INTRODUCTION

Several populations of dendritic cells (DCs) reside in distinct anatomical skin compartments during steady state (Haniffa et al., 2015). The epidermis is populated by Langerin⁺EpCAM⁺CD1a⁺ Langerhans cells (LCs) (Bigley et al., 2015; Borkowski et al., 1996; Fithian et al., 1981; Valladeau et al., 1999), whereas the dermis hosts several subsets of functionally distinct dermal DCs (dDCs) defined by their differential expression of CD141, CD1c, and CD14 (Chu et al., 2012; Haniffa et al., 2015). Skin DCs are promptly activated via toll-like receptor (TLR) signaling after tissue damage or microbial invasion (Yu et al., 2010). At sites of skin inflammation or infection, additional DCs can be recruited from the circulation (Pasparakis et al., 2014) and modulate local immune responses (Macleod et al., 2014; McLachlan et al., 2009).

Psoriasis is one example of a chronic inflammatory skin disease where DCs accumulate and produce cytokines capable of modulating the microenvironment. Scaly and well-demarcated plaques have been attributed to aberrant keratinocyte proliferation and infiltration of IL-17 and IL-22 producing T cells and neutrophils into the epidermis (Krueger et al., 2012; Lin et al., 2011; Lowes et al., 2008; Nestle et al., 2009). In the dermis, inflammatory DCs accumulate and contribute to the inflammatory environment through the production of tumor necrosis factor (TNF), inducible nitric oxide synthase, IL-12 (Brunner et al., 2013; Gutman-Yassky et al., 2007; Hansel et al., 2011; Lowes et al., 2005; Zaba et al., 2009), and IL-23 (Cai et al., 2011; Lee et al., 2004; Teunissen et al., 2012; Tonel et al., 2010; Yawalkar et al., 2009). In lesional epidermis, LCs display impaired migrational capacity (Cumberbatch et al., 2006; Shaw et al., 2010); elevated expression of CXCL9, CXCL10, and CCL20 (Fujita et al., 2011); and increased IL-23 production in response to TLR3 stimulation (Sweeney et al., 2016). Compared with LCs, epidermal DCs (eDCs) in lesional psoriasis display similar or higher gene expression of chemokines (Fujita et al., 2011). eDCs are found in direct contact with hyperproliferative keratinocytes; however, phenotypic and functional studies of these cells are scarce.

Here we show that both resident LCs and eDCs display proinflammatory profiles during active psoriasis. LCs responded to TLR4 and TLR7/8 activation in vitro by producing IL-23, whereas eDCs produced both IL-1β and IL-23. Despite the emergence of highly efficient therapies over the past few decades, psoriasis often recurs in previously affected sites. Our study reveals that eDCs are strictly confined to active disease, whereas LCs respond to TLR activation with IL-23 production during successful anti-TNF treatment.

RESULTS

A heterogeneous population of eDCs exceeds the number of LCs in lesional psoriasis

The presence and localization of professional antigen presenting cells in skin were visualized by their expression of...
CD74, the invariant chain of major histocompatibility complex class II, using confocal microscopy. As expected, epidermal CD74⁺ Langerin⁺ LCs containing Birbeck granules were detected in both healthy skin and lesional psoriasis (Figure 1a and b). Additionally, CD74⁺ cells lacking Langerin and Birbeck granules were observed in lesional psoriasis epidermis (Figure 1a and b). Both CD74⁺ Langerin⁺ and CD74⁺ Langerin⁻ cells were detected in close proximity to epidermal T cells (Supplementary Figure S1a online). To further characterize the CD74⁺ Langerin⁻ cells, epidermal and dermal cell suspensions were prepared by enzymatic tissue digestion (Supplementary Figure S1b). Virtually all epidermal HLA-DR⁺ cells expressed EpCAM in the healthy epidermis, whereas this population was sparse in healthy dermis (Figure 1c), with dermal EpCAM⁺ cells most likely reflecting LC migration from epidermis to draining lymph nodes. The majority of epidermal HLA-DR⁺ EpCAM⁺ cells in lesional psoriasis expressed CD11c but lacked CD141. Although a small subset of HLA-DR⁺CD11c⁺ CD141⁻ cells was present in lesional epidermis, further analysis of this subset was not possible due to the low density in limited clinical material (Figure 1c, Supplementary Figure S1c).

