

Original Article

Immunohistochemical analysis of Langerhans cells in chronic gingivitis using anti-CD1a antibody

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ABSTRACT

Background: The Langerhans cells (LCs) are dendritic cells (DCs) which belong to the group of antigen presenting cells (APCs). Their function is to recognize the antigen, capture it, and present it to the T lymphocytes; thus initiating an early immune response. The antigen presenting functional LCs may play an important part in initiation and development of gingivitis. The aim of this study was to analyze the density, intraepithelial distribution, and morphology of LCs in gingival epithelium among different age groups with chronic gingivitis and to compare it with that of normal gingiva.

Materials and Methods: Immunohistochemistry (IHC) was performed to study LCs in normal gingival epithelium ($n = 10$) and gingival epithelium in chronic gingivitis ($n = 30$) using anti-CD1a antibody. Mann-Whitney U test was performed to compare the density of LCs in normal gingiva with chronic gingivitis. The distribution of LCs in various layers of the epithelium within the three age groups was analyzed using Kruskal-Wallis test. P value less than 0.05 was considered as significant.

Results: The density of LCs in chronic gingivitis was significantly higher than that of normal gingiva. Comparing different age groups, the younger individuals had more number of LCs which were located in the superficial layers of gingival epithelium. In chronic gingivitis, higher number of LCs were located in deeper layers when compared with that of normal gingiva. Three morphological types of CD1a positive LCs were observed in normal gingiva, out of which the density of LCs with branched dendritic processes was highest in normal gingiva.

Conclusion: The LCs showed variable number, location, and morphology which indicated their adaptation for function in chronic gingivitis.

Key Words: Anti-CD1a, antigen presenting cells, dendritic cells, immunohistochemistry, Langerhans cells

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INTRODUCTION

It is believed that the gingival epithelial cells play an active role in host defenses by responding to bacteria in an interactive manner. Among the immune cells, intraepithelial lymphocytes together with

immunocompetent Langerhans cells (LCs) constitute an antigen trapping network.^[1] The LCs are a type of antigen presenting cells and their function is to recognize the antigen, capture it, and initiate an early immune response. LCs are bone marrow derived cells situated usually in the suprabasal layer of stratified squamous epithelia of oral mucosa and epidermis of skin.^[2]

LCs are located in the suprabasal and spinous layers of the sulcular and gingival epithelium.^[2] There is experimental evidence for differences in both LC number and in surface antigen expression between healthy and diseased gingiva. Consequently, LCs should represent “key” cells in pathogenesis of periodontal diseases.^[3] An increased number of

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LCs in gingival epithelium associated with the development of an oral microflora in germ-free mice has been demonstrated by Bos and Burkhardt.^[1] The relationship between an increased number of LCs and plaque accumulation in man during experimental gingivitis has been demonstrated which was confirmed by immunohistochemical methods. In this study LCs were found five times more in inflamed gingiva in comparison to healthy gingiva from same patients.^[4]

Recently, a qualitative study has been carried out to evaluate the number of LCs associated with gingival inflammation by the use of monoclonal antibodies reacting against two different antigens, human leukocyte antigen-DR (HLA-DR) and cluster of differentiation 1a (CD1a). The study also demonstrated an increase in number of LCs in moderate gingivitis but a decrease number of LCs in periodontitis compared with controls.^[5] LCs participate in the initial mechanism using the interleukin-1 (IL-1) produced by macrophages or keratinocytes and are important in presentation of antigen in all the phases of periodontal disease. The concept of immunostimulatory capacity of LCs is supported by new evidence that LCs infiltrate the lamina propria in chronic periodontitis.^[3]

It is believed that as periodontitis progresses, LCs collect in the lamina propria and become CD83+ mature dendritic cells (DCs).^[6] The authors suggested that these mature DCs play a role in the formation of 'oral lymphoid follicles' that develop in periodontitis. This is initiated in gingivitis by LCs or their progenitors in response to Pathogen Associated Molecular Patterns in the oral biofilm. In chronic gingivitis, immature DCs presumably migrate out of gingival epithelium, undergoing maturation as they traffic to lymph nodes, where a T cell response is elicited.

The number of Langerhans' cells in gingival tissues is a topic of much discussion. Many studies have reported that their number is increased in periodontitis,^[7] decreased in gingivitis, and periodontitis compared to healthy periodontal tissues.^[8] Some studies have also observed no quantitative change in periodontitis compared to gingivitis and healthy periodontal tissues.^[9] Less studies are conducted on the distribution of factor XIIIa(+) DCs and their possible role in periodontitis and gingivitis. Jotwani and Cutler^[10], demonstrated in their studies that the number of factor XIIIa(+)

DCs is increased in periodontitis compared to healthy gingival tissue. Thus, antigen presenting functional LCs may play an important part in initiation and development of gingivitis.

