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Factor XIIIa+ dendritic cells and S-100 protein+ Langerhans' cells in adult periodontitis

X. E. Dereka[1], K. I. Tosios[2], E. Chrysomali[3], E. Angelopoulou[3]
Departments of [1]Periodontology, [2]Basic Sciences and Oral Biology, and [3]Department of Oral Pathology, School of Dentistry, University of Athens, Athens, Greece

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Objective: The purpose of this study was to evaluate the presence of factor XIIIa+ dendritic cells and S-100 protein+ Langerhans' cells in the gingival epithelium and connective tissue of periodontal pockets, before and after non-surgical periodontal therapy.

Background: The microbial flora in periodontal pockets provokes complex immune reactions. Dendritic cells play a critical role in primary and secondary immune responses and are considered as antigen-presenting cells. Factor XIIIa positive dendritic cells and S-100 protein positive Langerhans' cells identified by immunoreactivity against factor XIIIa antigen and S-100 protein, respectively, are two distinct subpopulations of dendritic cells.

Methods: Fifty-four gingival tissue samples were obtained from periodontal pockets of initial depth 4–5 mm and ≥6 mm. Each group was subdivided in to three subgroups. The first subgroup consisted of samples taken on baseline day and used as control. The second and third subgroups included those obtained 1 month after plaque and calculus removal, and 1 month after scaling and root planing, respectively, additionally to oral hygiene instructions. The tissues were removed from the palatal gingiva under local anaesthesia during routine periodontal surgery. Immunohistochemical staining with antibodies against factor XIIIa and S-100 protein was performed to identify dendritic cells positive and Langerhans' cells positive, respectively.

Results: Factor XIIIa+ dendritic cell numbers decreased compared to controls after plaque and calculus removal, oral hygiene instructions and scaling and root planing in periodontal pockets of 4–5 mm, but not in those of ≥6 mm depth. S-100+ Langerhans' cell numbers decreased after periodontal treatment in the periodontal pockets ≥6 mm.

Conclusion: These results may reflect a tendency for reduction of these two distinctive subpopulations of dendritic cells after non-surgical periodontal therapy.

Xanthippi E. Dereka, 110 Vas. Sofias Str., 11527 Athens, Greece
Tel./Fax: +03 210 7773038
e-mail: dereka@otenet.gr

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Dental plaque microorganisms can produce potent virulent factors capable of causing destruction of the periodontal tissues. Pathogens of the microbial plaque or their antigenic products may penetrate the gingival epithelium and contact immunoreactive cells in the connective tissue, eliciting defensive reactions. Major constituents of the gingival immune system that play a critical role in primary and secondary immune responses

are dendritic cells. Immature and mature dendritic cells are found in the diseased gingiva (1, 2). They act as antigen-presenting cells, which can uptake, process and present foreign antigens to T-lymphocytes (3), that in concert with cytokines are important mediators of the pathogenesis of periodontitis (4–8). At the same time, the cytokine microenvironment controls the differentiation, maturation and activation of dendritic cells (3, 9).

Dendritic cells are identified in lymphoid and non-lymphoid organs, epidermis, epithelium and oral mucosa including gingiva (9–11). Dendritic cells containing the protransglutaminase-clotting enzyme factor XIIIa (12) represent a specific subpopulation of dermal dendritic cells that are found in close association with blood vessels. Factor XIIIa positive (+) dendritic cells have been shown to participate in oral reactive and neoplastic lesions (13) and, as potent antigen-presenting cells, have been found in increasing numbers in various chronic inflammatory conditions, including aphthous ulcers (14). Changes in the size, shape and distribution of factor XIIIa+ dendritic cells, as well as their increased presence and localization at perivascular areas, could indicate their involvement in the local inflammatory mechanisms (14).

Langerhans' cells constitute a subset of dendritic cells originating from bone marrow (12). Langerhans' cells have been found in healthy and diseased gingiva (15). Their role in the cellular immune response in moderate gingival inflammation is critical (16) and an increase of their number in gingival epithelium has been associated with microbial plaque accumulation (17). They are identified by their immunoreactivity against S-100 protein and CD 1a antigen.

