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Evaluation of the Diagnostic Value of Plasmacytoid Dendritic Cells in Differentiating the Lymphocytic Cicatricial Alopecias

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Key Words
Plasmacytoid dendritic cells · Lupus erythematosus · Lichen planopilaris and frontal fibrosing alopecia · Interferon · MxA · BDCA-2

Abstract
Background: Differentiating lymphocytic scarring alopecias may be difficult clinically as well as histopathologically. ***Objective:*** To investigate plasmacytoid dendritic cell (PDC) presence and distribution patterns and their diagnostic value in differentiating scarring alopecias of lupus erythematosus (LE) from lichen planopilaris (LPP) and frontal fibrosing alopecia (FFA). ***Methods:*** Seventeen LE-associated alopecia, 20 LPP and 10 FFA cases were immunohistochemically tested for PDC presence/distribution and activity. ***Results:*** LE-associated alopecia showed increased PDC content (≥10% PDCs in all cases and ≥50% in 94% of cases), PDC clusters (100% of cases), and deeper dermal and perieccrine distribution (100% of cases) with involvement of the dermoepidermal junction (DEJ, 94% of cases), while the majority of LPP and FFA had <10% PDC content that was mainly confined to the upper dermis surrounding the hair infundibulum with rare DEJ involvement and rare clustering. ***Conclusions:*** Specific PDC-related parameters may serve as a useful diagnostic adjunct in the differentiation between LE-associated alopecia versus LPP and FFA.

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Introduction

Differentiating the lymphocytic scarring alopecias, especially lupus erythematosus (LE) and lichen planopilaris (LPP), may be difficult as they may have overlapping clinical (fig. 1) and histopathological features [1]. Helpful microscopic clues for the diagnosis of scarring alopecia due to LE include basement membrane thickening, increased mucin deposition, and a superficial and deep perivascular and periadnexal lymphocytic infiltrate. However, these features, at times, may not be present. Differentiation of these entities from each other is crucial to patient management. There is currently no useful immunohistochemical marker that helps in differentiating these entities.

In the last few decades, plasmacytoid dendritic cells (PDCs) have been extensively studied and shown to correspond to a specialized dendritic cell population with plasma cell morphology. They express blood-derived dendritic cell antigen-2 (BDCA-2), interleukin-3 receptor alpha chain (CD123), and Toll-like receptor (TLR)7 and TLR9 within endosomal compartments and, when activated, are capable of producing large quantities of type I interferons (IFNs; IFN-α/β) against pathogenic agents or danger signals [2–5]. Plenty of evidence currently exists on a potential role of these cells in various human diseases affecting the skin including inflammatory, infectious and neoplastic [2–6]. Specifically, recent evidence suggests a significant role of PDCs in LE patho-

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Fig. 1. Examples of clinical presentations of LE-associated alopecia (**a**), LPP (**b**), and FFA (**c**). **Inset** Concomitant involvement of the face.

genesis [6, 7]. Although PDCs have been shown to be increased in all forms of cutaneous LE and lichen planus, their prominence and patterns of distribution in the two diseases were different [6–11]. PDCs were constantly more prominent and detected as distinct perivascular and periadnexal clusters in LE, while in lichen planus they were commonly increased in the upper dermis and in the epidermis adjacent to necrotic keratinocytes [6–11]. Recently, Walsh et al. [11] showed a significant diagnostic value of three criteria pertaining to PDCs (representation of ≥10% of inflammatory infiltrate, arrangement in clusters of ≥10 cells, presence at the dermoepidermal junction, DEJ) in differentiating hypertrophic LE from its mimickers. We have recently demonstrated the role of PDCs and their diagnostic value in the noncicatricial alopecias [12]. A recent study also demonstrated PDC recruitment into LPP lesions; however, no study has yet looked into the diagnostic value of PDC presence and distribution patterns in the lymphocytic cicatricial alopecias due to LE, LPP, and frontal fibrosing alopecia (FFA), the latter regarded by many as a clinical LPP variant (fig. 1) with indistinguishable microscopic features [1, 13–16].

