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# Origin of langerin (CD207)-expressing antigen presenting cells in the normal oral mucosa and in oral lichen planus lesions

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**Abstract**

The number of langerin-expressing antigen-presenting cells is higher in oral lichen planus than in normal oral mucosa. However, langerin may be expressed by several functionally different lineages of antigen presenting cells (APCs), and this has important implications for our understanding of the pathogenesis of oral lichen planus. The aim of this study was to determine the origin of the langerin-expressing APCs. To this end, we examined oral mucosal biopsies from healthy persons and patients with oral lichen planus using multicolor immunofluorescence. In normal oral mucosa, a substantial fraction of Langerhans cells expressed Ki-67, indicating that steady-state oral mucosal Langerhans cells are at least partially maintained by self-renewal. In oral lichen planus, the numbers of Langerhans cells were higher but proliferation was not altered, indicating that the higher cell numbers appeared to depend on recruited dendritic cell (DC)-precursors. Moreover, we found a markedly higher number of langerin$^+$ APCs within the lamina propria of oral lichen planus lesions. Such cells did not display monocyte- or macrophage markers, but rather showed a phenotype compatible with tissue-elicited IRF4$^+$ cDC2. Detailed understanding of how the oral mucosal APC network is regulated and the functional capacities of the different ontogenies may identify novel treatment targets for oral lichen planus.

**KEYWORDS**

dendritic cells, immunopathology, Langerhans cells, macrophages, oral lichen planus

## INTRODUCTION

Oral lichen planus is a chronic inflammatory disease of the oral mucosa with an overall estimated pooled prevalence of 0.89% among the general population and 0.98% among clinical patients [1]. Clinically, the disease typically appears bilaterally in the buccal mucosa, with characteristic white stripes in a grid-like pattern, sometimes with atrophic areas and ulcerations. The presence of bullae and plaque-variants are also described. The symptoms range from asymptomatic to pain and reduced quality of life [2]. Histologically, oral lichen planus is characterized by hyperkeratosis, basal cell vacuolization, and a dense mononuclear inflammatory infiltrate dominated by T cells just beneath the epithelium [3, 4]. Thus, although the pathogenesis of oral lichen planus is not completely resolved, it is thought to arise from an autoimmune

response involving T cells producing cytokines that induce a chronic inflammatory response and keratinocyte cell death [5].

Activation of $CD4^+$ T cells depends on the presentation of peptides in the context of MHC class II (MHCII) on antigen presenting cells (APCs) [6, 7]. Thus, APCs control the local activation of T cells, and may also contribute to recruitment and maintenance of the T-cell infiltrate in oral lichen planus lesions. Therefore, detailed understanding of the composition and functions of the mucosal APCs is important to understand the underlying immunopathology in patients with oral lichen planus.

The healthy oral mucosa contains a network of $MHCII^+$ APCs in both the epithelium and the underlying lamina propria [8, 9]. In the epithelium, Langerhans cells expressing langerin/CD207 and CD1a are the dominant population of APCs [9]. Such mucosal Langerhans cells share many features with their dermal counterparts, including the hallmark langerin-dependent Birbeck granules [10]. In the lamina propria, the majority of $MHCII^+$ cells are $CD14^+$ macrophages together with minor populations of spatially related dendritic cell (DC) subtypes [11]. The latter population comprises mainly $CD1c^+$ type 2 conventional DCs (cDC2) and minor sub-populations of APCs expressing CD1a or langerin.

Early studies reported increased numbers of epithelial Langerhans cells and Langerhans cell-like cells in the subepithelial infiltrates of oral lichen planus lesions [12]. This has been corroborated in more recent publications [13–15] even though there is significant discrepancy in the reported numbers and densities of such cells, probably due to methodological variations. Detailed interrogation of the mechanisms behind the alterations within the APC network, however, is still lacking. Langerin was previously considered to be specific for Langerhans cells, but it is now known that langerin may be expressed by other lineages of myeloid cells, including dendritic cells and monocyte-derived macrophages [16–20]. This has important implications for our understanding of the pathogenesis of oral lichen planus, because the control of immune responses crucially depends on the concerted action of functionally different subtypes of APCs that are specialized to respond to particular antigens and interact with specific T cell subsets. Although the ontogeny of human tissue APCs is not entirely understood, recent developments in the field indicate that the population of such cells is maintained by different precursors. For instance, whereas epidermal Langerhans cells appear to be replenished by self-renewal through local proliferation [21], mucosal macrophages are continuously replaced by elicited $CD14^+$ calprotectin$^+$ blood monocytes that differentiate into resident $CD14^+$ mature macrophages [22]. Moreover, tissue cDC2s display a very limited lifespan in mucosal tissues and are rapidly replaced by Interferon Regulatory Factor 4 (IRF4)-dependent blood precursors [23, 24].

The aim of this study was to examine the origin of langerin-expressing APCs in the normal oral mucosa and oral lichen planus lesions by immunophenotyping of APCs in tissue samples obtained from oral lichen planus patients and healthy persons.

## MATERIAL AND METHODS

### Patients

Archival formalin-fixed, paraffin-embedded (FFPE) blocks from patients presenting oral lichen planus lesions were obtained from the biobank at the Department of Pathology, Oslo University Hospital ($N = 11$). Histological evaluation was done by an experienced pathologist based on routinely H&E-stained formalin-fixed tissue specimens, according to the World Health Organization (WHO) diagnostic criteria for oral lichen planus [4]. None of the patients were under treatment for oral lichen planus. Normal oral mucosal biopsies were taken from clinically healthy buccal mucosa during third molar surgery or implant surgery ($N = 7$). These samples were taken from the distal part of the releasing incision in the buccal mucosa and formalin-fixed. Donor characteristics are summarized in Table S1. The mean age was 61 years (SD 16 years) and 45 years (SD 18 years) for patients with oral lichen planus lesions and donating normal oral mucosa, respectively, and the gender distribution in the groups was 8 F/3 M (oral lichen planus) and 3 F/4 M (normal oral mucosa). Additionally, three healthy volunteers were recruited and two 5 mm punch biopsies per patient taken from the buccal mucosa for use in flow cytometric analysis. The study was carried out with approval from the Regional Ethical Committee (REK Sørøst, Oslo, Norway, 2015/1247), and in accordance with the Declaration of Helsinki. Written consent was obtained from all donors.

