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Inflammatory chemokine profiles and their correlations with effector CD4 T cell and regulatory cell subpopulations in cutaneous lupus erythematosus

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ABSTRACT

We compared the chemokine/receptor expression in skin biopsies of discoid (SLE/DLE) and subacute lupus (SLE/SCLE) and correlated it with tissue and circulating effector CD4 T cells/regulatory cells. Skin biopsies and peripheral blood from 9 active SLE/DLE patients, 9 SLE/SCLE patients, 5 control SLE patients without cutaneous lesions and 10 control healthy donors were included. Clinical skin activity was measured by Cutaneous Lupus Erythematosus Disease Area and Severity Index scoring, and systemic activity was measured by a modified SLEDAI-2K excluding the cutaneous items. Pain and pruritus were evaluated by a 10-point visual analogue scale. To determine the frequencies of CXCL-10/CXCR3-, CCL2/CCR2-, CCL17/CCR4-, CCL20/CCR6-, CCL27/CCR10-, CXCL8/CXCR1-, CXCL13/CXCR5-, IL-22-, CD4/IL-17A-, CD4/IL-4-, CD4/IFN-γ-, CD123/IDO-, CD25/Foxp3-, and CD20/IL-10-expressing cells, double immunostaining procedures were performed. Circulating CD4+/CD161-/IL-22+, CD4+/CD161+/IL-17+, CD4+/CD25-/IL-4+, CD4+/CD25-/IFN-γ+, CD4+/CD25hi/Foxp3+, CD3+/CD19+/CD38hi/IL-10+, and CD123+/CD196+/IDO + cells were analyzed by flow cytometry.

Results: In the tissue, CXCL10, CXCR5, and CCL20 expression and IL-22+, CD4+/IL-17+, CD4+/IFN-γ + and CD123+/IDO + cell percentages were increased in SLE/DLE versus SLE/SCLE. Circulating CD4+/CD161-/IL-22+, CD4+/CD161+/IL-17+, CD4+/CD25-/IFN-γ+, CD19+/CD38hi/IL-10 + and CD123+/CD196+/IDO + cell percentages were higher in SLE/DLE versus SLE/SCLE. In the tissue, we found positive correlations between CXCR3 and CD4+/IL-17 + cells; CCR2 and CD4+/IFN-γ + cells; and CCR10 and CD123+/IDO + cells in the SLE/DLE patients and between CXCL13 and CD20+/IL-10 + cells in the SLE/SCLE patients. In the peripheral blood, we determined positive correlations between CXCR5 and CD4+/CD25-/IFN-γ + cells; CCL17 and CD4+/CD161-/IL-22 + cells; and CCL17 and CD4+/CD161+/IL-17 + cells in the SLE/DLE patients and between CXCR5 and CD3+/CD19+/CD38hi/IL-10 + cells; CCR2 and CD4+/CD25hi/Foxp3 + cells; and CXCR1 and CD4+/CD25hi/Foxp3 + cells in the SLE/SCLE patients. Positive correlations between the pain score and the expression of CCL2 and CCR6 expression were found in the SLE/SCLE patients.

In conclusion, the correlations between the expression of chemokines/receptors and subpopulations of effector/regulatory cells showed differential responses among the cutaneous pathologies.

1. Introduction

Cutaneous lupus erythematosus (CLE) is an inflammatory disease that encompasses diverse clinical features. Its pathogenesis is complex, multifactorial and still not defined. Although there is a broad spectrum of histological subsets, the overall histological finding in CLE is characterized by moderately dense to dense perivascular and periappendageal lymphocytic infiltrates in the papillary and reticular dermis with abundant mucin deposition in the reticular dermis [1].

The cell infiltration of the skin may be explained by the presence of

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Table 1
Demographic and clinical characteristics from patients with cutaneous lupus erythematosus.

	HD (n = 10)	SLE w/o CD (n = 5)	SLE/DLE (n = 9)	SLE/SCLE (n = 9)	*P* value
Demographics					
Age (years)					
Mean ± SD	40.0 ± 15.6	44.8 ± 16.4	30.9 ± 9.7	35.3 ± 13.1	
Median	42.0	44.0	29.0	34.0	
Range	(21 – 62)	(28 – 70)	(20 – 47)	(18 – 56)	
Sex					
(Female %)	90.0	80.0	100.0	88.9	
Disease duration (years)					
Mean ± SD	–	11.0 ± 6.8	7.7 ± 5.0	9.0 ± 8.5	
Median		11.0	9.0	5.0	
Range		(8 – 25)	(1 – 15)	(2 – 25)	
Laboratory variables					
Hemoglobin (g/dl)					
Mean ± SD	13.8 ± 2.0	13.9 ± 1.2	12.8 ± 1.3	12.3 ± 2.5	
Median	14.4	14.2	13.3	12.9	
Range	(11.0 – 15.5)	(12.7 – 15.6)	(10.5 – 14.5)	(8.6 – 15.1)	
Leucocytes (cells/μl)					
Mean ± SD	6050.0 ± 1720.2	6560.0 ± 2067.1	6700.0 ± 2241.7	6037.5 ± 2446.5	
Median	6100.0	6800.0	6100.0	6050.0	
Range	(4400 – 9100)	(4500 – 9400)	(4500 – 10900)	(1200 – 9500)	
Lymphocytes (%)					
Mean ± SD	30.9 ± 11.4	20.1 ± 6.7	23.2 ± 8.8	20.8 ± 10.9	
Median	28.8	17.0	21.5	19.5	
Range	(17.9 – 52.1)	(14.4 – 30.8)	(11.7 – 38.9)	(3.2 – 38.0)	
Monocytes (%)					
Mean ± SD	9.4 ± 2.2	6.4 ± 1.3	6.1 ± 2.3	6.2 ± 3.3	
Median	8.9	7.0	6.6	4.2	
Range	(6.6 – 13.2)	(4.6 – 7.5)	(3.4 – 10.0)	(2.4 – 10.0)	
Neutrophils (%)					
Mean ± SD	66.2 ± 17.1	72.2 ± 12.2	74.0 ± 6.1	71.8 ± 9.9	
Median	62.3	73.5	74.6	72.2	
Range	(50.0 – 90.2)	(52.5 – 84.6)	(63.2 – 81.0)	(54.9 – 90.0)	
Platelets (cells/μl)					
Mean ± SD	257,500.0 ± 24,501.7	225,400.0 ± 61,877.3	213,000.0 ± 83,479.0	207,000.0 ± 80,624.3	
Median	257,000.0	196,000.0	211,000.0	230,000.0	
Range	(234,000 – 282,000)	(168,000 – 308,000)	(99,000 – 355,000)	(45,000 – 278,000)	
Anti-dDNA (UI/ml)					
Mean ± SD	–	205.1 ± 119.6	237.1 ± 358.0	228.0 ± 324.5	
Median		238.9	96.7	94.7	
Range		(12 – 326)	(4 – 1055)	(7 – 957)	
C3 (UI/ml)					
Mean ± SD	–	95.2 ± 28.2	77.1 ± 23.9	76.7 ± 31.1	
Median		86.0	75.4	74.0	
Range		(69 – 139)	(45 – 111)	(42 – 127)	
C4 (UI/ml)					
Mean ± SD	–	13.4 ± 7.9	11.0 ± 4.4	15.2 ± 15.5	
Median		8.0	9.8	8.0	
Range		(7 – 23)	(7 – 19)	(6 – 54)	
ESR (mmHg)					
Mean ± SD	–	29.3 ± 27.8	32.5 ± 18.3	33.6 ± 19.4	
Median		22.5	25.0	36.0	
Range		(4 – 68)	(6 – 70)	(6 – 62)	
Clinical Variables					
CLASI activity score					
Mean ± SD	–	–	15.8 ± 6.8	21.9 ± 6.4	f*
Median			13.0	23.0	
Range			(8 – 29)	(14 – 35)	
Treatment					
Prednisone (mg/day)					
Mean ± SD	–	27.5	12.8 ± 9	25.28 ± 15	b**, f**
Median		27.5	11.25	30	
Range		(5–50)	(5–30)	(5–50)	
Prednisone (%)	–	40	88	100	
Antimalarial (%)	–	40	44	44	
DMARDs (%)	–	60	89	44	

(*continued on next page*)

Table 1 (*continued*)

	HD (n = 10)	SLE w/o CD (n = 5)	SLE/DLE (n = 9)	SLE/SCLE (n = 9)	*P* value
Cyclophosphamide (%)	–	20	11	11	

HD: healthy donors; SLE w/o CD: systemic lupus erythematosus without cutaneous disease; SLE/DLE: discoid lupus erythematosus; SLE/SCLE: subacute cutaneous lupus erythematosus. [a]HD versus SLE w/o CD; [b]HD versus SLE/DLE; [c]HD versus SLE/SCLE; [d]SLE w/o CD versus SLE/DLE; [e]SLE w/o CD versus SLE/SCLE; [f]SLE/DLE versus SLE/SCLE.

* $P \leq 0.05$.

** $P \leq 0.001$.

certain chemokine receptor and ligand interactions. Chemokines are a large family of chemotactic cytokines that participate in immune and inflammatory responses through the chemoattraction and activation of leukocytes. They are produced by a wide variety of cell types of both hematopoietic and nonhematopoietic origins and play key roles in the migration and activation of leukocytes *in vivo*. In addition, they are implicated in the modulation of other biological functions, including cell adhesion, phagocytosis, cytokine secretion, T cell activation, apoptosis, angiogenesis, proliferation and viral pathogenesis [2].

Nevertheless, the participation of chemokines in CLE has scarcely been evaluated, and studies have mainly focused on the Th1 response. For instance, increased mRNA expression of CXCL9 and CXCL10 in patients with discoid lupus (DLE), subacute lupus (SCLE) or lupus tumidus has been described [3]. In another study, patients with widespread active CLE skin lesions had significantly increased expression of CCR5 in peripheral blood mononuclear cells, whereas the expression of CCR3 in the patients was decreased when compared with that in healthy controls [4].

This study was undertaken to assess chemokines and their receptor profiles in DLE and SCLE and to correlate the disease activity CLASI score with chemokines/receptors and chemokines/receptors with CD4 T cells/regulatory cells in the skin and periphery.

