

The Role of Langerhans Cells in Autoimmune and Non-Autoimmune Inflammatory Conditions – A Case Control Study

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Abstract: Objective: The aim of this study is to compare the CD1a positive Langerhans Cells between lichen planus, non- autoimmune inflammatory reactions, and normal oral mucosa in order to understand and differentiate the pathogenic mechanisms between autoimmune and non-autoimmune inflammatory conditions. **Methodology:** The study group I comprised of histologically confirmed Lichen planus (n=20) and group II comprised of histologically confirmed chronic inflammatory hyperplasia of gingiva (n=20). The control group consisted of clinically appearing normal mucosa (n=10). Sections of 3 µm were cut from paraffin-embedded blocks. The immunohistochemical procedure was done using antiCD1a antibody. The total number of CD1a positive cells in the study and control groups were evaluated by counting three high power fields per lesional tissue. **Results:** There was a significant increase in CD1a count in lichen planus when compared to that of chronic inflammatory hyperplasia of gingiva and normal mucosa (p<0.001). The comparison between the chronic inflammatory condition of gingiva and normal mucosa was not statistically significant. **Conclusion:** Our findings suggest that the pathogenic mechanisms involved in autoimmune and non-autoimmune conditions are different. Dysregulation of Langerhans cells may be responsible for the initiation and progression of oral lichen planus.

Keywords: Lichen planus, gingivitis, Langerhans cell, Antigen Presenting cell, CD1a

Date of Submission: 14-06-2019

Date of acceptance: 29-06-2019

I. Introduction

Lichen Planus (LP) is a chronic inflammatory disease affecting the skin and areas in the oral cavity [1]. While taking into account the prevalence of Oral Lichen Planus (OLP) in the general population and in the Indian population, it was found to be 1-2% in the former and 2.6% in the later [2]. The disease is commonly seen in Asian population, predominantly in females with the age of onset between 3rd and 6th decades of life [3]. The clinical presentation of OLP ranges from mild painless white keratotic lesions to painful erosions and ulcerations [4]. OLP is a chronic mucocutaneous disease of unknown etiopathogenesis. Cell-mediated immunity which appears to play a major role in the pathogenesis of OLP is probably initiated through unmasking a keratinocyte self-peptide by the endogenous or exogenous factors in persons with a genetic predisposition to the development of the disease [5]. The factors predisposing to its development are genetics, dental materials, drugs, infectious agents, immunodeficiencies, psychological factors, trauma, diabetes, hypertension etc. The pathogenic mechanism leading to OLP is considered to be autoimmunity and being so, is refractory to treatment. Dendritic cells are those immune cells which are responsible for the initiation of autoimmunity. Langerhans cells(LCs) are a type of dendritic cells which are present in the oral mucosa. Their main function is to present antigens to T cells, in order to evoke a T cell response [6]. LCs act through certain molecules which they possess. One such a molecule is CD1a (Cluster of Differentiation 1a) which is capable of presenting various forms of self and microbial lipid antigens to T lymphocytes. The location of Langerhans cells in the epidermis allows the innate immune system to respond rapidly to foreign antigens in the epithelium and result in improved clearance of the antigen [6]. A dysregulated LC function is considered to be the initiating event in the pathogenesis of LP. Hence understanding its pathologic mechanism is important. The treatment of an autoimmune disease like OLP is only temporary, contributing towards the reduction of the discomforts occurring during the course of the disease [7]. Corticosteroids are the mainstay in the palliative management of OLP, which can be used topically, intralesionally or systemically [8]. The aim of the currently available OLP therapy is to alleviate symptoms. Understanding the role of LCs in the pathogenesis of lichen planus can help us

devise better intervention strategies. So we designed a study to understand the role of LC in the disease process of lichen planus by evaluating and comparing the presence of Langerhans cells in autoimmune and nonautoimmune inflammatory conditions. With this study, we will be able to unveil the importance of Langerhans cell in the pathogenesis of two different types of inflammatory responses.

