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ORIGINAL ARTICLE

Langerin-expressing and CD83-expressing cells in oral lichen planus lesions

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Abstract

Objective. Dendritic Langerhans cells (LCs) have been attributed a role in the pathogenesis of lichen planus as autoantigen-presenting cells initiating expansion of autoreactive T cells. Langerin and CD83, which are cell molecules expressed on LCs, are associated with antigen presentation. The present study examined expression of Langerin and CD83 molecules on LCs in patients with oral lichen planus (OLP). ***Material and methods.*** Biopsies were obtained from seven patients with OLP. Oral mucosa from seven healthy subjects served as controls. Monoclonal antibodies (mAbs) were used in standard immunohistochemical procedures to visualize CD1a-, Langerin-, and CD83-molecule-expressing cells. ***Results.*** CD1a+ and Langerin+ cells were found in significantly higher frequencies in OLP epithelium compared with healthy oral epithelium ($p < 0.01$ and $p < 0.05$, respectively); however, the frequency of CD83+ cells did not differ ($p > 0.05$). The connective tissue in OLP lesions showed significantly higher frequencies of CD1a+, Langerin+, and CD83+ cells compared with healthy connective tissue ($p < 0.01$, $p < 0.01$, and $p < 0.05$). CD1a+ and Langerin+ cells in OLP and healthy epithelium had a dendritic morphology. ***Conclusions.*** The study shows increased numbers of CD1a- and Langerin-expressing LCs in OLP compared with healthy controls. In the connective tissue, CD83+ cells with dendritic morphology were localized to regions of lymphocyte clusters. The presence of CD83+ dendritic cells in areas of lymphocyte clusters in the connective tissue of OLP lesions indicates the possibility of ongoing autoantigen presentation.

Key Words: *Autoimmunity, dendritic cells, human, oral mucosa, T lymphocytes*

Introduction

Lichen planus (LP) is a chronic inflammatory disease affecting skin and mucous membranes, including the oral cavity. Clinically, oral lichen planus (OLP) exhibits white hyperplastic striae with underlying redness and/or erosive/atrophic regions in the oral mucosa [1,2].

OLP affects from 0.1% to about 4% of the population [1]. The etiology of OLP is still unknown. However, OLP has several features of autoimmune diseases why dysfunction of the immune system has been ascribed a major role in the pathogenesis [1,4]. Viral infections have been proposed to contribute to disease development [2]. Recently, human herpesvirus type 7 has been suggested as being associated with the causation of skin LP [3].

The inflammatory cell infiltrate in OLP consists mainly of T cells [1]. It has been suggested that Langerhans cells (LCs) have a crucial role in LP with respect to presenting antigens to T cells [4–8]. LCs are a subpopulation of dendritic cells (DCs) residing in mucosal and skin epithelium [9]. DCs are "immunological sentinels" that are present in all organ systems in an immature form [10]. Immature DCs internalize antigens that may initiate the process of differentiation and maturation [10]. During maturation, LCs change from an immature phenotype characterized by high expression of CD1a and Langerin cell surface molecules and low expression of CD83 molecules into a mature phenotype characterized by downregulation of CD1a and Langerin cell surface molecules and upregulation of co-stimulating molecules CD80, CD83, and CD86 molecules [9,11].

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Interaction between antigen peptides on DCs and T cell receptors on the T cells, together with a second signal provided by interaction between co-stimulatory molecules, initiates T cell activation and a clonal expansion of antigen-specific T cells [10].

DCs also constantly engulf self-antigens [12–14]. If autoreactive T cells are present in the circulation, DCs may present these self-antigens to autoreactive T cells and thus initiate an autoimmune response [15,16]. The purpose of this study was to study presence and frequency of cell surface molecules on LCs related to (auto) antigen presentation and T cell activation in OLP.