The purpose of the present study was to determine the density, intraepithelial distribution, and morphological types of CD1a positive LCs in chronic gingivitis and compare the same with that of normal gingiva. The study also aimed at comparing the same parameters among different age groups of patients with chronic gingivitis.

MATERIALS AND METHODS

The gingival tissues obtained from the patients undergoing treatment in the Departments of Periodontia and Oral and Maxillofacial Surgery, Meenakshi Ammal Dental College and Hospital, Chennai, were used for this study. After explaining the surgical procedure, informed consent from all these patients was obtained by the concerned departments. The control group included ten cases of clinically normal gingiva of individuals who underwent surgical extraction of mandibular impacted teeth under local anesthesia. Gingival biopsies from the sites representing gingival overgrowth in diseased group ($n = 30$) and sites representing healthy gingiva ($n = 10$) in control group were collected from the respective departments. Diagnosis of both groups was based on clinical examination of oral cavity. The only criteria were clinical features like color, size, and contour of gingiva. Paraffin-embedded tissue sections of healthy and diseased gingiva were stained by hematoxylin and eosin (H and E) and the serial sections of the same were stained by immunohistochemical reagent (anti-CD1a for LCs). Clinical diagnosis of normal gingiva and chronically inflamed gingiva was confirmed by observing the H and E stained sections.

Inclusion criteria for the normal group were normal gingival epithelium and mild inflammatory component. Any sections showing pathological changes were excluded from the control group. The tissue sections from chronic gingivitis patients which showed greater inflammatory component along with changes in the gingival epithelium were included under the study group.

Immunohistochemical procedure

The immunohistochemistry (IHC) procedure was performed using monoclonal mouse antibody (Biogenex Life Sciences Private Limited and

Dako Cytomatio, Denmark). Tissue sections were deparaffinized in xylene and hydrated in graded concentration of ethyl alcohol. Antigen retrieval was done. The tissue sections were treated with blocking reagent 'power block reagent' (Biogenix) to block the endogenous peroxidase activity. The sections were further incubated with primary antibody anti-CD1a followed by incubation with secondary antibody (poly-horseradish peroxidase (poly-HRP)).^[11] Positive control sections included tissue sections of human skin specimen and were treated in the same manner as the test groups. Negative control sections which included one healthy and one diseased tissue were treated in the same manner as the test groups except that the primary antibody was omitted and substituted with phosphate buffered saline and a non-immune antibody (normal rabbit serum) at the same concentration.

Identification and counting of LCs

Following the immunohistochemical staining, all the stained sections of gingival epithelium were studied under light microscope. Any cells with dark brown stain within gingival epithelium were identified as CD1a positive LCs. The LCs were observed in three layers of gingival epithelium, that is, stratum basale, deep stratum spinosum, and superficial stratum spinosum. The LCs were of three morphological types and were labeled as type A cells with dark brown stained body and dendritic processes extending among keratinocytes, type B cells with a cell body without identifiable dendritic processes, and type C cells exhibiting only dark brown peripheral outline.

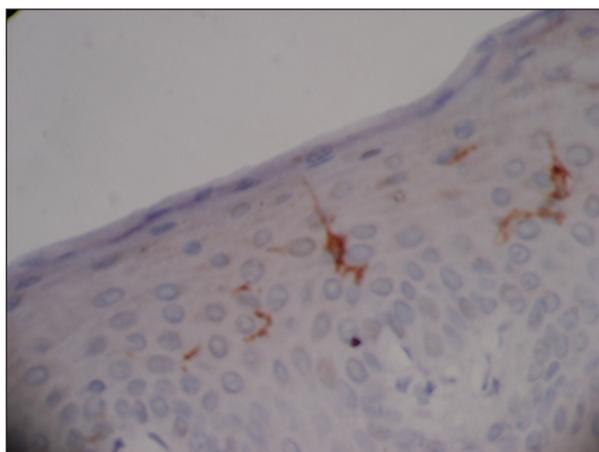


Figure 1: Photomicrograph of normal gingival epithelium showing CD1a positive Langerhans cells with branched dendritic process directed towards the superficial surface ($\times 10$, CD1a immunostaining)

Statistical analysis

Mann Whitney U test was performed to compare the density of LCs in normal gingiva with chronic gingivitis, the distribution of LCs in various layers of the epithelium and density of three different morphological types of LCs in normal gingiva and chronic gingivitis. The density of Langerhans among different age groups of individuals with chronic gingivitis and distribution of LCs in various layers of the epithelium within the three age groups was analyzed using Kruskal-Wallis test. P value less than 0.05 was considered as significant.

