



Deterioration of the Langerhans cell network of the human gingival epithelium with aging

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Summary Dendritic cells (DCs) are the professional antigen-presenting cells responsible for initiating of the immune response. Langerhans cells (LCs) are a type of DC that is a permanent resident of the oral epithelium. LCs are organized conforming a network in such a way as to maximize their surface area for efficient apprehension of antigens. To detect age-related changes in the LCs network, fragments of gingival epithelium spontaneously accompanying dental removals were processed by immunohistochemistry. Monoclonal antibody CD1a followed by biotinized immunoglobulin–streptavidin peroxidase were used to identify the LCs with the light microscope. LC density and LC types were analyzed according to their morphology and intraepithelial distribution. In the older age group (61–74 years) the density was significantly lower than in the younger age groups. Morphologically, LCs showed fewer dendritic-branching processes and had a rounded shape in the older age group.

Present observations indicate that the LC network changes markedly with aging. These results suggest that immunological defense of the oral tissue might be compromised in old age.

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Introduction

The oral mucosa is covered with a stratified epithelium formed by keratinocytes, Langerhans cells, lymphocytes and Merkel cells. The Langerhans cells (LCs) are representatives of dendritic cells lineage situated in the stratum spinosum of stratified squamous epithelia including that lining the mouth.

These dendritic cells belong to the family of immune cells able to capture intraepithelial pathogens and migrate to regional lymph nodes where they present them to naive T cells.¹

Quantitative differences in dendritic cells and a decline in T cell reactivity with aging have previously been reported as causes of decreased immune response to foreign antigens.²

An age-related decrease in human LC density has been demonstrated in the epidermis³ and in mucosal tissues such as conjunctival⁴ and human tonsil

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epithelium.⁵ However, few studies have examined age-related changes in the LC density of human oral epithelium.

The density of LC in the epithelium is not the only important factor in effective mucosal immunity. LCs are organized in such a way so as to maximize their surface area for efficient trapping of antigens.⁶ A comprehensive LC network is essential for the induction of an immune response to trap oral antigen.⁷ The purpose of this study was to evaluate age-related changes in the LC network of human gingival epithelium. In order to evaluate the gingival LC network, the morphology, density and intraepithelial distribution of LCs were examined in different age groups.

Material and methods

Twenty-seven fragments of gingival mucosa, which would otherwise have been discarded, were obtained after necessary tooth removal for orthodontic or prosthodontic reasons with the approval of the ethics committee of Cuyo Medical School, Mendoza, Argentina. Samples were taken only from the buccal surface of marginal gingival in order to avoid topographical variations.⁸ Only tissue from individuals with no evidence or history of mucosal disease was included.

Biopsies had been fixed in buffered 4% paraformaldehyde, processed and embedded in paraffin wax. Embedded tissue was oriented such that 7 μ m sections could be cut at 90° to the epithelial surface. Microscopic examinations were done and when there were pathological lesions, the sample was excluded. Thus, the total number of gingival tissue specimens studied was 25. According to age (14–74 years old), each gingival sample was included in one of the following groups: (a) group 1: 14–39 years ($n = 8$), (b) group 2: 40–50 years ($n = 7$), (c) group 3: 51–60 years ($n = 5$), and (d) group 4: 61–74 years ($n = 5$).

Immunohistochemical staining

LCs were identified with a monoclonal mouse anti-human CD1a (Immunotech, Marseille, France).⁹

Serial 7 μ m paraffin sections were desparaffinized in xylene and hydrated in graded ethanol concentrations to distilled water. Immunohistochemistry was performed using a streptavidin-biotinylated-immunoperoxidase reaction as described by Hsu et al.¹⁰ Endogenous peroxidase activity was blocked by immersion of the sections in 0.5% hydrogen peroxide and methanol for 5 min. The sections were incubated with the primary antibody CD1a

(1/100) for 30 min at room temperature. After washing, all sections were incubated in a 1/200 dilution of biotinylated anti-mouse immunoglobulin (DAKO) for 30 min and then with a streptavidin–biotin–peroxidase complex (30 min). CD1a positive cells were visualized after incubation in a solution of fresh 3-diaminobenzidine/hydrogen peroxide for 2 min. The sections were counterstained with Harris's haematoxylin.

After immunostaining, the gingival sheets were mounted and viewed with a Nikon UFX 2IIA light microscope.