Little is known about the distribution in periodontal tissues of dendritic cells, in particular factor XIIIa+ dendritic cells and S-100 protein positive Langerhans' cells, their local differentiation pathway and their possible role in the progression of periodontal diseases. The purpose of this study was to evaluate the presence of factor XIIIa+ dendritic cells and S-100+ Langerhans' cells in the gingival epithelium and connective tissue of periodontal pocket walls before and after non-surgical periodontal therapy.

Materials and methods

Patients

Gingival tissue samples ($N = 54$) were obtained from patients with adult periodontitis undergoing periodontal surgery. None of the patients had systemic diseases or any overt immunological abnormality; they had not been on any kind of medication and had not received any periodontal treatment during the last 6 months. The periodontal disease status was determined by probing depth, gingival index and radiographic examination.

According to the pocket depth during the initial clinical examination, two experimental groups were formed: 4–5 mm ($N = 18$) and ≥6 mm ($N = 36$). Each group was subdivided into three subgroups. The first subgroup consisted of samples taken during the first clinical examination (on baseline day) and used as control. The second and third subgroups included those obtained 1 month after microbial plaque and calculus removal, and 1 month after scaling and root planing, respectively, additionally to the oral hygiene instructions given to the patients. The tissue samples were removed under local anaesthesia from the palatal gingiva during routine periodontal surgery in the Department of Periodontology, School of Dentistry, University of Athens and immediately fixed in 10% neutral buffered formalin at room temperature (18–22 °C). The Faculty of Dentistry, University of Athens, approved the protocol of the study. Informed consent was obtained from all patients.

Immunohistochemistry

Immunohistochemical staining was accomplished with a standard streptavidin–biotin–peroxidase method with antibodies against factor XIIIa (clone AC 1A1, IgG_1; Biocare Medical, Walnut Creek, CA, USA) and polyclonal S-100 protein (Biocare Medical). Formalin-fixed and paraffin-embedded tissue sections 5-μm thick were deparaffinized and rehydrated. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide and nonspecific binding was blocked with a universal blocking reagent (HK085–5K) (Biogenex, San Ramon, CA, USA). The tissue sections were washed in phosphate-buffered saline solution. The primary antibodies were used at a dilution of 1:100. Incubation time for the primary antibodies was 1 h and for the secondary antibody (HK268-UK, 1:100) (Biogenex) 30 min at room temperature, according to the instructions of the supplier. A Strep-ABComplex/HRP (K0377) (Dako, Carpinteria, CA, USA) was applied and the specimens were stained with diaminobenzidine and counterstained with Mayers hematoxylin. The specificity of the antibodies was confirmed by substitution with respective isotype controls. Negative controls consisted of substitution of the primary antibody with rabbit or mouse serum.

Cell counts

Quantitative evaluation of immunolabelled cells in the gingival epithelium and connective tissue was performed independently by two observers. Intraepithelial S-100+ cells were counted only when the cell body and at least one dendritic process were stained, whereas factor XIIIa+ and S-100+ cells in the connective tissue did not present dendritic processes and were counted when the cell body was stained. In each case the number of positive cells regardless of staining intensity was estimated in five random high power fields (× 40) and the mean number of cells in the five fields was calculated.

Statistical methods

The intragroup and intergroup differences for factor XIIIα positive dendritic cells and S-100 protein positive Langerhans' cells number were analyzed by one-way ANOVA test at $p < 0.05$ statistical significance level.

Results

Factor XIIIa+ dendritic cells

Dendritic cells were clearly identified by their immunoreactivity to anti-factor XIIIa. Spindle- or dendritic-shaped factor XIIIa positive cells were observed in the connective tissue beneath the basal stratum of the epithelium, around blood vessels (Figs 1 and 2). In some cases, the cytoplasmic processes were extending between the vascular endothelial cells. The main aggregations of factor XIIIa+ dendritic cells were localized in areas with dense inflammatory infiltration where the connective tissue was loosely organized. No positive cells were found in the gingival epithelium.