Similar densities of epidermal LCs were detected in lesional psoriasis, nonlesional psoriasis, and healthy skin (Figure 2a). In contrast, eDCs were strictly confined to lesional psoriasis with a density four times higher than LCs (Figure 2a). In accordance with previous findings, dermal HLA-DR⁺ CD11c⁺ cells accumulated in lesional psoriasis as compared with nonlesional psoriasis and healthy skin (Figure 2a). Gene expression analysis of sorted cells confirmed that epidermal HLA-DR⁺ EpCAM⁺ cells expressed LANGERIN, Ecadherin, TROP2, and CD1A genes that phenotypically define LCs. These LC-associated genes were absent or expressed at a significantly lower level in eDCs. Instead, eDCs expressed similar levels of CLEC9A, CD206, and CD163 as dermal DCs, with higher CD68 expression (Figure 2b, Supplementary Figure 2a online). Flow cytometric analysis confirmed that healthy and lesional LCs expressed high levels of CD1a and lacked CD14 expression (Figure 2c, Supplementary Figure S2c online). In lesional psoriasis, approximately 30% of eDCs and dDCs expressed CD14, compared with 10% in healthy dermis (Figure 2c, Supplementary Figure S2b). Interestingly, the frequency of CD1a⁺CD1c⁻ cells was increased in eDCs compared with dDCs, indicating phenotypic differences between the epidermal and dermal DC populations. Additionally, the frequency of CD1a⁺CD1c⁺ cells was higher in dDCs compared with eDCs, although this did not reach statistical significance (Figure 2c). In line with previous studies phenotypically defining Tip-DCs and slan-DCs (Hansel et al., 2011; Lowes et al., 2005; Zaba et al., 2009), TNF⁺ CD1c⁻CD11c⁺ DCs were detected in lesional dermis (Supplementary Figure S2d). A proportion of eDCs expressed TNF, together with CD1a and CD1c, revealing a phenotypic heterogeneity of TNF⁺ DCs in the different anatomical compartments within the skin. In comparison with epidermal LCs, eDCs also expressed a distinct pattern of CC and CXC chemokine receptors (CCR and CXCR) with increased expression of CCR2 and CXCR4 but similar CCR6 expression (Figure 2d). In agreement with the gene expression data, protein expression of CCR2 was three-fold higher in lesional eDCs compared with lesional LCs (Figure 2e). Thus, although eDCs expressed phenotypic markers that indicate a heterogeneous population with several subsets of DCs, we found that eDCs were phenotypically distinct from dDCs and LCs in lesional psoriasis.

eDCs express IL-17 polarizing genes involved in psoriasis inflammation

To investigate the inflammatory profile of LCs and eDCs, the expression of 37 genes associated with inflammatory
responses (Supplementary Table 1 online) was analyzed by real-time PCR in sorted cells from healthy and lesional skin. LCs and eDCs extracted from lesional psoriasis displayed increased expression of the S100A8 and S100A9 genes associated with psoriasis, compared with LCs from healthy skin. Interestingly, lesional eDCs expressed several genes involved in keratinocyte activation and neutrophil recruitment, exemplified by IL1B, IL6, IL8, CXCL1, CCL17, and TRAIL (Figure 3a and b). Additionally, eDCs expressed a number of regulatory ligands and cytokines, exemplified by IL10 (Figure 3b). The IL-17 polarizing cytokine IL23A was increased in lesional LCs compared with healthy LCs, which warranted further investigation even though the observed difference did not reach statistical significance. Although the T-cell stimulatory cytokine IL15 was upregulated both in psoriasis LCs and eDCs (6.6- and 5.3-fold upregulation), IL15RA, which potentiates IL-15 stimulation, was exclusively expressed by eDCs (Figure 3b). Gene expression analysis of LCs and eDCs revealed that both populations displayed increased expression of different inflammatory mediators with the potential to modulate the epidermal milieu and promote psoriasis pathology.

TLR4 and TLR7/8 stimulation increase IL-23 and IL-1β expression in LCs and eDCs in active psoriasis lesions

To verify elevated expression of genes relevant to psoriasis pathology we next investigated intracellular cytokine expression in LCs, eDCs, and dDCs. Baseline expression of IL-23 and IL-1β was higher in lesional eDCs and LCs compared with healthy LCs, and IL-10 was selectively detected in eDCs, in line with the gene expression data (Figure 4a).