### Immunofluorescence staining

Four-micron thick FFPE sections were deparaffinized prior to heat-induced epitope retrieval in 0.05% citraconic anhydrid (Sigma-Aldrich) for 15 min at 100˚C using a decloaking chamber (Biocare Medical) and cooled on ice. Staining was preceded by a blocking step of 30 min with 5% serum matching the species of the secondary antibody, after which the sections were incubated overnight at 4˚C with the primary antibody. After washing, the sections were incubated with matched secondary antibodies for 2 h at room temperature. Primary and secondary antibodies used are listed in Table S2A. Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI; Molecular Probes) before coverslips were mounted with polyvinyl alcohol mounting medium containing DABCO (Sigma-Aldrich). Control stainings were carried out, both with isotype-matched antibodies (Table S2A) and after omission of the primary antibodies. All the control stainings were negative.

Stained cells were counted by the same investigator on images obtained from a Nikon E90i fluorescence microscope with a 20x objective. A rectangular viewing frame was used, covering an area of 0.13 $mm^2$ at 20x magnification. The following procedure was used to count langerin$^+$ MHCII$^+$, Ki-67$^+$, and MHCII$^+$ cells: For the epithelium, the entire area visible in the biopsy was counted. The epithelial area and epithelial surface length (ESL) were then determined with ImageJ software (National Institute of Health), and cell densities calculated as cells/$mm^2$ and cells/ESL. For the lamina propria, a one frame high zone was counted, situated beneath the basal lamina over the whole length of the biopsy, and cell densities expressed as cells/$mm^2$. For the other markers used in the study, three randomly selected fields were counted. For the epithelium, the lower line of the counting frame was grossly aligned with the basal lamina and for the lamina propria the upper line of the frame was aligned with the basal lamina. Confocal images were acquired with an Olympus iX81 FluoView 1000 inverted confocal microscope, equipped with a PlanApo 60 x/1.30 oil and the binary pictures were pseudocolored. High resolution images (Figure S2) were acquired with an Olympus SpinSR10 spinning disk confocal super resolution microscope equipped with a Yokogawa CSU-W1 SoRa. Images were acquired with a 60 x Plan Apo 1.42 NA objective. Tile scans (Figures S3, S6) were performed with an Andor Dragonfly equipped with a fusion stitcher. The Andor Dragonfly was built on a Nikon TiE inverted microscope equipped a 60 x/1.40 NA oil immersion objective.

## Flow cytometry

For flow cytometry, two 5 mm punch biopsies were obtained from the buccal mucosa of healthy volunteers. Biopsies were incubated in Dulbecco's modified Eagle's medium containing 1.25 mg/mL of dispase (GIBCO) and incubated overnight at 4°C. The epithelium was then separated from the underlying connective tissue under a microscope, cut into small pieces and digested under stirring for 60 min at 37 °C in RPMI, supplemented with 0.25 mg/mL Liberase TL (Roche) and 20 U/mL DNase I (Roche). The digested cell suspensions were then passed through a 100 $\mu$m filter, washed in PBS, and stained with fluorescently labeled antibodies (Table S2B) together with FcR blocking reagent (Miltenyi Biotec) on ice for 15 min. Dead cells were excluded based on TO-PRO-1 staining (Molecular Probes). Flow cytometry was performed on a LSR Fortessa (BD Biosciences), and data were analyzed using FLOWJO 10 software (FlowJo LCC).

## Statistical evaluation

Estimates of cell counts and cell fractions are presented as mean values ± SD. Differences observed between normal oral mucosa and oral lichen planus lesions were assayed by two-tailed t tests for independent samples. However, for samples with minimal spread of values, the Mann Whitney test were used. P values lower than 0.05 were considered to indicate statistical significance.

# RESULTS

## MHCII$^+$ and langerin$^+$ cells

We first stained tissue sections of biopsies from normal oral mucosa and oral lichen planus lesions with antibodies to MHCII, and estimated the cell density based on cell numbers per $mm^2$ of cross-sectioned epithelium. We found significantly higher numbers of MHCII$^+$ APCs both in the epithelium and lamina propria of oral lichen planus lesions compared with normal oral mucosa (Figure S1A). The numbers of APCs were increased by 2.6 and 13.3-fold in the epithelium and lamina propria, respectively.

We then assessed the numbers of epithelial Langerhans cells (MHCII$^+$ langerin$^+$) (Figure 1A), estimated as both cells/$mm^2$ of cross-sectioned epithelium (Figure 1B) and cells/mm ESL (Figure S1B). Nearly all langerin$^+$ cells expressed MHCII (Figure S1C, S3B), both in the epithelium and in the lamina propra. This was corroborated by flow cytometry on digested healthy human oral epithelial sheets (Figure S1D). The number of Langerhans cells was significantly higher ($P < 0.05$) in oral lichen planus than in normal oral mucosa, both in terms of density (Figure 1B) and cells/mm ESL (Figure S1B). The number of Langerhans cells per $mm^2$ was 2.6-fold higher in oral lichen planus lesions than in normal oral mucosa (231 cells/$mm^2$ ± 96 for oral lichen planus versus 89 cells/$mm^2$ ± 34 in normal oral mucosa) and 1.8-fold higher when counted as cells/mm ESL in oral lichen planus lesions than in normal oral mucosa (47 cells/mm ESL ± 22 versus 26 cells/mm ESL ± 9), respectively (Figure S3). Moreover, Langerhans cells constituted the major fraction of MHCII$^+$ cells within the epithelium of both normal oral mucosa and oral lichen planus lesions, and this fraction was not significantly different between the two groups (Figure 1C).

