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ORIGINAL RESEARCH

# CD1a-labeled Langerhans Cells in Gingival Pyogenic Granuloma and Fibroma Lesions: An Immunohistochemical Study

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## ABSTRACT

**Background:** Role of Langerhans cells is well investigated in pathological conditions of oral tissues. No studies have been reported so far describing possible role of Langerhans cells in reactive gingival overgrowths. This study aims at evaluation of density of Langerhans cells in common localized gingival overgrowths.

**Materials and methods:** Thirty-two specimens of localized gingival overgrowths (19 fibromas and 13 pyogenic granulomas) were subjected to immunohistochemistry with anti-CD1a antibody for demonstration of Langerhans cells. Langerhans cells in each specimen were counted as number of cells/high power field. The density of Langerhans cells thus obtained in study groups was compared. It was also compared with the same in control group.

**Results:** Mean number of CD1a-labeled cells in control group was 2.24 ± 1.11 cells/HPF. The mean number of CD1a-labeled cells was 2.39 ± 1.80 cells/HPF in pyogenic granuloma group, while in irritation fibroma group the mean number was as 1.71 ± 1.96 cells/HPF. The difference in the density of Langerhans cells among two study groups was not significant.

**Conclusion:** Langerhans cells were an integral component of epithelium in both types of reactive lesions. A wide range in density of Langerhans cells was observed in both groups.


**Keywords:** CD1a, Fibroma, Immunohistochemistry, Langerhans cells, Pyogenic granuloma.

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## INTRODUCTION

Langerhans cells (LCs) are dendritic nonkeratinocytes which form a network within stratified squamous epithelium of oral mucosa. They are derived from bone marrow and migrate into the epithelium. They constitute 2 to 8% of the intraepithelial cell content, interlinking via their dendritic processes and forming a single layer the 'reticuloendothelial trap'.[1] As antigen-presenting cells, they play an important role in the immune mechanisms associated with oral mucosa.

Pyogenic granuloma is a common reactive lesion of oral cavity. It is said to be an exaggerated tissue response to a minor injury or a low grade irritation. Lawoyin et al suggested that microulcerations produced by calculus, overhanging edges or rough margins of restorations may allow percolation of antigenic substances from low virulent microorganisms colonizing the gingival surface, into the lamina propria. This may evoke an exaggerated vascular response in the connective tissue resulting in formation of pyogenic granuloma.[2]

Irritation or traumatic fibroma is also known as fibrous epulis.[3] Two distinctive pathways for evolution of this lesion are possible. According to the first mechanism, it is apparently a hyperplasia of connective tissue fibroblasts and collagen fibers reactionary to chronic irritation. Microscopically, it shows a circumscribed mass of dense and collagenized connective tissue. It is said to arise due to trauma induced inflammation and therefore exhibits scattered chronic inflammatory cells. Increased mass of submucosal connective tissue in this lesion results in atrophy of overlying stratified squamous epithelium. Another process suggested for pathogenesis of irritation fibroma is fibrous maturation of preexisting pyogenic granuloma.[4]

Distribution, morphology, quantity and functions of Langerhans cells are altered in variety of oral and maxillofacial diseases.[5] About five times as many CD1a-positive Langerhans cells are seen in clinically inflamed gingiva as compared with the healthy gingiva.[1] Reduced number of Langerhans cells due to the effect of calcium channel blocking medications initiates altered tissue homeostasis which results in tissue overgrowth as gingival hyperplasia.[6] It appears that cytokines secreted

by Langerhans cells and injured keratinocytes play an important role in the immune mechanisms involved in various lesions. Thus, there is a little evidence to suggest that Langerhans cells play an important role in immune response and pathogenesis of reactive and inflammatory lesions of gingiva. However, the distribution and functional contribution of Langerhans cells in reactive gingival overgrowths is not clearly understood. This study was conducted with the aim to evaluate the density of Langerhans cells in common reactive gingival overgrowths, i.e. pyogenic granuloma and irritation fibroma.

## MATERIALS AND METHODS

### Samples

The study protocol was approved by the institutional ethics committee. A total of 32 specimens of localized gingival overgrowths, of which 19 were diagnosed as fibroma and 13 as pyogenic granuloma, were selected from banked tissue blocks prepared by the Department of Oral Pathology and Microbiology, MGV's KBH Dental College and Hospital, Nashik, India.