So, taking advantage of the availability of multiple markers (especially BDCA-2) for immunohistochemical PDC detection on formalin-fixed paraffin-embedded tissues [17], we intend in the current study to investigate the occurrence and pattern of distribution of PDCs in lymphocytic scarring alopecias due to LE, LPP and FFA to evaluate their diagnostic value in the histopathological differentiation of these entities.

Materials and Methods

The Institutional Review Board of the American University of Beirut Medical Center approved the study. Archival materials with diagnosis of LE, LPP and FFA were retrieved from the dermatopathology database at the Dermatology Department at our institution. A total of 17 cases of LE, 20 cases of LPP, and 10 cases of FFA were included in the study. Only straightforward cases that fit the clinicopathological features of LE, LPP and FFA were chosen for the study. Cases in which the diagnosis was not certain were excluded from the study. The histological sections of all cases were re-reviewed and the diagnoses were confirmed. Clinical information was extracted and all patient data were de-identified.

Immunohistochemical Analysis

Immunohistochemical analysis was performed on 5-mm-thick sections obtained from formalin-fixed, paraffin-embedded tissue. Following de-paraffinization and rehydration steps, antigen retrieval was performed using citrate buffer and steamer. Slides were treated with 3% hydrogen peroxide in methanol for 10 min to block the endogenous peroxidase. Samples were then incubated with appropriate blocking serum. Antibodies to BDCA-2 (mouse IgG1, clone 124B3.13, dilution 1:50, Dendritics, Lyon, France) and myxovirus protein A (MxA, M143, University of Freiburg, Freiburg, Germany; dilution 1:100; provided by Prof. Haller) were used. While anti-BDCA-2 antibody is a specific marker of PDCs, anti-MxA antibody

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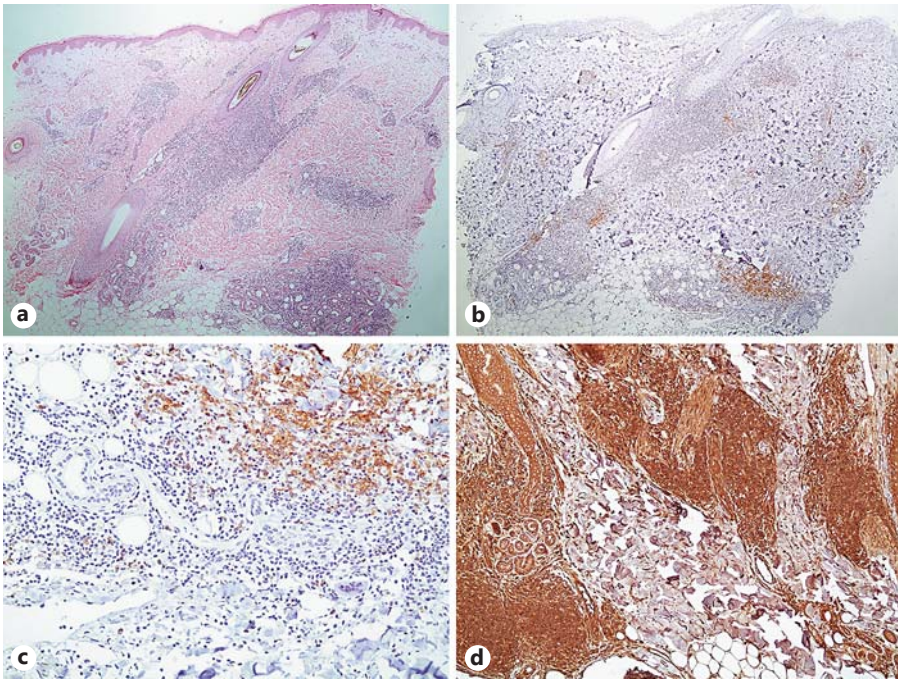

