

## CLINICAL REPORT

# The Proportion of Lymphocytic Inflammation with CD123-positive Cells in Lupus Erythematosus Profundus Predict a Clinical Response to Treatment

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**Lupus erythematosus profundus is a rare inflammatory disorder of subcutaneous fat in patients with lupus erythematosus. Previous reports suggested that plasmacytoid dendritic cells, which expressed CD123 and CD303 antigens, play a central proinflammatory role in the pathogenesis of lupus erythematosus. To find the factors that determine the response to treatment, we analysed 23 skin specimens from the patients with lupus erythematosus profundus. The patients with considerable lymphocytic inflammation with high percentages of CD123<sup>+</sup> cells in dermis and subcutaneous fat significantly responded to the systemic corticosteroid therapies. On the other hand, the patients with minor lymphocytic inflammation with low percentages of CD123<sup>+</sup> cells showed poor response to treatments. The mean percentage of CD123<sup>+</sup> cells in patients who showed good response to therapy was significantly higher than those that showed poor response ( $p=0.027$ ). These results suggest that the clinical response to treatment of lupus erythematosus profundus could be predicted from the histological features. Key words: lupus erythematosus profundus; plasmacytoid dendritic cells; CD123; CD303.**

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Lupus erythematosus profundus (LEP) is an inflammatory disorder of the subcutaneous fat in patients with lupus erythematosus (LE). LEP accounts for approximately 1–3% of patients with cutaneous LE (1, 2). The typical clinical presentations are deep subcutaneous nodules or plaques. It may be observed in patients with discoid lupus erythematosus (DLE), systemic lupus erythematosus (SLE) or as an isolated phenomenon without systemic or other cutaneous findings (3, 4). Most commonly the proximal limbs, the trunk and head and neck are involved. Most patients are adults between 20 and 60 years old, with a female to male ratio of approximately 2:1.

Previous reports suggested that plasmacytoid dendritic cells (pDCs) and type I interferon (IFN) play a central proinflammatory role in the pathogenesis of LE (5–7). Plasmacytoid DC have been identified as the main IFN-producing cells in LE and expressed high level of CD123 antigen, which is also known as IL-3 receptor  $\alpha$  chain. Alternatively, CD303 (BDCA-2), a type II transmembrane C-type lectin, was reported to be strictly expressed on pDCs. Elevated serum levels of type I IFN in patients with SLE and increased levels of type I IFN in skin lesions of DLE were also reported previously (8–10).

In this study, we investigated clinical, histopathological and immunophenotypical features of LEP, and assessed the association between the histological features and clinical manifestations. Furthermore, we examined the proportion of CD123<sup>+</sup> cells in LEP and association between these and the clinical response to treatment.

## PATIENTS AND METHODS

### *Clinical assessment and patient material*

Twenty-three patients with LEP and 17 patients with SLE who visited Kumamoto University Hospital between 2004 and 2013 were investigated. The diagnosis of LEP was confirmed by both clinical and histological findings. Diagnosis of SLE was defined as the presence of at least 4 items of American College of Rheumatology (ACR) criteria (4). We analysed their age, sex, duration of disease, clinical symptoms, lesions, histopathologic sections, therapy and clinical response to the treatment. Clinical and laboratory data reported in this study were obtained at the time of sampling of tissue or serum. LE is classified into 5 types, as listed below (5). cutaneous LE (CLE): cutaneous manifestation only, intermediate LE (ILE) I: basically CLE, associated with slight systemic and/or laboratory findings, ILE II: basically SLE, not satisfying the criteria of SLE, SLE I: lacking systemic and/or laboratory findings listed below, SLE II: associated with renal involvement, central nervous system (CNS) involvement, thrombocytopenia, haemolytic anaemia and/or serositis. Institutional review board approval and written informed consent were obtained before patients were entered into this study according to the Declaration of Helsinki.

### *Antinuclear antibodies*

Antinuclear antibodies (ANA) were detected by indirect immunofluorescence using Hep-2 cells as the substrate and double immunodiffusion, as described previously (6).

### Histology

Twenty-three skin samples were obtained from the lesions clinically suggesting panniculitis. Seventeen skin samples were obtained from patients of SLE. Haematoxylin-eosin stained sections were examined and Peters and Su histologic criteria were used for the diagnosis of LEP (3): lymphocytic aggregates, hyaline degeneration of the fat, lobular or septal panniculitis, lymphocytic vasculitis, and calcification criteria. Lesions of DLE were seen in about half of cases. In this study, we examined the presence of the following morphologic criteria: (i) Epidermal involvement: atrophy and/or flattening of the epidermis, vacuolar degeneration of the basal layer, necrotic keratinocytes, and hyperkeratosis. (ii) Dermal involvement: pattern of infiltration, involvement of the hair follicles, mucin deposition, and presence of fibrosis and/or sclerosis. (iii) Subcutaneous involvement: pattern of infiltration (lobular or septal) and presence or absence of the features: fibrosis of the septae, hyaline fat necrosis, calcium deposits, vasculitis, granulomatous reaction and lymphocytic nuclear dust.

