflammatory human gingival tissues, the CD4+ T-cells, CD1a+ immature

DCs, and CD83+ mature DCs can be differently expressed according to the inflammation type and inflammation severity.

There are some ways out which will be clarified about DCs[21]. It remains to be clarified how subpopulations of DCs and B-Cells are involved in local and systemic antigen presentation. And, it is needed to identify their precursors *in situ* to understand the immunopathogenesis of periodontitis, and to design immunotherapeutic agents.

In conclusion, on the basis of our results, data demonstrated that expression of DCs tended to increase in inflammatory gingival tissues compared with the healthy gingival tissues. Therefore, further studies will be required to investigate the morphology and role of this DCs subpopulation in human inflammatory as well as periimplant gingival tissues.

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