II. Materials and Methods

This case-control study was done at Mar Baselios Dental College, Kerala, India and the study samples were collected at the out-patient clinic. The study group was divided into 2 groups. Group I comprised of 20 histopathologically confirmed cases of lichen planus and Group II comprised of 20 histopathologically confirmed cases of chronic inflammatory hyperplasia of gingiva. The 10 control subjects were patients indicated for other minor oral surgical procedures who had clinically normal appearing mucosa.

Study design: Case-control study

Study location: Out-patient clinic of Mar Baselios Dental College, Kerala, India

Study duration: January 2013 to December 2014

Sample size: The total number of cases was 40 which were divided into two groups of 20 each and the number of control subjects was 10

Subjects and Selection method:

Selection of study groups

Group I- Subjects with histopathologically confirmed oral lichen planus (n=20).

Inclusion criteria

- All the clinical forms of lichen planus.
- Subjects of age group 20-70 years

Exclusion criteria

- Non autoimmune inflammatory lesions
- Subjects with deleterious oral habits and associated oral lesions.
- Systemic diseases and disorders(chronic heart disease, diabetes mellitus) and medically compromised patients(AIDS, blood dyscrasias)

Group II – Subjects with clinically and histopathologically confirmed chronic inflammatory hyperplasia of gingiva (n=20)

Inclusion criteria

- Non auto-immune inflammatory lesions
- Subjects of age group 20-70 years

Exclusion criteria

- Subjects with deleterious oral habits and associated oral lesions.
- Autoimmune inflammatory lesions
- Systemic diseases and disorders(chronic heart disease, diabetes mellitus) and medically compromised patients(AIDS, blood dyscrasias)

Selection of control group

Group III- Patients with clinically apparent normal mucosa (n=10)

Inclusion criteria

- Patients matched in age and sex with those of the study groups
- Patients indicated for third molar extractions and alveoloplasty. (Excess discarded tissue during flap reduction will be collected as control sample)

Exclusion criteria

- Subjects with deleterious oral habits
- Subjects with oral lesions
- Systemic diseases and disorders(chronic heart disease, diabetes mellitus) and medically compromised patients(AIDS, blood dyscrasias)

In order to avoid the confounding factors, the histologically normal appearing areas of the mucosa within the study sample (Group I and Group II) sections were selected as internal controls. Ethical clearance was obtained from the Institutional Review Board and an informed consent was obtained from participants of the study.

Procedure Methodology

The 20 tissue samples from each study group and 10 control tissues embedded in paraffin blocks, were serially sectioned in 3µm thickness and collected in APES (3-aminopropyl triethoxy silane) coated slides for immunostaining of CD1a monoclonal antibody. Antigen retrieval was done in a domestic pressure cooker. The

antigen retrieval buffer used was citrate buffer at a pH of 6.2. The wash buffer used was Tris-buffer. The immunohistochemical staining procedures were carried out at room temperature, within a humidifying chamber. The tissue sections were covered with primary antibody, anti CD1a with a dilution of 1:100 (BioGenex Life Sciences Pvt Ltd)TM. The slides were incubated for 1 hour 30 minutes at room temperature in the humidifying chamber. After subsequent applications of Super enhancer and Secondary antibody (SS Label Solution), the sections were covered with a mixture of DAB (3,3'-diaminobenzidine) chromogen and DAB (3,3'-diaminobenzidine) buffer for 10 minutes. The slides were then treated with Harris hematoxylin. A known positive tissue (skin tissue) section was stained in each batch in order to ensure the sensitivity of the antibody. Primary antibody step was omitted from the known positive tissue (skin tissue) and sections were run in each batch to ensure the specificity of the antibody.