2. Materials and methods

2.1. Patients

This study was a cross-sectional study conducted in a tertiary care center between February 2015 and December 2016. We included patients with cutaneous lupus in the DLE and SCLE subsets. To be eligible, patients had to meet the ACR classification criteria for systemic lupus erythematosus (SLE) [5] and have an active lupus-specific lesion compatible with DLE or SCLE. The diagnosis of CLE was established by consensus by a rheumatologist and a dermatologist and was also biopsy proven. In addition, patients should not receive any topical treatment, including steroids, other than emollients within the last 4 weeks. However, patients could maintain their basic systemic treatment, such as oral steroids or immunosuppressors. Patients were excluded if they had any concomitant cutaneous lesion not attributed to lupus or had an overlapping autoimmune condition.

As controls, we included patients with SLE without cutaneous lesions (SLE w/o CD) (who may or may not have received steroids or immunosuppressors) and healthy donors (HD) matched by age ± 5 years. The healthy controls did not have any autoimmune diseases or concurrent infections and did not receive prednisone or immunosuppressive therapy.

Clinical skin activity was measured by the Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI), a validated index to quantify disease severity [6]. As this index measures both activity and damage, for the present study, we only used the activity domain that ranges from 0 to 70 (higher scores are indicative of more severe disease). We also scored a modified SLEDAI-2K that excluded the cutaneous items to assess systemic activity [7]. Pain and pruritus were evaluated by means of 10-point Visual Analogue Scales (VAS).

In addition, patients' clinical records were carefully reviewed according to a preestablished protocol to retrospectively collect demographic data as well as clinical and serological features.

2.2. Tissue samples and blood samples

Skin punch biopsies (4 mm diameter) were performed, and the tissue samples were fixed in formalin and evaluated with hematoxylin-eosin staining for the presence of classic histological CLE features. Then, the rest of each specimen was stored for immunohistochemistry. Microscopic review for diagnosis was conducted in a blinded manner by two independent observers (DALl and JFC). Overall, most of the CLE biopsies corresponded to photoexposed areas localized in the thorax, arms or scalp. Control tissue biopsies were also taken from photoexposed areas and, when possible, from the same anatomical region.

In addition, fifteen milliliters of venous blood from each subject was obtained by venipuncture in tubes with EDTA.

2.3. Standard laboratory assessments

Evaluations included blood chemistry, anti-double stranded DNA (ds-DNA) antibody levels determined by ELISA, and C3 and C4 levels quantified by ELISA.

2.4. Immunohistochemistry for chemokines

To identify chemokine- and receptor-expressing cells, 4-μm-thick sections of formalin-fixed, paraffin-embedded tissue were placed on positively charged slides. The sections were deparaffinized with xylene and rehydrated with a graded series of ethanol and water washes before staining. Following standard dewaxing and rehydration, enzyme antigen retrieval was performed (Enzo Life Sciences, Inc., Farmingdale, NY, USA). The tissue samples were blocked with a peroxidase solution. Then, nonspecific background staining was prevented with an IHC background blocker (Enzo Life Sciences). To determine the subpopulations of CXCL10+/CXCR3+ (Santa Cruz Biotechnology, Santa Cruz, CA, USA/Abcam, Cambridge, UK), CCL2+/CCR2+ (Santa Cruz Biotechnology), CCL17+/CCR4+ (Abcam/Santa Cruz Biotechnology), CCL20+/CCR6+ (Santa Cruz Biotechnology), CCL27+/CCR10+ (Santa Cruz Biotechnology/Abcam), CXCL8+/CXCR1+ (Santa Cruz Biotechnology/Abcam) and CXCL13+/CXCR5+ (Abcam) cells, simultaneous detection was performed (MultiView (mouse-HRP/rabbit-AP) Enzo Life Sciences). The procedure was a sequential double-staining procedure in which the IHC universal negative control reagent, used as a negative control (Enzo Life Sciences), and 10 μg/mL mouse monoclonal/rabbit polyclonal antibodies were incubated for 30 min at room temperature. The slides were washed and then incubated with the appropriate PolyView IHC reagent (mouse-HRP and rabbit-AP) for 20 min. Finally, antigens were visualized using horseradish peroxidase (HRP)/3,3′-diaminobenzidine (DAB), and the second antigen was visualized with alkaline phosphatase (AP)/Permanent Red. The tissue samples were counterstained with Harris hematoxylin and mounted with permanent mounting medium. All stained cells were assessed independently of staining intensity by estimating the percentage of positively stained vs. negatively stained cells in four randomly selected fields (320x) in each tissue sample in the superficial and deep dermis

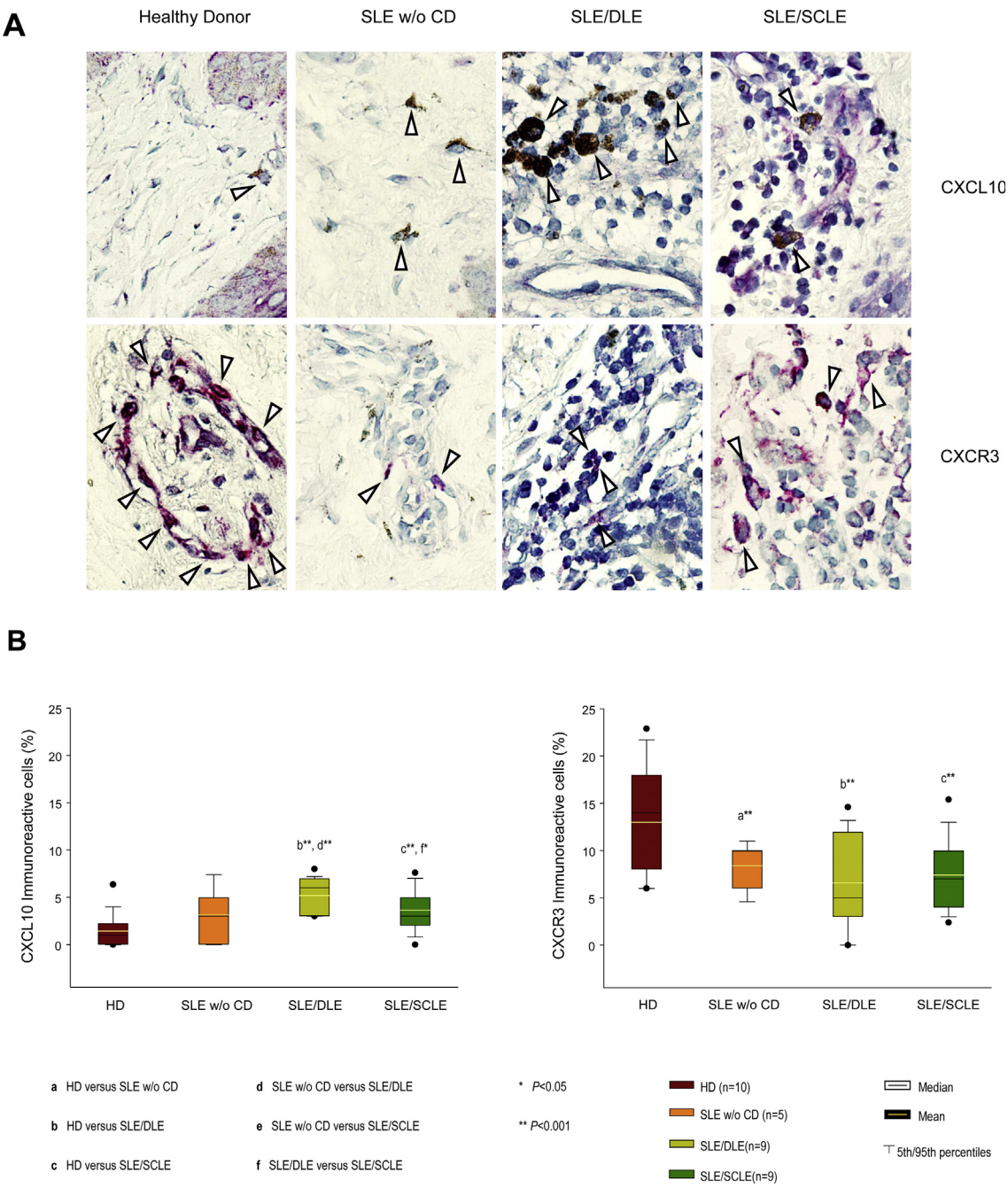

Fig. 1. Representative immunostaining for (A) CXCL10 (*upper panel*) and CXCR3 (*lower panel*) in tissue biopsies from healthy donors and SLE w/o CD, SLE/DLE and SLE/SCLE patients. Arrowheads indicate CXCL10 (in brown) and CXCR3 (in red). (C) Representative immunostaining for CCL2 (*upper panel*) and CCR2 (*lower panel*) in tissue biopsies. Arrowheads indicate CCL2 (in brown) and CCR2 (in red). (E) Representative immunostaining for CCL27 (*upper panel*) and CCR10 (*lower panel*) in tissue biopsies from healthy donors and SLE w/o CD, SLE/DLE and SLE/SCLE patients. Arrowheads indicate CCL27 (in brown) and CCR10 (in red). (G) Representative immunostaining for CXCL13 (*upper panel*) and CXCR5 (*lower panel*) in tissue biopsies from healthy donors and SLE w/o CD, SLE/DLE and SLE/SCLE patients. Arrowheads indicate CXCL13 (in red) and CXCR5 (in brown). (I) Representative immunostaining for CXCL8 (*upper panel*) and CXCR1 (*lower panel*) in tissue biopsies from healthy donors and SLE w/o CD, SLE/DLE and SLE/SCLE patients. Arrowheads indicate CXCL8 (in red) and CXCR1 (in brown). (K) Representative immunostaining for CCL17 (*upper panel*) and CCR4 (*lower panel*) in tissue biopsies from healthy donors and SLE w/o CD, SLE/DLE and SLE/SCLE patients. Arrowheads indicate CCL17 (in brown) and CCR4 (in red). (M) Representative immunostaining for CCL20 (*upper panel*) and CCR6 (*lower panel*) in tissue biopsies from healthy donors and SLE w/o CD, SLE/DLE and SLE/SCLE patients. Arrowheads indicate CCL20 (in red) and CCR6 (in brown). The original magnification was 600X. (B, D, F, H, J, L, N) Percentage of immunoreactive cells per microscopic field. The results are expressed as the mean (horizontal yellow line), median (horizontal black line), and 5th/95th percentiles of positive cells. $*P < 0.05$ and $**P < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and were reported as the percentage of immunoreactive cells. The results are expressed as the mean ± standard error of the mean (SEM) of the cells. Negative control staining was performed with normal human serum diluted 1:100 instead of a primary antibody and the IHC universal negative control reagent specifically designed to work with rabbit, mouse, and goat antibodies (IHC universal negative control reagent, Enzo Life Sciences). The reactive blank was incubated with phosphate-buffered saline-egg albumin (Sigma-Aldrich) instead of a

