

# Immunohistochemical Analysis of S100-Positive Langerhans Cells in the Healthy Gingiva and Periodontal Disease

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*Langerhans cells are antigen presenting cells, located in the stratified squamous epithelium of the skin and oral epithelium. A high density of Langerhans cells in gingival epithelium associated with an inflammatory process has been demonstrated in the literature by numerous studies, although the relationship with inflammation was less investigated. The aim of our study was to evaluate the distribution, density, morphology of Langerhans cells in both gingival epithelium and the associated inflammatory infiltrate in the lamina propria of the gingiva. The relationship between Langerhans cells and inflammatory process was also assessed. The present study included a number of 51 gingival biopsies. 12 of these didn't show significant changes on routine examination. In all of the other 39 cases the presence of inflammatory lesions was noticed. The inflammatory infiltrate was described by using hematoxylin eosin staining and quantitative score, with values between 0 and 3. Langerhans cells were immunohistochemically highlighted by using S100 antibody and quantified with hot spot method. We found an increase of Langerhans cells density in all cases of inflammation as compared with healthy gingival tissue. The highest density was found in the 13 cases with severe inflammation. At these last cases was noticed a migration tendency of Langerhans cells from infiltrate inflammatory area to basal layer of the epithelium. The results of this study indicated that Langerhans cells number is higher in inflammatory gingival tissue comparative with normal tissue, and the density of these cells is in relation with the severity of the inflammatory process.*

*Keywords: Langerhans cells, antigen presenting cells, S100 protein, human gingival epithelium*

All covering and lining tissues contain various numbers of non-epithelial immune-competent cells. In stratified squamous epithelia, the prominent immune reactive cell is the Langerhans cell [1].

Langerhans cells were described by Paul Langerhans in 1868, as the cell with dendritic morphology, located in the stratified squamous cornified epithelium of the skin. Langerhans cells were found later in all stratified epithelia. Langerhans cell body is normally located in the suprabasal layer of the epidermis and oral epithelium, but branched processes extend in both superficial and deep directions [2]. The Langerhans cells originate from the bone marrow and then migrate into the epithelium to perform the function of antigen recognition and presentation [3].

In 1985, DiFranco and collaborators [4] demonstrated by immunohistochemistry with HLA-DR, S100 and OKT6 antibodies, the presence of Langerhans cells in the gingival epithelium, both clinically healthy and inflamed gingiva. Many data showed that Langerhans cell density varies with age and location [5]. A study of Katz *et al.*, in 1979 [6], suggests that the origin of Langerhans cells is to the bone marrow. From the bone marrow they migrate into the epithelium, where they arrive at seven weeks of gestation [7]. In epithelium, they function as antigen presenting cells and play an important role in the local defense mechanisms.

Lombardi and collaborators described two types of Langerhans cells according to their morphology. Langerhans cells, type I are pyramidal in shape and are located in the suprabasal layer of the epithelium. The type

II Langerhans cells are spherical and are present in the basal layer of the epithelium [8].

A high density of Langerhans cells in gingival epithelium associated with an inflammatory process has been demonstrated by numerous studies in the literature. Experimental studies in mice have shown that the number of Langerhans cells in gingival epithelium increased with the development of the inflammatory process [9,10]. Langerhans cell density is significantly increased in gingivitis compared with healthy gingiva. These data suggest that Langerhans cells initiates the development of lymphoid follicles in the oral mucosa as a response to pathogen-associated molecular patterns [11,12]. Few data are available on the S100- positive dendritic cells in the lamina propria of gingiva in patients with periodontal disease.

The aim of our study was to evaluate the distribution, morphology and density of Langerhans cells in both the gingival epithelium and the associated inflammatory infiltrate in the lamina propria of the gingiva. The relationship between the previous parameters of Langerhans cells and inflammatory infiltrate was also analyzed.