RESULTS

In the present study, we observed that in the normal gingiva group, density of LCs per square millimeter of gingival epithelium was 74.56 ± 39.45 [Figure 1]. The density of LCs was significantly higher in gingival epithelium of chronic gingivitis patients [Figure 2] when compared to that of normal gingiva group ($P < 0.01$) [Table 1]. The density of LCs was found to be higher in younger patients with normal gingiva. Evaluating the location of LCs in gingival epithelium of chronic gingivitis group, it was observed that higher density of LCs was present in stratum basale and deep stratum spinosum ($P < 0.05$) [Figures 3 and 4]. Comparison of location of LCs among different age groups, it was observed that in the younger age group, higher number of LCs were observed in the superficial stratum spinosum and lowest in the stratum basale. Whereas in chronic gingivitis patients, the density of LCs in the deep

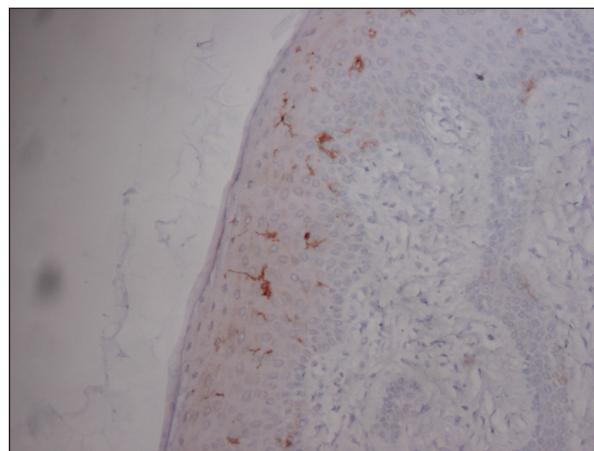


Figure 2: Photomicrograph of gingival epithelium in chronic gingivitis showing increased density of CD1a positive Langerhans cells ($\times 10$, CD1a immunostaining)

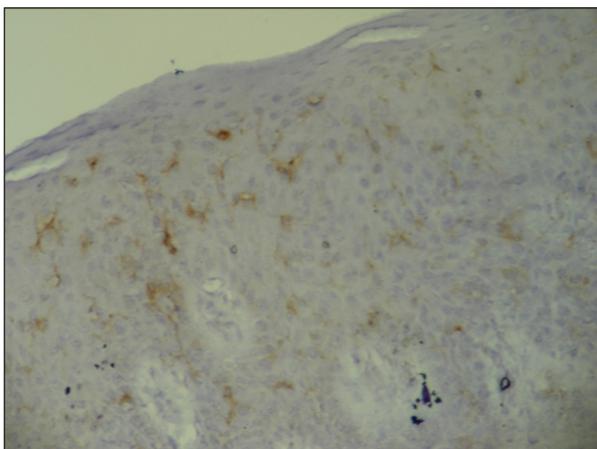


Figure 3: Photomicrograph of gingival epithelium in chronic gingivitis showing predominance of CD1a positive Langerhans cells within stratum spinosum (x20, CD1a immunostaining)

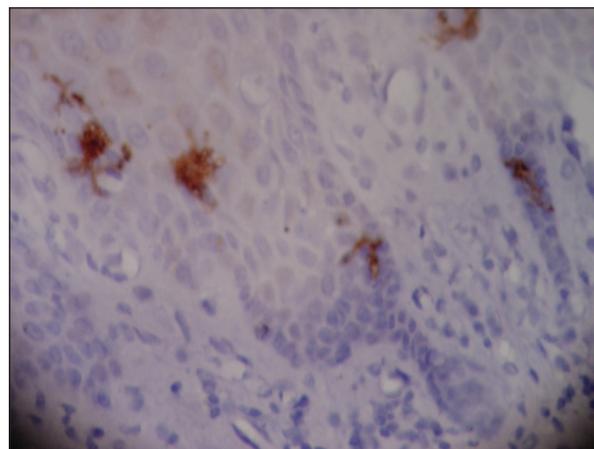


Figure 4: Photomicrograph of gingival epithelium in chronic gingivitis showing CD1a positive Langerhans cells within stratum basale (x40, CD1a immunostaining)