Scoring criteria

The evaluation of CD1a positive cells was done according to a procedure proposed by McCartan BE et al [9]. Three random high power fields were selected from the lesional area of the histological section. In each high power field, the number of CD1a positive cells were counted separately in the epithelium and subepithelial connective tissue. Similarly, CD1a positive cell count was done in histologically normal appearing areas of sections from Group I and II to be considered as internal control. CD1a positive cell count was also done from three random high power fields of control sample tissue sections.

Statistical evaluation

Data analysis was done by SPSSTM software version 16.0. Data were presented as Median(IQR). Kruskal-Wallis test, Mann-Whitney test and Wilcoxon Signed Rank test were done to compare the cases with controls. 'p' value less than 0.05 was considered to be statistically significant. Inter-observer variability was evaluated using intra-class correlation coefficient. Correlation level of >0.7 was considered to be the accepted level of interobserver agreement.

III. Results

1. Study group demographics

The average age of Group I subjects in this study was found to be 42.75±16.28 years. Our study group I comprised of 65% females and 35% males with a female to male ratio of 1.85:1. The site of occurrence of lichen planus in this research is the buccal mucosa. While assessing the distribution of clinical types of lichen planus, our study group showed reticular OLP (90%) and erosive OLP (10%) types. On evaluating the clinical features in Group I subjects (n=20), skin lesions were seen only in 10% and the chief complaint of burning sensation in oral cavity was seen in 65% of the subjects. The mean duration of lichen planus in the study sample was 12.95 [1-72] months.

The mean age of subjects under Group II with chronic inflammatory hyperplasia of gingiva, selected in our study was 50.65±15.37 years. The study group II consisted of 75% females and 25% males with a female to male ratio of 3:1. The mean duration of lesion of selected subjects with chronic gingival inflammation in this study was 5.2 [1-24] months.

2. Comparison of Langerhans cells between study groups(Group I & Group II) and external control

The density of CD1a positive cells in the epithelium and subepithelial connective tissue of Group I subjects were more when compared to that of Group II subjects thus showing a statistically significant difference between the two groups with p<0.001 [Table 1, Figures b & d].

A significant increase of Langerhans Cells in epithelium and subepithelial connective tissue of study Group I (Oral lichen planus) was found when compared to that of normal mucosa with p<0.001 [Table 2, Figures b & f] whereas the comparison made between study Group II (chronic inflammatory hyperplasia of gingiva) and normal mucosa did not reveal any statistically significant difference [Figures d & f].

3. Comparison of Langerhans cells between study groups (Group I & Group II) and internal control

When the internal controls were compared with Group I and Group II subjects separately, an increase was noted in the former with p<0.001 [Table 3], whereas not much difference was noted with the later.

On comparing the CD1a counts in the epithelium and subepithelial connective tissue between the internal controls of the study groups I and II, no significant difference was noted.

Table 1: Comparison of Epithelial and Sub-epithelial Connective tissue CD1a counts between study groups (Group I and Group II)

CD1a	Category	N	Median(IQR)	P value
Epithelium	Group I	20	57.00 (36.50, 67.75)	<0.001*
	Group II	20	20.50 (8.50, 38.75)	
Subepithelial Connective Tissue	Group I	20	32.50 (16.25, 43.50)	<0.001*
	Group II	20	5.00 (3.00, 14.50)	

*p≤0.05 was considered to be statistically significant

Table 2: Comparison of Epithelium and subepithelial connective tissue CD1a counts between Group I and external control