To investigate an association between the histopathological features and the clinical manifestations, we evaluated the patterns of infiltrated lymphocytes and classified the histopathological patterns of the cases into 3 types listed below. Type 1: involves all layers of the skin (associated with DLE), type 2: involves dermis and fat only, type 3: involves only fat tissue. These samples were estimated independently by the 2 observers in a blinded manner (A.M. and S.F.). Some researchers have used the term LEP as a synonym for lupus panniculitis, and some have used the term LEP to designate the patients who have both lupus panniculitis and DLE lesions, and the term lupus panniculitis to refer to those having only subcutaneous lesions (11, 12). In this study, we used the term LEP for lupus panniculitis including the cases that have only subcutaneous lesions.

### Direct immunofluorescence study

For the direct immunofluorescence staining, 4 µm sections were cut in a cryostat at -20°C and then placed on special slides. Direct immunofluorescence studies were performed using fluorescein isothiocyanate labelled anti-human IgG, IgM, IgA, complement C3 and fibrin (Dako, Tokyo, Japan) according to the instructions.

### Immunohistochemistry

Skin samples were fixed in 10% neutral-buffered formalin and embedded in paraffin. Staining with mouse anti-human CD123 antibody (clone 7G3; BD Biosciences, CA, USA) was performed as a surface marker of pDCs. Deparaffinised sections were retrieved by incubation with citrate buffer, pH9, for 5 min in an autoclave. Endogenous peroxidase activity was inhibited after which sections were incubated with 5% normal goat serum for 20 min at room temperature. Anti-human CD123 antibody was used at a concentration of 1:50 and incubated with the specimens overnight in a humidifying chamber. After excess antibody was washed off with PBS, samples were incubated with horseradish peroxidase (HRP)-labelled goat anti-mouse antibody (Nichirei, Tokyo, Japan) for 60 min. The reaction was visualised by the DAB substrate system (Dojindo, Kumamoto, Japan). Slides were counterstained with Mayer's haematoxylin, and mounted using aqueous medium. Primary isotype monoclonal mouse antibodies (clone MG2a-53; abcam, Tokyo, Japan) was used as negative control. Stained sections were evaluated in the percentages of positive cells for CD123 staining among the infiltrated mononuclear cells. The cells were counted in 5 different 400-fold magnification fields under a light microscope.

### Double-colour immunofluorescence staining

Double-colour immunofluorescent staining of cryostat sections of skin samples was performed using anti-CD303 mAb (clone AC144; Miltenyi Biotec, CA, USA) and anti-CD123-PerCP-Cy 5.5 mAb (clone 7G3; BD Biosciences, CA, USA). Staining with non-conjugated primary mAb was revealed by secondary staining with Alexa Fluor 488-conjugated anti-mouse IgG Ab (Invitrogen, Oregon, USA). The sections were finally mounted with a medium containing 4,6-diamidino-2-phenylindole (DAPI) (VECTER; CA, USA) for nuclear fluorescent staining, and viewed under fluorescence microscope. Primary isotype monoclonal mouse antibodies (clone MOPC-31C; BD Pharmingen, Tokyo, Japan and clone MG2a-53; abcam, Tokyo, Japan) were used as negative controls.

### Statistical analysis

Statistical analysis was carried out with Mann-Whitney's U test and Kruskal-Wallis test using the statistical software Excel (Microsoft Corp., Redmond, WA, USA) and Statcel (OMS Publishing Inc, Tokyo, Japan). Correlation analysis was done by Spearman's rho test. *p*-value <0.05 was considered statistically significant.

## RESULTS

### Clinical findings

The profiles of 23 patients were shown in Table S1<sup>1</sup>. The majority of the patients (74%) were over 30 years of age, mean age at diagnosis was 36.8 years (range 12–59 years). A female to male ratio was 20:3. Proximal limbs were the most commonly involved sites (61%) followed by head and/or neck (35%) and trunk (8%). The majority of clinical symptoms was indurated erythema or indurated plaques. Duration of symptoms varied from 1 month to 14 years (up to 6 months: 61%, more than 6 months: 39%). Five patients (21%) fulfilled the ACR criteria for SLE.