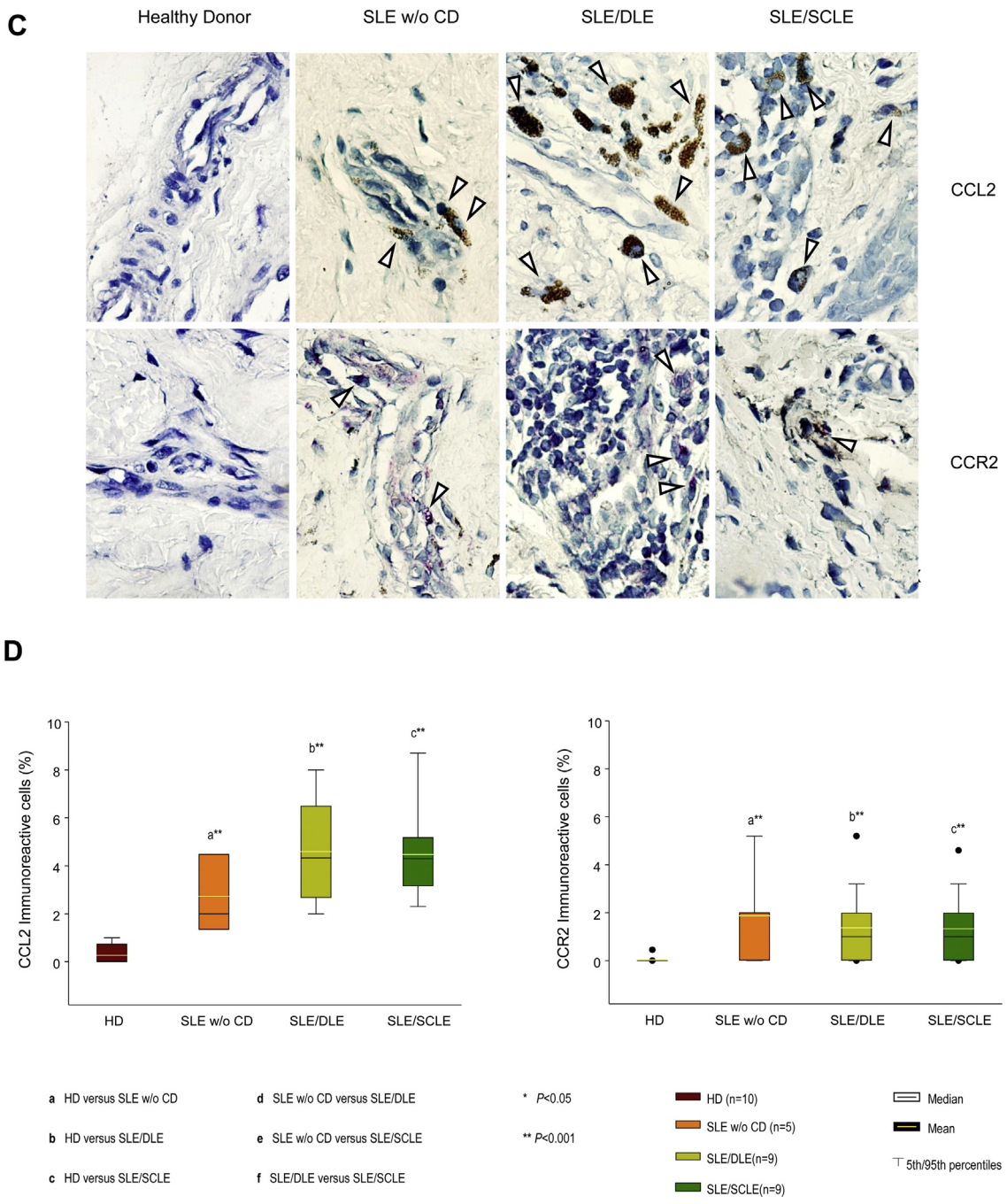

Fig. 1. (*continued*)

primary antibody. All controls were used to exclude nonspecific staining or endogenous enzymatic activities [8].

2.5. Immunohistochemistry for effector CD4 T cells and regulatory cell subpopulations

IL-22-expressing cells were assessed in 4-μm-thick sections of available formalin-fixed, paraffin-embedded tissue. Endogenous peroxidase activity and binding to nonspecific proteins were blocked with 3% H_2O_2 and 3% normal serum, respectively. The tissue samples were incubated with 10 μg/mL goat polyclonal anti-human IL-22 antibody (Santa Cruz Biotechnology). Binding was identified with a biotinylated donkey anti-goat IgG antibody (ABC Staining System; Santa Cruz Biotechnology). The slides were incubated with HRP-streptavidin, followed by the peroxidase substrate DAB (Sigma-Aldrich) for 10 min. The sections were counterstained with hematoxylin, dehydrated with alcohol and xylene, and mounted in resin.

To determine the subpopulations of CD4 + /IL-17A + cells (potentially Th17 cells), CD4 + /IL-4 + cells (potentially Th2 cells), CD4 + /IFN-γ + cells (potentially Th1 cells), CD25 + /Foxp3 + regulatory T cells (potentially Tregs), CD20 + /IL-10 + B cells (potentially Bregs), and CD123 + /IDO + plasmacytoid dendritic cells (potentially pDCregs), simultaneous detection was performed (MultiView (mouse-HRP/rabbit-AP) Enzo Life Sciences). A rabbit polyclonal anti-CD4, anti-CD20, anti-CD25 IgG (Santa Cruz Biotechnology), or anti-CD123 IgG antibody (Abcam pcl, CA, UK) or a mouse monoclonal anti-IL-17A, anti-IL-4, anti-IFN-γ, anti-IL-10, anti-Foxp3 IgG1 or anti-IDO IgG antibody (Santa Cruz Biotechnology) at 10 μg/mL was visualized using HRP/DAB or alkaline phosphatase (AP)/Permanent Red as appropriate. The tissue samples were counterstained with hematoxylin and mounted in

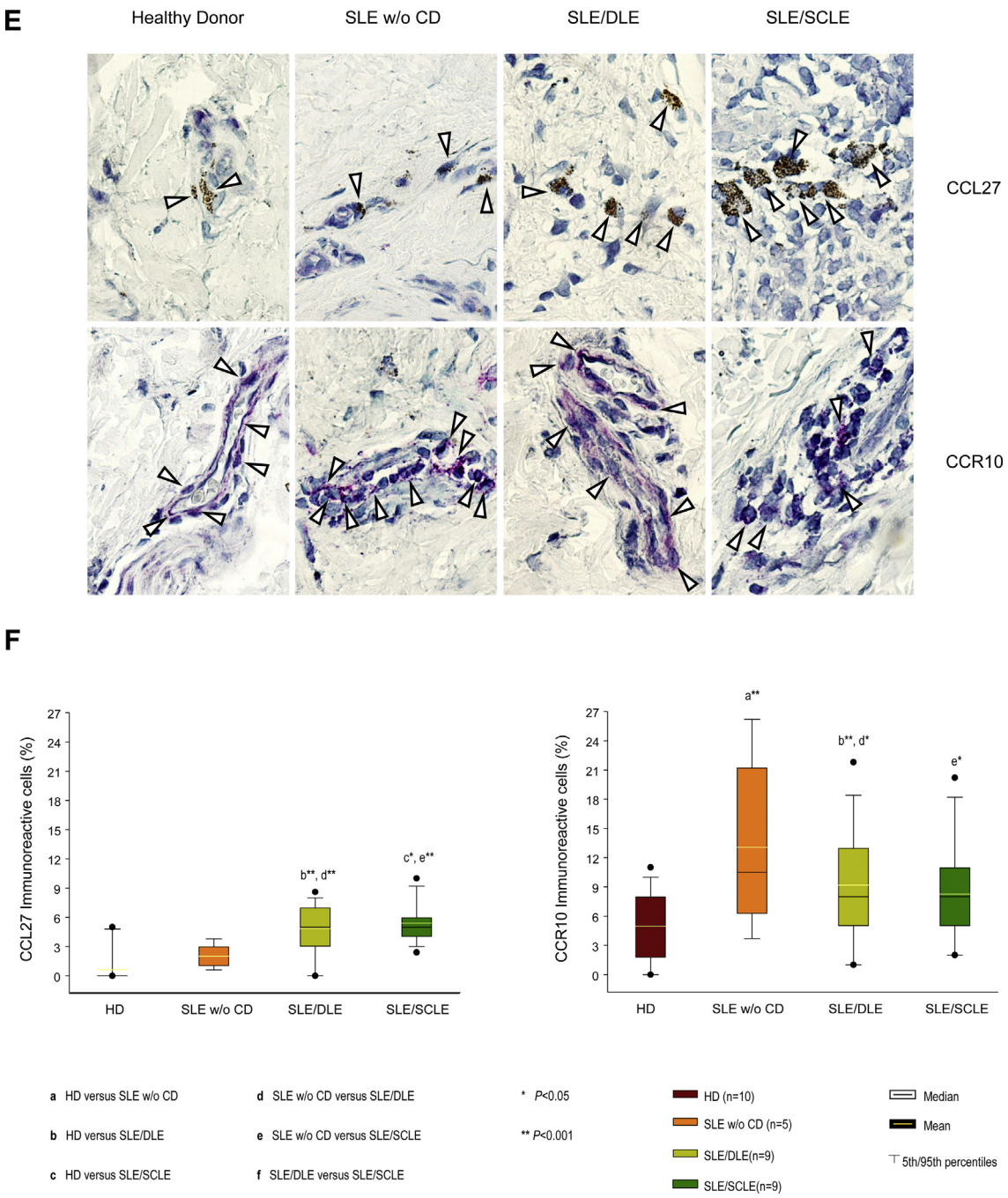

Fig. 1. (*continued*)

aqueous mounting medium. Double-positive cytokine-expressing cells were assessed in three fields (320x) and were reported as the percentage of immunoreactive cells. The results are expressed as the mean ± standard error of the mean (SEM) of the cells quantified by Image Pro Plus version 5·1·1 [8].

2.6. Peripheral blood mononuclear cell isolation

Fifteen milliliters of venous blood was obtained from each subject. Peripheral blood mononuclear cells (PBMCs) were obtained by gradient centrifugation with Lymphoprep (Axis-Shield PoC AS, Oslo, Norway).