The mean values of factor XIIIa+ cells in each subgroup are shown in Table 1. On baseline day, the quantitative assessment of factor XIIIa+ cells showed that increased pocket depth (4–5 mm vs. ≥ 6 mm) was accompanied by an increase in cell number (9.30 ± 4.82 vs. 12.40 ± 3.73, $p < 0.05$). A decrease of the factor XIIIa+ counted cells was recorded in the samples obtained from patients with periodontal pockets of 4–5 mm depth after plaque, calculus removal and oral hygiene instructions, and a further decrease of the cell number was evident after scaling and root planing and oral hygiene instructions compared to controls. In the group of periodontal pockets of ≥6 mm depth, the number of factor XIIIa+ cells was not statistically decreased after plaque, calculus removal and oral hygiene instructions, or after scaling and root planing compared to controls.

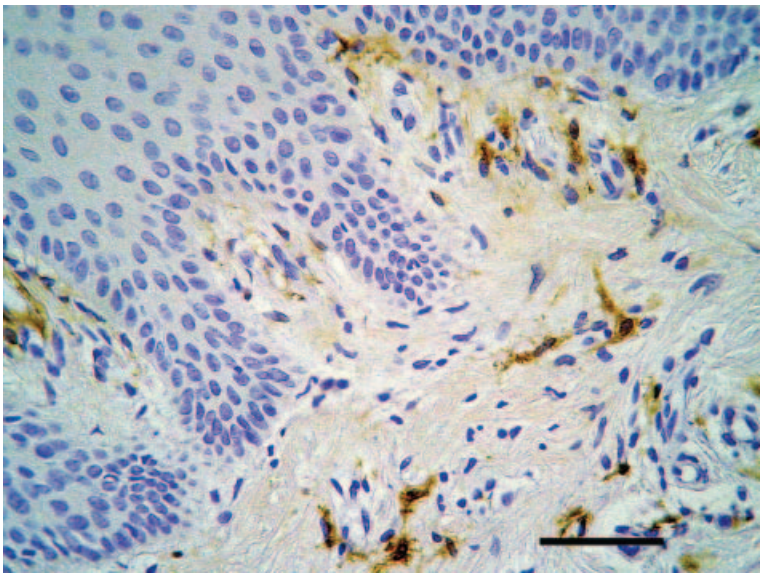

Fig. 1. Immunohistochemical staining for factor XIIIa+ dendritic cells in the subepithelial gingival connective tissue. Final magnification 400 ×. Bar = 100 μm.

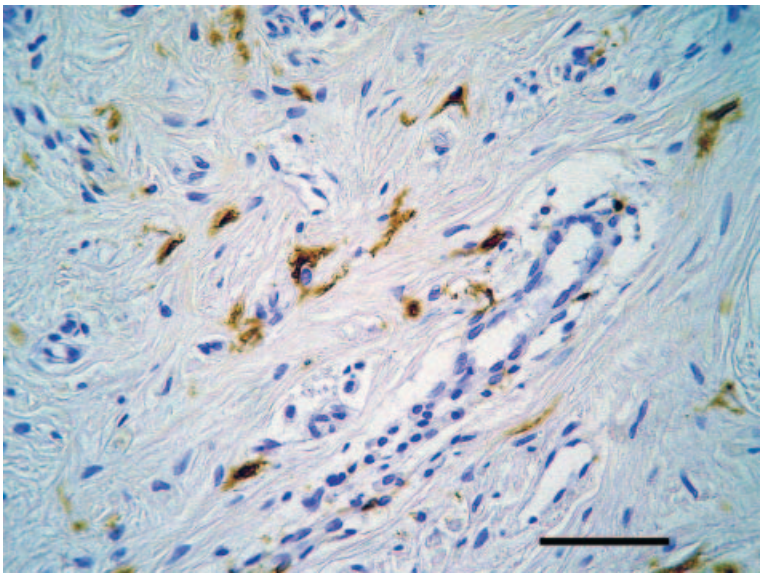

Fig. 2. Immunohistochemical staining for factor XIIIa+ dendritic cells. Dendritic cells are distributed around blood vessels. Final magnification 400 ×. Bar = 100 μm.