Digital photographs of the CD1a+ LCs were taken using a networked Nikon DN100 Digital camera.

In order to identify different LCs, we considered two types of cells in relation to cytoplasmic processes in each group. Those cells with 1 or more branched-cytoplasmic-processes, which we call (type 1 cells) and those without branched-cytoplasmic-processes (type 2 cells). Intraepithelial dendritic cells containing dark brown reaction products were identified as CD1a+ Langerhans cells and their density was expressed in line with Sasaki, as the cell number per mm² of epithelial section area.¹¹ The mean values and standard deviation were computed for each group.

As negative control, the primary antibody was substituted with normal rabbit serum used at 1/100 dilution.

Statistical analysis

Two-way ANOVA was used for statistical analysis (GraphPad Prism Software). If ANOVA analysis indicated a significant difference ($P < 0.05$) among the groups, then the Tukey's multiple comparisons test was used.

The analysis of digital images was performed using the public domain Scion Image developed at the US National Institutes of Health and available on the internet at: <http://www.rsb.info.nih.gov/nih-image>.

Results

Langerhans cell density according to morphology

Immunohistochemical examination revealed numerous clearly defined CD1a positive dendritic cells (LCs). These dendritic-shaped CD1a+ cells were observed in the gingival epithelium and no positive cells were found in connective tissue. Intraepithelial LCs presented a brown-dark stained body with cytoplasmic processes extending among keratinocytes

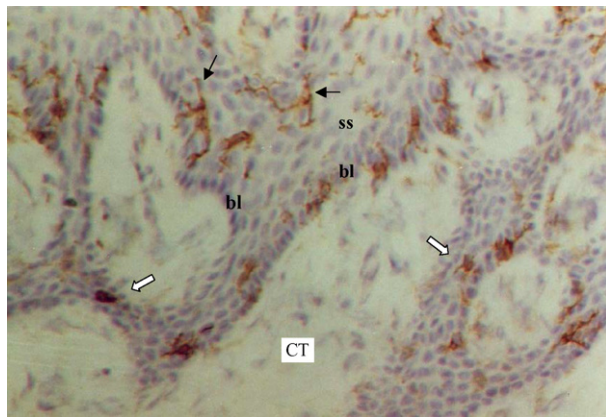


Figure 1 Immunohistochemical stain with anti CD1a monoclonal antibody. Numerous type 1 branched-Langerhans cells (black-arrows) can be observed in the gingival epithelium of an 18 year-old-woman. White arrows indicate less branched-type 2 LCs. Notice the absence of stained cells in connective tissue (CT) and both LCs types located in basal layer (bl) as well as in stratum spinosum (ss) (100 $\times$).

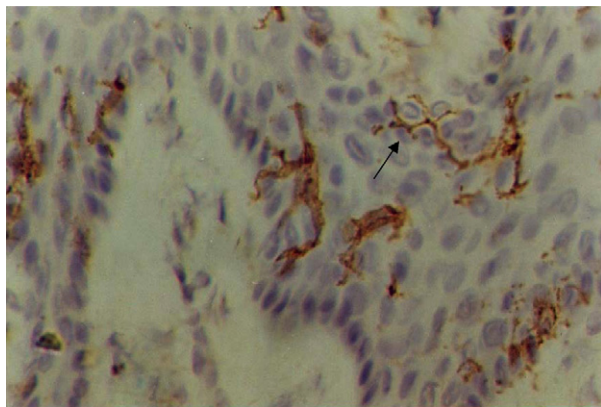


Figure 2 Higher magnification of type 1 cell (18 year-old-woman, belonging to group 1) to show the branched LC processes (arrow) (400 $\times$).

(Fig. 1). Type 1 LCs, almost always presented pyramidal shaped bodies with long and branched processes (Fig. 2) while the type 2 cells displayed a rounded cell appearance with shorter and scarce branching dendritic processes as shown in Figs. 1 and 7.

The mean values of type 1 and 2 CD1a+ cell in each age group are shown in Table 1. In the young age group (14–39 years) 70% of the CD1a+ cells found were type 1 cells. Inter-group comparison revealed that the density of type 1 CD1+ cells in the group 1 (14–39 years) was significantly higher than all other age groups ($P < 0.001$).

Although type 2 Langerhans cells were found in all the studied groups, only in the group of older age (61–74 years) they turn out to be a majority.