2.7. Flow cytometry

PBMCs were labeled with 5 μL of anti-human CD3 FITC-labeled, anti-human CD4 PECy5-labeled, and anti-human CD161 APC-conjugated monoclonal antibodies (BD Biosciences, San Jose, CA); anti-human CD3 FITC-labeled and anti-human CD4 PECy5-labeled monoclonal antibodies (BD Biosciences); anti-human CD19 APC-labeled and anti-human CD38 PECy5-labeled monoclonal antibodies (BD Biosciences); or anti-human CCR6 PerCP/Cy5.5-conjugated and anti-human CD123 FITC-labeled monoclonal antibodies (BD Biosciences) in separated tubes for 20 min at 37 °C in the dark. The cells were permeabilized with 200 μL of Cytofix/Cytoperm solution (BD Biosciences) at 4 °C for 30 min. Intracellular staining was performed with anti-human IL-22 PE-labeled, IL-17A PE-labeled, IL-4 PE-labeled, IFN-γ PE-labeled, Foxp3 PE-labeled, IL-10 PE-labeled, or IDO PE-labeled mouse monoclonal antibodies (BD Biosciences) for 30 min at 4 °C in the dark. Electronic gates were made for CD3 + /CD4 + /CD161 − cells, CD3 + /CD4 + /CD161 + cells, CD3 + /CD4 + /CD25 − cells, CD3 + /CD4 + /

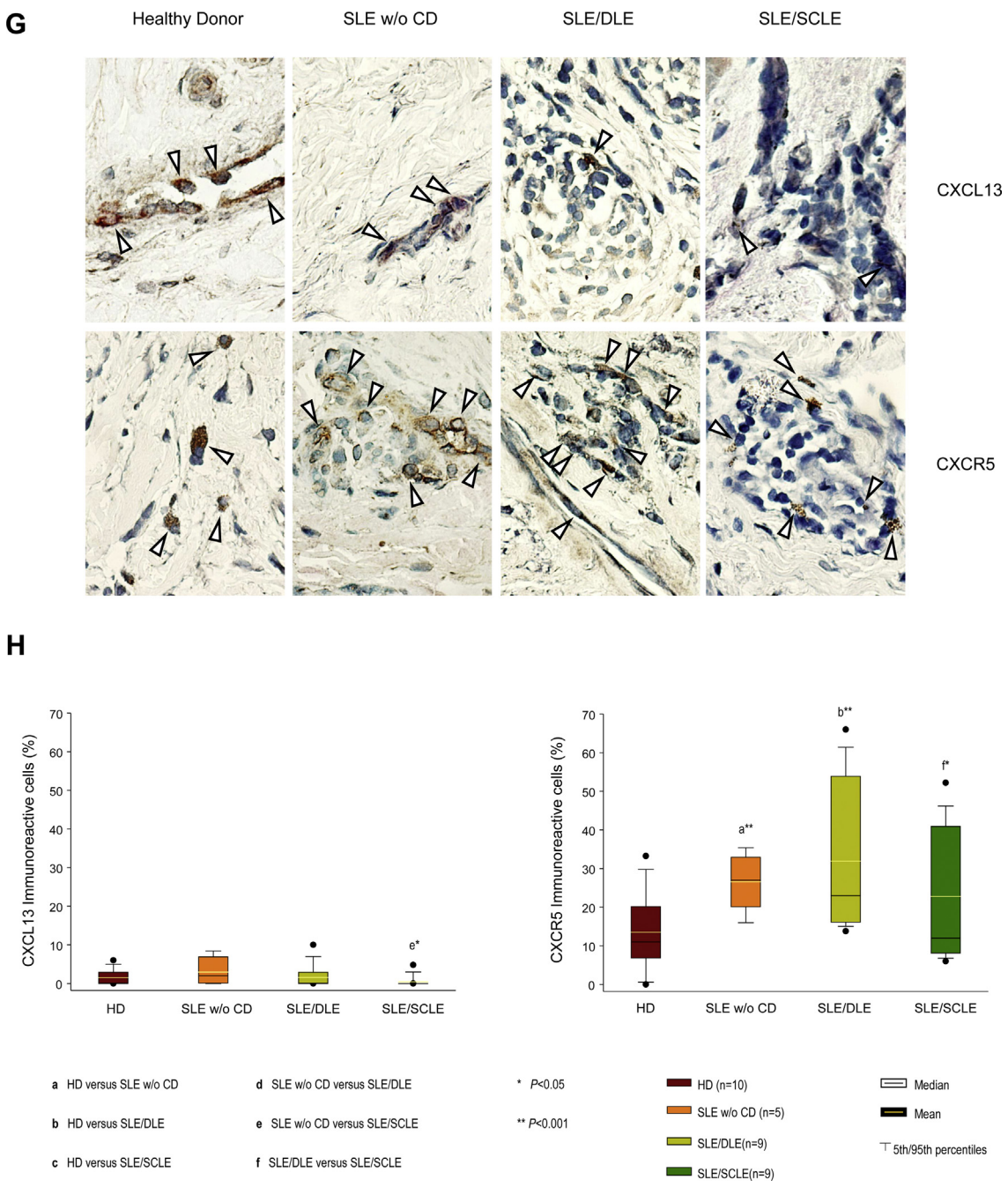

Fig. 1. (*continued*)

CD25hi cells, CD19 + /CD38hi cells, and CD123hi/CD196 + cells. The results are expressed as the relative percentages of IL-22-, IL-17A-, IL-4-, IFN-γ-, Foxp3-, IL-10-, and IDO-expressing cells in each gate. As isotype controls, IgG1 FITC-labeled/IgG1 PE-labeled/CD45 PECy5-labeled mouse IgG1 *kappa* antibodies (BD Tritest, BD Biosciences) were used to set the thresholds and gates on the flow cytometer. We ran an unstained (autofluorescence control) and permeabilized PBMC sample. The autofluorescence control was compared to single-stained positive control cells to confirm that the stained cells were on scale for each parameter. In addition, BD CaliBRITE 3 beads were used to adjust instrument settings, set fluorescence compensation, and check instrument sensitivity (BD CaliBRITE, BD Biosciences).

Fluorescence minus one (FMO) controls were stained in parallel using the panel of antibodies with sequential omissions of each antibody, except for the anti-IL-22, anti-IL-17A, anti-IL-4, anti-IFN-γ, anti-Foxp3, anti-IL-10, and anti-IDO antibodies, which were replaced by an isotype control rather than simply omitted. Finally, T cell subsets were analyzed by flow cytometry with an Accuri C6 (BD Biosciences). A total of 500,000–1,000,000 events was recorded for each sample and analyzed with FlowJo X software (TreeStar, Inc.) [8].

2.8. Ethical considerations

This study was performed in accordance with the principles expressed in the Declaration of Helsinki. It was approved by the Ethical and Medical Committee of the Instituto Nacional de Ciencias Médicas y Nutrición (Ref. 822), and written informed consent was obtained from all subjects.

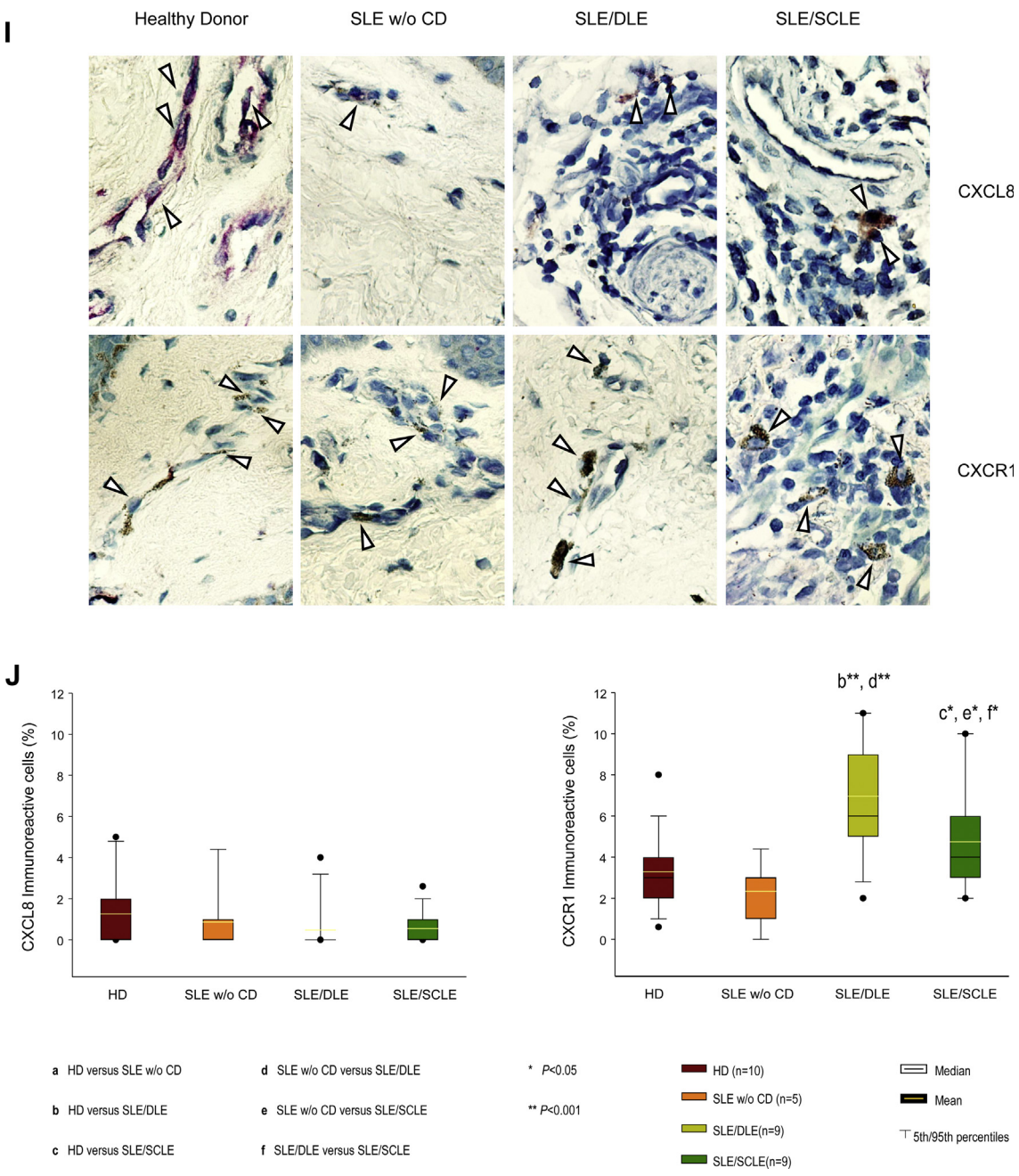

Fig. 1. (*continued*)

2.9. Statistical analysis

Descriptive statistics were performed, and categorical variables were compared using the χ^2 test or Fisher's exact test. Immunohistochemical statistical analysis was performed using one-way analysis of variance on ranks by the Holm-Sidak method and Dunn's test for all pairwise multiple comparison procedures. We report non-parametric correlations using Spearman coefficients between (1) the CLASI activity score and chemokines/receptors; (2) the tissue chemokine/receptor expression and CD4 T/regulatory cells; and (3) the tissue chemokine/receptor expression and circulating CD4 T/regulatory cells.