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Fig. 2. Representative LE-associated alopecia case. **a** Superficial and deep perivascular and periadnexal inflammatory infiltrate with dermal fibrosis and loss of sebaceous glands. Hematoxylin-eosin stain. ×40. **b**, **c** BDCA-2 immunostaining highlighted numerous PDCs (>10% of the mononuclear infiltrate) arranged both as single cells and clusters and distributed in superficial and deep perivascular and periadnexal locations. **b** ×40. **c** ×100. **d** MxA immunostaining was intense and diffuse involving the superficial and deep epithelial structures and the inflammatory cells. ×100.

was used to assess for the activity of PDCs as MxA is an IFN-α/β-inducible intracellular protein well established as a surrogate marker for local type I IFN production [18]. All stained slides were reviewed independently by first and senior authors (R.S. and O.A.) to ensure consistency of interpretation. PDC content was scored on sections stained for BDCA-2 and reported as a percentage of total mononuclear infiltrate: 0 (very rare positive cells), 1 (<10% of positive cells), 2 (10–50% of positive cells), and 3 (>50% of positive cells). We focused on three main parameters pertaining to PDCs including PDC content (≥10% or PDC score of 2 or 3 vs. <10% or PDC score of 0 or 1), PDC cluster arrangement with clusters defined as ≥10 PDCs, patterns of PDC distribution (superficial or deep, perivascular, perifollicular, perieccrine, and/or at DEJ). MxA staining was scored as: 0 = negative, 1 = weak/patchy staining, and 2 = intense staining. Normal skin tissue served as negative control and cases of inflamed molluscum contagiosum served as positive control [19].

Statistical Analysis

Statistical analysis was performed by using the Mann-Whitney test to analyze statistical differences in PDC and MxA scores between LE, LPP and FFA groups. Fisher's exact test was used to analyze statistical differences in the PDC distribution pattern between LE, LPP and FFA. A two-tailed p value of <0.05 will be considered statistically significant.

Results

All patients had their lesions on the scalp. Patients with LE (14 women and 3 men) had a mean age of 45 years. Patients with LPP (18 men, 2 women) had a mean age of 64 years. Patients with FFA were all women and had a mean age of 47 years.

PDCs in LE, LPP and FFA

This was assessed using antibodies against BDCA-2, which is a marker expressed on the surface of PDCs. PDCs were identified as medium-sized round cells. PDCs were present in all LE, LPP and FFA cases (fig. 2–4). There was no statistically significant difference in the mere presence of PDCs in these three entities. However, comparisons of PDC-related variables between LE (fig. 2) and LPP (fig. 3), LE and FFA, and LE and LPP/FFA showed significant differences. These results are outlined in table 1.

First, PDC content (demonstrated by PDC score) was significantly higher in LE cases (fig. 2) as compared to LPP (fig. 3) and in LE cases as compared to FFA (fig. 4): PDCs were more than 10% of the mononuclear infiltrate (PDC score of 2 or 3) in all (100%) cases of LE-associated alopecia, while only 6 of 20 (30%) LPP cases and 2 of 10 (20%) FFA cases showed a similar content of PDCs. PDC clusters were also observed significantly more in LE-associated alopecia cases (17 of 17 cases; 100%) compared to LPP (3 of 20 cases; 15%) and FFA (0 of 10 cases; 0%) cases. In addition, the distribution of PDCs was different. While PDCs were present in a superficial perivascular and peri-infundibular location in all three entities, only LE-associated alopecia cases also showed deeper dermal