Material and methods

Subjects and biopsies

Seven patients with a clinical diagnosis of OLP were included in the study. Oral mucosal lesions not related to dental restorations and displaying white hyperplastic striae with underlying redness and/or erosive/atrophic regions and a histopathological diagnosis of lichenoid reaction were classified as OLP. Control specimens ($n = 7$) were obtained from healthy oral mucosa of patients undergoing oral surgery for removal of impacted wisdom teeth or installation of implants. Following informed consent from these patients, healthy oral mucosal specimens were obtained from them. The local Ethics Committee approved the collection of healthy specimens. In OLP patients, biopsies from oral mucosal lesions were taken as part of the diagnosing procedure. Patient characteristics are described in Table I. Following excision, the tissue samples were placed in Histocon® (Histolab, Bethlehem Trading Ltd, Göteborg, Sweden) and within 30 min were snap-frozen in embedding medium (OCT Compound; Miles, Ind., USA). An oral pathologist performed examination and diagnosis of tissue specimens.

Antibodies

The following monoclonal antibodies (mAbs) were used: Anti-CD1a (NA1/34; Serotec Ltd, Oxford, UK), anti-CD83 (HB15A17.11; Serotec Ltd, Oxford, UK), and anti-Langerin (12D6; Novocastra Laboratories Ltd, Newcastle upon Tyne, UK).

Immunohistochemical staining

Cryostat sections 4 μm thick were prepared and air-dried for 15 min. The sections were then fixed in acetone for 5 min at 4°C and air-dried for 20 min. All subsequent incubations were followed by extensive rinsing for at least 5 min in Tris-HCL buffer (pH 7.6) containing 0.9% NaCl (Tris-buffered saline (TBS)). Endogenous peroxidase activity was inhibited by incubating the sections in 0.3% H_2O_2 in TBS for 5 min. Sections were then incubated with goat serum diluted 1:50 in 4% bovine serum albumin (BSA) in TBS for 30 min at room temperature. Incubation with the mAbs diluted in 4% BSA in TBS was then performed at 4°C overnight, except for mAb against CD1a, which was incubated for 30 min at room temperature.

Sections treated with mAb to CD1a, CD83, and Langerin were incubated for 30 min at room temperature with DAKO EnVision (peroxidase anti-mouse; DAKO A/S, Glostrup, Denmark). Sections were developed for 10 min for CD1a and for 20 min for CD83 and Langerin using the DAB kit (Vector Laboratories Inc., Burlingame, Calif., USA) and further counterstained with Mayer's haematoxylin for 2 min.

Immunohistochemical stainings were done with isotype-matched irrelevant primary antibodies mouse immunoglobulin G2a (IgG2a) Kappa (UPC10); Sigma, St. Louis, Mo., USA) and mouse IgG2b (No. X0931; DAKO A/S, Glostrup, Denmark).

Quantitative analysis was performed on two sections per biopsy. Digitalized images were obtained in a light microscope from six high-power fields (HPFs, ×125). The sections were then analysed with Easy Image 3000 (Tekno Optik AB, Göteborg, Sweden). In the epithelium and the inflammatory infiltrate in the connective tissue, all nucleated, positively stained, cells were counted manually and area determined with the aid of computer software. Results are expressed as the number of positively stained cells/mm^2.

Statistical analysis

Analysis of differences between groups was performed using the Mann-Whitney U-test. A p-value <0.05 was considered statistically significant.

Results

Specificity of staining

Sections of OLP lesions and healthy controls incubated with or without isotype-matched, irrelevant primary mAbs were negative. Sections of tonsils showed positive staining with anti-CD1a, anti-CD83, and anti-Langerin mAbs.

CD1a-expressing Langerhans cells

CD1a-positive cells were detected in both epithelium and connective tissue in sections of OLP lesions and healthy oral mucosa (Figure 1A, B). The epithelium and connective tissue of OLP lesions had a significantly higher number of CD1a-positive LCs per area unit compared with healthy mucosa ($p < 0.01$) (Figure 2A, B). In epithelium of OLP and healthy oral mucosa, CD1a-positive cells were forming an intra-epithelial network of DCs (Figure 1A, B).