Statistical analyses were performed using the Sigma Stat 11.2 program (Aspire Software International, Leesburg, VA, USA). Data are expressed as the median, range, and mean ± standard deviation (SD)/standard error of the mean (SEM). *P* values less than or equal to 0.05 were considered significant.

3. Results

3.1. Clinical and demographic characteristics of patients

We included 18 patients with cutaneous lupus, 9 with SLE/DLE and 9 with SLE/SCLE without another autoimmune comorbidity. As controls, we included 5 patients with SLE w/o CD and 10 healthy donors. The demographic, laboratory and clinical data of the patients and controls are shown in Table 1. Compared with the other groups, the group comprising patients with SLE/SCLE had the highest CLASI scores ($P = 0.03$) as well as the highest-modified SLEDAI scores ($P = 0.02$).

3.2. Expression of chemokines and their receptors

The expression of CXCL10 was increased in the SLE/DLE patients compared with the SLE/SCLE and SLE w/o CD patients and healthy

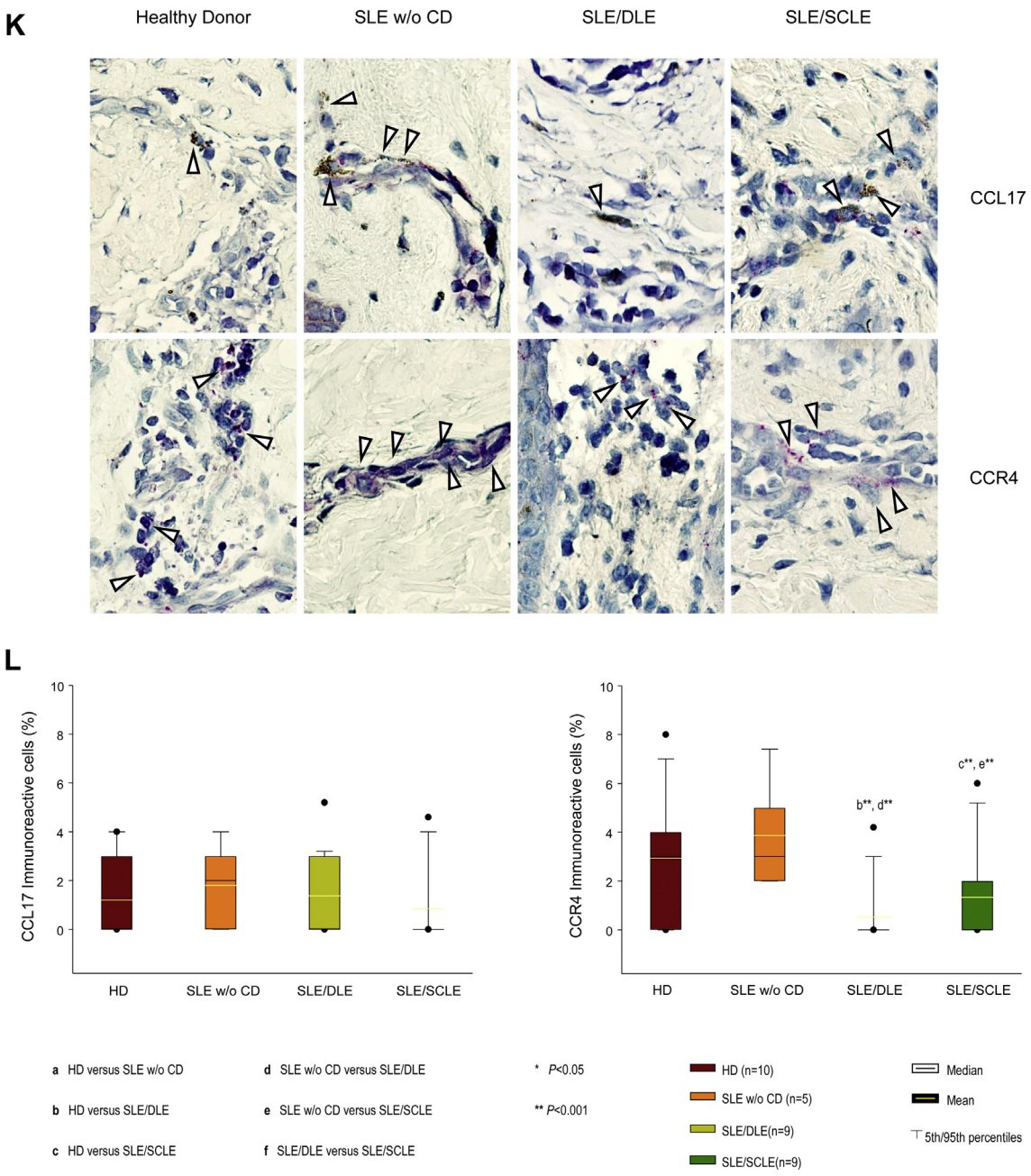

Fig. 1. (*continued*)

donors (Supplementary Table 1). Conversely, the expression of its receptor CXCR3 was conspicuously higher in the healthy control group than in the other groups (Fig. 1A and B; Supplementary Table 1).

Tissue from all lupus patients (with or without CLE) had higher expression of CCL2 than that from the healthy controls (Supplementary Table 1). Similarly, the expression of CCR2 was practically absent in the healthy controls. We did not find any difference when comparing the SLE/DLE subgroup versus the SLE/SCLE subgroup (Fig. 1C and D; Supplementary Table 1).

The number of CCL27 + cells was higher in the skin of the SLE/DLE and SLE/SCLE patients than in that of the healthy controls and SLE w/o CD patients (Table 2). However, there was no difference when we compared the SLE group without skin involvement with the healthy control group. Moreover, the number of cells that expressed CCR10 was increased in the SLE w/o CD and SLE/DLE groups compared with the healthy control group (Fig. 1E and F; Supplementary Table 1).

The tissue samples from the patients with SLE w/o CD showed a higher proportion of CXCL13 + cells than those from the SLE/SCLE patients (Supplementary Table 1). However, noticeably higher expression of the CXCL13 receptor CXCR5 was observed in the SLE/DLE group than in the healthy control and SLE/SCLE groups (Fig. 1G and H; Supplementary Table 1).

We also found higher expression of CXCR1 in the SLE/DLE and SLE/SCLE groups than in the SLE w/o CD and healthy control groups, with the highest expression in the SLE/DLE group (Supplementary Table 1). There were no significant differences in the expression of CXCL8 among the groups (Fig. 1I and J; Supplementary Table 1). We studied CCL17 and did not observe any differences among the groups (Supplementary Table 1). However, lower CCR4 immunoreactivity was found in the SLE/DLE and SLE/SCLE groups compared with the SLE w/o CD and healthy control groups (Fig. 1K and L; Supplementary Table 1).

Finally, the CCL20 immunoreactivity was higher in the SLE group than in the healthy control and SLE/DLE groups (Supplementary Table 1), whereas CCR6 was mainly expressed in both the SLE/DLE and

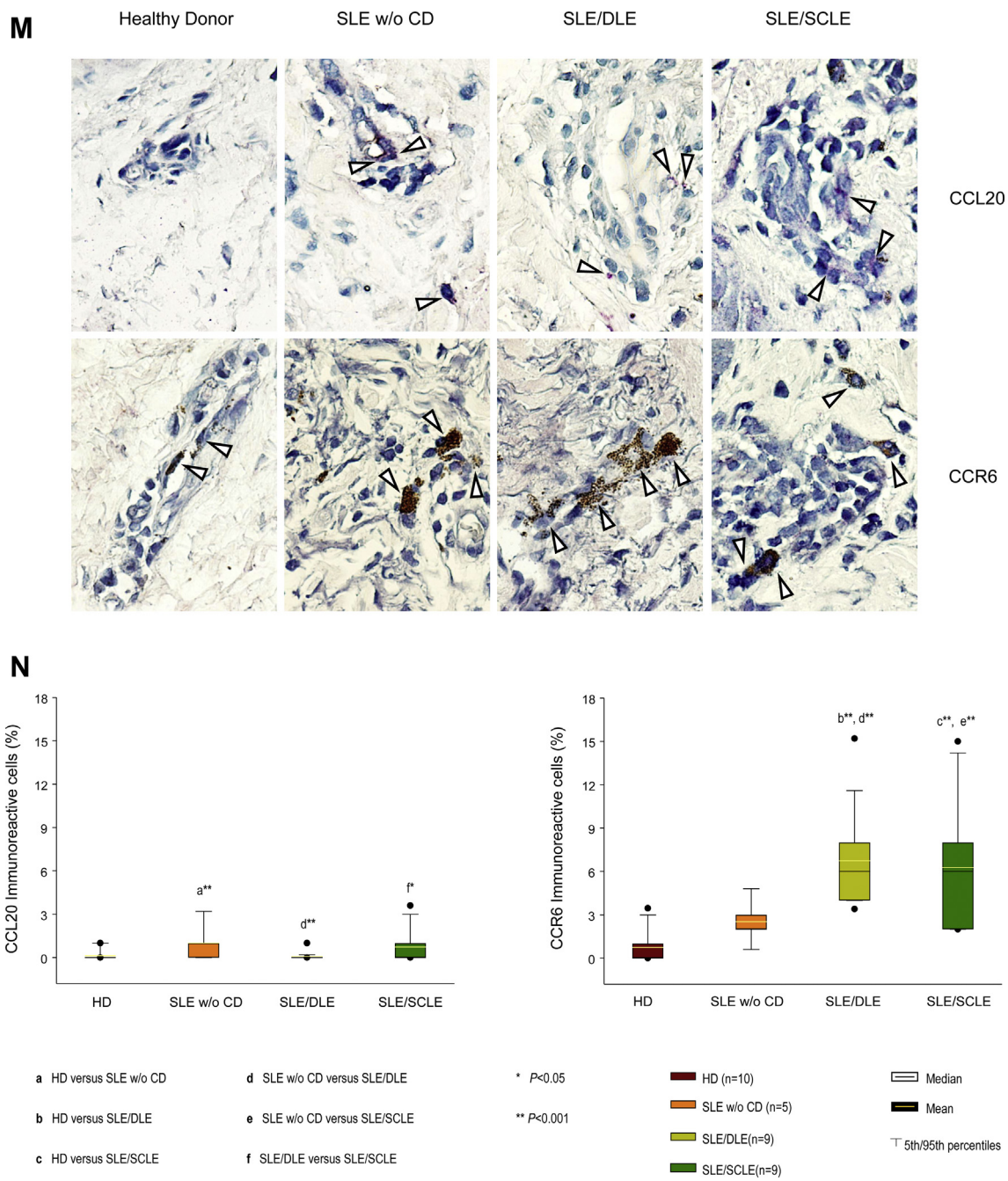

Fig. 1. (*continued*)

SLE/SCLE groups and not in the SLE w/o CD and healthy control groups (Fig. 1M, N; Supplementary Table 1).