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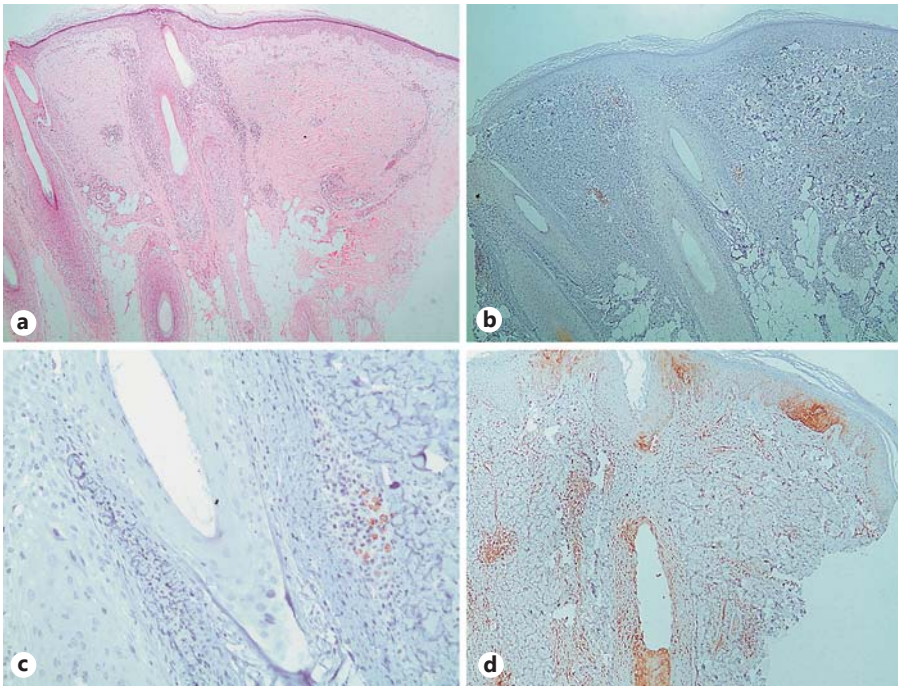

Fig. 3. Representative LPP case. **a** Predominantly lichenoid peri-infundibular inflammatory infiltrate with focal hypergranulosis and loss of sebaceous glands. Hematoxylin-eosin stain. ×40. **b**, **c** BDCA-2 immunostaining highlighted scattered PDCs (<10% of the mononuclear infiltrate) mainly distributed in peri-infundibular locations. **b** ×40. **c** ×200. **d** MxA immunostaining was weak and patchy involving the superficial epithelial structures and inflammatory cells. ×40.