Table I. Patient characteristics

Patient no.	Age (years)/sex	Diagnosis	Biopsy site	Oral involvement	Systemic diseases	Medication
1	64/F	OLP[a] erythematous	buccal mucosa	buccal mucosa/tongue	–	oestrogen
2	46/M	OLP erythematous	buccal mucosa	buccal mucosa	–	–
3	52/M	OLP erythematous	buccal mucosa	buccal mucosa/lip/gingiva	–	–
4	31/M	OLP reticular	buccal mucosa	buccal mucosa	–	–
5	54/F	OLP erythematous	buccal mucosa	buccal mucosa	migraine	–
6	72/M	OLP erythematous	–	gingiva, buccal mucosa, floor of the mouth	–	–
7	26/M	OLP erythematous	buccal mucosa	buccal mucosa/gingiva	psoriasis, allergy to cat and pollen	–
8	29/M	H[b]	buccal mucosa	–	–	–
9	58/F	H	buccal mucosa	–	–	–
10	72/M	H	gingiva	–	–	metoprolol, aspirin, atorvastatin
11	31/F	H	buccal mucosa	–	–	oestrogen
12	67/F	H	buccal mucosa	–	–	–
13	24/M	H	buccal mucosa	–	allergy to pollen	–
14	50/M	H	buccal mucosa	–	–	–

[a]Oral lichen planus; [b]healthy oral mucosa.

Langerin-expressing Langerhans cells

Langerin-positive cells were detected in both epithelium and connective tissue in sections of OLP and healthy mucosa (Figure 1C, D). The Langerin-expressing LCs per area unit were significantly more frequent in both epithelium and connective tissue in OLP lesions compared with healthy mucosa ($p < 0.05$) (Figure 2A, B).

CD83-expressing cells

CD83-positive cells were detected mainly in connective tissue and rarely in epithelium (Figure 1E, F). The number of CD83-positive cells per area unit in epithelium of OLP lesions and healthy oral mucosa did not differ significantly ($p > 0.05$) (Figure 2A). In connective tissue, the number of CD83 molecule-expressing cells was low but significantly more abundant in OLP compared with healthy oral mucosa ($p < 0.01$) (Figure 2B).

Cell morphology

CD1a-positive LCs in general had a dendritic appearance and were located in the suprabasal part of the epithelium (Figure 1A, B). CD1a-positive cells with dendritic morphology were found scattered in the connective tissue of OLP lesions (Figure 1A). Solitary CD1a-positive cells were seen in healthy connective tissue (Figure 1B). Langerin-positive cells within the epithelium and connective tissue sometimes, but not always, exhibited a dendritic morphology. The cells were located in the suprabasal portion of the epithelium. CD83-positive cells also exhibited dendritic morphology, but were not as pronounced as the CD1a-expressing cells. CD83-positive cells were mainly found in lymphocyte-rich areas of the inflammatory cell infiltrate. CD1a-, Langerin-, and CD83- positive cells were sometimes located in clusters of cells with lymphocyte morphology (Figure 3A–C).

Discussion

The present study shows that CD1a- and Langerin-expressing LCs are more abundant in epithelium of OLP lesions compared with healthy oral epithelium. In the inflammatory cell infiltrate present in the connective tissue of OLP lesions, there is an increase in numbers of CD1a-, CD83-, and Langerin-expressing cells compared with healthy oral mucosa.

In T cell-mediated autoimmune diseases it has been suggested that autoantigens presented by DCs to T cells cause expansion of autoreactive T cell clones, which causes tissue damage [10]. Activation of T cells requires professional antigen-presenting cells such as DCs, macrophages and B cells [10,17]. Dendritic cells are the most potent antigen-presenting cells for helper T cells as well as cytotoxic T cells [10]. The immunopathological changes seen in OLP support the theory that T cells play a key part in the disease process. Evidence has been presented that DCs play an important part in activation of autoreactive T cells [18]. Several studies have described the increased numbers of CD1a molecule-expressing LCs in epithelium affected by OLP compared with healthy oral epithelium [8,19,20]. The results of the present study, with increased frequency of CD1a-positive LCs, confirm these earlier findings. CD1a and Langerin are LC-specific cell surface markers [21]. Langerin is a recently characterized cell molecule associated with antigen uptake in immature LCs [21]. It has been suggested that Langerin molecules are closely associated with Birbeck's granules – an LC-specific organelle that takes part in antigen processing [22,23].