3.3. CD4 effector T cell and regulatory cell subpopulations in the tissue from patients with cutaneous lupus

3.3.1. Proinflammatory/antifibrogenic cytokine expression

The IL-22 + cell percentage was significantly increased in the SLE/DLE tissue samples compared to the SLE/SCLE, SLE w/o CD, and healthy control tissue samples, while the IL-22 + cell number was increased in the SLE/SCLE group versus the SLE w/o CD and healthy donor groups (Fig. 2A; Supplementary Table 2).

The IL-17A + /CD4 + T cell frequency was conspicuously higher in the tissue samples from the patients with SLE/DLE than in the SLE/SCLE patient or healthy donor tissue samples. Moreover, a higher immunoreactive cell number was found in the tissue from the SLE/SCLE group compared with that from the SLE w/o CD and healthy donor groups (Fig. 2B; Supplementary Table 2). The tissue from the SLE/DLE patients had a significantly higher percentage of IFN-γ + /CD4 + T cells than that from the SLE/SCLE patients, SLE w/o CD patients, or healthy donors. In addition, the number of IFN-γ + /CD4 + T cells in the skin from the SLE/SCLE patients was increased compared with that in the skin from the SLE patients and healthy donors (Fig. 2D; Supplementary Table 2).

3.3.2. Anti-inflammatory/profibrogenic cytokine expression

Tissue from the SLE/DLE and SLE/SCLE patients had significantly higher IL-4 + /CD4 + T cell percentages than that from the SLE w/o CD patients and healthy donors. There were no statistically significant differences between the SLE/DLE and SLE/SCLE patient groups

Table 2
Spearman's correlation between chemokine/chemokine receptor and effector $CD4^+$ T cell and regulatory cell subpopulations from tissue and periphery of patients with cutaneous lupus erythematosus.

			Tissue							Periphery						
			Th22	Th17	Th2	Th1	Treg	Breg	pDCreg	Th22	Th17	Th2	Th1	Treg	Breg	pDCreg
Group																
Healthy Donors	CXCL10	Rho; *P*	NS	NS	NS	NS	NS	NS	**−0.63; 0.04**	NS	NS	NS	NS	NS	NS	NS
	CCL2	Rho; *P*	NS	NS	NS	**−0.79; 0.006**	NS	**−0.70; 0.02**	NS	NS	NS	NS	NS	NS	NS	NS
	CCR6	Rho; *P*	NS	NS	NS	NS	NS	NS	NS	**−0.66; 0.03**	NS	NS	NS	**0.65; 0.03**	NS	NS
	CCL20	Rho; *P*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**−0.71; 0.02**	NS
	CCL27	Rho; *P*	NS	NS	NS	NS	NS	NS	**−0.81; 0.004**	**−0.80; 0.005**	NS	NS	NS	NS	NS	NS
	CCR10	Rho; *P*	NS	NS	NS	NS	**−0.79; 0.006**	NS	NS	NS	NS	NS	NS	NS	NS	**−0.75; 0.01**
	CXCL8	Rho; *P*	**−0.67, 0.03**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	CXCL13	Rho; *P*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.64; 0.04**
SLE																
	CCR2	Rho; *P*	NS	NS	NS	NS	**0.90; 0.03**	NS	NS	NS	NS	NS	NS	NS	NS	NS
	CCR4	Rho; *P*	NS	NS	NS	NS	**−0.94; 0.01**	**−0.97; 0.005**	NS	NS	NS	NS	NS	NS	NS	**0.97; 0.005**
	CCL27	Rho; *P*	NS	**−0.91; 0.02**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	CCR10	Rho; *P*	NS	NS	NS	NS	NS	NS	NS	NS	**0.97; 0.005**	NS	NS	NS	NS	NS
	CXCL8	Rho; *P*	NS	NS	NS	NS	NS	NS	NS	NS	**−0.94; 0.01**	NS	NS	NS	NS	NS
SLE/DLE	CXCL10	Rho; *P*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**−0.74; 0.02**
	CXCR3	Rho; *P*	NS	**0.74; 0.02**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	CCL2	Rho; *P*	NS	**−0.69; 0.03**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	CCR2	Rho; *P*	NS	NS	NS	**0.71; 0.03**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	CCR4	Rho; *P*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**−0.71; 0.03**
	CCL17	Rho; *P*	NS	NS	NS	NS	NS	NS	NS	**0.69; 0.03**	**0.67; 0.04**	NS	NS	NS	NS	NS
	CCR10	Rho; *P*	NS	NS	NS	NS	NS	NS	**0.73; 0.02**	NS	NS	NS	NS	NS	NS	NS
	CXCL8	Rho; *P*	**−0.73; 0.02**	NS	NS	NS	NS	NS	NS	NS	**−0.73; 0.02**	**−0.73; 0.02**	NS	NS	NS	NS
	CXCR5	Rho; *P*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.72; 0.02**	**−0.73; 0.02**	NS	NS
	CXCL13	Rho; *P*	NS	NS	NS	NS	NS	**−0.70; 0.03**	NS	NS	NS	NS	NS	NS	NS	NS
SLE/SCLE	CCL2	Rho; *P*	NS	NS	NS	NS	**−0.68; 0.04**	**−0.71; 0.03**	NS	**−0.89; 0.002**	NS	NS	NS	NS	NS	**−0.73; 0.04**
	CCR2	Rho; *P*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.73;0.04**	NS	NS
	CCR4	Rho; *P*	**−0.76; 0.01**	NS	NS	NS	NS	**−0.82; 0.007**	NS	**−0.85; 0.007**	NS	NS	NS	NS	NS	NS
	CCR6	Rho; *P*	NS	NS	NS	NS	NS	NS	NS	**−0.74; 0.03**	NS	NS	NS	NS	NS	NS
	CCL27	Rho; *P*	NS	NS	NS	**−0.66; 0.05**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	CXCR1	Rho; *P*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.72; 0.04**	NS	NS
	CXCR5	Rho; *P*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.74; 0.03**	NS

NS: non-significant.

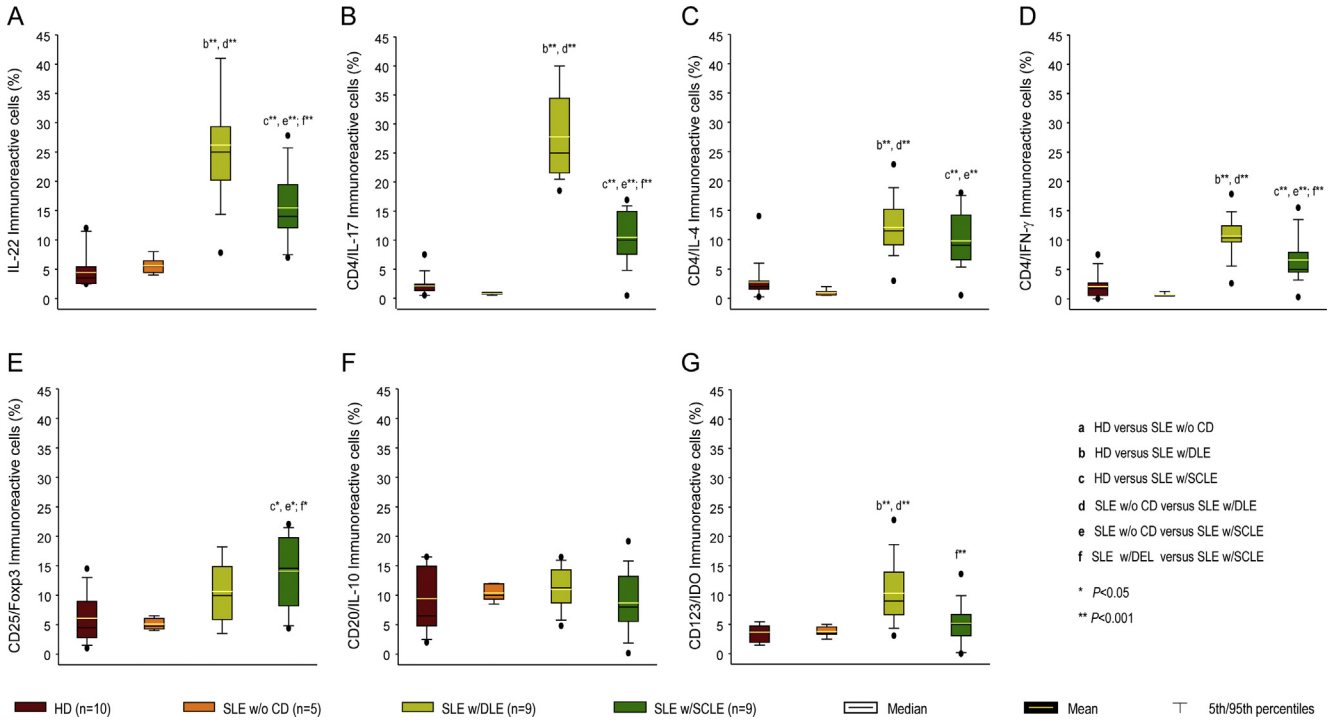

Fig. 2. Percentage of immunoreactive CD4 effector T cells and regulatory cells per microscopic field in the skin tissue. The results are expressed as the mean (horizontal yellow line), median (horizontal black line), and 5th/95th percentiles of positive cells. *$P < 0.05$ and **$P < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 2C; Supplementary Table 2).

3.3.3. Regulatory cells

The percentage of Tregs in tissue from the SLE/SCLE patients was higher than that in tissue from the SLE/DLE patients, SLE w/o CD patients and healthy donors. Nonetheless, there were no significant differences between the SLE/DLE patients and healthy donors (Fig. 2D; Supplementary Table 2).

There were no statistically significant differences in the IL-10+/CD20 + B cell percentage among the groups (Fig. 2F; Supplementary Table 2).

