Original Article

Distribution of Dendritic Cells and Langerhans Cells in Peri-implant Mucosa

Jagadish Reddy Gooty, Deepthi Kannam[1], Vikram Reddy Guntakala, Rajababu Palaparthi

Department of Periodontology and Oral Implantology, Kamineni Institute of Dental Sciences, Narketpally, Hyderabad, Telangana, [1]Consultant Periodontist and Implantologist, Hyderabad, Telangana, India

Abstract

Background: Peri-implant diseases leading to the failure of dental implants is concern in the field of dentistry. Difference in immune response around peri-implant tissues with healthy tissue might be responsible for the hidden cause of peri-implant diseases. Hence, in the current study, the dispersion of the dendritic cell (DC) subpopulations and Langerhans cells (LCs) was evaluated in healthy peri-implant mucosa (HPIM) and healthy mucosa (HM) to know the imbalance in immune homeostasis. **Subjects and Methods:** A total of 15 nonsmoker participants were selected for the study. First sample of the HM was obtained before the implant placement (Group I) and second sample of peri-implant mucosa was obtained at the time of placement of the gingival former (Group II). Immunochemistry was used to quantify DCs and LCs in the samples. **Statistical Analysis Used:** To analyze the distribution of cells in the epithelium and lamina propria, Wilcoxon matched pairs test was used. **Results:** Mean numbers of CD1a (LCs) in the epithelium and lamina propria of Group I and Group II were 25.2 ± 6.41 and 27.47 ± 10.26 and 19.27 ± 7.27 and 12.46 ± 3.04, respectively. Mean numbers of factor XIIIa (DCs) in the epithelium and lamina propria in Group I and Group II were 30.37 ± 5.42 and 86.93 ± 13.99 and 50.47 ± 7.27 and 124.33 ± 10.27, respectively. Statistically significant differences in the number of cells in the epithelium and lamina propria of Group I and Group II were noted ($P = 0.001$ and $P = 0.001$). **Conclusions:** CD1a-positive LCs were more in the epithelium rather than lamina propria in Group II. Higher numbers of factor XIIIa-positive DCs were observed in the lamina propria than epithelium in Group I and II.

Keywords: *Dendritic cells, epithelium, Langerhans cells, titanium*

Introduction

The ease of rehabilitation of lost tooth due to various reasons with implant-supported prosthesis positioned on the principles of osseointegration has made implant dentistry formidable breakthrough in the last decade. Many well-contained longitudinal studies have provided statistical survival rate but also with some failures. Whatever might be the reasons for the failure, biological or mechanical imbalance of immune homeostasis around the implant soft tissue is inevitable for the peri-implant disease.

The oral mucosa is conquered by commensal and pathogenic flora challenging the host in maintaining immune homeostasis. The presence of antigens and lymphocytes does not always leads to immunity; dendritic cell (DC) systems, and third path, of antigen-presenting cells (APCs) are the discoverers and mediators of the immune response. Presentation of the antigen from an oral pathogen precisely into the gingival mucosa in rat model of periodontitis induced a rapid and protective humoral immune response, suggesting gingival mucosa may be a significant site of capturing and presenting antigens locally.[1]

DCs, professional APCs, act as an intertwining link between innate and adaptive immune responses by demonstrating antigen to T-cells in the immature $CD83^+$ state.[2,3] Langerhans cells (LCs), finest represented immature DCs, are usually residing over the basal layers of the epithelial cells in the gingiva.[4] These cells represent a central colony of the immune system.

Interstitial DCs (IDCs) are subpopulation of DCs communicate the protransglutaminase-clotting enzyme factor XIIIa + establish in close union with the blood vessels.[5] Their participation, morphological changes and increase in numbers in oral reactive and neoplastic lesions,[6] and aphthous ulcers[7] suggest

How to cite this article: Gooty JR, Kannam D, Guntakala VR, Palaparthi R. Distribution of dendritic cells and langerhans cells in peri-implant mucosa. Contemp Clin Dent 2018;9:548-53.