### Laboratory findings

Antinuclear antibodies were positive in 13 of 19 cases (68%). Anti-double-stranded DNA antibodies were positive in 3 of 23 cases (13%). Peripheral blood counts abnormalities including anaemia, thrombocytopenia, and leucopenia were observed in 3 of 23 patients (13%).

### Histopathologic findings

Skin biopsies were performed in all patients. The histopathologic findings in the lesions clinically suggesting panniculitis are characterised by lymphocytic panniculitis, hyaline degeneration of the fat, perivascular lymphocytic infiltration in dermis and lymphoid nodular structures in the lower dermis and subcutaneous tissue. Lesions of DLE were observed in 12 of 23 cases (52%). Mucin depositions were seen in 8 of 23 cases

<sup>1</sup><http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1777>

(35%). In our histopathological classification, 12 cases (52%) were classified into type 1, 7 cases (30%) were type 2 and 4 cases (17%) were type 3. (Table S1<sup>1</sup>) There was not a correlation between the duration of symptom and the inflammatory cell infiltrates ( $p=0.54$ ). Some cases that have long duration of symptom showed a large number of lymphocytic infiltrates. The cases that have long duration did not show the histopathological findings of scar tissue.

#### Direct immunofluorescence study

We analysed the results of the direct immunofluorescence studies in 17 cases of LEP. Nine of 17 cases (53%) showed the immunoglobulin deposition along the basement membrane. In most cases, IgM deposition was observed. In 4 cases, the diagnosis of SLE was made. In 2 cases the diagnosis of LEP was made solely (Table S1<sup>1</sup>).

#### Treatment and clinical response

Twenty-one LEP patients were treated with systemic corticosteroid (0.5 mg/kg/day) and 2 patients were treated with topical corticosteroids. Antimalarial treatments are not available in Japan. In all patients, the erythema diminished. Subcutaneous indurations were in remission in 18 patients, and in 5 patients the lesions did not show any changes. Those 5 patients were classified into type 2 (3/7) and type 3 (2/4) in our histopathological classification. All patients classified into type 1 significantly responded to the treatments ( $p=0.029$ ). Moderate to strong lymphocytic infiltrations were seen in all cases of type 1. Scarce to strong lymphocytic infiltrations were seen in type 2 and 3, and the cases of type 2 and 3 who presented with minor lymphocytic inflammation and hyalinised fat necrosis showed poor response to the treatments.

In most of cases with dense lymphocytic inflammation in dermis and subcutaneous fat showed satisfactory improvements in response to systemic corticosteroid therapy (Fig. 1B). On the other hand, the cases with minor lymphocytic inflammation and hyalinised fat necrosis showed poor response to systemic corticosteroid therapy (Fig. 1A).

#### Immunohistochemical examination

The cells positive for CD123 were found in inflamed dermis and adipose tissues (Fig. 2A). The percentages of positive cells for CD123 were listed in Table S1<sup>1</sup>. In our histopathological classification, the mean percentage of positive cells for CD123 of patients classified into type 1 was 18.6%, type 2 was 8.9% and type 3 was 3.5%. The prevalence of CD123<sup>+</sup> cells in type 1 was higher than that in type 3 ( $p=0.019$ ). On the other hand, there was no statistical difference among the patients that clinically diagnosed as SLE (12.9%), ILE (12.6%) and CLE (11.7%). The mean percentage of CD123<sup>+</sup> cells in patients who showed good response to therapy was 14.2% and 5.0% for the patients who did not respond. The percentage of CD123<sup>+</sup> cells in patients who showed good response to therapy was significantly higher than that in the patients who did not respond ( $p=0.027$ ) (Table I).

Additionally, we examined 17 bioptic sections of SLE concerning a presence of CD123 positive cells. Vermi et al. (13) examined the frequency of cutaneous pDCs, positive for CD123 and CD303 in LE. They judged the cases that showed more than 1% of positive cells as positive case. They showed that cutaneous pDC infiltration was more frequent in CLE compared to SLE (96.4% vs 72.2%), and the percentage of positive cells in mononuclear infiltrates were significantly higher in CLE compared to SLE. Consistent with the previous study, the frequency of pDCs in LEP was higher than those in SLE (94.7% vs 64.7%). The mean percentage of positive cells for CD123 in LEP was 12.3% (range 0.9–31.3%) and 4.7% in SLE (range 0–15.5%). Furthermore, the percentage of CD123 positive cells in LEP was significantly higher than that in SLE ( $p=0.008$ ).