In the lamina propria, we observed a much higher number of langerin$^+$ cells in the oral lichen planus specimens (Figure 1B), with a 42.8-fold higher number (310 cells/$mm^2$ ± 192) in oral lichen planus lesions than in control specimens (7 cells/$mm^2$ ± 9). These cells were also uniformly MHCII$^+$ (Figure S1C, Figure S3B) and constituted 17.6% ± 10.8 of the mucosal MHCII$^+$ APCs in the oral lichen planus lesions (Figure 1C). Using super-resolution microscopy, we found that langerin appeared to be enriched within these cells in intracellular organelles compatible with the Birbeck granules observed in Langerhans cells (Figure S2). The langerin$^+$ cells in the lamina propria were mostly scattered within the upper

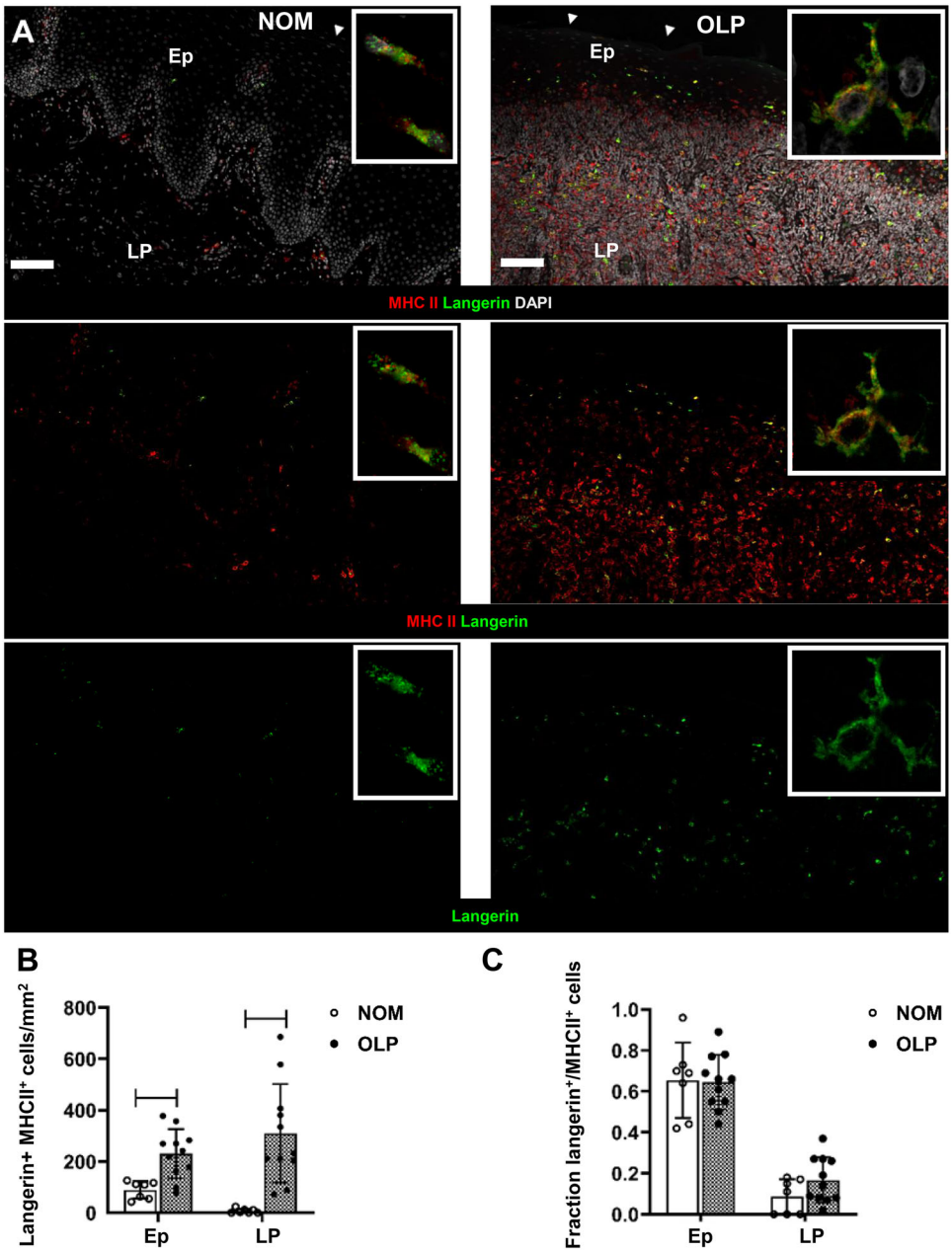


**FIGURE 1** **Langerhans cells and lamina propria langerin$^+$ cells in healthy buccal mucosa and in oral lichen planus lesions**. (A) Biopsies were immunostained with anti-MHC II (red) and langerin (green) antibodies and incubated with DAPI (white) for nuclear localization (original magnification 20 x). Inserts show examples of langerin$^+$ and MHC II$^+$ Langerhans cells in normal oral mucosa and oral lichen planus lesions (original magnification 60 x). Ep: Epithelium LP: lamina propria. (B) Numbers of langerin$^+$ MHCII$^+$ cells/mm$^2$ in the epithelium (Ep) and lamina propria (LP), in oral lichen planus and normal oral mucosa. (C) Fractions of Langerhans cells within total MHCII$^+$ cells in normal oral mucosa and oral lichen planus. Horizontal brackets show statistically significant differences between normal oral mucosa and oral lichen planus (t test; $P < 0.05$). Arrow heads mark the surface of the epithelium. Scale bar: 50 $\mu$m

and middle parts of the inflammatory infiltrates, unevenly distributed throughout the width of the infiltrate with intermitting focal accumulations (Figure 1A, Figure S3B). Co-staining for CD3 and CD4 revealed that the langerin$^+$ cells were closely intermingled with mucosal T cells (Figure S4).