S-100 protein+ Langerhans' cells

S-100 protein+ Langerhans' cells were found in the gingival epithelium and the underlying connective tissue (Figs 3 and 4). On baseline day, the number of S-100+ Langerhans' cells in the epithelium was less than that in the connective tissue in both experimental groups. The number of intraepithelial S-100+ Langerhans' cells was greater in the group of ≥6 mm than the positive cells found in the group of 4–5 mm periodontal pockets. In the experimental group of 4–5 mm periodontal pocket depth, the number of intraepithelial S-100+ Langerhans' cells significantly increased 1 month after the non-surgical phase of the treatment. The presence of S-100+ Langerhans' cells in the subgroups after plaque, calculus removal and oral hygiene instructions and after scaling and root planing followed a reverse shift in the pockets of ≥6 mm relative to the respective subgroups in the pockets of 4–5 mm, where their number was significantly decreased (Table 2).

The number of positive Langerhans' cells in the gingival connective tissue showed a similar variation with the exception of the 4–5 mm group, where 1 month after plaque, calculus removal

Table 1. Numbers of factor XIIIa+ dendritic cells in the gingival connective tissue

Periodontal pocket depth	Controls[a]	After OHI[b]	After OHI and SRP[c]
4–5 mm ($n = 18$)	9.30 ± 4.82 ($n = 6$)	5.10 ± 4.12 ($n = 6$)	4.20 ± 3.06 ($n = 6$)
≥ 6 mm ($n = 36$)	12.40 ± 3.73 ($n = 12$)	12.00 ± 3.11 ($n = 12$)*	11.46 ± 3.88 ($n = 12$)*

Mean values are representative of the means ± SD. OHI, oral hygiene instructions; SRP, scaling and root planing.
[a]Baseline day.
[b]One month after OHI and removal of plaque and calculus.
[c]One month after OHI and SRP.
*Statistically significant difference between the subgroups of 4–5 mm and ≥ 6 mm depth pocket ($p < 0.05$).

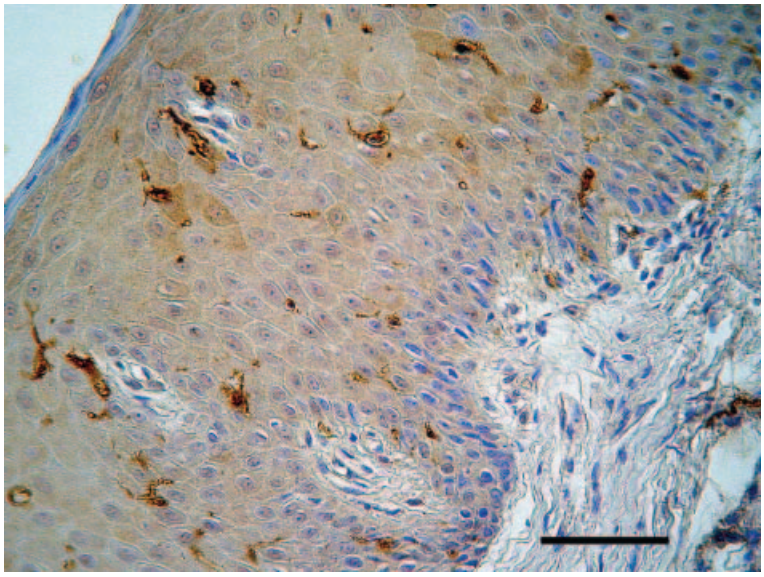

Fig. 3. Immunohistochemical staining for S-100 protein+ Langerhans' cells in the gingival epithelium. Final magnification 400 ×. Bar = 100 μm.