With informed consent, 32 gingival tissues without clinical evidence of inflammation were retrieved from the patients undergoing surgical removal of wisdom teeth and crown lengthening procedures. These specimens constituted the control group.

### Staining Procedure

All specimens were fixed in 10% neutral buffered formalin for at least 24 hours and were processed for paraffin embedding. Three micrometer thick sections were sliced from the tissue blocks. At least one section of each specimen was stained with hematoxylin and eosin and was examined microscopically (Figs 1 and 2). The other sections were subjected to immunohistochemistry.

Immunohistochemistry procedure was carried out using polymer labeling technique (Dako EnVision). Sections were dewaxed in xylene followed by dehydration in increasing grades of alcohol. Sections were rinsed in distilled water. Antigen retrieval was carried out in a decloaking chamber (Biocare) with 10 mM citrate buffer solution at 125°C temperature for 30 seconds followed by 90°C for 10 seconds. Slides were cooled naturally and brought to room temperature. Endogenous peroxidase was blocked by using 0.3% hydrogen peroxide in methanol at room temperature for 10 minutes. Sections were rinsed with phosphate buffered saline briefly and incubated with appropriately diluted primary antibody (monoclonal mouse antihuman antibody to CD1a, Clone: O10, Dako North America, Inc. Carpinteria, CA, USA) for 60 minutes. Sections were rinsed with phosphate buffered saline and incubated with the polymer for 30 minutes. Sections were again rinsed with phosphate buffered saline. Diaminobenzidine was used as chromogen in hydrogen peroxide for 10 minutes. Finally, the sections were counterstained with Mayer's hematoxylin (HiMedia Laboratories Pvt. Ltd., Mumbai, India) and mounted with DPX mounting medium (Merck Specialities Private Limited, Mumbai, India). Omission of the primary antibody constituted the negative control. Palatine tonsil tissue was used as a positive control.

### Quantification of CD1a-positive Langerhans Cells

The number of immunolabeled cells in 10 high power fields was calculated in each specimen, by two masked examiners. Number of CD1a-labeled Langerhans cells, in each specimen, was counted as mean number of CD1a-positive cells/high-power field.

Counts of CD1a-labeled cells were restricted to cells exhibiting positive (brown) cytoplasmic staining by

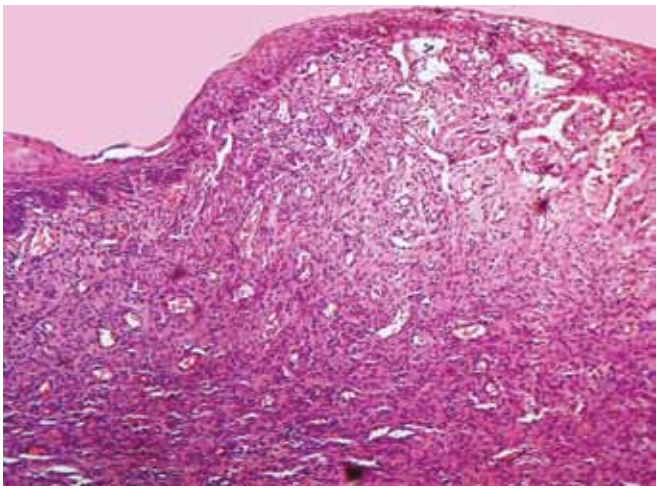


**Fig. 1:** Photomicrograph of a gingival pyogenic granuloma (hematoxylin and eosin stain: 100×)

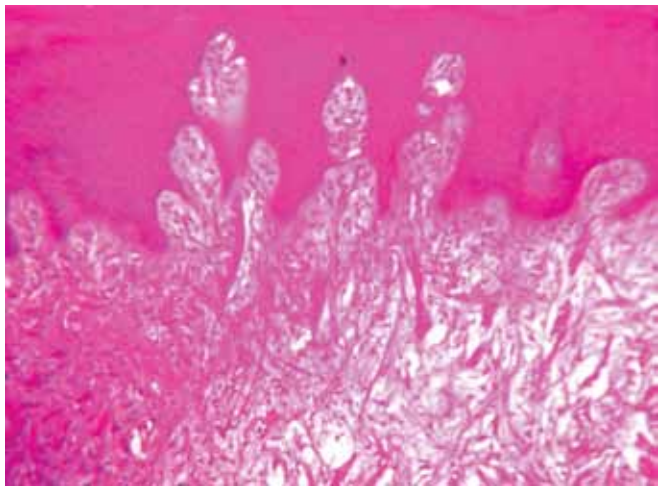


**Fig. 2:** Photomicrograph of a gingival fibroma (hematoxylin and eosin stain: 100×)

chromogen, a well-defined nucleus and body with at least two well-visualized dendrites (Figs 3 and 4).