## Expression of proliferation marker Ki-67 in langerin$^+$ cells

We then co-stained for langerin and the proliferation marker Ki-67 (Figure 2A). Whereas a significant fraction of epithelial Langerhans cells in normal oral mucosa expressed Ki-67

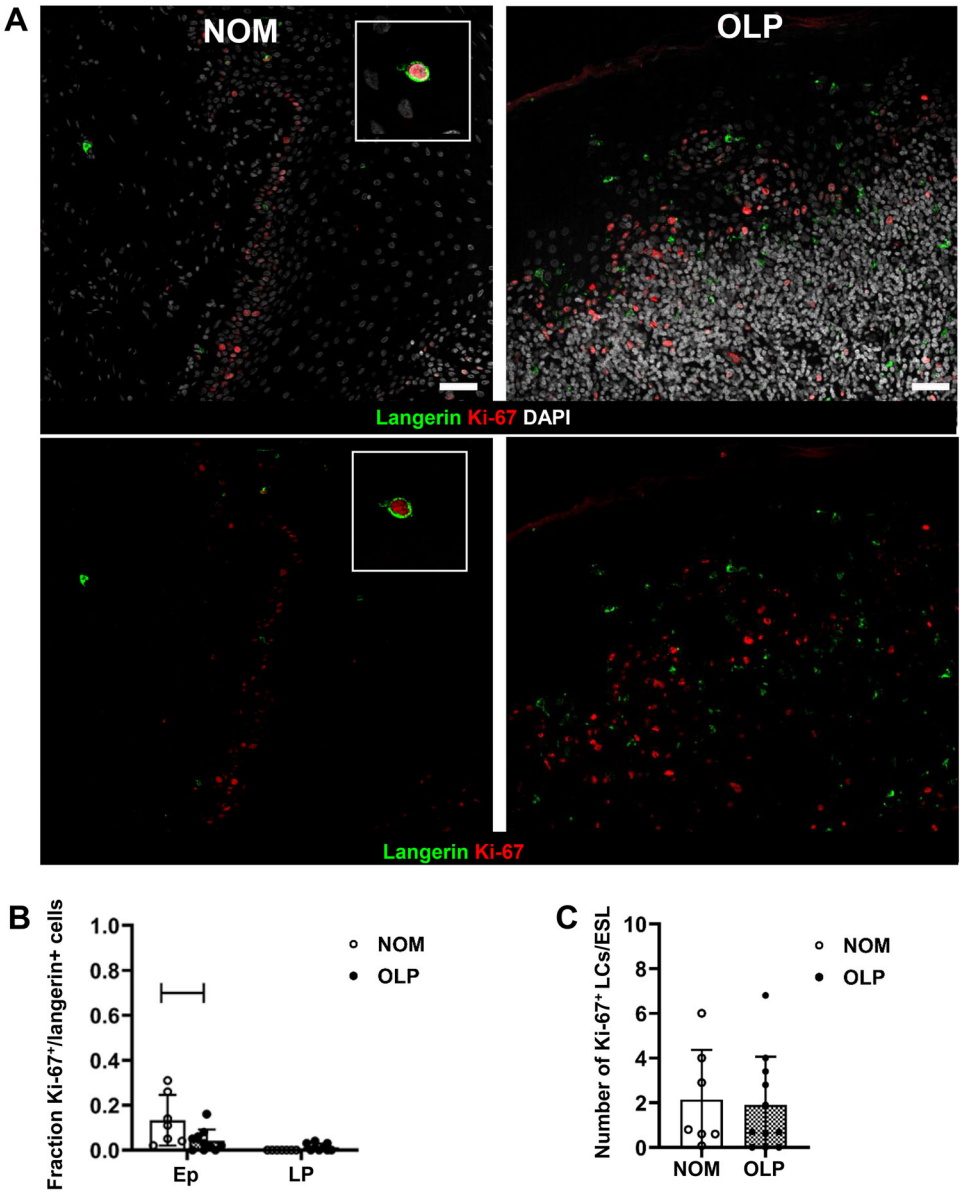


**FIGURE 2** **Expression of Ki-67 in langerin$^+$ cells in epithelium and lamina propria of normal oral mucosa and oral lichen planus**. (A) Biopsies were immunostained with anti-langerin (green) and anti-Ki-67 (red) antibodies and incubated with DAPI (white) for nuclear staining (original magnification 20 x). Inserted images show an example of a Ki-67$^+$ nucleus in langerin$^+$ Langerhans cell in normal oral mucosa and oral lichen planus lesions (original magnification 60 x). (B) Fractions of Langerhans cells that co-express Ki-67 within total langerin$^+$ cells in normal oral mucosa and oral lichen planus lesions. (C) Numbers of langerin$^+$ Ki67$^+$ cells within epithelial surface length. Ep: Epithelium, LP: Lamina propria. Horizontal bracket indicates statistically significant difference between normal oral mucosa and oral lichen planus (t test; $P < 0.05$). Scale bar: 50 $\mu$m

(13.3% ± 11.3), this fraction was lower in oral lichen planus (4.2% ± 5.0) (Figure 2B). However, the absolute number of Ki-67$^+$ langerin$^+$ cells/ ESL in oral lichen planus (1.7 ± 2.2) was not significantly different from normal oral mucosa (2.4 ± 2.0) (Figure 2C). Only a small fraction of langerin$^+$ MHCII$^+$ cells in the lamina propria of oral lichen planus and normal oral mucosa stained for Ki-67 (Figure 2B).

## Myeloid marker expression of langerin$^+$ cells

We next examined the expression of the macrophage markers CD14, calprotectin (S100A8/S100A9) and CD68 on langerin-expressing cells. In normal oral mucosa epithelium, 47.5% ± 0.2 of the Langerhans cells expressed CD14 (Figure S5), and this was corroborated with flow cytometry on healthy human oral epithelial sheets (Figure S1D). In oral lichen planus epithelium, however, CD14 and CD68 were barely