Address for correspondence:
Dr. Jagadish Reddy Gooty, Room No 211, Kamineni Institute of Dental Sciences, Sreepuram Narketpally, Nalgonda, Hyderabad - 508 254, Telangana, India.
E-mail: drjagadishreddy@gmail.com

Access this article online

Website: www.contempclindent.org

DOI: 10.4103/ccd.ccd_688_18

Quick Response Code:

their entanglement in local inflammatory immunemediated mechanisms.[7]

Both LCs and IDCs are found in the gingival tissue.[8] The figure of LCs in the gingival epithelium during inflammation is a subject matter of contemplation with increased numbers,[9] decreased numbers,[10] and no quantitative change[11] due to variation in the grade of disease. The presence of $CD1A^+$ LCs more frequently has been observed in the oral epithelium than in the sulcular/junctional epithelium without difference between various peri-implant conditions and aggressive periodontitis.[8]

Most of the dental implants are made up of titanium or its alloys. Although titanium is an inert material, biocorrosion has been detected in the surrounding tissues when it is in contact for a significant period of time and the dispersion of the DC molecules has been controlled by titanium ions.[12]

The author hypothesized that the difference in the host immune response to the healthy mucosa (HM) around the titanium dental implants and the HM might be associated with the difference in number of LCs and IDCs which might be due to the titanium ions surrounding the implants. Thus, in the current study, the distribution of LCs and IDCs in the HM and peri-implant mucosa was compared quantitatively using immunohistochemical analysis.

Subjects and Methods

A total of 15 participants who were nonsmokers and systemically healthy were selected from the outpatient department of periodontics. The Institutional Review Board validated the study protocol and every participant on personal basis was enlightened about the intention of the study and consigns their penned acquiescence in their vernacular language (KIDS/IEC/2013/28). Participants whose systemic illness known to affect the outcome of periodontal therapy, individuals allergic to medications, pregnant or lactating women, patients using tobacco in any form, and individuals with unacceptable oral hygiene were excluded from the study.

The first sample of HM (Group I) was obtained before the implant placement and the second sample of healthy peri-implant mucosa (HPIM) (Group II) was obtained at the time of implant exposure before placement of the gingival former.

Immunohistochemical staining procedure

Reagents prepared

Tris-buffered saline solution (pH: 9.0–9.2) was prepared by adding 6.2 gm of Tris and 0.75 gm of ethylenediaminetetraacetic acid to 1000 ml of distilled water in a glass beaker.

Phosphate-buffered saline (PBS, pH: 7.2–7.6) was prepared by adding 3.4 gm of sodium dihydrogen orthophosphate, 12 gm of di-sodium hydrogen phosphate, and 8.5 gm of sodium chloride to 1000 ml of distilled water in a glass beaker.

Substrate diaminobenzidine (DAB) chromogen solution was prepared by adding 1 ml DAB buffer with two drops DAB chromogen in a mixing vial. The above buffer solutions were prepared freshly every day before the procedure.

Sections of 3 μm thickness were prepared from the paraffin wax-embedded tissue blocks of samples randomly selected from the archival material with a rotary microtome. Superfrost glass slides were used to hold all the sections (Group I and II) and deparaffinization was done using hot air oven at 100°C for a time period of 10 min. Two changes were given with xylene, various grades of alcohol, that is, 100%, 80%, and 70% each for 5 min to rehydrate the tissue sections. The sections were rinsed off in running water for 2–5 min.

Antigen retrieval

Slides were placed in Coplin jar and then filled with Tris-buffered saline. Pretreatment with microwave was done at 100°C for 5 min with a frequency of 4–5 times. Slides were taken out and bench cooled to room temperature. This technique helps in reversal of the masked epitope and to restore the epitope–antibody binding, which is caused by cross-linked methylene bridges between proteins during fixation by formaldehyde.

During the staining procedure, precautions were taken such that tissue sections do not get dry. Reagents which were initially stored in the refrigerator were heated to room temperature (24°C–28°C) before use.