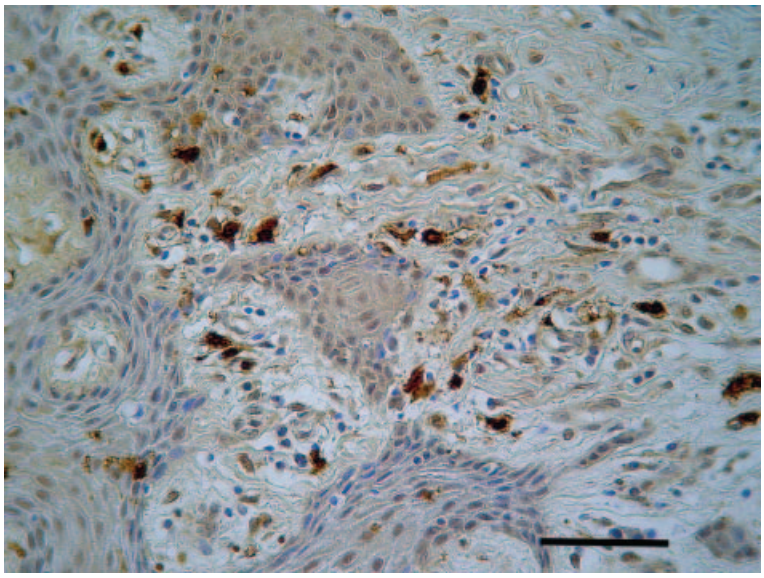

Fig. 4. Immunohistochemical staining for S-100 protein+ Langerhans' cells in the underlying connective tissue. Final magnification 400×. Bar = 100 μm.

and oral hygiene instructions, the Langerhans' cell numbers slightly decreased, whereas 1 month after scaling and root planing it increased (Table 3). In the experimental group of ≥6 mm pockets, a reduction of the Langerhans' cell numbers was seen after the non-surgical treatment, although the number of positive cells was significantly ($p < 0.05$) decreased only in the subgroup after plaque, calculus removal and oral hygiene instructions.

Discussion

This study was performed in order to quantitative assess the presence of factor XIIIa+ dendritic cells and S-100+ Langerhans' cells in the gingival epithelium and connective tissue of periodontal pockets of different depths after non-surgical periodontal treatment and to investigate the impact on the cell number of the stage of the periodontal disease. Previous studies have correlated the presence of Langerhans' cells in healthy and diseased gingiva with clinical indices (17, 18) or reported variation in Langerhans' cells' morphology and density between gingivitis and periodontitis (19, 20). The results of those studies are inconsistent because the number of Langerhans' cells has been reported to increase in diseased compared to healthy gingiva and decrease in gingivitis and periodontitis samples compared to control group, respectively. Other studies have contemplated on the similarities between adult periodontitis and contact hypersensitivity reactions (1, 9) and concluded that the increased density of dendritic cells induced by *Porphyromonas gingivalis* is accompanied by activation and maturation of dendritic cells, leading to the release of cytokines and stimulation of T-cells. Data of a recent study indicated that chronic periodontitis is associated with a significant increase in the number of dermal dendritic cells compared to healthy gingival specimens (21).

The present study showed that the number of factor XIIIa+ dendritic cells in the gingival connective tissue increased with increasing pocket depth and that removal of the supragingival plaque and oral hygiene instructions,

Table 2. Numbers of S-100+ Langerhans' cells in the gingival epithelium

Periodontal pocket depth	Controls[a]	After OHI[b]	After OHI and SRP[c]
4–5 mm ($n = 18$)	0.16 ± 0.4 ($n = 6$)	1.79 ± 2.2 ($n = 6$)	3.88 ± 2.2 ($n = 6$)*
≥ 6 mm ($n = 36$)	2.7 ± 3.8 ($n = 12$)	1.15 ± 1.48 ($n = 12$)	0.60 ± 0.88 ($n = 12$)**

Mean values are representative of the means ± SD. OHI, oral hygiene instructions; SRP, scaling and root planing.
[a]Baseline day.
[b]One month after OHI and removal of plaque and calculus.
[c]One month after OHI and SRP.
*Significant difference ($p < 0.05$) compared to the control subgroup.
**Significant difference ($p < 0.05$) between the subgroups of 4–5 mm and ≥ 6 mm depth pocket.