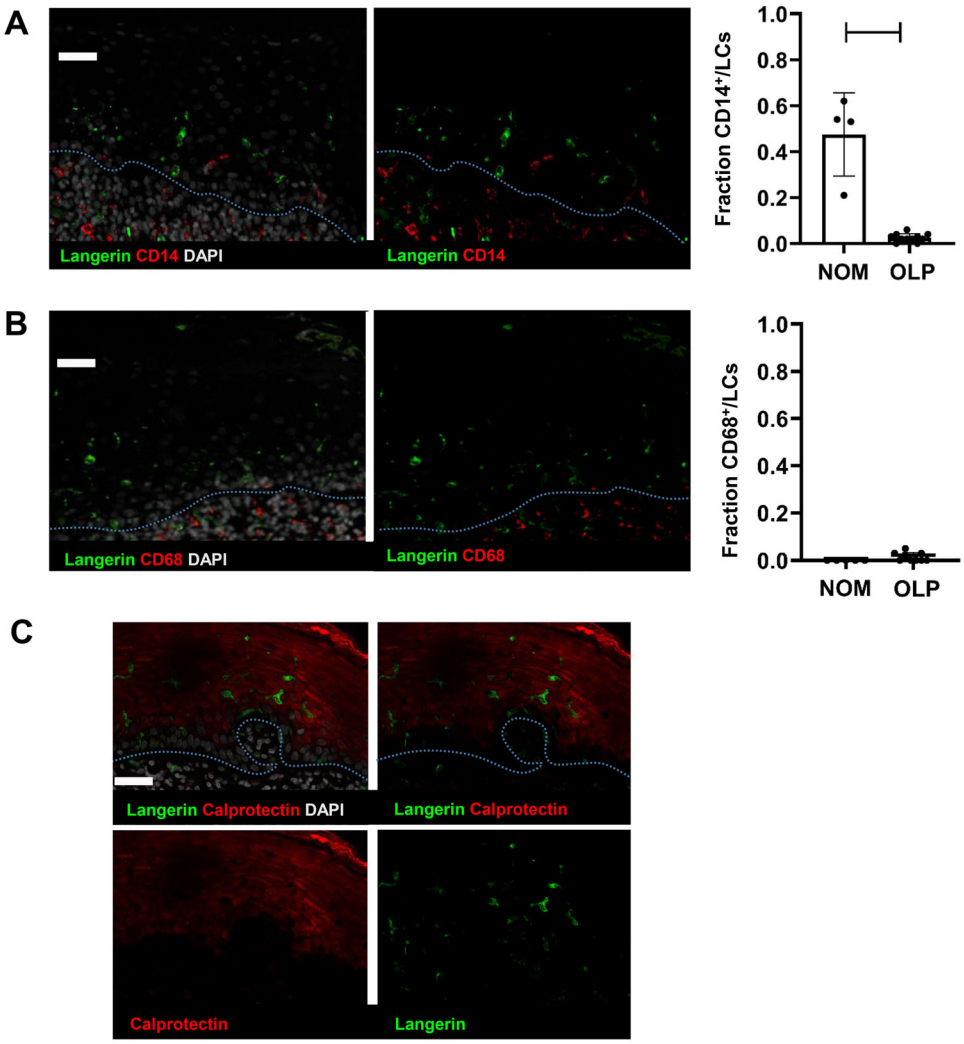


**FIGURE 3** **Expression of CD14, CD68 and calprotectin in Langerhans cells in the epithelium of normal oral mucosa and oral lichen planus lesions**. Biopsies were immunostained with anti-langerin combined with (A) anti-CD14, (B) anti- CD68 and (C) anti-calprotectin, and incubated with DAPI for nuclear staining. Only pictures of oral lichen planus are shown. (A, B) Confocal images of epithelium in oral lichen planus lesions with (left) and without (right) nuclear staining (original magnification 20 x). Box plots show fractions of langerin$^+$ cells that co-express CD14 and CD68. The horizontal bracket indicates statistically significant difference between normal oral mucosa and oral lichen planus (t test; $P < 0.05$). (C) Confocal image of epithelium of oral lichen planus lesion, triple- double- and single stained as indicated. The stippled lines indicate the position of the basal membrane. Scale bar: 50 $\mu$m

detectable on Langerhans cells (Figure 3A and B). Calprotectin expression appeared to be confined to keratinocytes in this compartment (Figure 3C).

In the lamina propria of oral lichen planus lesions, we found a high number of cells that expressed the pan macrophage marker CD14 (data not shown). However, CD14 was undetectable on lamina propria langerin$^+$ APCs in both oral lichen planus and normal oral mucosa (Figure 4A). Furthermore, staining for CD68, a lysosomal protein highly expressed by macrophages, revealed a similarly higher number of CD68$^+$ cells in oral lichen planus lesions than in normal oral mucosa (Figure S6). As for CD14, however, CD68 expression was not seen in langerin$^+$ cells (Figure 4B, Figure S6). Similarly, when assessing the expression of calprotectin, which is expressed by recently elicited monocytes but gradually down-regulated during macrophage differentiation, calprotectin$^+$ cells in the lamina propria of oral lichen planus were highly enriched compared with normal oral mucosa, but langerin$^+$ cells co-expressing calprotectin constituted only a minor fraction (Figure 4C).

Tissue staining for the transcription factor IRF4 revealed nearly no staining in normal oral mucosa, but in oral lichen planus, numerous cells displayed nuclear IRF4 staining (Figure S7). Co-staining of langerin and IRF4 revealed that among Langerhans cells, 16% ± 13 expressed IRF4 (Figure 5A). Furthermore, 51% ± 17 of lamina propria langerin$^+$ cells showed expression of this transcription factor (Figure 5B).