## Experimental part

### Material and method

Our study includes a total of 51 gingival biopsies taken from patients with age between 6 and 80 years. 12 cases showed no significant changes in the gingiva, and in the remaining 39 cases, the inflammatory lesion was noted by clinical exam. The specimens were fixed in 10% buffered formalin for 48 h and paraffin embedded. Five

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mm thick step sections were performed for each case. Slides from each case were stained with hematoxylin-eosin method, for the pathological diagnosis. We performed an enzyme pretreatment (Bond Enzyme Pretreatment Kit, Leica Biosystems, Newcastle upon Tyne, UK) for 10 minutes. Endogenous peroxidase blocking was realized with 3% hydrogen peroxide for 5 minutes. This step was followed by incubation for 15 minutes with S100 protein (Novocastra, Newcastle upon Tyne, UK, rabbit polyclonal, ready to use) as primary antibody. The Bond Polymer Refine Detection System (Leica Biosystems, Newcastle upon Tyne, UK) was used for visualization. 3, 3'-diaminobenzidine dihydrochloride was applied as chromogen and hematoxylin was used for counterstaining. The entire immunohistochemical procedure was developed with Leica Bond-Max (Leica Biosystems, Newcastle upon Tyne, UK) autostainer.

Image acquisition and analysis were performed using Nikon Eclipse E600 microscope and Lucia G software for microscopic image analysis. The local research ethics committee approved the protocol of the study and informed consent was obtained from all subjects according to the World Medical Association Declaration of Helsinki.

The inflammatory infiltrate of the gingival tissue was scored as 0 (absent), +1 (isolated inflammatory cells, less than 10/x400), +2 (aggregates of inflammatory cells in the lamina propria only), and +3 (aggregates of inflammatory cells in the lamina propria associated with intraepithelial lymphocytes). Quantification of Langerhans cells in the lamina propria and epithelium was made by hot spot method. Three hot spot areas with high density of Langerhans cells were chosen at low power magnification. The Langerhans cells were counted at x400 magnification in the epithelium and inflammatory infiltrate from the lamina propria. The arithmetical media of the three hot spots was the final result.

Clinical criteria to evaluate inflammatory changes, according to Loe and Silness [18], were the following: stage 0: apparently healthy gingiva; stage 1= mild inflammation, discrete change of color and texture (slightly edema) without bleeding to the touch with periodontal probe; stage 2= moderate inflammation, erythema, edema, swelling which gives a glossy aspect to gingiva, bleeding to the pressure with periodontal probe; stage 3= severe inflammation, marked redness and edema, tendency to spontaneous bleeding, some ulcers.

## Results and discussions

The absence of inflammatory infiltrate was noticed in 12 of the 51 cases included in our study. In these cases, Langerhans cells were noticed in small numbers to the epithelium, in the stratum spinosum. In 39 cases we noticed the presence of inflammatory infiltrate constituted mostly by lymphocytes. The neutrophilic granulocytes and macrophages, few plasma cells and eosinophilic granulocytes were also present. In the cases of severe inflammatory infiltrate we noticed, as a particular aspect, the presence of epithelial cells islands in the lamina propria. Focal necrosis was found in the cases with high-density of neutrophils only.

Thus from the 51 cases included in our study, we identified the inflammatory score 0 in 12 cases, score 1 (17 cases), score 2 (9 cases) and score 3 (fig. 1) in 13 cases. A correlation between the clinical criteria and microscopic description of inflammatory infiltrate was also noticed.

The morphology, density, and distribution of Langerhans cells in both gingival epithelium and inflammatory infiltrate of the lamina propria were identified by

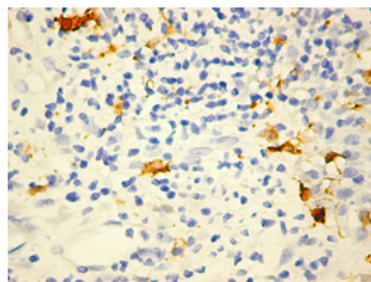


Fig. 1. Inflammatory infiltrate, score 3, numerous Langerhans cells. S100 immunostaining, ob. X 20

immunohistochemical technique, for each case included in our study.

In stratified squamous parakeratinized epithelium of the gingiva, Langerhans cells were distributed in both, the spinous and the basal layer. The morphology of Langerhans cells in the two layers of the epithelium was different. Thus, the Langerhans cells from the basal layer of the epithelium are round, almost spherical in shape and cytoplasmic extensions are few and short (fig. 2).