Following antigen retrieval, washing was done in PBS. Time taken for this step was 2 min for about three times. Excess buffer was then rinsed off using water.

Peroxide block was used to cover the tissue sections for about 15–20 min at room temperature. Washing with PBS stream was done gently for three times with a 30-s time gap for each wash.

Power block was used to cover the tissue sections for 5 min at room temperature, and later, the excess power block was drained off. Following the power block, the sections were covered with prediluted CD1a and factor VIIIa primary antibody, respectively. Later, the sections were incubated at room temperature for a time period of 1 h. Again, washing with gentle PBS stream was performed with a 30-s time gap for each wash and the excess buffer was rinsed off.

Super enhancer was then added to the tissue sections and the sections were placed in incubation chamber for 20 min at room temperature. Washing the sections with PBS stream was performed gently for four times with a 30-s time gap for each wash and the excess buffer was rinsed off. Incubation of the sections with polymer human resource planning was done for 30 min in a closed chamber at room temperature. Gentle stream of PBS washing was done for

three times with a 30-s time gap for each wash, and the excess buffer was rinsed off.

Slides were taken out from the incubation chamber and the tissue sections were immersed in DAB chromogen substrate and later incubated again for 7–10 min, followed by gentle PBS stream three times with a 30-s time gap for each wash. Later, the sections were washed under distilled water for four times.

The tissue sections were then counterstained by immersing them in Harris hematoxylin for 2 min and rinsed under tap water. Dehydration was performed using various grades of alcohol, that is, 70%, 80%, and 100% alcohol each for 5 min. Immersion of sections in xylene was done for the purpose of clearing and the slides were mounted with Digital Picture Exchange (DPX) and observed under microscope.

Interpretation of results

The existence of brown-colored end product at the site of target antigen was considered as positive immunoreactivity. The cytoplasm and nucleus of the cells present in the tissue sections were stained positive by the specific antibodies used.

Histomorphometric quantification procedure was performed for LCs by counting the cells that were CD1a positive in HM [Figure 1] and HPIM [Figure 2]. Identification of LCs was based on nucleic and cytoplasmic staining and their dendritic shape and the DCs by counting the cells that were positive for factor XIIIa in HM [Figure 3] and HPIM [Figure 4]. Counts of the cells were restricted to the immunolabeled cells. The cells were counted in five fields, which were randomly selected under × 100 magnifications, that is, epithelium and lamina propria of each slide.

Statistical analysis

Wilcoxon matched pairs test was used to compare the distribution of CD1a (LC) and factor XIIIa (DC) in both the epithelium and lamina propria in Group I and Group II.

Results

Fifteen participants, six males and nine females, with a mean age of 32–46 years were constituted in the study [Table 1 and Graph 1].

The number of CD1a in epithelium and lamina propria of Group I and Group II were 25.2 ± 6.41, 27.47 ± 10.26, 19.27 ± 7.27, and 12.46 ± 3.04, respectively. Statistically significant difference in a mean number of cells in the epithelium and lamina propria between Group I and Group II was noted (P = 0001 and P = 0.001). Although a positive relation was found in the cells in the epithelium and lamina propria in Group I, there was no statistically significant difference (P = 0.470), but a significant difference was observed in Group II (P = 0.023) [Table 2 and Graph 2].

The number of factor XIII a (DC) in the epithelium and lamina propria in Group I and Group II were 30.37 ± 5.42 and 86.93 ± 13.99 and 50.47 ± 7.27 and 124.33 ± 10.27, respectively. Statistically significant difference in the number of cells in the epithelium and lamina propria of Group I and Group II was noted (P = 0001 and P = 0.001) [Table 3 and Graph 3].