## STATISTICAL ANALYSIS

The mean value of counts of two examiners was used for statistical analysis. Unpaired t-test was applied to search the significant difference in the mean number of Langerhans cells between control and study groups. The significance of differences in mean number of Langerhans cells between groups and within groups was assessed by one-way analysis of variance (ANOVA).

For all the statistical methods, probability value of 0.05 was considered as significant.

## RESULTS

Dendritic cells showing positive immunoreactivity for CD1a antibody were detected in all specimens of control group. Whereas 24 out of 32 (75%) of specimens of reactive gingival overgrowth revealed CD1a-labeled Langerhans cells (Tables 1 and 2). Langerhans cells were located in suprabasal regions of epithelium in most of the specimens. In few specimens of pyogenic granuloma group, CD1a-labeled cells were clustered near the tip of connective tissue papillae (Figs 5A and B). Mean number of CD1a-labeled cells in control group was 2.24 ± 1.11 cells/HPF. Both groups of reactive gingival overgrowths demonstrated Langerhans cells in their epithelial components, but there was a wide range in their density in both groups. Table 3 shows mean number of CD1a-labeled cells in fibroma group and pyogenic granuloma group. The mean number of CD1a-labeled cells was 2.39 ± 1.80 cells/HPF in pyogenic granuloma group; while in

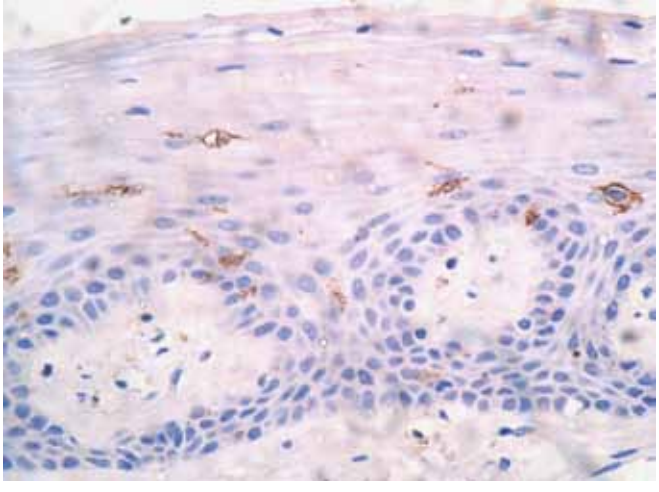


**Fig. 3:** A gingival fibroma demonstrating CD1a-labeled Langerhans cells in suprabasal region of epithelium (polymer labeling method: 450×)

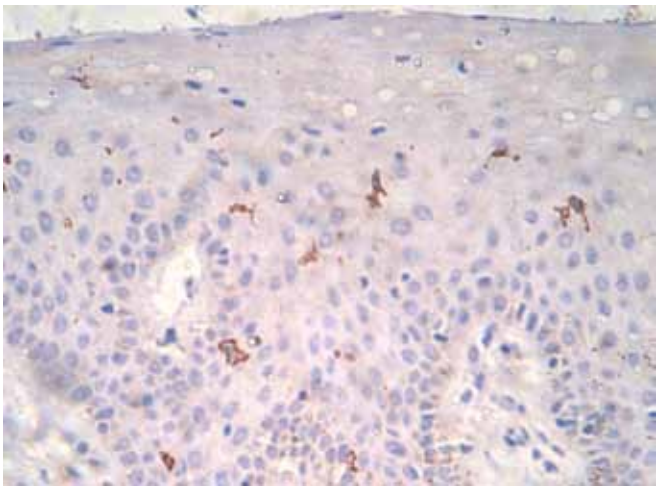


**Fig. 4:** A gingival pyogenic granuloma demonstrating CD1a-labeled Langerhans cells in suprabasal region of epithelium (polymer labeling method: 450×)