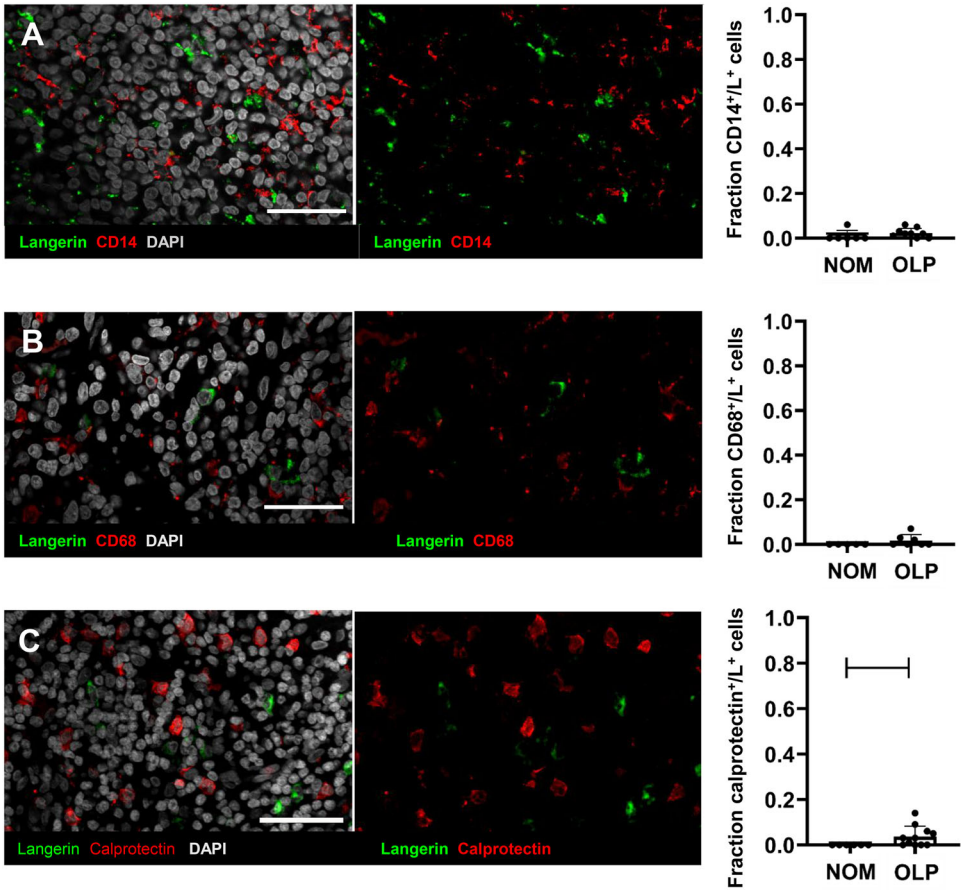


**FIGURE 4** **Expression of CD14, CD68 and calprotectin in langerin$^+$ MHCII$^+$ cells in the lamina propria of normal oral mucosa and oral lichen planus lesions**. Biopsies were immunostained with anti-langerin (green) combined with (A) anti-CD14 (red), (B) anti- CD68 (red) and (C) anti- calprotectin (red), respectively, and incubated with DAPI for nuclear staining. Only pictures of oral lichen planus are shown. Images show the inflammatory infiltrate in the lamina propria of oral lichen planus lesions with (left) and without (right) nuclear staining (original magnification 20 x). Box plots show fractions of langerin$^+$ (L$^+$) cells that co-express CD14, CD68 or calprotectin. The horizontal bracket indicates statistically significant difference between normal oral mucosa and oral lichen planus (Mann Whitney-test; $P < 0.05$). Scale bar: 50 $\mu$m

## DISCUSSION

Langerin-expressing APCs are found both in the epithelium and the lamina propria in normal oral mucosa, and the results of the present study support the observations that their numbers are higher in oral lichen planus [15, 25, 26] than in normal oral mucosa. The aim of this study was to investigate the origin of such cells.

In the epithelium, langerin expression is confined to Langerhans cells, which are specialized APCs in the epidermis and other stratified squamous epithelia characterized by their high expression of CD1a and molecules involved in epithelial cell adhesion, including E-cadherin, EpCAM, and tight junction proteins [23]. Notably, Langerhans cells express high levels of the c-type lectin langerin that is crucial for development of Birbeck granules, hallmark organelles found in Langerhans cells [27]. Even though several studies have reported increased numbers of Langerhans cells in oral lichen planus [15, 25, 26], the mechanism behind the Langerhans cell expansion in oral lichen planus has not been examined. It is currently well established that human dermal Langerhans cells are able to self-renew through proliferation in situ [28, 29], but mouse studies indicate that oral mucosal Langerhans cells instead can be derived from DC-precursors and monocytes [30].

In the oral epithelium of normal oral mucosa, we observed that a notable fraction of Langerhans cells expressed the proliferation marker Ki-67, indicating that human oral mucosal Langerhans cells have the capacity for local self-renewal. However, approximately 50% of the Langerhans cells in normal oral mucosa expressed CD14, which is compatible with a monocyte origin. This suggests a model in which the oral epithelium harbors a population of self-renewing Langerhans cells that are integrated within a dynamic Langerhans cell pool derived from hematopoietic cells [23], which largely displays a monocyte origin.