#### Double-colour immunofluorescence examination

Plasmacytoid DCs have been identified as the main IFN-producing cells in LE and expressed high levels of CD123 antigen, which is also known as IL-3 receptor  $\alpha$  chain. Alternatively, CD303 (BDCA-2), a type II transmembrane C-type lectin was reported to be strictly expressed on pDCs. Vermi, et al. (13) and Farkas, et al. (14) showed that pDCs positive for CD123 were

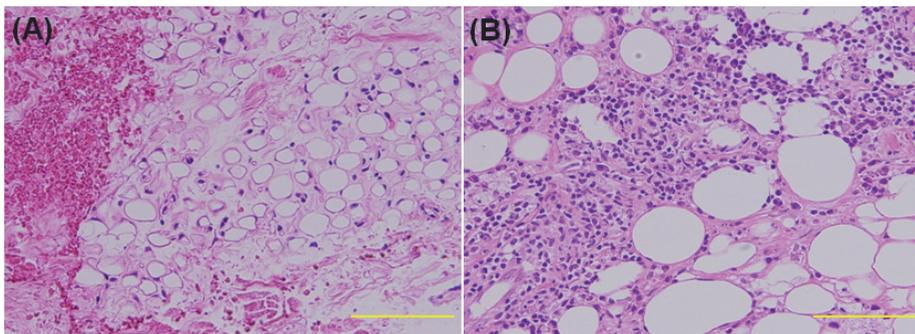


Fig. 1. Histopathological findings. (A) The case with minor lymphocytic inflammation and hyalinised fat necrosis ( $\times 100$ ). (B) The case with strong lymphocytic panniculitis and slight hyaline degeneration of the fat ( $\times 200$ ). Bars: 200  $\mu$ m (A), 100  $\mu$ m (B).

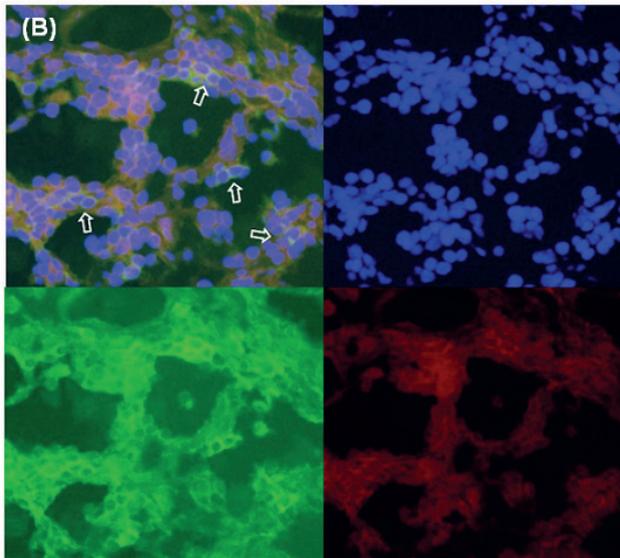
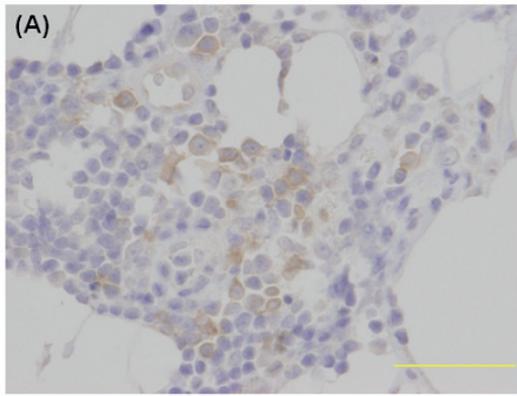


Fig. 2. Representative staining patterns for CD123 in lupus erythematosus profundus (LEP) skin lesions (A), and double-colour immunofluorescence staining for CD123 and CD303 (B). (A) Plasmacytoid morphologic cells staining positive for CD123 were observed in LEP skin lesions ( $\times 400$ ). Bar: 50  $\mu$ m. (B) Representative staining with Alexa Fluor 488-conjugated (green) anti-CD303 mAb, PerCP-Cy 5.5-conjugated (red) CD123 mAb and DAPI (blue). Confocal images were merged to demonstrate co-localisation (yellow, positive cells are indicated by arrows). The cells positive for CD123 were also positive for CD303 ( $\times 400$ ).

actively producing IFN- $\alpha/\beta$  in CLE and SLE tissue samples. Furthermore, Dzionek et al. (15) demonstrated that purified pDCs from healthy volunteers, positive for CD303, produced a large amounts of IFN- $\alpha/\beta$  with the IFN- $\alpha/\beta$ -inducing agents, e.g. serum from a SLE patient, and the ligation of CD303 using anti-CD303 mAb potently suppressed induction of IFN- $\alpha/\beta$  production. Therefore we performed a double-colour immunofluorescence staining for CD123 and CD303. We found that the cells positive for CD123 were also positive for CD303 in the tissue samples of LEP (Fig. 2B). It suggested that the pDCs in our cases could produce IFN- $\alpha/\beta$ .