**Table 1:** Distribution of CD1a-labeled Langerhans cells in pyogenic granuloma

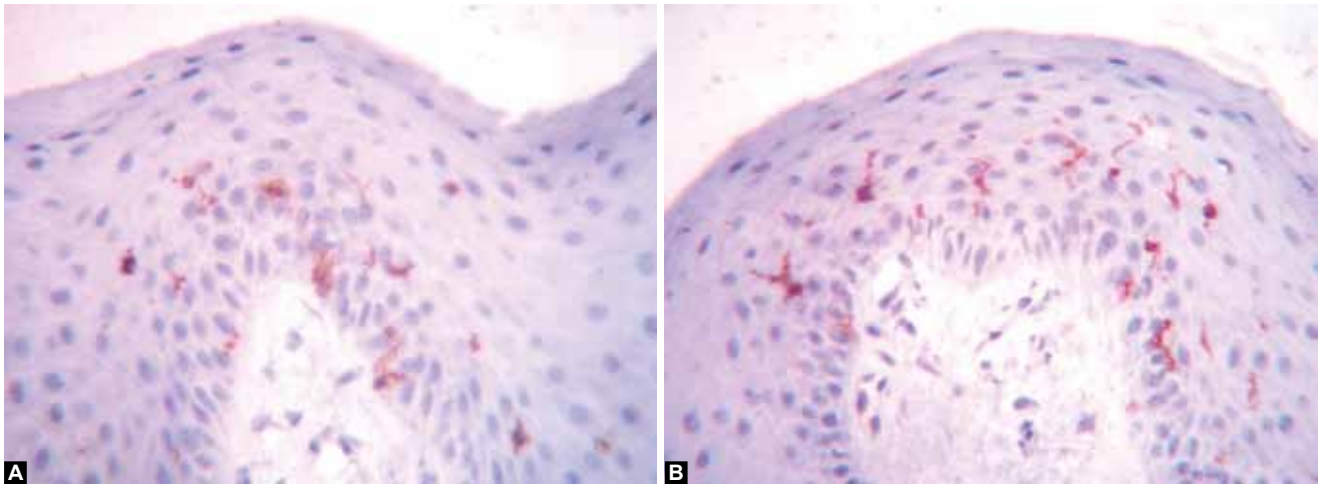
| *Case no.* | *Age/gender* | *Location* | *Mean CD1a-positive cells/HPF* |
|---|---|---|---|
| 1 | 32/F | Mandible | 4.5 |
| 2 | 35/M | Maxilla | 3.2 |
| 3 | 60/M | Maxilla | 2.9 |
| 4 | 11/F | Maxilla | 0.5 |
| 5 | 9/F | Maxilla | 2.4 |
| 6 | 41/F | Mandible | 3.2 |
| 7 | 40/M | Mandible | 2.8 |
| 8 | 30/F | Mandible | 6.5 |
| 9 | 41/M | Maxilla | 1.5 |
| 10 | 56/M | Maxilla | 0.3 |
| 11 | 12/M | Maxilla | 0.0 |
| 12 | 42/M | Maxilla | 2.2 |
| 13 | 35/F | Mandible | 1.1 |

F: Female; M: Male

**Table 2:** Distribution of CD1a-labeled Langerhans cells in irritation fibroma

| *Case no.* | *Age/gender* | *Location* | *Mean CD1a-positive cells/HPF* |
|---|---|---|---|
| 1 | 20/M | Maxilla | 2.5 |
| 2 | 40/M | Maxilla | 1.5 |
| 3 | 30/M | Maxilla | 3.7 |
| 4 | 37/M | Maxilla | 1.5 |
| 5 | 32/F | Mandible | 2.3 |
| 6 | 65/F | Maxilla | 0.0 |
| 7 | 18/F | Mandible | 0.0 |
| 8 | 76/M | Maxilla | 1.0 |
| 9 | 8/M | Maxilla | 1.6 |
| 10 | 35/M | Maxilla | 0.0 |
| 11 | 28/M | Mandible | 6.2 |
| 12 | 30/F | Mandible | 1.7 |
| 13 | 29/F | Maxilla | 0.0 |
| 14 | 37/F | Mandible | 3.5 |
| 15 | 80/F | Mandible | 0.9 |
| 16 | 38/F | Mandible | 0.0 |
| 17 | 44/M | Maxilla | 0.0 |
| 18 | 34/M | Maxilla | 0.0 |
| 19 | 19/M | Mandible | 6.1 |