Table 1: Density of Langerhans cells based on location

Layers of epithelium: Study group	n	Density of LCs/mm ² (Mean ± SD)	U-value	P-value
Stratum basale:				
Normal gingiva	10	12.48±20.03	-2.22	0.02*
Chronic gingivitis	30	40.83±43.12		
Deep stratum spinosum:				
Normal gingiva	10	32.64±19.06	-2.76	0.006*
Chronic gingivitis	30	66.88±37.65		
Superficial stratum spinosum:				
Normal gingiva	10	39.68±19.15	-1.28	0.199
Chronic gingivitis	30	36.48±8.72		

*P < 0.05 is considered to be significant using Mann-Whitney U test

stratum spinosum of younger age group was higher when compared to other two age groups ($P < 0.01$) [Table 2].

Regarding the morphological types of LCs, highest number of type A LCs [Figure 5] were observed in normal gingival epithelium. The density of type C LCs [Figure 6] was markedly increased in chronic gingivitis compared to that of normal gingiva and this finding was statistically highly significant ($P < 0.001$) [Table 3]. In chronic gingivitis patients, densities of type A and type B LCs was minimal in the superficial stratum spinosum. This finding was statistically highly significant ($P < 0.01$).

Type A cell body with dendritic processes; type B cell body; and type C peripheral outline.

DISCUSSION

In the previous studies, number of Langerhans in normal gingival epithelium were; 0.05–3.0 cells/mm² (DiFranco *et al.*^[5]), 3.0 cells/mm² (Saglie *et al.*^[12]), 35.7 ± 3.8 cells/mm² (Nurmenniemi *et al.*^[13]), and 73.0 cells/mm² (Séguier *et al.*^[8]). As there are no large scale studies done, the standard value for the density of LCs in normal gingival epithelium is not available from the literature.

In our study, the total number of LCs in gingival epithelium was significantly increased in chronic gingivitis group when compared to that of normal gingiva group and high statistical significance was observed. This was consistent with previous studies.^[1,2,13] Newcomb *et al.*,^[14] and Walsh *et al.*,^[15] stated that LCs in the gingival epithelium are very responsive to the accumulation of bacterial plaque and they migrate to the inflamed site in large numbers during gingivitis.^[9,16] Cury *et al.*, found that the number of LCs in the oral epithelium and interstitial DCs in the lamina propria are increased in gingivitis compared to periodontitis. This may be contributed to the different patterns of host immune response in these diseases.^[17]

LCs may result from different bacterial antigens eliciting different responses or may reflect different responses to similar plaque antigens penetrating the surface of oral epithelium.^[15] This indicated that the LCs responded to external stimulus, most likely plaque antigens diffusing through the epithelium.

It is suggested that the increase in number of LCs in some pathological conditions, as shown in previous studies, could occur only within first few weeks of gingival inflammation.^[12] As the disease progresses, the LCs carrying the antigen, leave the gingival epithelium and migrate towards the connective tissue in greater numbers contributing to their decreased number in the gingival epithelium.^[8,18,19] There was a wide range of variation in the number of LCs in

gingivitis (44.8-278.4 LCs/mm²). This may be due to the fact that individuals with early gingivitis may be the ones with larger number of cells than those with late gingivitis. Comparison of density of LCs in stratum basale and deep stratum spinosum of gingival epithelium among normal and chronic gingivitis groups showed a statistically significant increase in the LCs among chronic gingivitis group. The decrease in the LCs in the superficial layers in elderly age group may be due to a decreased host immune response which aids in the progression of the disease.^[20] It has also been demonstrated in previous studies that in normal elderly patients, maximum number of LCs were located in deep stratum spinosum and very low numbers were observed in stratum granulosum.^[17,20]

Increased antigenic load in gingival epithelium could play a role in selective presence of functional LCs, that is, the LCs with branched dendritic processes.^[21] The LCs exhibiting dendritic morphology indicated an efficient antigen trapping function. The second type of LCs are non-dendritic and are less efficient in antigen capture. Another possibility could be that the LCs carrying the antigenic peptides adapt a spherical morphology to migrate to deeper layers, reach the connective tissue, and enter the lymphatics to reach the draining lymph nodes.^[4] Once within connective tissue they are no longer CD1a positive LCs, rather are known as CD XIIa(+) interstitial DCs^[7,10,22] and further within lymphatics as veiled cells. DCs can assume a different morphology, phenotype, and

Table 2: Density of Langerhans cells in different age groups with chronic gingivitis based on location