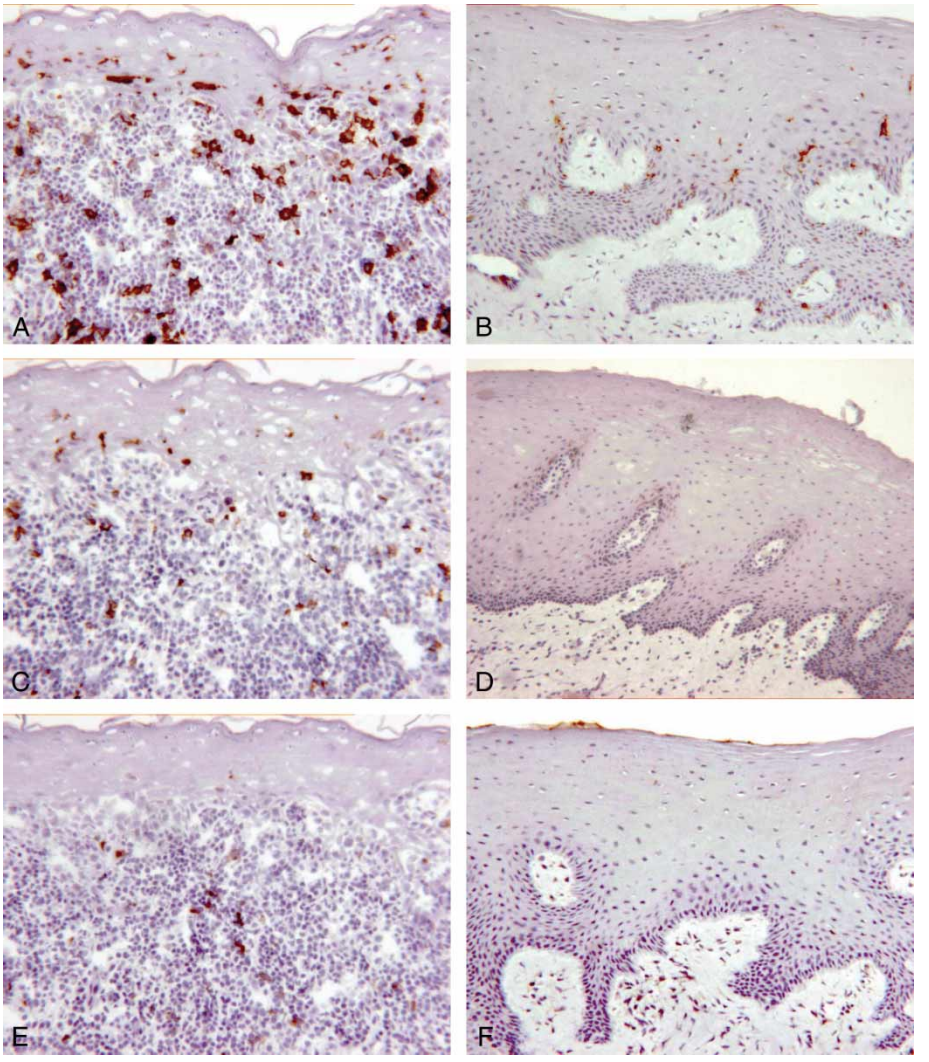

Figure 1. Distribution and phenotype of CD1a-, Langerin-, and CD83-expressing cells stained with labeled polymers (EnVision™) in sections from oral lichen planus and healthy mucosa (×125). CD1a positive cells in oral lichen planus (A) and healthy mucosa (B). Langerin-expressing cells in oral lichen planus (C) and healthy mucosa (D). CD83-expressing cells in oral lichen planus (E) and healthy mucosa (F).