M: Male; F: Female



**Figs 5A and B:** CD1a-positive Langerhans cells clustered near the tip of connective tissue papilla (polymer labeling method: 450×)

**Table 3:** Mean number of CD1a-labeled cells/high-power field in the groups studied

| *Groups* | *Mean CD1a-labeled cells in lesions examined (cells/HPF)* |
|---|---|
| Control (n = 32) | 2.24 ± 1.11 |
| Irritation fibroma group (n = 19) | 1.71 ± 1.96 |
| Pyogenic granuloma group (n = 13) | 2.39 ± 1.80 |

irritation fibroma group, the mean number was as 1.71 ± 1.96 cells/HPF.

## DISCUSSION

The stratified squamous epithelium of oral mucosa is important as a physical barrier against antigens. Langerhans cells are antigen presenting cells capable of priming naïve helper/cytotoxic T cells to undergo clonal expansion, thus initiating an adaptive immune response.[7] Quantity of Langerhans cells in gingival epithelium has been shown to decrease with progression from gingivitis to periodontitis.[8] Their lower number in periodontitis may decrease the defense response to periodontal bacteria resulting in sustained immune response and tissue injury.[7] Gingival enlargement as an effect of various medications has been attributed to the altered tissue homeostasis resulting from altered cytokine profile, due to reduced number of Langerhans cells.[6]

Results of the present study show that gingival pyogenic granulomas and irritation fibromas possess Langerhans cells in epithelium. Their number in these lesions show wide range and there were no significant differences between mean numbers of Langerhans cells between control group and study group as well as between subgroups of the study group.

It is evident that Langerhans cells increase, i.e. recruited or proliferate in response to antigenic exposure.[5,9,10] Epicutaneous exposure to antigen downregulates E-cadherin expression on the surface of Langerhans cells, thus favoring their migration toward lymph nodes.[1] Being a condition resulting from exaggerated immune response to antigens, it is possible that pyogenic granuloma may show increased numbers of Langerhans cells in initial stages. Density of Langerhans cells may decrease or become normal after their migration toward connective tissue and lymph nodes. Reduced number of Langerhans cells alters cytokine profile which alters Th1/Th2 ratio, resulting in altered tissue homeostasis, which may be responsible for fibrosis.[6] This may explain fibrous maturation of long standing pyogenic granulomas.

Some lesions first present as pyogenic granulomas which subsequently may undergo fibrosis. In contrast to this, some lesions directly go through hyperplasia of connective tissue cells and fibers resulting in irritation fibroma. Thus, immunological response of host tissue and nature of antigenic material may affect the density of Langerhans cells and the course of a reactive process in different persons. Further investigations are warranted to assess the presented concepts.

Thus, the role of Langerhans cells in irritation fibromas remains to be elucidated. The variation in number of oral epithelial LCs in different lesions may either result from different antigens eliciting different responses or merely reflect different responses against similar antigens.[9] As suggested by Seguier et al, the presence of distinct subsets of intraepithelial lymphocytes could control the supply of LCs and thus maintain the immune response or could reduce the number of LCs in order to prevent overstimulation of the immune system.[11]

Physiological variation in density of Langerhans cells do exist. Numbers of LCs vary from one oral focus to another and from one patient to another.[12] Their density changes markedly with aging and oral habits, which may affect the results in some cases.[13-15]

Results of the present study show that there is wide range in density of Langerhans cells in gingival pyogenic granuloma and irritation fibroma lesions. Studies on larger samples with longitudinal study design are required which can significantly improve our knowledge about immunological mechanisms involved in these lesions. Furthermore, studies with categorization and selection of cases of reactive lesions in various stages, such as lesions under initial phase, long standing or recurrent lesions, pyogenic granuloma lesions occurring in hormonally primed gingiva and other reactive lesions of gingiva, are needed to explain the role played by Langerhans cells in reactive lesions of gingiva.

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