Table 1: Mean age of the participants and distribution of males and females in Group I and Group II

Number of participants	Mean age/years	Number of males	Number of females
15	32.46	6	9

Table 2: Comparison of distribution of CD1a Langerhans cells in epithelium and lamina propria in Group I and Group II by Wilcoxon matched pairs test

	Epithelium	Lamina propria	*P**
Group I	25.20±6.41	27.47±10.26	0.470
Group II	19.27±7.27	12.46±3.04	0.023*
Group I versus Group II (*P*)	0.001*	0.001*	

*$P \leq 0.05$ is significant

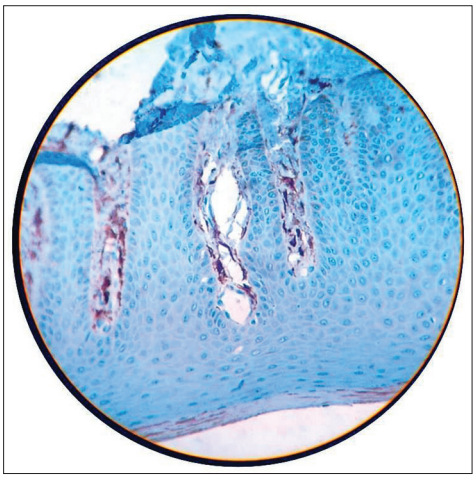


Figure 1: Immunohistological picture showing CD1a Langerhans cells in the healthy mucosa

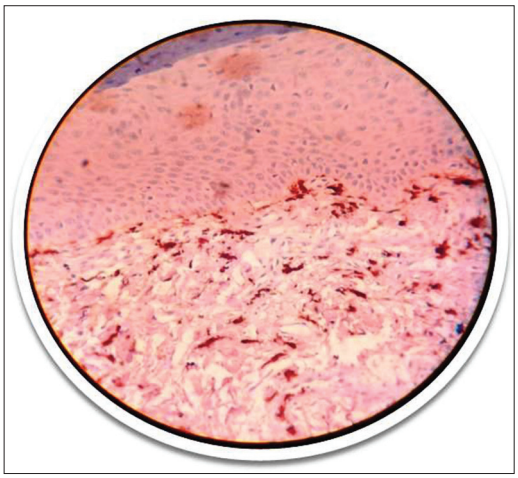


Figure 2: Immunohistological picture showing CD1a Langerhans cells in the healthy peri-implant mucosa

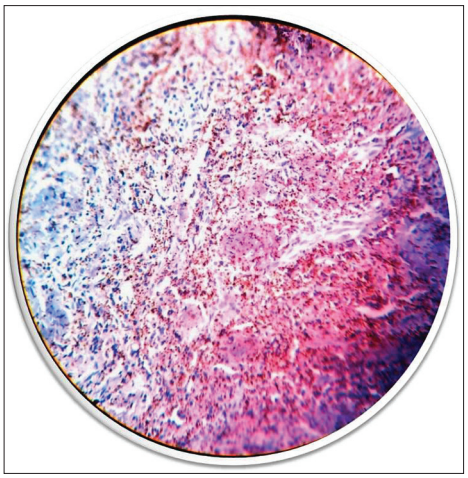


Figure 3: Immunohistological picture showing factor XIIIa dendritic cells in the healthy mucosa