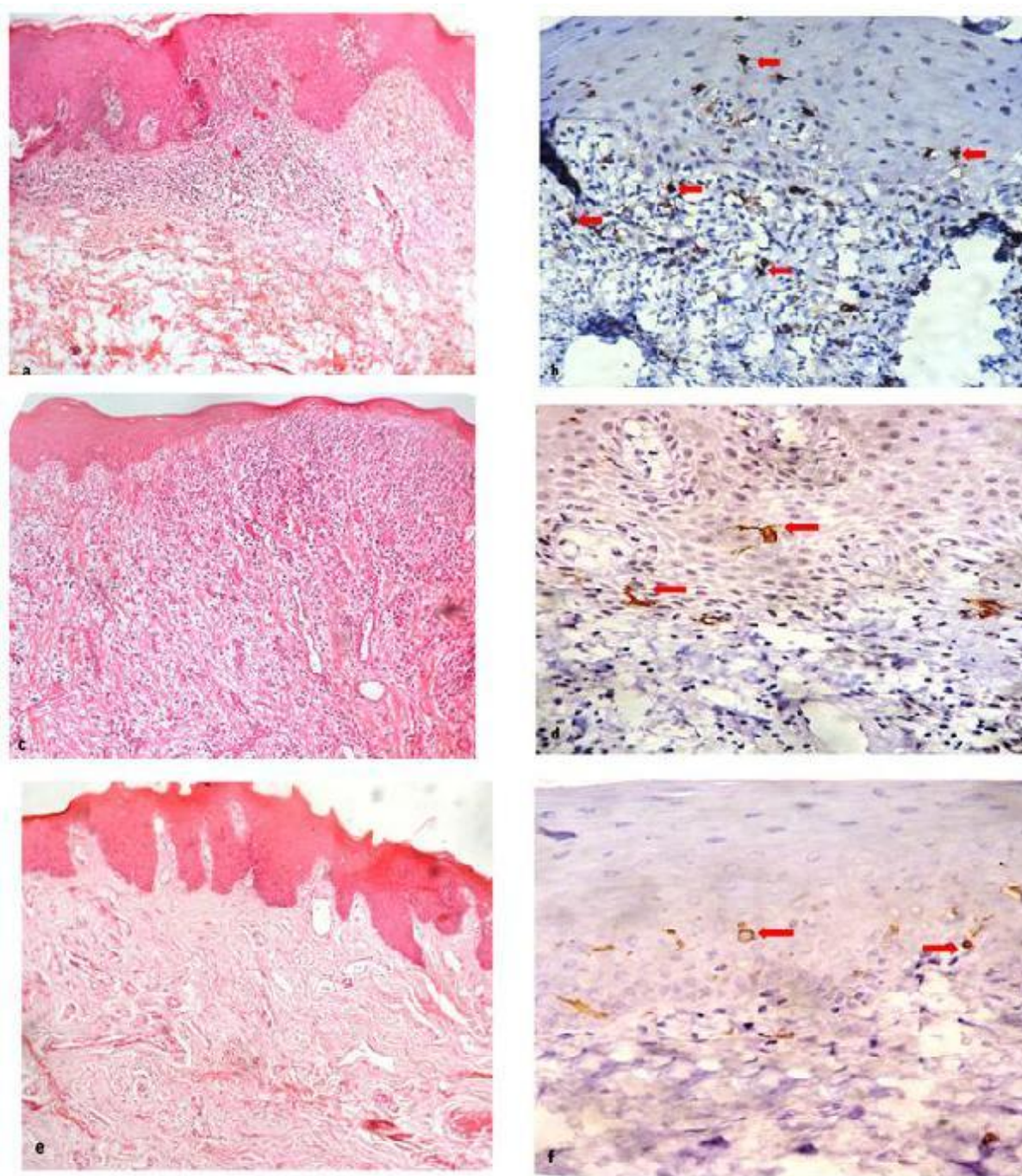
CD1a	Category	N	Median (IQR)	P value
Epithelium	Group I	20	57.00(36.50,67.75)	<0.001*
	Control	10	14.50(10.75,14.50)	
Subepithelial Connective Tissue	Group I	20	32.50(16.25,43.50)	<0.001*
	Control	10	6.00(1.00,9.25)	

*p≤0.05 was considered to be statistically significant

Table 3: Comparison of Epithelium and subepithelial connective tissue CD1a counts between Group I and internal control