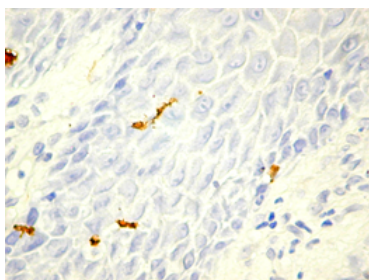


Fig. 2. Langerhans cells from the basal layer of the epithelium with round shape, S100 immunostaining, ob. X 40

Another issue in these cases was related to the morphology of Langerhans cells in the inflammatory infiltrate. Besides the two shapes, round and pyramidal, previously described a spindle shape was observed. The Langerhans cells found in the spinous layer of the epithelium are pyramidal in shape with long cytoplasmic extensions which insinuated through epithelial cells (fig. 3).

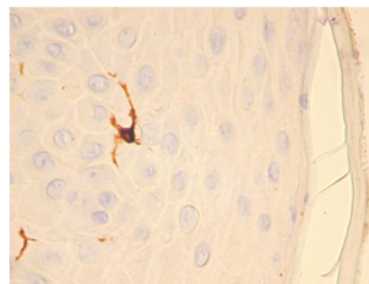


Fig. 3. The Langerhans cells present in the spinous layer of the epithelium are pyramidal in shape with long cytoplasmic extensions. S100 immunostaining, ob. X 40

The inflammatory infiltrate in the lamina propria contains round and pyramidal shaped Langerhans cells with extensions that insinuates among inflammatory cells. The pyramidal Langerhans cells from the inflammatory infiltrate showed longer and more numerous cytoplasmic extensions than those of round shape.

We found differences in the density of Langerhans cells depending on the location and severity of inflammatory infiltrate. Thus, in the cases where the inflammatory infiltrate was located at a distance from epithelial tissue, the number of Langerhans cells in the lamina propria and intraepithelial was significantly lower as compared to cases with inflammatory infiltrate located near the gingival epithelium. In these cases 2-3 Langerhans cells / field in the inflammatory infiltrate of the lamina propria and 4-5 Langerhans cells / field in the epithelium were noticed. In the epithelium they were distributed predominantly in the intermediate layer and occasionally in the basal layer. In all cases of the inflammatory infiltrate located near the gingival epithelium, the number of Langerhans cells was



higher in both the epithelium and inflammatory infiltrate regardless its score.

In the cases with severe inflammatory infiltrate and subepithelial localisation, an increased number of Langerhans cells was noticed just below the epithelial basal membrane. In these cases 14-16 Langerhans cells / field were counted. The number of Langerhans cells decreases as we move away from the epithelium. Thus in areas with inflammatory infiltrate located at distance from the epithelium an average of 2-3 Langerhans cells / field was counted. In all cases with severe and subepithelial inflammatory infiltrate, the number of Langerhans cells in the basal layer was significantly higher than in the cases where the inflammatory infiltrate was minimal or located away from the epithelium. In these cases 12-13 Langerhans cells / field in the basal layer, with round shape all of them, and few cytoplasmic extensions were found. The Langerhans cells were observed in the spinous layer, but less than in the basal layer.

In the cases with severe inflammation we noticed Langerhans cells at the interface between epithelium and lamina propria. These could explain the high density of Langerhans cells in the epithelium (fig. 4).

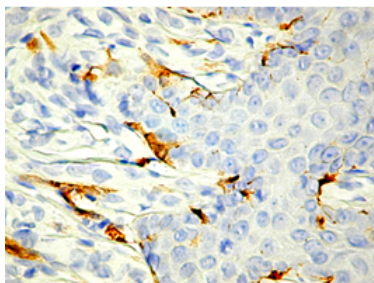


Fig. 4. The tendency of Langerhans cells to migrate from the inflammatory infiltrate to the basal layer of gingival epithelium in cases with severe inflammation. S100 immunostaining, ob. X 40

In one case we noticed more intraepithelial Langerhans cells and only few in the subepithelial inflammatory infiltrate.

We found no differences in the density of Langerhans cells in relation with sex and age of patients.

Morphological description of the Langerhans cells found in the epithelium, noticed also in the cases included in our study is according with those made by Lombardi and colleagues in a study from 1993 [14].