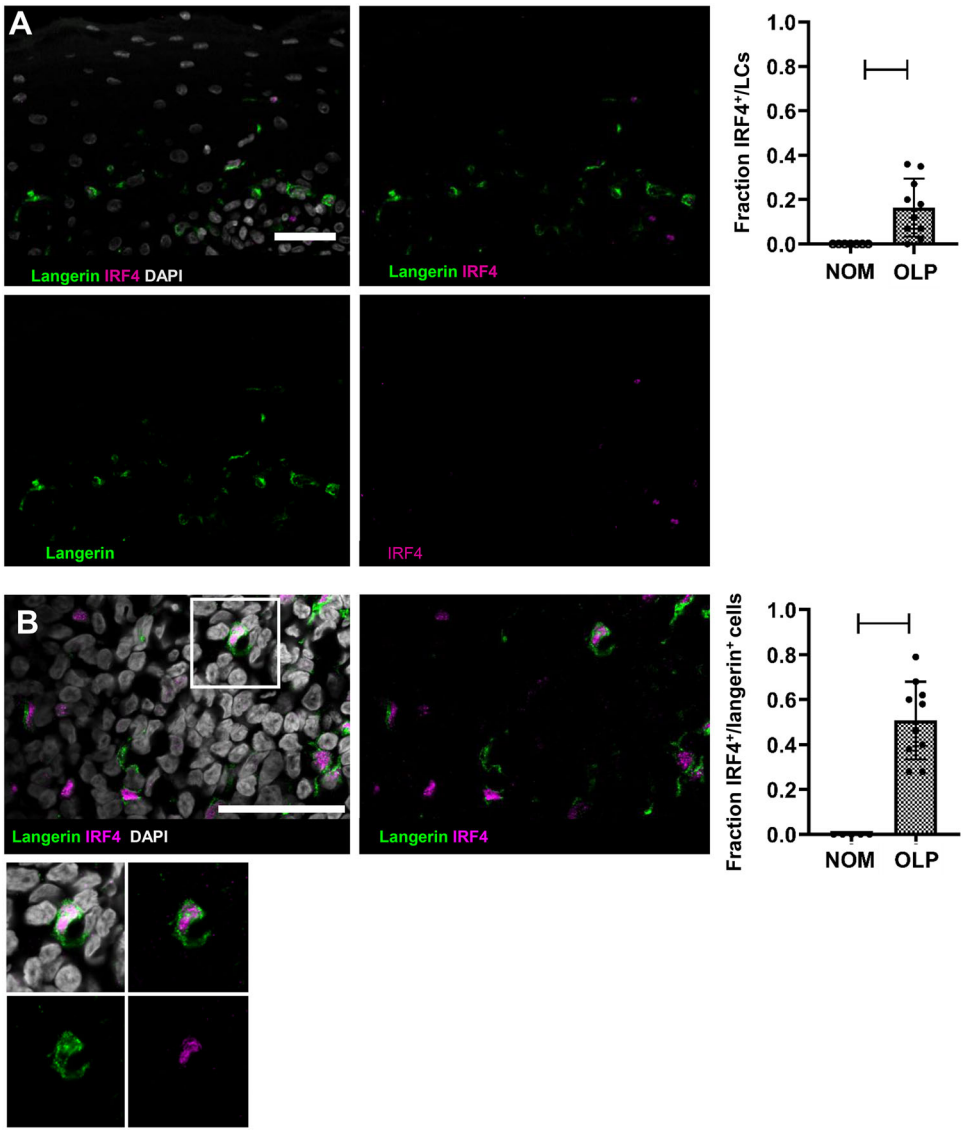


**FIGURE 5** **Expression of IRF4 in langerin$^+$ cells in the epithelium (A) normal oral mucosa and (B) oral lichen planus lesions**. Biopsies were immunostained with anti-langerin (green) and anti-IRF4 (magenta) antibodies, and incubated with DAPI for nuclear staining (original magnification 20 x for the epithelium, 60x for the lamina propria). (A) Confocal images of normal oral mucosa, with (upper left) and without (upper right) DAPI, and single channels for Langerin and IRF4 (lower row). (B) Confocal images of oral lichen planus lesion with (left) and without (right) DAPI. The box plots show the fraction of langerin$^+$ cells that co-expresses IRF4 in normal oral mucosa and oral lichen planus lesions. Small images in (B) show a part of the large pictures (white square). The horizontal brackets indicate statistically significant differences between normal oral mucosa and oral lichen planus (Mann Whitney test; $P < 0.05$). Scale bar: 50 $\mu$m

In the epithelium of oral lichen planus, we found a significant 2-fold higher number of Langerhans cells in oral lichen planus than in normal oral mucosa, in line with earlier studies [12–15]. This difference was significant regardless of estimation based on the cross-sectional epithelial area or the surface length of the epithelium, showing that the higher number is not the result of reduced epithelial thickness observed in oral lichen planus [31, 32]. Although the fraction of proliferating Langerhans cells was lower in oral lichen planus lesions, the density of Ki-67$^+$ Langerhans cells was not different from that seen in normal oral mucosa. In contrast to normal oral mucosa, the Langerhans cells in oral lichen planus lesions were mostly negative for CD14, but a significant fraction expressed the transcription factor IRF4. This indicates that the increased Langerhans cell numbers in oral lichen planus are due to an increased influx of precursor cells with a cDC2 phenotype, that mature to Langerhans cells locally.

The functional implications of such remodeling of the Langerhans cell network in oral lichen planus remain to be explored. Steady-state Langerhans cells have conventionally been considered as the first line of defense against invasive

pathogens and important in the maintenance of tissue homeostasis by rapidly scavenging debris from dying cells [33]. However, recent data indicate that Langerhans cells are also important immune regulators by activating and propagating regulatory T cells (Tregs) in barrier tissues. Notably, when stimulated by pathogens, such Langerhans cells activate effector memory T (Tem) cells and limit Treg stimulation [34]. Moreover, Langerhans cells differentiated from cDC2 display a proinflammatory phenotype, by producing high levels of inflammatory cytokines including TNF$\alpha$ and IL-6 [18]. Thus, the cDC2-related Langerhans cells observed in oral lichen planus may promote tissue damage by both activating pathogenic effector T cells and by secreting cytokines that promote epithelial damage. Future work should thus explore whether the macrophage-like and DC-related Langerhans cells observed in normal oral mucosa and oral lichen planus, respectively, display different immunostimulatory or regulatory potential. In addition, the role of the tissue microenvironment in recruiting Langerhans cell-precursors should be assessed. Mouse models indicate that sequential action of bone morphogenetic protein 7 (BMP7) and TGF-$\beta$ underpin epithelial translocation and differentiation of Langerhans cells in healthy oral mucosa [35], but how this is regulated in oral lichen planus remains elusive.

In the lamina propria of normal oral mucosa, only few langerin$^+$ cells were detected, in line with previous observations [9, 15]. In the sub-epithelial lamina propria of oral lichen planus lesions, however, a prominently higher number of langerin$^+$ MHCII$^+$ cells was seen. Only very few cells expressed Ki-67, indicating a low level of local proliferation. Therefore, their increased numbers appear to depend on precursors recruited from the blood. Delineation of such precursor cells is complex due to overlapping expression of phenotypic markers among tissue APCs [23, 36]. In particular, tissue macrophages and cDC2 are hard to distinguish due to shared expression of several canonical cDC2-markers, including CD1c/BDCA1 and CD11c [20, 23, 24, 37]. However, tissue macrophages retain expression of several monocyte markers, thus staining for CD14, CD163, or CD68 can be used for phenotyping [22, 37, 38]. Recent comparative gene expression analyses of APC subtypes have shown that APCs can be robustly classified based on lineage and expression of key transcription factors [39–41]. Although cDC2s appear to share several transcription factors with other DC subsets [23], IRF4 is regarded to be lineage-defining for such cells [40, 42]. Therefore, we stained the langerin-expressing cells in the lamina propria of oral lichen planus for CD14, CD68, calprotectin, and IRF4.