There was not a significant difference among the values for type 2 CD1a+ cells in all age groups (inter-group analysis, $P > 0.05$). However, intra-group analysis indicated that in the oldest group (61–74 years) 69% of the cells belonged to type 2 CD1a+.

Considering the total number of both cellular types, the healthy gum epithelium in young samples (14–39 years-old group) displayed 25 ± 3 (mean $\pm$ S.D.) LC/mm², a density that decreases with aging.

The density was significantly lower in the 61–74 year-old group (7 ± 2.9 LC/mm²; mean $\pm$ S.D.) compared to any of the 14–39 ($P < 0.001$), 40–50 ($P < 0.001$) and 51–60 ($P < 0.05$) year old groups.

Intraepithelial distribution

Histomorphometric analysis was done on epithelial thickness of the gingival mucosa with a computer-supported image processing system. We considered three gingival epithelium zones, the stratum basale, the stratum spinosum, and a third zone which included the stratum granulosum. The results and statistical analysis are shown in Table 2.

Although, in young individuals (groups 1 and 2), most of LCs were located in the suprabasal layer (stratum spinosum) ($P < 0.001$, intra and inter group statistical analysis) we found numerous cells in the basement membrane closely related and also in the superficial layers (stratum granulosum) with long cytoplasmic processes (Fig. 3). Although LC bodies were located in the stratum granulosum, we found their LC processes in the stratum corneum usually directed towards the epithelial surface (Fig. 4). In group 3 (51–60 years) and elderly individuals of group 4, LCs were located basically in spinosum strata (Figs. 5 and 6). The density of CD1a+ cells

Table 1 Gingival epithelium, type 1 and 2 CD1a+ Langerhans cells density in different age groups (expressed as cell/mm²)

Cell type	Group 1 (14–39 years)	Group 2 (40–50 years)	Group 3 (51–60 years)	Group 4 (61–74 years)
1	17.9 ± 2.6 (n = 8)	13.7 ± 2.4 (n = 7)***	7.6 ± 1.8 (n = 5)***	2.2 ± 0.4 (n = 5)***
2	7.1 ± 2.5 (n = 8)	8.4 ± 1.7 (n = 7)	5.8 ± 1.3 (n = 5)	4.8 ± 2.6 (n = 5)
Total	25 ± 3.0 (n = 8)***	22.1 ± 3.4 (n = 7)***	13.4 ± 2.9 (n = 5)**	7.0 ± 2.9 (n = 5)

Means values are representatives of the means $\pm$ S.D. Statistical significance level: ** $P < 0.01$ and *** $P < 0.001$.

Table 2 CD1a+ LCs density in different epithelial gingival layers

Layer	Group 1 (14–39 years) <i>N</i> = 8	Group 2 (40–50 years) <i>N</i> = 7	Group 3 (51–60 years) <i>N</i> = 5	Group 4 (61–74 years) <i>N</i> = 5
Stratum basale	9.6 ± 1.4	8 ± 2.1	1.2 ± 1.3 ^{***}	1.0 ± 1 ^{***}
Stratum spinosum	18 ± 2.5 ^{***}	17 ± 2.6 ^{***}	8.6 ± 2.0	7.2 ± 1.8
Stratum granulosum	3.75 ± 1.7	3.7 ± 1.7	0.2 ± 0.4 [*]	0.06 ± 0.1 [*]

Means values are representatives of the means ± S.D. *N*: number of patients.

^{*} Significant difference ($P < 0.05$) compared to group 1.

^{***} Significant difference ($P < 0.001$) between the groups 1–2 and 3–4.

in basal and granulosum layers was significantly lower compared to same layers of groups 1 and 2 ($P < 0.001$ and < 0.05 , respectively).

In all examined groups most of stratum basale-LCs were seen around connective tissue papillae (Fig. 7).

No differences between male and female were detected.