CD1a	Category	N	Median (IQR)	P value
Epithelium	Group I	20	57.00 (36.50, 67.75)	0.005*
	Internal control	20	39.10 (20.75, 51.00)	
Subepithelial Connective tissue	Group I	20	32.50 (16.25,43.50)	0.003*
	Internal control	20	13.50 (10.00, 22.75)	

*p≤0.05 was considered to be statistically significant



(Figure a: H&E stained section of Lichen Planus under 10X view. Figure b: IHC section of Lichen planus showing Langerhans cells (red arrow) under 40X view. Figure c: H&E stained section showing chronic gingival inflammation under 10X view. Figure d: IHC section of chronic gingival inflammation with Langerhans cells (red arrow) under 40X view. Figure e: H&E stained section of normal mucosa under 10X view. Figure f: IHC section of normal mucosa with Langerhans cells (red arrow) under 40X view.)

IV. Discussion

The autoimmune etiology of lichen planus poses a major challenge to clinicians as well as the patient who demands a permanent cure. But, lichen planus remains a persistent problem, refractory to treatment [6].

Langerhans cells (LCs) are found to play an important role in activating immune reaction against foreign antigens and inducing tolerance towards self-antigens. Hence the dysregulation of Langerhans cell activity can be an impact factor in the pathogenic mechanism of OLP. Recent studies have examined the distribution of CD1a positive LCs in various normal human oral tissues like vestibular area, buccal mucosa, palate, lingual tissues, sublingual area, and gingiva. Studies have shown that highest number of LCs are found in

buccal mucosa [10]. In lichen planus lesional epithelium, LCs form a plexus of interdigitating cells into which the keratinocyte self-antigens are trapped [9]. When these antigens are presented to T lymphocytes, the autoreactive T cells are activated leading to the destruction of basal keratinocytes [11]. This finding implies the role of Langerhans cells in this study on the initiation of the inflammatory process in lichen planus [12]. A significant increase of LCs in the subepithelial connective tissue which was noticed in this study can be attributed to the migration of epithelial LCs through the connective tissue to the lymph nodes. A study by Gustafson et al in 2007, have concluded that an increased count of LCs found in both epithelium as well as lamina propria in OLP is by Activin A, a member of TGF β 1 family which was expressed in both epithelium and lamina propria of lichen planus [13]. Activin A can induce the differentiation of CD14+ monocytes into Langerhans cells so that there will be a continuous recruitment of LCs in OLP [14].

Compromised oral hygiene leads to the accumulation of plaque and pathogenic bacterial antigens which evoke an inflammatory response. The relatively slow turnover rate of gingiva compared to the doubling time of bacteria may provide enough time for bacteria to spread beyond the epithelia [15]. LCs participate in the initial mechanism of gingival inflammation [16]. In chronic gingival inflammation, proteolytic destruction of E-cadherin cell adhesion molecules by bacterial pathogens occurs, resulting in the loss of retention of the Langerhans cells on the epithelial cells [17]. There will be a migration of the dendritic cells into the lymphoid organ through the connective tissue [18]. Also, during the process of antigen presentation, LCs loses the CD1a molecules on their surface [19]. These factors may be responsible for the lowered CD1a count in chronic gingival inflammation.

Conditions like systemic bacterial infections, hypersensitivity reactions and chronic inflammatory diseases of skin and mucosa can alter the LC counts [20]. These factors can act as confounding factors when comparisons are made with an external control. Hence non-inflamed unaffected areas adjacent to the lesional site were selected as the internal control for our study groups. The statistical difference noted when the lesional sites in subjects with oral lichen planus were compared with the internal controls, admits the role of Langerhans cells in the pathogenesis involved in subjects with oral lichen planus. The comparison done among the internal controls for both the study groups have not shown any statistically significant difference. Hence the confounding factor based on lesional site in this study can be eliminated, focusing more on the difference in the pathogenic mechanisms involved in autoimmune and non-autoimmune inflammatory conditions.