Langerhans cells have dendritic morphology and are located in stratified squamous epithelium of the skin, oral mucosa, gastrointestinal tract and female genital tracts, where they form about 2-8% of the epithelial cell population [14]. Although they are located in the epithelium, it has been shown to be a dynamic cell population, derived from the bone marrow [6]. Our study proved the presence of S100 positive Langerhans cells in both healthy and inflammatory gingival tissue. Data from the literature showed a high number of Langerhans cells in the cases of inflammatory processes in the oral cavity (gingivitis, periodontitis, stomatitis), and in the cases of malignant tumors in the same area also.

In the cases of oral cancer it was observed an increased number of Langerhans cells, macrophages and CD8+ lymphocytes in the oral epithelium [15, 16]. In squamous cell carcinomas a strong relationship between the Langerhans cells and lymphocytes was found, both in terms of numbers and their distribution in the epithelium. These observations suggest that in the oral squamous cell carcinoma the Langerhans cells present tumor antigens to lymphocytes and play a similar role as in the case of allergic reactions [17]. Recent data also suggests that mast cell accumulation at the periphery of tumor areas and the release of proangiogenic factors may represent a tumor-

host interaction that probably favors tumor progression [18].

The number of Langerhans cells in gingival tissue is a controversial topic. Thus there are studies in the literature that reported an increase in the Langerhans cells number in periodontitis, others showed a decrease of the Langerhans cells number in gingivitis and periodontitis comparative to healthy tissue [19]. Some studies showed that there are no quantitative differences in gingivitis compared to healthy periodontal tissue [19]. In our study we noticed an increase of Langerhans cells number in both, epithelium and inflammatory infiltrate in all cases, regardless of the inflammatory process severity. The highest number was noted in the cases with severe inflammation, where the inflammatory infiltrate was located close to the epithelium.

It was shown that the number of Langerhans cells is increased in gingivitis comparative to periodontitis and suggest that numerical differences contribute to the different response of the host to these disorders [20]. TNF alfa is an eloquent test for cytotoxic detection that can early detect signs of inflammation by measuring the endotoxine from the serum [21].

Some datas showed that gingival epithelial Langerhans cells are distributed in the basal and suprabasal layer from both healthy and inflammatory gingival tissue [22,19]. The same location was noticed in the cases included in our study, but in addition we found the Langerhans cells in inflammatory infiltrate.

On the other hand, Derek et al.[23] suggests that the increase of Langerhans cells number in the gingival epithelium is the result of their migration from the lamina propria, probably as a response to a high bacterial aggression. We noticed a tendency of Langerhans cells to dispose at the interface between epithelium and lamina propria, fact that could explain the high density of Langerhans cells in the epithelium. This aspect was found in the cases of severe inflammation (score +3) and when the inflammatory infiltrate was arranged immediately below the basal membrane of epithelial tissue only. In the cases of moderate or low inflammation, we did not notice the disposition of Langerhans cells at the border between epithelium and lamina propria.

It has been shown that to both, humans and animals Langerhans cell density varies according to age, sex and anatomical region [24, 25, 26]. In our study we observed no significant difference in the density of Langerhans cells by age and sex, according with Mathur et al. [27]. A decrease of Langerhans cell density with age, was reported in skin [28, 29] and in the oral epithelium. A reduced number of Langerhans cells in the lingual epithelium from cadavers [30] and in human gingival epithelium was found [31, 32].

According to Lombardi et al. [8], we noticed in our study two types of Langerhans cells: pyramidal shape cells in the parabasal layer and round shape in the basal layer of the epithelium. Besides the two types previously mentioned it was noticed that Langerhans cells with spindle morphology were present in the epithelium and inflammatory infiltrate also.

Langerhans cells with more numerous and longer cytoplasmic extensions were described in the exocervix epithelium and suggest that those cells are more active [33]. Compared to this type, we noticed different aspect of cytoplasmic extensions and cells morphology in the gingival epithelium.

## Conclusions

As a result of the our observational study we concluded that the number of Langerhans cells is higher in the

inflammatory gingival tissue compared to the number of Langerhans cells we found in the healthy one, but their density is directly related to the severity of the inflammatory process that affects the gingiva.

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