Table I. The percentage of positive cells for CD123 in the patients

	Mean, %	<i>p</i> -value
Histopathological classification		
Type 1 ( <i>n</i> =9)	18.6	0.019 <sup>a</sup>
Type 2 ( <i>n</i> =6)	8.9	
Type 3 ( <i>n</i> =4)	3.5	
Clinical diagnosis		
Systemic lupus erythematosus ( <i>n</i> =5)	12.9	0.94 <sup>a</sup>
Intermediate lupus erythematosus ( <i>n</i> =7)	12.6	
Cutaneous lupus erythematosus ( <i>n</i> =7)	11.7	
Clinical response to treatment		
Remission ( <i>n</i> =14)	14.2	0.027 <sup>b</sup>
No change ( <i>n</i> =5)	5.0	

*p*-values were determined by <sup>a</sup>Kruskal-Wallis test and <sup>b</sup>Mann-Whitney's *U* test. *p*<0.05 was considered statistical significance.

## DISCUSSION

In this study we investigated clinical and histopathological features in LEP. First, we evaluated our patients' clinical manifestations. The majority of the patients (74%) were over 30 years of age, mean age at diagnosis was 36.8 years (range 12–59 years). The remaining 26% of the patients were under 20 years of age compared to the reports which implicate that paediatric cases are rare (1, 16). Similar to the previous studies, proximal limbs were the sites most commonly involved (61%). This is an important clinical feature to distinguish the diagnosis with other panniculitis (1, 16). Duration of symptoms varied from 1 month to 14 years (mean period 18 months). There was no statistical difference between the response to therapy and the duration of symptoms (*p*=0.67). Five patients (21%) fulfilled the ACR criteria for SLE, and the majority of cases were not in serious condition. In the literature, SLE have been known to develop in approximately 50% of patients with LEP (1, 17, 18). Our study showed lower frequency of SLE, and this observation was also seen in recent studies (1, 19, 20).

We have also presented an association between the histological features and the clinical response to treatment in LEP. Most cases showed satisfactory improvements in response to systemic corticosteroid therapy or topical application of corticosteroid. Five cases showed poor response to systemic corticosteroid therapy and they were classified into types 2 or 3 by our histopathological classification. Their histological features were minor lymphocytic inflammation and hyalinised fat necrosis. On the other hand, the cases with strong lymphocytic inflammation in perivascular dermis and in subcutaneous fat, all of type 1 and half of type 2 and 3 showed significant improvement in response to the therapy. Additionally, pDCs positive for CD123 and CD303 have been found in inflamed dermis and adipose tissue, thus has been suggested that pDCs have an important role in LEP in the same way as in other cutaneous LE

skin lesions (9). Furthermore, the percentage of CD123<sup>+</sup> cells in patients who showed good response to therapy was significantly higher than that in the patients who did not respond ( $p=0.027$ ). Thus, evaluating the presence of CD123<sup>+</sup> cells might be useful for predicting the response to therapy. In addition, Vermi et al. (13) reported that the percentages of pDCs correlated with the abundance of the inflammatory infiltrates in the skin lesions of LE. Therefore, we performed the statistical correlation analysis between the lymphocytic infiltrates and the pCD infiltrates. The results showed that the percentage of infiltrating pDC did not correlate with the numbers of lymphocytic infiltrates ( $p=0.16$ ). On the other hand, the numbers of infiltrating pDCs correlated with the numbers of lymphocytic infiltrates ( $p=0.006$ ). In our experiments, it was not revealed if the infiltrate of pDCs is a cause or an effect of the infiltrate of lymphocytes. Further studies are needed to figure out the mechanism of the observation. However, some reports have demonstrated that pDCs can drive the cutaneous immigration of other leukocytes via the production of type I IFN (9, 13).

In conclusion, LEP could be classified into 3 types histopathologically and the prevalences of CD123<sup>+</sup> cells are significantly different between the groups. The patients with considerable lymphocytic inflammation with high percentage of CD123<sup>+</sup> cells in the dermis and subcutaneous fat responded to systemic corticosteroid therapy. On the other hand, the patients with minor lymphocytic inflammation with low percentage of CD123<sup>+</sup> cells showed poor response to systemic therapy. The clinical response to treatment of LEP could be predicted from the histological features. Further studies are needed to elucidate this proposition.

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