Age groups	Layers	n	Density of LCs/mm ² (Mean ± SD)	H-value	P-value
10-20 years	Stratum basale	12	36.53±36.47	8.08	0.01*
	Deep stratum spinosum		81.86±44.07		
	Superficial S. spinosum		38.66±47.76		
21-30 years	Stratum basale	8	30.72±48.72	2.19	0.33
	Deep stratum spinosum		46.00±24.48		
	Superficial stratum spinosum		56.00±73.03		
31-50 years	Stratum basale	10	54.08±47.016	9.30	0.01*
	Deep stratum spinosum		65.60±32.22		
	Superficial stratum spinosum		18.24±19.78		

*P = 0.01 is considered to be highly significant using Kruskal-Wallis test

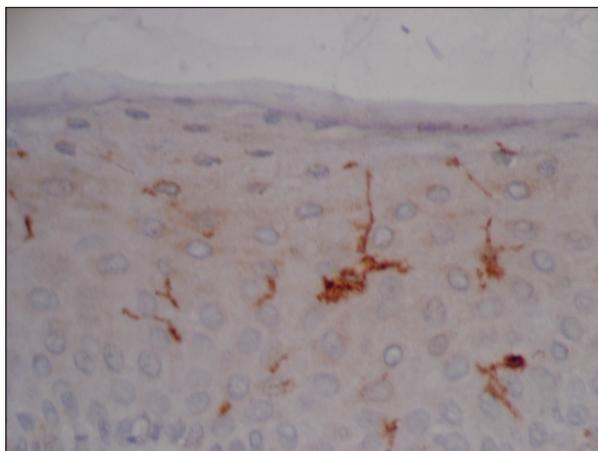


Figure 5: Photomicrograph of gingival epithelium showing CD1a positive type A Langerhans cells with branched dendritic processes (×40, CD1a immunostaining)

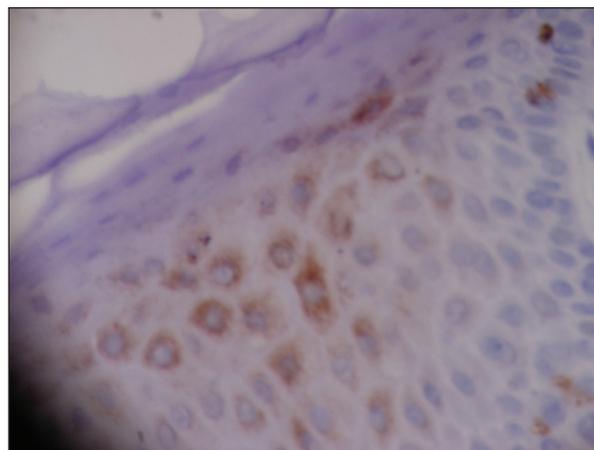


Figure 6: Photomicrograph of gingival epithelium showing CD1a positive type C Langerhans cells with dark brown stained peripheral cytoplasmic outline (×40, CD1a immunostaining)

Table 3: Density of different morphological types of Langerhans cells

Morphological type: Study group	n	Density of LCs/mm ² (Mean ± SD)	U-value	P-value
Type A				
Normal gingiva	10	41.28±26.49	-0.93	0.348
Chronic gingivitis	30	61.26±45.79		
Type B				
Normal gingiva	10	17.60±14.09	-0.81	0.416
Chronic gingivitis	30	26.56±24.12		
Type C				
Normal gingiva	10	11.84±14.86	-3.36	0.001*
Chronic gingivitis	30	62.18±46.11		

*P < 0.01 is considered to be highly significant using Mann-Whitney test

function depending on the state of maturation of DCs.^[4,23] LCs observed with only CD1a positive peripheral outline may be because of presence of LCs with dendritic processes but giving a cross sectional appearance of dendritic processes alone encircling the keratinocytes.

CONCLUSION

In the present study, the density of LCs was increased in chronic gingivitis when compared to the normal gingiva with high statistical significance. The density of LCs was higher in superficial stratum spinosum in normal gingiva and in younger patients supporting a better immune defense function of gingival LCs whereas in chronic gingivitis it was higher in deep stratum spinosum suggesting their migration towards deeper layers. In normal gingiva, higher density of LCs with long dendritic processes was observed. When age groups were compared, more density of branched LCs was found in younger age group indicating a better immune surveillance function as compared to the elderly group with chronic gingivitis, and most of these cells were located in deep stratum spinosum. Thus, present work suggests that gingival LCs in elderly patients could present an early maturation in response to the pathogens in the form of their decreased number in gingival epithelium and altered morphological patterns.

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