Presence of Langerin molecules on LCs indicates an immature phenotype, with high endocytic capacity and low T cell-stimulating capacity [23]. By contrast, mature DCs expressing the co-stimulatory molecules CD80, CD83, and CD86 indicate low endocytic capacity but high T cell-stimulating capacity [9]. There is no complete overlap between CD1a and Langerin expression. Valladeau et al. [21] report that 15–35% of CD1a positive cells express Langerin and that variation in maturation stage is reflected in different expression of these cell molecules.

To date, only one study has assessed Langerin expression on LCs in OLP and healthy oral mucosa [24]. The present study supports the results of Santoro et al. [24], of increased numbers of CD1a and of Langerin-expressing LCs in epithelium and connective tissue of OLP compared with healthy controls. In this study, both OLP epithelium and the inflammatory cell infiltrate in the connective tissue had significantly increased numbers of CD1a- and Langerin-expressing LCs compared with healthy oral mucosa.

To our knowledge, CD83 molecule-expressing cells in OLP lesions have not been previously reported. The results show that in our samples CD83 molecule-expressing cells were present but were not more abundant in OLP epithelium compared with healthy oral epithelium. However, in the connective tissue, cells carrying CD83 were significantly more abundant compared with healthy tissue, although low absolute numbers of positive cells. DCs are extremely capable of stimulating T cells [25]. Thus, a major influx of DCs is not required to initiate and maintain T cell stimulation. Expression of maturation marker CD83 in the inflammatory cell infiltrate of OLP indicates presence of mature antigen-presenting cells since CD83 is predominantly expressed by the dendritic cell lineage, although not strictly lineage specific [26]. Macrophages have the

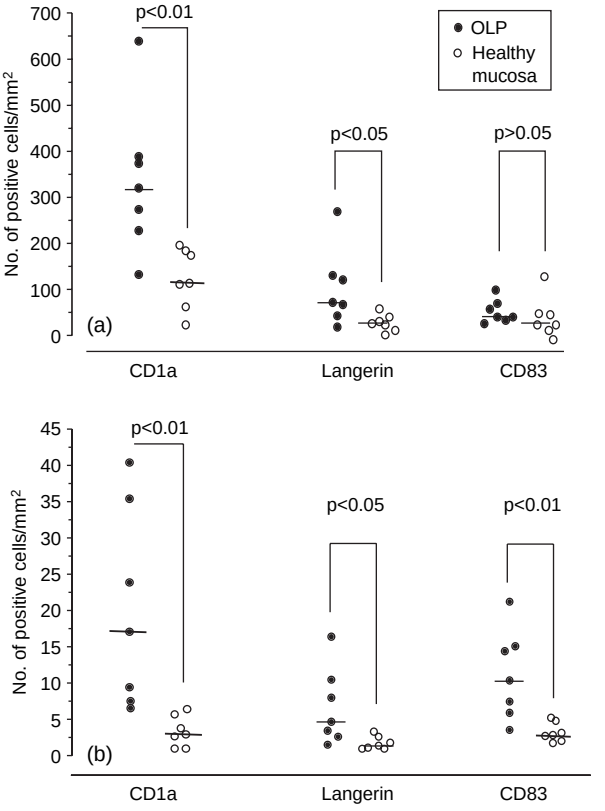

Figure 2. Number of cells expressing CD1a-, Langerin-, and CD83 molecules in epithelium (A) and connective tissue (B) of oral lichen planus (filled circle) and healthy mucosa (open circle). Each symbol denotes one patient and bars denote the median values.