Discussion

There is strong evidence showing LCs-density variation in relation to age, sex and different body regions both in humans and animals^{12–14} although Mathur et al., using ATPase staining, did not find significant difference.¹⁵ LCs can be selectively visualized at the light microscopic level through

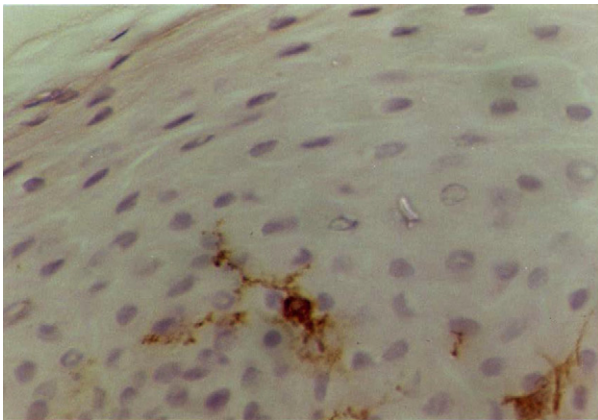


Figure 3 Type 1 LC of a 17 year-old-woman projecting branched dendritic processes between the keratinocytes (400×).

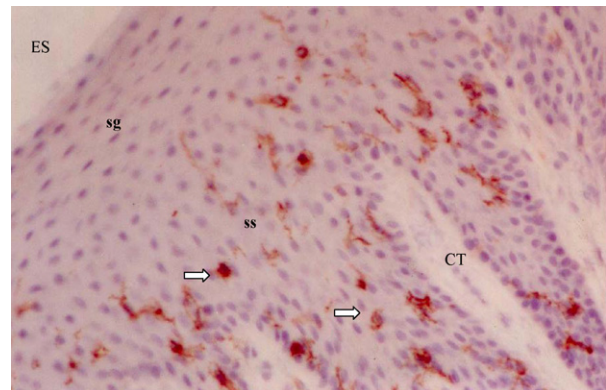


Figure 5 Section of gingival epithelium from a patient of group 2 (40–50 years) showing numerous CD1a+ cells. White arrows indicate some rounded LCs (type 2) located in the stratum spinosum (ss) (100×). ES, epithelial surface; CT, connective tissue; sg, stratum granulosum.

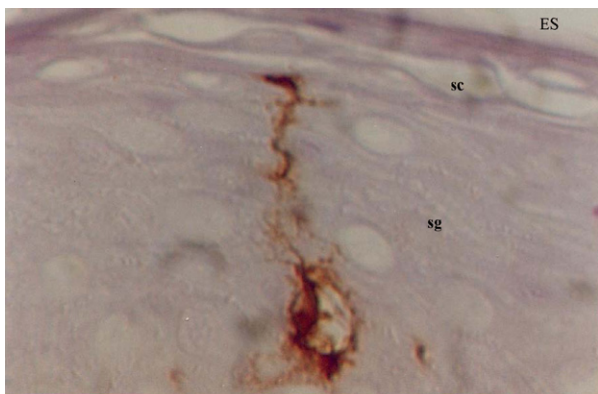


Figure 4 Another example of type 1 LC in which the body is located in the stratum granulosum (sg) with a long process directed to the epithelial surface (ES) (400×).

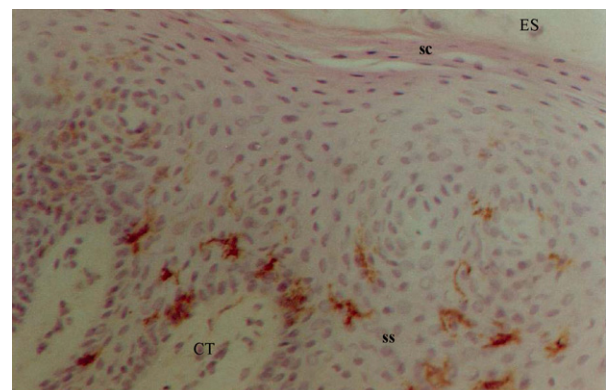


Figure 6 Light micrograph representative of a group 3 (51–60 years) showing numerous CD1a+ cell preferably located in suprabasal layers (stratum spinosum) (100×).

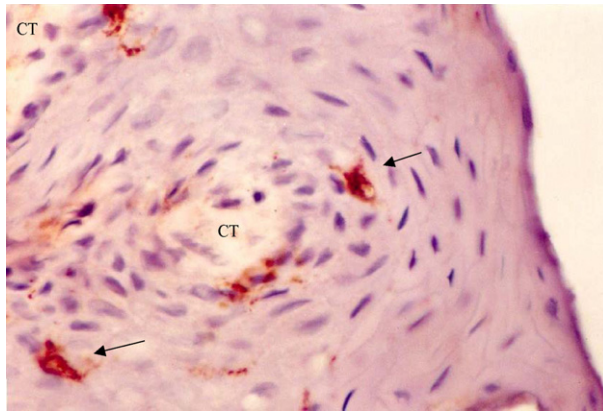


Figure 7 Sixty-one-year-old man (group 4). The gingival epithelium shows scarce and round-shaped type 2 LCs (400 $\times$).