Psoriasis often flares during viral or bacterial upper respiratory infections (Fry and Baker, 2007), and we next determined if eDCs and LCs respond to ligands of TLR4, which senses bacterial polysaccharides, or TLR7 and 8, which sense viral or endogenous single-stranded RNA (Yu et al., 2010). Short-term stimulation with the TLR4 ligand lipopolysaccharide or the TLR7/8 ligand resiquimod induced IL-23 expression in LCs and both IL-23 and IL-1β in eDCs from lesional psoriasis. In contrast, expression of IL-10 in eDCs was not affected by TLR4 or TLR7/8 activation (Figure 4a, Supplementary Figure S3 online). eDCs displayed cytokine production similar to dDCs in lesional psoriasis with both single- and double-expressing IL-23 and IL-1β cells after resiquimod stimulation (Figure 4a and b). Considering the
abundance of eDCs and the combined expression of TNF together with IL-17 polarizing cytokines IL-1β and IL-23, our results suggest that eDCs amplify the local inflammatory environment during active disease.

eDCs are strictly confined to macroscopic psoriasis inflammation

Modern treatments for psoriasis normalize the skin morphology (Boehncke and Schön, 2015), but the disease often recurs in previously affected sites, indicating a localized disease memory (Cheuk et al., 2014; Suarez-Farinas et al., 2011). Although the number of dermal DCs decreases in resolved lesions (Gunther et al., 2013; Johnson-Huang et al., 2010; Lowes et al., 2005; Malaviya et al., 2006; Zaba et al., 2007), these cells retain the capacity to produce IL-23 and TNF-α (Gunther et al., 2013), indicating that dDCs might contribute to localized disease memory. Anti-TNF (Etanercept) and UVB treatment induces effective, but seldom complete disease control, which allows sampling from remaining active disease during or after treatment. To investigate if eDCs follow macroscopic pathology during disease resolution, resolved and nonresolved skin lesions were sampled during systemic anti-TNF or after UVB treatment. Four subjects (three anti-TNF treated and one UVB treated) with residual disease could be identified and biopsies were collected from resolved and nonresolved lesions (Figure 5a). In resolved lesions, the number of DCs in the dermis remained two- to five-fold higher than in healthy demis (data not shown). Strikingly, eDCs were absent in resolved epidermis after UVB or during anti-TNF treatment, indicating strict confinement of eDCs to clinical disease.

LCs in resolved lesions remain poised to express IL-23

A clinical challenge when treating psoriasis is the recurrence of disease in previously affected areas. To investigate if LCs retain proinflammatory properties after disease resolution, the gene expression profile in LCs sorted from clinically resolved lesions after 25–30 sessions of UVB treatment or during anti-TNF treatment (Table 1) was analyzed. A few genes of interest to psoriasis pathology remained differentially expressed in LCs from skin successfully treated with anti-TNF or UVB compared with healthy LCs (Figure 5b). IL15 and CCR2 expression was upregulated in LCs from UVB-treated skin compared with healthy controls (Figure 5c). IL23A expression was elevated in LCs from anti-TNF-treated patients, albeit normalized in UVB-treated samples in line with a previous report (Johnson-Huang et al., 2010) (Figure 5b and c). To determine whether LCs also retained the capacity to produce IL-23 in response to TLR stimulation, LCs from patients undergoing anti-TNF treatment with almost complete disease resolution were analyzed (Table 1). Despite the increased expression of IL23 in anti-TNF-treated LCs, less than 2% of LCs from healthy and resolved skin expressed IL-23 (Figure 5d) ex vivo. Interestingly, after TLR7/8 stimulation, LCs from anti-TNF-treated skin responded with a higher abundance of eDCs and the combined expression of TNF together with IL-17 polarizing cytokines IL-1β and IL-23, our results suggest that eDCs amplify the local inflammatory environment during active disease.