We observed that langerin expression was hardly detectable in either recently elicited macrophages (as characterized and detected by calprotectin staining) or in mature macrophages (as characterized and detected by CD14 and CD68 staining). This thus indicates that blood monocytes do not contribute to the langerin$^+$ cells in the oral lichen planus lesions, even though it is well established that they avidly upregulate langerin in vitro in response to GM-CSF together with IL-4 and TGF-$\beta$ [16, 17]. Instead, we found that the majority of lesional langerin$^+$ MHCII$^+$ cells showed nuclear staining for IRF4, indicating that they are related to cDC2 [24, 42]. cDC2s have indeed been shown to be able to rapidly induce high expression of langerin in vitro in response to GM-CSF, TSLP, or TGF-$\beta$ [18–20], and may even display cytoplasmic Birbeck granules following such treatment [18]. In fact, we also observed langerin-staining organelles that are compatible with Birbeck granules within the lesional langerin$^+$ cells, thus indicating a function in receptor-mediated endocytosis within these cells.

A hallmark of cDCs is their capacity to migrate to regional draining lymph nodes [41], but in the current type of study, it is not possible to assess the migration patterns of the langerin$^+$ cDCs. However, our co-staining of langerin with T cell markers revealed that these cells were closely intermingled within the leukocytic infiltrates. It is therefore conceivable that langerin$^+$ DCs are involved in local T cell activation in the mucosa. Studies of other human inflammatory disorders, including allergic rhinitis and celiac disease, indeed indicate that mucosal tissue DCs may provide efficient local activation of pathogenic T cells [43, 44]. The nature of the T cell responses underlying oral lichen planus is not resolved [45–49], but the most supported model is that autoreactive CD8+ T-cells are involved in degeneration of the epithelial basal cells. However, the inflammatory infiltrate also contains substantial numbers of different CD4$^+$ T-helper cell subsets that can shape the immune response through the production of chemokines and cytokines. Many studies have suggested a role of differently polarized T-helper subsets in the pathogenesis of oral lichen planus, but the relative contribution of these is not established [45–48]. cDC2 have been shown to promote a wide range of T cell responses, including Th1 and Th17 cells [23], and may also provide antigen cross-presentation to CD8 T cells [50]. Interestingly, a recent study reported improvement of oral lichen planus after treatment with the IL-17-inhibitor secukinumab [49]. Moreover, targeting cytokines associated with the Th17 axis using anti-IL-23 or anti-IL-12/IL-23 lead to marked and prolonged improvements of mucosal lesions in oral lichen planus. Although the study encompassed a limited number of subjects, this is compatible with a role for Th17 cells as drivers of the disease. In inflammatory settings, cDC2 produce high levels of cytokines critical for T cell responses, including IL-12 and IL-23 [23]. Thus, cDC2s recruited to oral lichen planus-lesions may be important activators of the pathogenic T cells in the mucosa, and provide pro-inflammatory factors that maintain the tissue inflammation.

A limitation of the present study could be that the study groups were not fully age- and gender-matched: age showed

a trend to be lower in the healthy group (t test, $P > 0.05$; data not shown) and the gender distribution in the groups was 8 F/3 M (oral lichen planus) and 3 F/4 M (normal oral mucosa). However, in statistical terms, age did not appear to be a confounding factor in our evaluations as calculations of Kendall $\tau$ correlation coefficients between age and all dependent variables within the healthy group revealed no statistically significant associations ($P > 0.05$; data not shown). Furthermore, Mann Whitney tests within the healthy group showed no statistically significant differences for the studied variables when male and female volunteers were compared ($P > 0.05$; data not shown). Regarding Langerhans cells, this is in line with earlier observations that age and gender have no effect on oral mucosal Langerhans cell density. Numbers of Langerhans cells were indeed found to remain stable with increasing age in a study assessing the density of $CD1a^+$ oral Langerhans cells in a large tissue material from 91 subjects spanning 16–96 years of age [51]. Moreover, no significant differences in the numbers of oral mucosal Langerhans cells between women and men were found. In a similar approach, analyses of HLA-$DR^+$ Langerhans cells in samples from 41 subjects spanning 16–98 years of age revealed no significant differences in Langerhans cell numbers in relation to age and gender [52]. Therefore, differences in age and gender in the current groups are not likely to be confounding factors in the interpretation of the results on Langerhans cells. Available data on effects of age and gender on human interstitial DCs is scarce. However, several studies report that the numbers of circulating cDC2-precursors are unaltered in relation to age and gender [53]. Furthermore, when analyzing the composition of cDCs in the human gut, no significant changes in the frequencies of cDCs were reported in older individuals [41, 54], thus indicating that mucosal DCs are not expanded merely as a function of increasing age. Moreover, our visual examinations of the non-inflamed connective tissue areas surrounding the oral lichen planus lesions revealed no overt increase in the cDC2-density as compared with normal oral mucosa (not shown). Thus, it is likely that the vastly (> 40 x) higher number of cDC cells seen in oral lichen planus lesions is mainly driven by the local inflammation.

Taken together, we found that a substantial fraction of Langerhans cells appears to proliferate in healthy human oral epithelium, thus indicating that steady-state oral mucosal Langerhans cells are, at least partially, maintained by self-renewal. In oral lichen planus, the number of proliferating Langerhans cells was not altered, despite increased density of Langerhans cells, suggesting an increased influx of precursor cells that mature to Langerhans cells locally, most likely from cDC2s. Moreover, we found a large increase of $langerin^+$ APCs within the lamina propria of oral lichen planus patients. Such cells did not display monocyte or macrophage markers, but rather showed a phenotype compatible with tissue-elicited cDC2. Future efforts should be directed at detailed understanding of how the oral mucosal APC network is regulated and the functional capacities of the different ontogenies.

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## CONFLICTS OF INTEREST

The authors state no conflicts of interest.

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