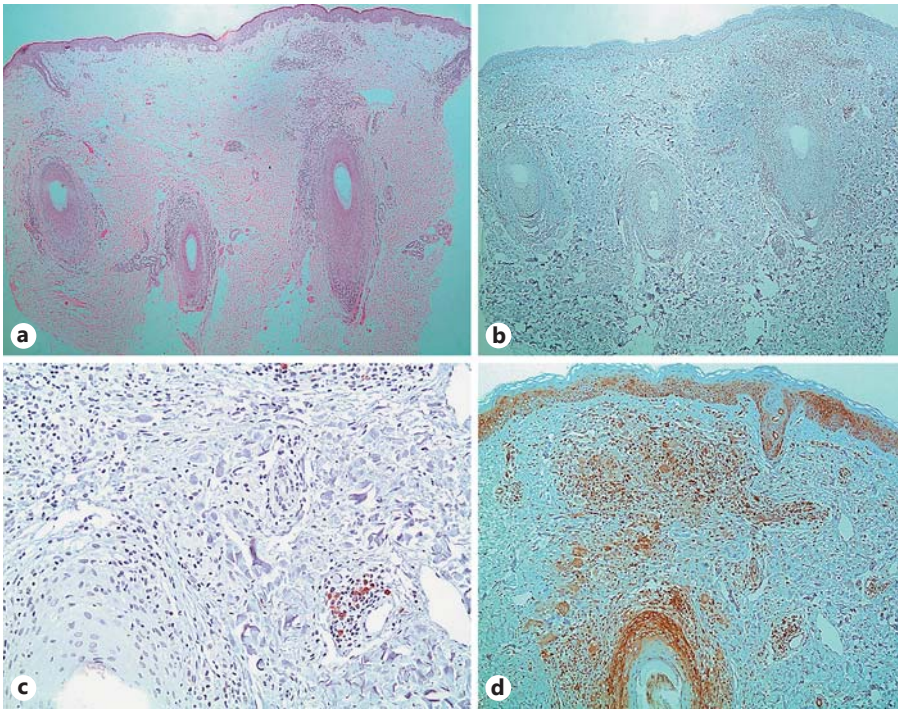

Fig. 4. Representative FFA case. **a** Predominantly peri-infundibular inflammatory infiltrate with perifollicular fibrosis and loss of sebaceous glands. Hematoxylin-eosin stain. ×40. **b**, **c** BDCA-2 immunostaining highlighted scattered PDCs (<10% of the mononuclear infiltrate) mainly distributed in peri-infundibular location. **b** ×40. **c** ×200. **d** MxA immunostaining was weak and patchy involving the superficial epithelial structures and inflammatory cells. ×100.

PDC infiltrate, especially in a perieccrine distribution (17 of 17 cases; 100%). PDCs were also observed at the DEJ in the majority of LE-associated alopecia cases (16 of 17 cases; 94%), whereas only 2 of 20 (10%) LPP cases and 0 FFA cases showed this feature.

Expression of MxA

MxA is a protein induced by type 1 IFNs and its tissue expression is a surrogate marker of local tissue type 1 IFN production [14, 15]. In parallel with the occurrence of PDCs, all cases of LE (fig. 2), LPP (fig. 3) and FFA (fig. 4)

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Table 1. Frequency and activity of PDCs in lupus alopecia versus LPP, FFA, and LPP/FFA

	LE cases (n = 17)	LPP cases (n = 20)	p value (LE vs. LPP)[3]	FFA cases (n = 10)	p value (LE vs. FFA)[3]	LPP/FFA cases (n = 30)	p value (LE vs. LPP and FFA)[3]
Frequency of PDC infiltration	17 (100)	20 (100)	1	10 (100)	1	30 (100)	1
PDC score[1]							
0	0 (0)	0 (0)	<0.0001	0 (0)	<0.0001	0 (0)	<0.0001
1	0 (0)	14 (70)		8 (80)		22 (73.3)	
2	1 (6)	5 (25)		2 (20)		7 (23.3)	
3	16 (94)	1 (5)		0 (0)		1 (3.3)	
PDC clusters	17 (100)	3 (15)	<0.0001	0 (0)	<0.0001	3 (10)	<0.0001
Deep dermal PDCs	17 (100)	0 (0)	<0.0001	0 (0)	<0.0001	0 (0)	<0.0001
PDCs at DEJ	16 (94)	2 (10)	<0.0001	0 (0)	<0.0001	2 (7)	<0.0001
Perieccrine PDCs	17 (100)	0 (0)	<0.0001	0 (0)	<0.0001	0 (0)	<0.0001
MxA score[2]							
0	0 (0)	0 (0)	<0.0001	0 (0)	<0.0001	0 (0)	<0.0001
1	0 (0)	15 (75)		9 (90)		24 (80)	
2	17 (100)	5 (25)		1 (10)		6 (20)	

Values represent number of cases (%).

[1] $BDCA2^+$ PDC content was scored as percentage of total mononuclear infiltrate: 0 (no positive cells), 1 (<10% positive cells), 2 (10–50% positive cells), and 3 (>50% positive cells).

[2] MxA staining was scored as: 0 = negative, 1 = weak/patchy, and 2 = intense/diffuse.