Finally, the SLE/DLE patients had the highest percentage of CD123+/IDO + pDCregs (Fig. 2G; Supplementary Table 2).

3.4. CD4 effector T cell and regulatory cell subpopulations in the peripheral blood from patients with cutaneous Lupus

3.4.1. Proinflammatory/antifibrogenic cytokine expression

The relative percentages of circulating CD4+/CD161-/IL-22+, CD4+/CD161+/IL-17A+, and CD4+/CD25-/IFN-γ + cells in the SLE/DLE patients were higher than those in the SLE/SCLE patients, SLE w/o CD patients and healthy donors. Moreover, the levels of the CD4+/CD161-/IL-22+, CD4+/CD161+/IL-17A+, and CD4+/CD25-/IFN-γ + subsets were increased in the SLE/SCLE patients versus the SLE patients and healthy donors (Figs. 3A and 4.A, B and D; Supplementary Table 3).

3.4.2. Anti-inflammatory/profibrogenic cytokine expression

Regarding the anti-inflammatory/profibrogenic CD4+/CD25-/IL-4 + cell percentage, there were significant increases in the SLE/DLE and SLE/SCLE patients compared to the healthy donors (Fig. 3A and 4C; Supplementary Table 3), while the CD4+/CD25-/IL-4 + cell percentage was lower in the SLE/DLE and SLE/SCLE patients than in the SLE w/o CD patients. Furthermore, there were no differences in the CD4+/CD25-/IL-4 + cell relative percentages between the SLE/SCLE and SLE/DLE patients (Fig. 3A and 4C; Supplementary Table 3).

3.4.3. Regulatory cells

The percentage of Forkhead box P3-expressing CD4 T cells showed a statistically significant decrease in the SLE/SCLE and SLE w/o CD patients compared with the healthy donors. There were no statistically significant differences between the SLE/DLE and SLE/SCLE patients (Fig. 3B and 4E; Supplementary Table 3).

In addition, the relative percentage of IL-10-producing B cells was higher in the SLE/DLE patients than in the SLE/SCLE and SLE w/o CD patients and healthy donors (Fig. 3B and 4F; Supplementary Table 3).

The percentage of IDO-expressing CD123+/CD196 + cells was conspicuously higher in the SLE/DLE and SLE w/o CD patients than in the SLE/SCLE patients and healthy donors (Fig. 3B and 4G; Supplementary Table 3).

3.5. Correlations in cutaneous lupus erythematosus among chemokines/receptors, the CLASI activity score, pain and pruritus

We found positive correlations between the pain score and CCL2 (Spearman's rho: 0.77, $P = 0.01$) or CCR6 expression (Spearman's rho: 0.75, $P = 0.02$) in the SLE/SCLE patients. We did not find any significant correlations with the CLASI activity score.

3.6. Correlations between effector CD4 T cell/regulatory cell subpopulations and chemokine/chemokine receptor expression

3.6.1. CD4/IL-22 and chemokine/chemokine receptor expression

In the periphery, a positive correlation was determined in SLE/DLE between the CD4+/CD161-/IL-22 + cell subset and the expression of CCL17 (Spearman's rho: 0.69, $P = 0.03$), while negative correlations were found in SLE/SCLE between CD4+/CD161-/IL-22 + cells and the expression of CCL2 (Spearman's rho: −0.89, $P = 0.002$), CCR4 (Spearman's rho: −0.85, $P = 0.007$), and CCR6 (Spearman's rho: −0.74, $P = 0.03$). In the SLE w/o CD patients, the correlations between

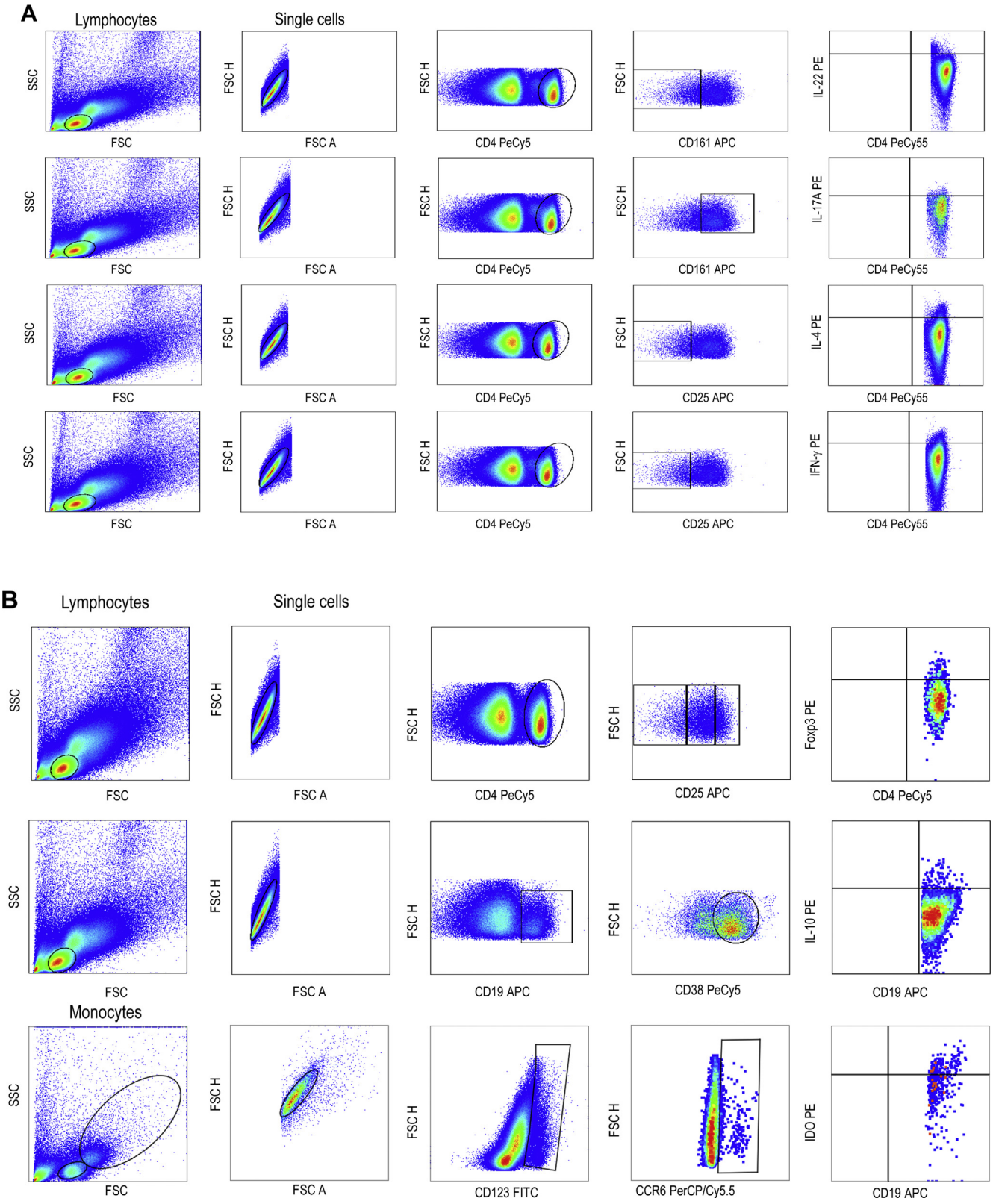

Fig. 3. (A) Representative gating strategy for each cell subpopulation of CD4 effector T cells. (B) Representative gating strategy for each cell population of regulatory cells in a DLE patient.

CD4 + /CD161-/IL-22 + cells and chemokine/chemokine receptor expression were not significant (Table 2).

In the tissue, a negative correlation was found in SLE/DLE between the CD4 + /IL-22 + cell subset and the expression of CXCL8 (Spearman's rho: −0.73, $P = 0.02$) and in SLE/SCLE between CD4 + /IL-22 + cells and CCR4 (Spearman's rho: −0.76, $P = 0.01$). In the SLE w/o CD patients, the correlations between CD4 + /IL-22 + cells and chemokine/chemokine receptor expression were not significant