The presence of two out of the many different subsets of CD4+ T helper cells (Th cells) – Th1 and Th2 have two distinct cytokine profiles [21]. The Th1 differentiation is initiated by cytokines like IL-12 (Interleukin-12) and IFN- γ (Interferon- γ) produced by dendritic cells [22]. MHC class II Antigen presentation in OLP is mediated by Langerhans cells or keratinocytes [9]. According to Farthing et al in 1990, there are increased number of LCs in OLP with upregulated MHC class II expression [23]. When the LCs present the antigen associated with MHC Class II to CD4+ helper T cells, it stimulates the secretion of cytokines IL2 and IFN- γ . The anti-keratinocyte auto-reactive CD8+ cytotoxic T cells are activated by means of (i) antigen associated with MHC class I on basal keratinocytes and (ii) Th1 CD4+ cell-derived IL-2 and IFN- γ . The IFN- γ secretion by Th1+CD4+ T-cells may stimulate TNF- α secretion by CD8+ cytotoxic T cells. A simultaneous antigen presentation to CD8+ and CD4+ T cells along with MHC Class I and II are required for the development of a persistent CD8+ cytotoxic T cell activity in OLP. This triggers the keratinocyte apoptosis. Studies have reported that cytokine secretions by CD4+ T cells may further skew the immune reaction towards a Th1 cytokine profile in OLP. Th1 differentiation of CD4+ T-cells is stimulated by CD-80 expression and IL-12 secretion by MHC Class II LCs, thereby forming a closed loop [9]. These findings show that LCs play an important role in Th1 biasing in the pathogenesis of OLP.

An important cytokine which is defective in OLP is the TGF- β (Transforming Growth Factor- β) [24]. The antigen-specific CD4+ TGF- β secreting regulatory T-cell suppress immune responses to self-antigens and prevent autoimmunity [25]. TGF- β 1 down-regulates Langerhans cell IL-12 production, thus blocking Th1 differentiation of CD4+ T cells and cytotoxic T cell responses [26]. It was suggested in various studies that, TGF- β 1 deficiency may predispose to autoimmune lymphocytic inflammation as seen in OLP [27,9]. Hence OLP pathogenesis can also be attributed to a defect in TGF- β 1 immunosuppressive pathway involving: (i) insufficient numbers of TGF- β 1 secreting Th3 regulatory T cells, (ii) blockage of TGF- β 1 secretion, (iii) secretion of non functional TGF- β 1, (iv) defective or inadequate TGF- β 1 receptor expression, or (v) defective intracellular signalling downstream from the TGF- β 1 receptors [9]. An elevated amount of TGF- β seen in chronic gingival inflammatory lesion was reported in a study conducted by Ali B.F et al in 2013. In conventional inflammatory reactions, the action of TGF- β helps in the resolution of inflammation. The increase in TGF- β can down-regulate the differentiation of Th1 cells and promote resolution of tissue inflammation [28].

V. Conclusion

In our study, we have identified Langerhans cells to be a key factor involved in the autoimmune pathogenesis of Oral lichen planus (OLP). When OLP was compared with non-autoimmune inflammatory responses, it was found that LCs play an important role in the initiation and persistence of LP lesions via (1) constant activation of autoreactive T cells and (2) skewing the inflammatory cytokine profile towards Th1 type. Our findings suggest that two different pathogenic mechanisms are active in the autoimmune and nonautoimmune inflammatory conditions. Recognising the LC's role in the pathogenesis of oral lichen planus can help us to devise intervention strategies and implement better therapeutic interventions targeting these cells. Further studies on the cytokine profiles and the role of Langerhans cells in regulating the inflammatory response needs to be done in order to manage Oral Lichen Planus.

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Tessy Lukose. "The Role of Langerhans Cells in Autoimmune and Non-Autoimmune Inflammatory Conditions – A Case Control Study." *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, vol. 18, no. 6, 2019, pp 13-19.