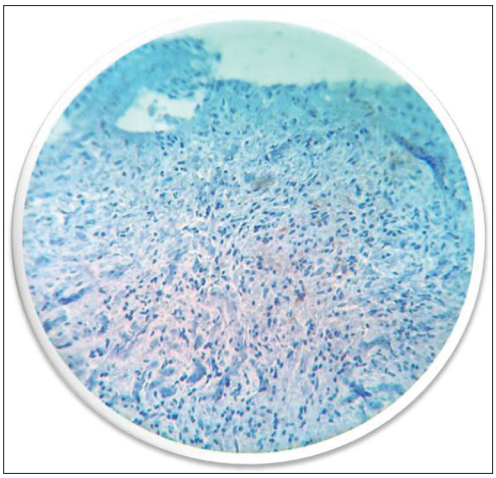
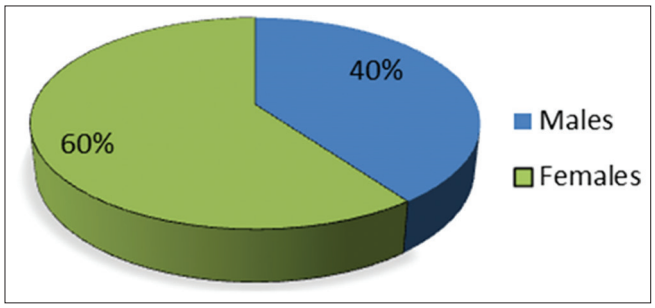
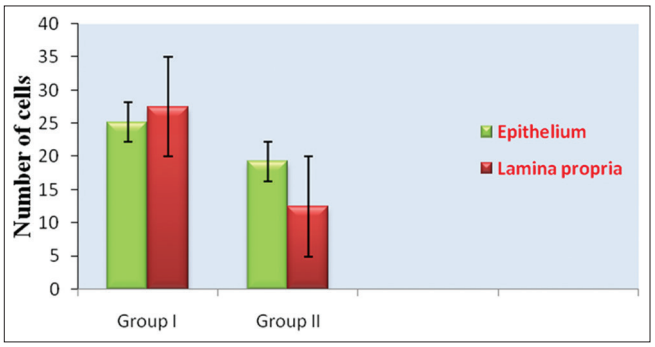


Figure 4: Immunohistological picture showing factor XIIIa dendritic cells in the healthy peri-implant mucosa



Graph 1: Distribution of males and females in Group I and Group II



Graph 2: Comparison of distribution of CD1a Langerhans cells in the epithelium and lamina propria in Group I and Group II

Table 3: Comparison of distribution of factor XIIIa dendritic cells in epithelium and lamina propria in Group I and Group II by Wilcoxon matched pairs test

	Epithelium	Lamina propria	*P**
Group I	30.37±5.42	86.93±13.99	0.001*
Group II	50.47±7.27	124.33±10.27	0.001*
Group I versus Group II (*P*)	0.001*	0.001*	

**P*≤0.05 is significant

Discussion

Periodontitis is a complex multifactorial inflammatory disease delineates by loss of connective tissue and underlying alveolar bone. The immune balance in the periodontal environment is rapidly monitored by the innate immune system which involves anatomical barriers, secretory molecules, and cellular elements such as DCs equipped with receptors to identify oral bacteria.[13] DCs are phagocytic cells that are akin to the dendrites of the nerve cells exhibiting long finger-like processes.[14]

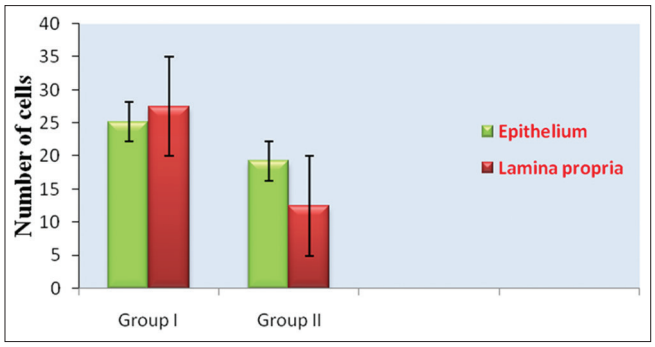
DCs dwell in an immature state with a higher level of phagocytic capacity and have a competence to act as a guard that survey the tissues mainly to invade microbes. At the time of infection, DCs target these trespassers and a maturation process is initiated by various mechanisms. The term professional APCs was awarded to DCs because of this scope to coeval antigen through major histocompatibility complex Class II, activating naive T-cells, indicating their important role in the initiation of adaptive immunity.[15]

Depending on various anatomical location, function, and expression of distinct cellular markers various DCs subsets can be identified. According to Mizumoto and Takashima, LCs explicit CD1a molecule at unusually high levels with practically no perceptible CD1b and only prudent CD1c expression.[16]

Hence, in the present study, CD1a marker was used because of its effectiveness in the identification of LCs being a specific marker.