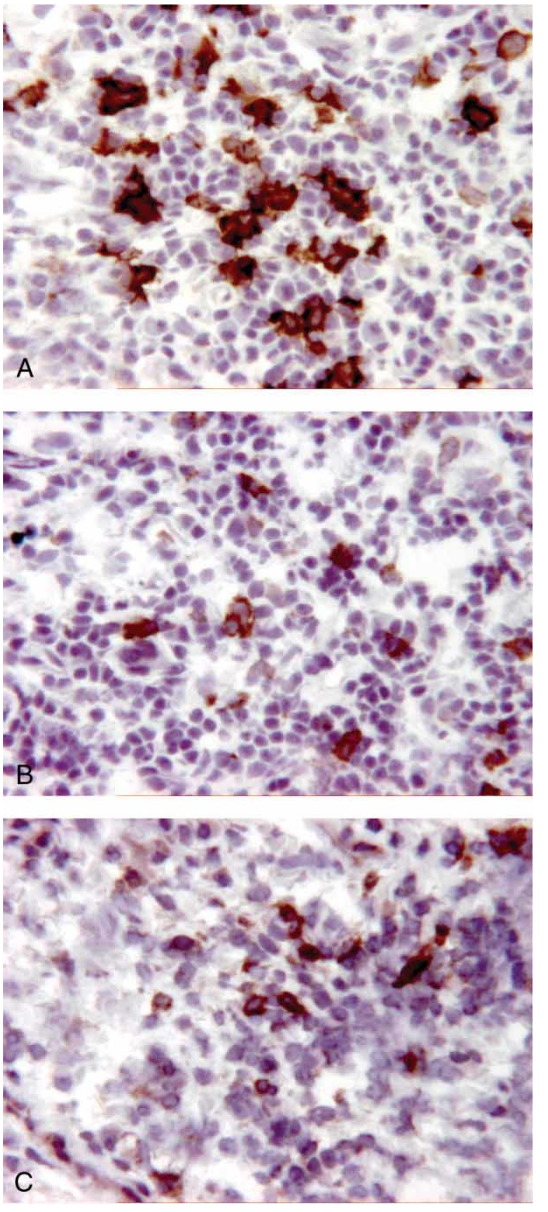

Figure 3. CD1a-expressing cells (A), Langerin-expressing cells (B), and CD83-expressing cells (C) localized to regions of lymphocytes in oral lichen planus ($\times 320$). Sections stained with labeled polymers (EnVision^{TM}).

capacity to express CD83 in pathological conditions [27]. B cells may express CD83 in inflamed tissue [28]. However, B cells have been reported not to be present to a major extent in OLP [29].

Occasionally, CD83 molecule-expressing cells in OLP lesions were seen in clusters of lymphocytes. This may reflect ongoing autoantigen presentation by DCs or macrophages to T cells. However, Deguchi et al. [30] recently reported increased frequency of epidermal and dermal CD1a-positive LCs in skin LP compared with healthy control skin, but no significant increase in CD68-positive macrophages in skin LP compared with healthy controls. Therefore, macrophages do not seem to be the major antigen-presenting cell in lichen planus. Cell–cell interaction between DCs and T cells is necessary for antigen-specific T cell stimulation [31]. Hence, co-localization of DCs and T cells in the inflammatory cell infiltrate of OLP lesions is a prerequisite for (auto)antigen presentation. The findings support results by Hasséus et al. [8], who described clustering of CD1a-expressing LCs and CD86-expressing cells with lymphocytes in OLP.

Notably, in the present study, Langerin-positive LCs were localized in the basal region of the epithelium. Immature LCs localized to this region provide the possibility for autoantigen uptake and subsequently for maintaining autoimmune inflammation.

The number of patients in the present study is small. The patients included in the study did not report any diseases and were not on ongoing immunomodulating medication. Bearing in mind the difficulty in recruiting patients without diseases and medication influencing immune responses, the present patient material is small but well defined. But studies with larger numbers of OLP patients are needed to confirm the results reported in this study.

In summary, the results of this study show increased numbers of CD1a- and Langerin-expressing LCs in OLP compared with healthy controls. Presence of CD83 positive dendritic cells in areas of lymphocyte clusters in the connective tissue of OLP

lesions may indicate local autoantigen presentation by mature DCs to T cells.

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