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Figure 3. Inflammatory gene expression in sorted LCs and eDCs in lesional psoriasis and healthy skin. (a) Relative gene expression heat map of inflammatory genes in sorted LCs from healthy epidermis (n = 9), and LCs and eDCs from lesional psoriasis (n = 9). Columns: individual donors, rows: individual genes. (b) Bar graphs comparing selected gene expression in LCs from healthy skin with LCs and eDCs from lesional psoriasis. Housekeeping gene RPLPO. Mean ± SD is shown. eDC, epidermal dendritic cell; LC, Langerhans cell; SD, standard deviation.
frequency of IL-23 expression compared with healthy stimulated LCs (Figure 5d) albeit to a lower extent compared with LCs extracted from lesional psoriasis. In contrast, IL-1β production was not affected by stimulation and did not exceed what was present in healthy LCs (Figure 5d), in line with the normalized gene expression profile (Figure 5b). As shown by confocal imaging (Figure 5e), LCs were found in close contact with T cells in resolved lesions in patients treated with anti-TNF therapy, emphasizing that even moderate alterations in cytokine production may affect resident T cells in resolved lesions.

**DISCUSSION**

A flexible and compartmentalized immune system is necessary to maintain skin homeostasis and immunity, but aberrant activation of the system may induce focal pathology. Psoriasis is a chronic immune-mediated skin disease with profound epidermal alterations, in part driven by interactions between activated keratinocytes (Bonish et al., 2000; Chiricozzi et al., 2011; Harper et al., 2009), infiltrating T cells (Cheuk et al., 2014; Conrad et al., 2007; Hijnen et al., 2013), and neutrophils (Lin et al., 2011; Wetzel et al., 2006). Here we report dynamic changes in the composition of eDCs with the potential to modulate the epidermal inflammation during different clinical phases of psoriasis.

The role of LCs in psoriasis remains elusive but proinflammatory properties are indicated by expression of CXCL9, CXCL10, and CCL20 (Fujita et al., 2011). We found similar densities of LCs in healthy skin, nonlesional, lesional, and resolved psoriasis in agreement with various reports (Cumberbatch et al., 2006; Guttman-Yassky et al., 2007) and in contrast to others (Glitzner et al., 2014; Gunther et al., 2012). The discrepancy in the reported density of LCs may result from the use of different markers and experimental protocols to evaluate LCs by histology and flow cytometry. Additionally, the density of LCs changes during the evolution of psoriasis plaques (Bieber and Braun-Falco, 1989) and potentially in different parts of individual plaques. In active psoriasis, we observed that LCs expressed IL23, IL1B, and IL15 genes associated with psoriasis inflammation. Furthermore, LCs responded to short-term TLR4 and TLR7/8 stimulation with increased protein production of IL-23, in line with a previous study investigating the effect of TLR3 ligation (Sweeney et al., 2016). Thus, LCs may contribute to the local inflammatory microenvironment in response to microbial stimulation or endogenous ligands.

Recurrent psoriasis in fixed spots of the skin is a clinical reality that has been attributed to the persistence of tissue alterations (Suarez-Farinas et al., 2011) and retention of tissue resident IL-23R^CCR6^CD103^CD8^ T (T_{RM}) cells
poised to produce IL-17 (Cheuk et al., 2014). LCs from UVB- or anti-TNF-treated lesions displayed increased expression of IL15 and IL23, and both cytokines are capable of in situ stimulation of pathogenic T RM cells. Although moderate, LCs from anti-TNF-treated lesions responded to TLR7/8 activation with increased IL-23 production. Considering that LCs reside close to epidermal T cells, LCs could potentially initiate local inflammation mediated by keratinocytes and IL-23R expressing T RM cells. One proposed mechanism for UVB treatment is the induction of LC emigration (Duthie et al., 2000; Mohammed et al., 2016). Conflicting data on the density of epithelial LCs after UVB treatment have been published (Erkin et al., 2007; Tjoe et al., 2003), potentially reflecting sample collection at different times in relation to the termination of UVB treatment. LCs collected from UVB-treated skin displayed elevated CCR2 and IL15 expression, corresponding to transient inflammatory CCR2+ LCs that are recruited from circulation to replenish the local pool of LCs at sites of inflammation (Eidsmo et al., 2009; Ginhoux et al., 2006; Merad et al., 2002; Nagao et al., 2012). A dramatic increase in the number of CD11c+ cells is obvious in both epidermis and dermis in active psoriasis lesions. Lesional dermal CD11c+ cells produce IL-23 and IL-12 (Cai et al., 2011; Lee et al., 2004; Teunissen et al., 2012; Tonel et al., 2010; Yawalkar et al., 2009), and Tip-DCs and slan-DCs are the main producers of inducible nitric oxide synthase and TNF (Brunner et al., 2013; Guttman-Yassky et al., 2007; Hansel et al., 2011; Lowes et al., 2005; Zaba et al., 2009). We found similar frequencies of TNF producing cells in eDCs and dDCs. However, TNF+ eDCs coexpress CD1c and CD1a, whereas dermal TNF+ cells lacked these markers, in agreement with previous studies characterizing Tip-DCs (Zaba et al., 2009) and slan-DCs (Hansel et al., 2011).
In summary, our data indicate that both LCs and eDCs contribute to the proinflammatory environment in psoriasis. Importantly, eDCs were 10 times more numerous than LCs and showed increased IL-1β expression. Thus, eDCs may be recruited to sites of inflammation where they amplify the tissue responses and contribute to psoriasis pathology. Our results suggest that resident and infiltrating DCs act together to influence the inflammatory microenvironment in psoriasis, which opens up for topical treatments targeting depletion of pathogenic DCs within individual psoriasis plaques.