Oral epithelium consists of LCs, similar to skin dermis and subsets of DCs expressing the C-type lectin. Increased number of LCs are found in the sulcular epithelium, whereas rarely found in the junctional epithelium showing their uneven distribution in the gingival epithelium.[17]

This study demonstrates decrease in number of LC CD1a cells in the epithelium of peri-implant mucosa distinguish to that of HM. Significant difference of cells in the epithelium might explain the different immune responses



Graph 3: Comparison of distribution of factor XIIIa dendritic cells in the epithelium and lamina propria in Group I and Group II

in the HM and peri-implant mucosa. Similar to the study conducted by Bullon *et al*.,[8] in the present study, also LCs were found frequently in the oral epithelium.

According to Dereka *et al*., DCs consisting of protransglutaminase-clotting enzyme factor XIIa symbolize a specific subpopulation of the dermal DCs prevailing in union with the blood vessels.[18] Factor XIIIa marker was used for identification of DCs in the current study.

Significant escalation in factor XIIIa DCs in the epithelium of peri-implant mucosa was observed distinguish to HM stating their role in reducing fibroblast proteolytic activity favoring gingival enlargement.[19]

Geijtenbeek *et al*. concluded that DC-SIGN–ICAM-2 and DC-SIGN–ICAM-3 interplay organize chemokine-induced transmigration of DCs traversing space for both resting and activated endothelium. Therefore, the essential role in astonishing trafficking capacity of DCs is DC-SIGN, which is in addition backed by the expression of DC-SIGN on forerunners in blood and on immature and mature DCs,[20,21] similar to the results of the present study.

Lower number of LCs CD1a in the lamina propria of peri-implant mucosa may be attributed to the decreased immune response stimulation, pronounced inflammatory response and as a part of controlling the titanium particle-induced peri-implant infection. Titanium particles when present for a longer period of time even results in the reduction of DCs.[12]

The authors finding was confirmed in the study, wherein larger number of neutrophils and macrophages are seen in peri-implant mucosa resulting in a higher inflammatory response ultimately leading to the tissue destruction when compared to the teeth in which the disease has been induced by ligatures.

Increased factor VIIIa DCs in peri-implant mucosa may be attributed to the healing that occurs after the placement of implant, during which the peri-implant mucosa undergoes remodeling of the matrix.

Degradation of type I collagen by gingival fibroblasts factor XIIIa + DCs, strong cytokine producers, promoting the increase of tumor necrosis factoralpha (TNFα) and of interleukin8 expression.[22] Drug-induced gingival enlargement in immunosuppressed patients is due to reduced number of factor XIIIa + cells resulting in decreased secretion of TNF-α, reducing fibroblast proteolytic activity, and favoring gingival enlargement.[19]

Conclusions

Within the limitations of the present study, the following conclusions were drawn:

Divergent immune responses in the gingival and peri-implant tissues may be justified by lower number of LCs and higher number of DCs in the epithelium and lamina propria. This difference in distribution of the cells in the peri-implant tissue is to control the inflammatory reaction. Inflammatory response around peri-implant tissues, as hypothesized by authors, might be due to biocorrosion of titanium dental implants, releasing titanium ions around peri-implant tissues. Due to this inflammatory reaction, higher number of factor XIIIa DC causing collagen degradation were observed.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Kawai T, Eisen-Lev R, Seki M, Eastcott JW, Wilson ME, Taubman MA, *et al.* Requirement of B7 costimulation for th1-mediated inflammatory bone resorption in experimental periodontal disease. J Immunol 2000;164:2102-9.
2. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998;392:245-52.
3. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, *et al.* Immunobiology of dendritic cells. Annu Rev Immunol 2000;18:767-811.
4. van Loon LA, Krieg SR, Davidson CL, Bos JD. Quantification and distribution of lymphocyte subsets and Langerhans cells in normal human oral mucosa and skin. J Oral Pathol Med 1989;18:197-201.
5. Cerio R, Griffiths CE, Cooper KD, Nickoloff BJ, Headington JT. Characterization of factor XIIIa positive dermal dendritic cells in normal and inflamed skin. Br J Dermatol 1989;121:421-31.
6. Regezi JA, Nickoloff BJ, Headington JT. Oral submucosal dendrocytes: Factor XIIIa+and CD34+dendritic cell populations in normal tissue and fibrovascular lesions. J Cutan Pathol 1992;19:398-406.
7. Natah SS, Hayrinen-Immonen R, Malmstrom M, Kontinnen YT. Factor XIIIa-positive dendrocytes are increased in number and size in recurrent aphthous ulcers. J Oral Pathol Med 1997;26:408-13.
8. Bullon P, Fioroni M, Goteri G, Rubini C, Battino M. Immunohistochemical analysis of soft tissues in implants with healthy and peri-implantitis condition, and aggressive periodontitis. Clin Oral Implants Res 2004;15:553-9.
9. Saglie FR, Pertuiset JH, Smith CT, Nestor MG, Carranza FA Jr., Newman MG, *et al.* The presence of bacteria in

the oral epithelium in periodontal disease. III. Correlation with Langerhans cells. J Periodontol 1987;58:417-22.
10. Séguier S, Godeau G, Brousse N. Immunohistological and morphometric analysis of intra-epithelial lymphocytes and Langerhans cells in healthy and diseased human gingival tissues. Arch Oral Biol 2000;45:441-52.
11. Gemmell E, Carter CL, Hart DN, Drysdale KE, Seymour GJ. Antigen-presenting cells in human periodontal disease tissues. Oral Microbiol Immunol 2002;17:388-93.
12. Chan EP, Mhawi A, Clode P, Saunders M, Filgueira L. Effects of titanium (iv) ions on human monocyte-derived dendritic cells. Metallomics 2009;1:166-74.
13. Benakanakere M, Kinane DF. Innate cellular responses to the periodontal biofilm. Front Oral Biol 2012;15:41-55.
14. Steinman RM, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. J Exp Med 1973;137:1142-62.
15. Steinman RM. Dendritic cells: Understanding immunogenicity. Eur J Immunol 2007;37 Suppl 1:S53-60.
16. Mizumoto N, Takashima A. CD1a and langerin: Acting as more than Langerhans cell markers. J Clin Invest 2004;113:658-60.
17. Inaba K, Witmer-Pack M, Inaba M, Hathcock KS, Sakuta H, Azuma M, *et al*. The tissue distribution of the B7-2 costimulator in mice: Abundant expression on dendritic cells *in situ* and during maturation *in vitro*. J Exp Med 1994;180:1849-60.
18. Dereka XE, Tosios KI, Chrysomali E, Angelopoulou E. Factor XIIIa+ dendritic cells and S-100 protein+ Langerhans' cells in adult periodontitis. J Periodontal Res 2004;39:447-52.
19. Cury PR, Arsati F, de Magalhães MH, de Araújo VC, de Araújo NS, Barbuto JA, *et al.* Antigen-presenting cells in human immunosuppressive drug-induced gingival enlargement. Spec Care Dentist 2009;29:80-4.
20. Geijtenbeek TB, Krooshoop DJ, Bleijs DA, van Vliet SJ, van Duijnhoven GC, Grabovsky V, *et al.* DC-SIGN-ICAM-2 interaction mediates dendritic cell trafficking. Nat Immunol 2000;1:353-7.
21. Geijtenbeek TB, Torensma R, van Vliet SJ, van Duijnhoven GC, Adema GJ, van Kooyk Y, *et al.* Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. Cell 2000;100:575-85.
22. Meikle MC, Atkinson SJ, Ward RV, Murphy G, Reynolds JJ. Gingival fibroblasts degrade type I collagen films when stimulated with tumor necrosis factor and interleukin 1: Evidence that breakdown is mediated by metalloproteinases. J Periodontal Res 1989;24:207-13.