[3] Statistical analysis was performed using Mann-Whitney test with two-tailed p value of <0.05 considered statistically significant. Fisher's exact test was used to analyze statistical differences in PDC distribution pattern and PDC clusters between lupus alopecia vs. LPP, FFA, and LPP/FFA; p values in the table relate to comparisons between LE-associated alopecia vs. LPP alone, FFA alone, and LPP and FFA together.

showed positive staining of MxA usually within the epithelial structures and inflammatory cells. Intensity of MxA staining (demonstrated by MxA score), however, differed with all LE cases showing intense diffuse MxA staining compared to 5 of 20 (25%) LPP cases and 1 of 10 (10%) FFA cases. The remaining LPP and FFA cases showed patchy staining (table 1; fig. 3, 4).

LPP and FFA biopsies showed essentially similar results in all studied parameters of PDC content, clustering, and distribution as well as MxA expression. Combining LPP and FFA cases still showed statistically significant differences in these criteria when comparing them to LE-associated alopecia cases (table 1).

Discussion

PDCs, also known as 'natural IFN-producing cells', are the most potent type I IFN producers, secreting up to 1,000 times more IFN-α/IFN-β than other cell types [2, 6, 12, 19]. Essentially absent from normal skin, PDCs have been demonstrated in a variety of infectious, inflammatory and neoplastic cutaneous diseases including viral infections, psoriasis, connective tissue diseases, solid cancers and lymphomas [2, 6, 12, 20, 21]. Their prominent role in the different LE forms affecting the skin has been well studied and is linked to their ability to activate both the innate and adaptive immune systems [6, 11]. Sunlight-induced keratinocytic injury and release of self-antigens are thought to trigger PDC recruitment to the skin and type I IFN production. Moreover, PDCs can mature under the influence of certain cytokines allowing them to stimulate CD4-positive T cells to produce interleukins and IFN-γ [22].

Differentiating the lymphocytic scarring alopecias, especially LE and LPP, may be difficult as they share overlapping clinical and histopathological features [1]. This study showed that by BDCA-2 staining for PDCs and using three specific parameters, PDC content with a cutoff at 10% of mononuclear infiltrate, presence of PDC clusters (clusters defined as ≥10 PDCs), and distribution pattern of PDCs, differentiation between the LE-associated alopecia versus LPP and/or FFA can be made with high accuracy. While LE-associated alopecia showed increased PDC content (≥10% PDCs in all cases and ≥50% in 94% of cases), PDC clusters (100% of cases), and deeper dermal and perieccrine distribution (100% of cases) with DEJ involvement (94% of cases), the majority of LPP and FFA had <10% PDC content mainly confined to upper dermis surround-

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ing the hair infundibulum with rare DEJ involvement and rare clustering (table 1). The latter findings of PDCs in LPP were similar to those shown in a recent study [13].

In our study, we also looked at PDC activity via MxA staining. LE-associated alopecia showed intense and diffuse MxA staining in all cases as compared to patchier staining in LPP and FFA (table 1) in a distribution pattern similar to that seen with BDCA-2 (fig. 2–4). Thus, MxA staining can be used as an additional parameter in differentiating these three entities.

First described by Kossard et al. [23], FFA is characterized by progressive recession of the frontal hairline mainly affecting postmenopausal women. The pathogenesis of LPP and FFA has been closely linked and, based on histological and immunohistochemical features, FFA has generally been regarded as a variant of LPP [15]. This study further supports this view, as all FFA showed similar infiltration and distribution patterns of PDCs to LPP.

Study limitations include its retrospective nature, which may lead to a lack of clinical data; however, this was avoided by reviewing each case and extracting the needed information.

In conclusion, the presence, distribution pattern, and activity of PDCs in LE-associated alopecia appear to be reminiscent of that in the other forms of cutaneous LE. These PDC-related parameters, along with the appropriate clinicopathological correlation, can have diagnostic value in confirming LE-associated alopecia and differentiating it from its mimickers. Our findings thus support the current practice of some dermatopathology laboratories which employ the immunohistochemical detection of PDCs as an adjunct for the diagnosis of suspected LE cases.

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Disclosure Statement

No conflict of interest declared.

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