**MATERIAL AND METHODS**

**Clinical samples**

Four-millimeter punch biopsies were collected from lesional, non-lesional, or healed (Etanercept- or UVB-treated) skin from patients at the Psoriasis Association Clinic (Sundbyberg, Sweden) or the Department of Dermatology, Karolinska Hospital (Stockholm, Sweden). Resolved skin was defined as skin lacking scaling and macroscopic inflammation and was identified by photographs taken before the start of treatment, residual hyperpigmentation, or reliable patient history. Skin from healthy volunteers undergoing reconstructive surgery was obtained from AdVita Clinic (Stockholm, Sweden) or from Karolinska University Hospital (Stockholm, Sweden). Patient characteristics are shown in Table 1. The study was approved by the Stockholm Regional Committee of Ethics and performed according to the Declaration of Helsinki. Signed consent forms were collected from all donors.

**Confocal microscopy**

Frozen 8-μm-thick sections of cryopreserved skin punch biopsies were stained as previously described (Cheuk et al., 2014; Eidsmo et al., 2009) with the antibodies shown in Supplementary Table S2 online. Images were acquired by Zeiss LSM780 (Zeiss, Oberkochen, Germany) and analyzed with Fiji-ImageJ.

**Transmission electron microscopy**

Punch biopsies from three donors were fixed in 3% paraformaldehyde (Agar Scientific, Stansted, UK), transferred to 2.5% glutaraldehyde (Ladd Research, Williston, VT) and postfixed in 2% osmium tetroxide (TAAB Laboratories, Reading, UK), followed by dehydration and embedding in LX-112 (Ladd Research). Ultrathin sections (50–60 nm) were cut by a Leica EM UC 6 (Leica Microsystems, Wetzlar, Germany). Sections were contrasted with uranyl acetate followed by lead citrate and examined in a Tecnai 10 transmission electron microscope (FEI, Hillsboro, OR) at 100 kV. Digital images were taken by using a Voleta camera (Olympus Soft Imaging Solutions, Münster, Germany).

**Preparation of epidermal cell suspension**

Whole skin biopsies were incubated in 5 U/ml Dispase (Life Technologies, Carlsbad, CA) overnight at 4 °C, and the epidermis and dermis were separated. Epidermis and dermis were incubated with 570 U/ml Collagenase III (Worthington, Lakewood, NJ) with 5 μg/ml DNAse (Roche, Basel, Switzerland) for 90 minutes at 37 °C. The epidermal and dermal cell suspensions were obtained after mechanical disruption by pipetting or using Medicon (BD Biosciences, San Jose, CA), respectively.

**Flow cytometry, cell sorting, RNA extraction, and quantitative RT-PCR**

Cell sorting was performed from epidermal and dermal cell suspensions from four to five punch biopsies per donor stained with conjugated antibodies (Supplementary Table S2) using MoFlo XDP (Beckman Coulter, Brea, CA). Cells were collected in QiAziol lysis reagent (Qiagen, Venlo, the Netherlands) and total RNA was extracted.
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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2016.11.033.

REFERENCES


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