Table 3. Numbers of S-100+ Langerhans' cells in the gingival connective tissue

Periodontal pocket depth	Controls[a]	After OHI[b]	After OHI and SRP[c]
4–5 mm ($n = 18$)	2.38 ± 3.09 ($n = 6$)	2.00 ± 2.34 ($n = 6$)	3.91 ± 3.61($n = 6$)
≥ 6 mm ($n = 36$)	3.50 ± 3.09 ($n = 12$)	1.14 ± 0.90 ($n = 12$)*	1.25 ± 1.16 ($n = 12$)

Mean values are representative of the means ± SD. OHI, oral hygiene instructions; SRP, scaling and root planing.
[a]Baseline day.
[b]One month after OHI and removal of plaque and calculus.
[c]One month after OHI and SRP.
*Significant difference ($p < 0.05$) compared to the control subgroup.

as well as scaling and root planing, reduced cell density only in those biopsies obtained from 4–5-mm deep pockets. It was also shown that in the gingival epithelium of the control specimens the number of S-100 protein+ Langerhans' cells was increased in the periodontal pockets of ≥6-mm depth, whereas after the non-surgical phase of the periodontal treatment the cell density decreased. The number of the Langerhans' cells in the gingival connective tissue of ≥6-mm pockets followed the same variation and was decreased after the non-surgical phase of the periodontal treatment.

These findings could reflect differences of periodontal disease severity and demonstrate a tendency for reduction of factor XIIIa+ and S-100+ cells by non-surgical periodontal therapy. The decrease in the number of factor XIIIa+ dendritic cells 1 month after therapeutic intervention could be due to the fact that removal of microbial plaque and calculus results in reduction of the antigenic effect and therefore reduced dendritic cell presence. Disarrangement in the cytokine microenvironment that controls dendritic cells' function and occurs during the non-surgical periodontal treatment could also influence to the results of our study (22). Furthermore, it is well known that stimulation of the immune response leads to recruitment of T-lymphocytes, which bind mature dendritic cells for antigen presentation to occur. These findings may confirm the important role that T-lymphocytes play in early gingival defence reactions against microbial invasion, as it was proposed for the dental pulp inflammation (23). Bacteria effectively induce dendritic cell maturation and enhance the antigen presenting function of dendritic cells, and chemokines released by dendritic cells stimulated by bacteria are active in attracting T-cells (24).

In our material, a limited number of Langerhans' cells and a large number of dermal dendritic cells were seen in the gingival epithelium and connective tissue, respectively. Similar results in previous studies (1, 9) were explained by the hypothesis that dermal dendritic cells might be derived from Langerhans' cells exposed to antigenic stimulation. However, it is established that Langerhans' cells and dermal dendritic cells are derived from different progenitors and represent different cell populations (3), closely co-operating in immunopathological conditions. Furthermore, multiple dendritic cell subsets have been found in gingival tissues from healthy and chronic periodontitis subjects (21). The fact that there are certain prerequisites for Langerhans' cells to induce the local immune response during disease could explain the restricted number of Langerhans' cells compared to dendritic cells (25), i.e. a specific cytokine microenvironment, as it has been found that interleukin-10 inhibits Langerhans' cells antigen-presenting ability (26).

A possible limitation in the interpretation of our results is that the samples were derived from different individuals, and age, gender and immune status may affect reactions against periodontal pathogens and, consequently, could alter dendritic cell activity in general. In addition, smoking was not estimated in our sample, and it is well known that smoking can also affect the local immune response, modifying the inflammatory and immune process against the microbial challenge (27).

The results of this study provide further evidence for the presence of factor XIIIa+ dendritic cells and S-100 protein+ Langerhans' cells in the gingival epithelium and connective tissue of periodontal pockets of adult periodontitis. Additional studies should elucidate the role of those cells in the pathogenesis of gingival inflammatory diseases.

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