histochemical procedures and, most importantly, through immunolabeling ones. Among the histochemical reactions, the demonstration of ATPase activity is an excellent method to detect LCs but its specificity has been questioned.¹⁶ The demonstration of ATPase activity in cells clearly varies depending upon the technique employed. This could explain the different results obtained. Anti-CD1a and anti Langerin/CD207 immunolabeling are considered the most reliable assays to identify intraepithelial LCs.^{9,17}

Many studies suggest that the immune system weakens with aging^{18,19} and that might be related to a decrease in the number or reactivity of immune cells such as lymphocytes or dendritic cells (Langerhans cells).

An age-related decrease in epidermal LC density was found in mice skin by Choi and Sauder and Sprecher et al.^{20,21} In human skin, Gilchrist et al. and Thiers et al. reported a reduction in LC density associated with aging.^{22,23} However, there have been few studies of human mucosal epithelium. Sasaky, for example (1994) reported an age-related decrease of the LC density in the lingual epithelium from cadaver samples.¹¹ In agreement with these findings, in previous studies, we found an age-related decrease in the LC density in the human gingival epithelium.²⁴

In addition, this data suggests that the immunological defenses of the skin and oral mucosa decrease with age.

Dendritic LCs resident in stratified squamous epithelia are highly effective at capturing and processing foreign antigens. In the junctional gingival epithelium, Lombardi et al. had described two LC types. The first type, with a spherical morphology, is weakly stained for CD1a (T6). The other type exhibits dendrites of moderate length and number with a

varied fluorescence intensity against CD1a (T6) antigen.²⁵ On the other hand, Figueroa and Caorsi described, in human exocervix, different LC types in relation to the cytoplasmic processes. They suggested that the different types of LCs represent different degrees of activity, those with the largest number of processes might display a high antigen-binding activity.²⁶

In agreement with Lombardi et al., we distinguish two LC types but we were not able to find staining differences with the immunohistochemical technique employed. Besides in the study, we observed that most of the cells belonged to LCs type with long and branched cytoplasmic-processes (dendrites-like). The present data suggests that this situation seems to differ drastically in older people where the LC dendrites were shorter and with less ramified processes. In agreement with Figueroa and Caorsi our findings may show that antigen-binding activity of LCs in gingival epithelium could deteriorate with aging.

Although LCs have been described as located basically in the suprabasal (stratum spinosum) region of stratified epitheliums, our data is consistent in demonstrating that type 1- branched-LCs of the young individuals were more spread than those of the elderly. They were present between basal keratinocytes as well as in the stratum spinosum even in the epithelial superficial layers. Only in the elderly LCs appeared exclusively in the suprabasal region of gingival epithelium. According to the specific LCs location in the epithelium it could be speculated that the immuno-surveillance ability of the whole LCs set is less active in elderly people.

Spatially, LCs are distributed so as to form a dendritic-intraepithelial network. Considering the density, the number of cytoplasmic branched extensions and the cellular distribution, as a whole, our findings show that the cells shape a network within the whole thickness of the epithelium, especially in the youngest patients. With age, the reduction in the cell number, the most scarcely branched extensions as well as narrower intraepithelial distribution, suggest an age-related deterioration of the cellular network, which would mark the limited possibility to take contact with new antigens.

Dewar et al. has shown, in mice, that when the neonatal LC network is poorly developed due to shorter dendritic-like processes, it is functionally immature and the antigen applied through this "functionally immature network" results in antigen specific immunosuppression.²⁷ According to our findings the damaged network described in this report might behave as a network of immature cells as described by Dewar.

On the other hand, several studies have also demonstrated that exposure to antigen in mucosal

sites can result in the production of secretory IgA in other mucosal tissues.^{28,29} These migratory pathways constitute a common mucosal immune system. Following this line of reasoning a deterioration in the LC network in human gingival epithelium with aging, as demonstrated here, could be related to a decrease in the immunological defenses in other mucosal sites such as the intestine or urinary system. Although the present results may help to explain the age-related decline of the immune system, clearly more studies of antigen presenting cells are necessary to understand the impact of aging in oral tissues.

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