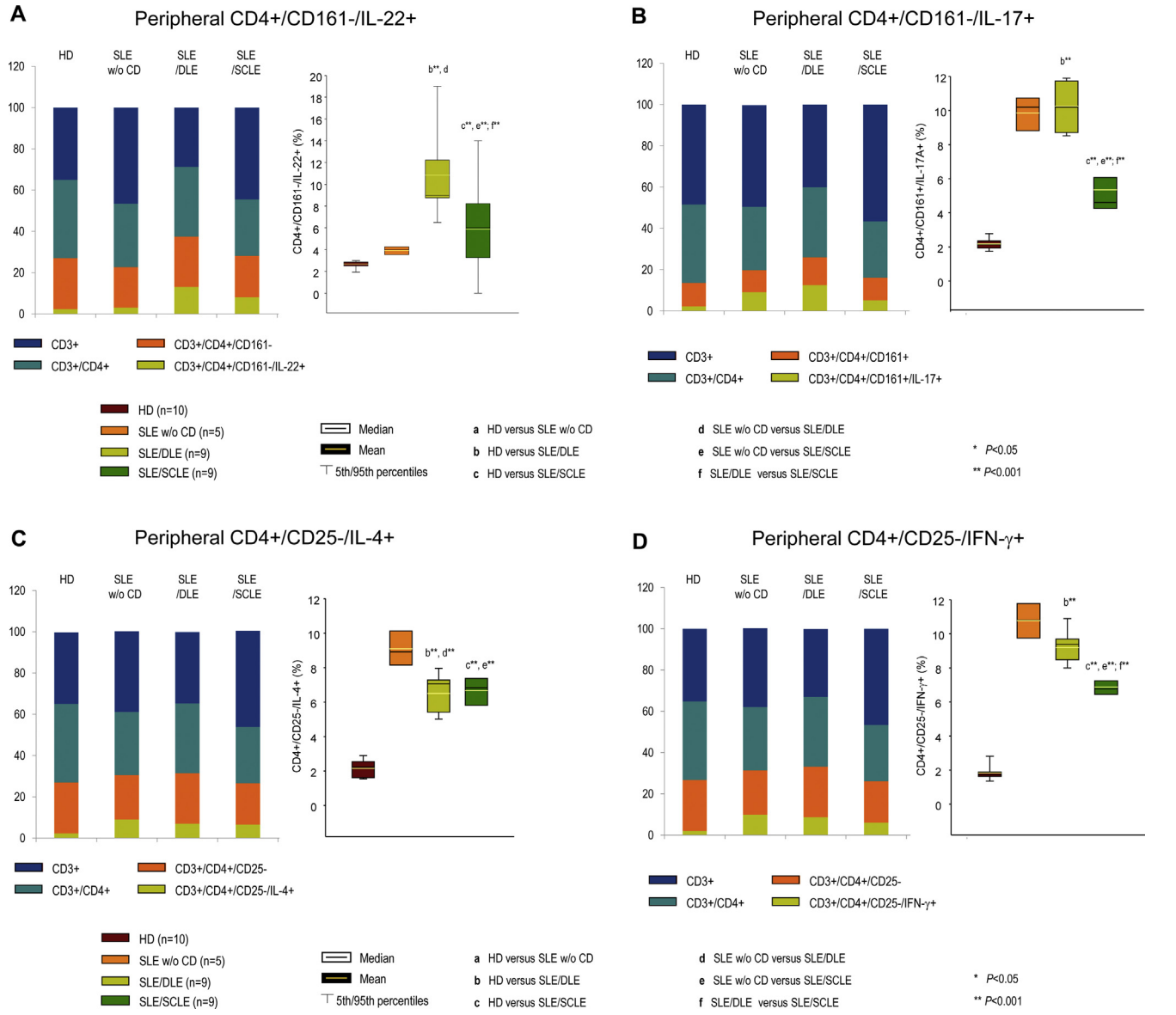

Fig. 4. Percentages of circulating (A) CD4+/CD161-/IL-22 + cells, (B) CD4+/CD161+/IL-17A + cells, (C) CD4+/CD25-/IL-4 + cells, (D) CD4+/CD25-/IFN-γ + cells, (E) CD4+/CD25hi/Foxp3 + cells, (F) CD19+/CD38hi/IL-10 + cells, and (G) CD123+/CD196-/IDO + cells. The results are expressed as the mean (horizontal yellow line), median (horizontal black line), and 5th/95th percentiles *$P < 0.05$ and **$P < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Table 2).

3.6.2. Th17 cells and chemokine/chemokine receptor expression

In the periphery, a positive correlation was determined in SLE/DLE between the CD4+/CD161+/IL-17A + cell subset and the expression of CCL17 (Spearman's rho: 0.67, $P = 0.04$), while a negative correlation was found between the CD4+/CD161+/IL-17A + cell subset and the expression of CXCL8 (Spearman's rho: −0.73, $P = 0.02$). In the SLE/SCLE patients, the correlations between CD4+/CD161+/IL-17A + cells and chemokines were not significant. In the SLE w/o CD patients, a negative correlation was found between the CD4+/CD161+/IL-17A + cell subset and the expression of CXCL8 (Spearman's rho: −0.94, $P = 0.01$), while a positive correlation was found with CXCR10 (Spearman's rho: 0.97, $P = 0.005$) (Table 2).

In the tissue, a positive correlation was found in SLE/DLE between the IL-17A-expressing CD4 T cell subset and the expression of CXCR3 (Spearman's rho: 0.74, $P = 0.02$), while a negative correlation was found between the CD4+/IL-17A + cell subset and the expression of CCL2 (Spearman's rho: −0.69, $P = 0.03$). In the SLE patients, a negative correlation was found between the CD4+/IL-17A + cell subset and the expression of CCL27 (Spearman's rho: −0.91, $P = 0.02$) (Table 2).

3.6.3. Th2 cells and chemokine/chemokine receptor expression

In the periphery, a negative correlation was determined in SLE/DLE between IL-17- and IL-4-expressing CD4 T cells and the expression of CXCL8 (Spearman's rho: −0.73, $P = 0.02$; Table 2).

3.6.4. Th1 cells and chemokine/chemokine receptor expression

In the periphery, a positive correlation was determined in SLE/DLE between the CD4+/CD25-/IFN-γ + cell subset and the expression of CXCR5 (Spearman's rho: 0.72, $P = 0.02$), while the correlations between the CD4+/CD25-/IFN-γ + cell subset and the expression of chemokines/chemokine receptors were not significant in the SLE/SCLE or SLE patients (Table 2).

In the tissue, a positive correlation was found in SLE/DLE between the IFN-γ-expressing CD4 T cell subset and the expression of CCR2

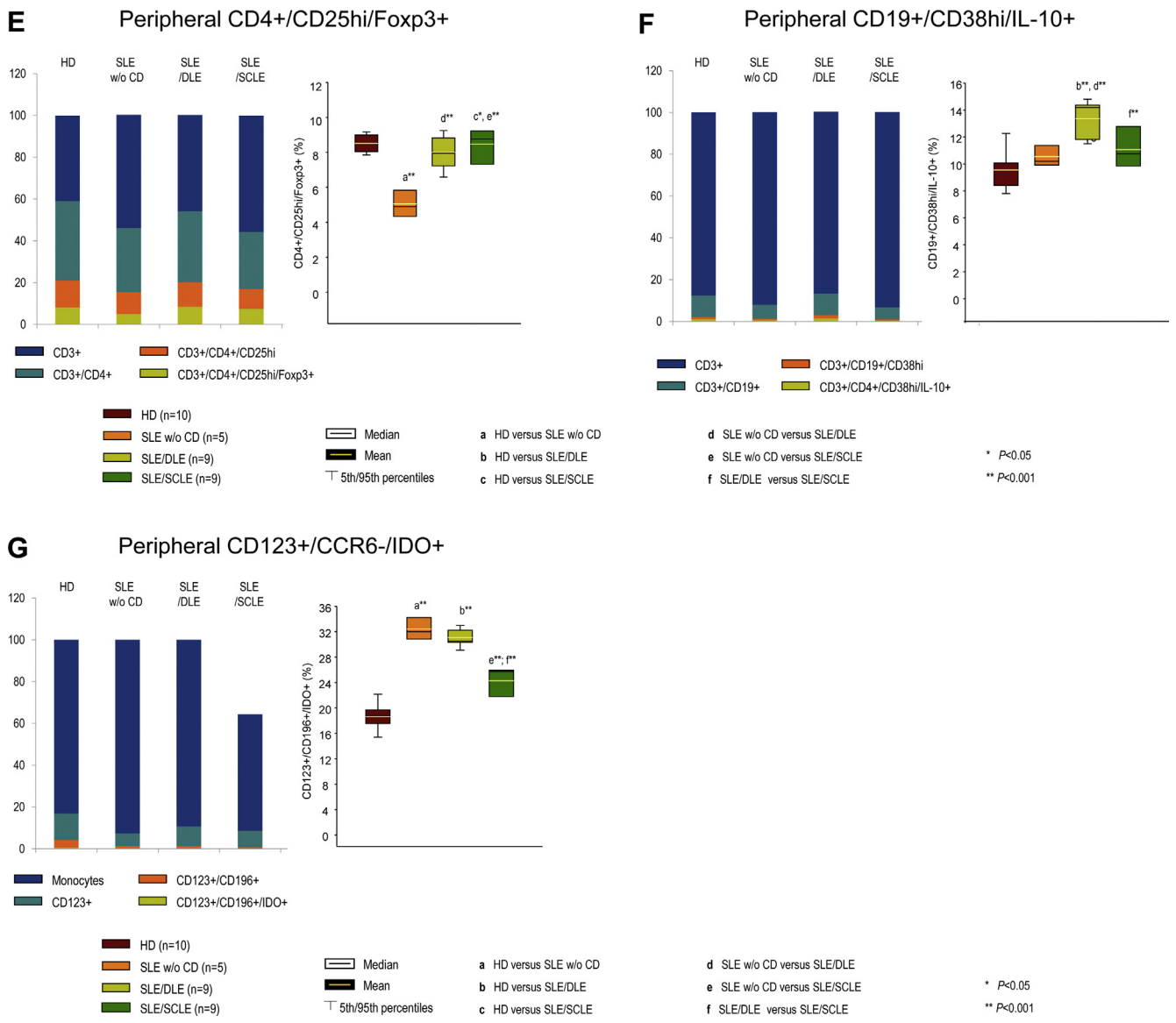

Fig. 4. (*continued*)

(Spearman's rho: 0.71, $P = 0.03$), while a negative correlation was determined in SLE/SCLE between the CD4+/IFN-γ + cell subset and the expression of CCL27 (Spearman's rho: −0.66, $P = 0.05$). In the SLE w/o CD patients, the correlations between CD4+/IFN-γ + cells and chemokine/chemokine receptor expression were not significant (Table 2).

3.6.5. Forkhead box P3-expressing CD4 T cells and chemokine/chemokine receptor expression

In the periphery, positive correlations were determined in SLE/SCLE between the CD4+/CD25hi/Foxp3 + cell subset and the expression of CCR2 (Spearman's rho: 0.73, $P = 0.04$) or CXCR1 (Spearman's rho: 0.74, $P = 0.03$). A negative correlation was determined in SLE/DLE between CD4+/CD25hi/Foxp3 + subset and the expression of CXCR5 (Spearman's rho: −0.73, $P = 0.002$) (Table 2).

In the tissue, a negative correlation was found in SLE/SCLE between the Foxp3-expressing CD4 T cell subset and the expression of CCL2 (Spearman's rho: −0.68, $P = 0.04$), while correlations between Foxp3-expressing CD4 T cells and the expression of chemokines/chemokine receptors were not significant in SLE/DLE. In the SLE w/o CD patients, a positive correlation was found between Tregs and the expression of CCR2 (Spearman's rho: 0.90, $P = 0.03$), and a negative correlation was found between Tregs and CCR4 expression (Spearman's rho: −0.94, $P = 0.01$) (Table 2).

3.6.6. IL-10-producing B cells and chemokine/chemokine receptor expression

In the periphery, a positive correlation was determined in SLE/SCLE between CD19+/CD38hi/IL-10 + cells and the expression of CXCR5 (Spearman's rho: 0.74, $P = 0.03$). The correlations between the CD19+/CD38hi/IL-10 + cell subset and chemokine/chemokine receptor expression in SLE/DLE and SLE were not significant (Table 2).

In the tissue, negative correlations were found in SLE/SCLE between the IL-10-producing, CD20-expressing B cell subset and the expression of CCL2 (Spearman's rho: −0.71, $P = 0.03$), CCR4 (Spearman's rho: −0.82, $P = 0.007$) or CXCL13 (Spearman's rho: −0.70, $P = 0.03$). In SLE/DLE a negative correlation was determined between IL-10-producing CD20-expressing B cell subset and the expression of CXCL13 (Spearman's rho: −0.70, $P = 0.03$). However, in the SLE w/o CD patients, a negative correlation was found between the IL-10-producing CD20-expressing B cell subset and the expression of CCR4 (Spearman's rho: −0.97, $P = 0.005$) (Table 2).

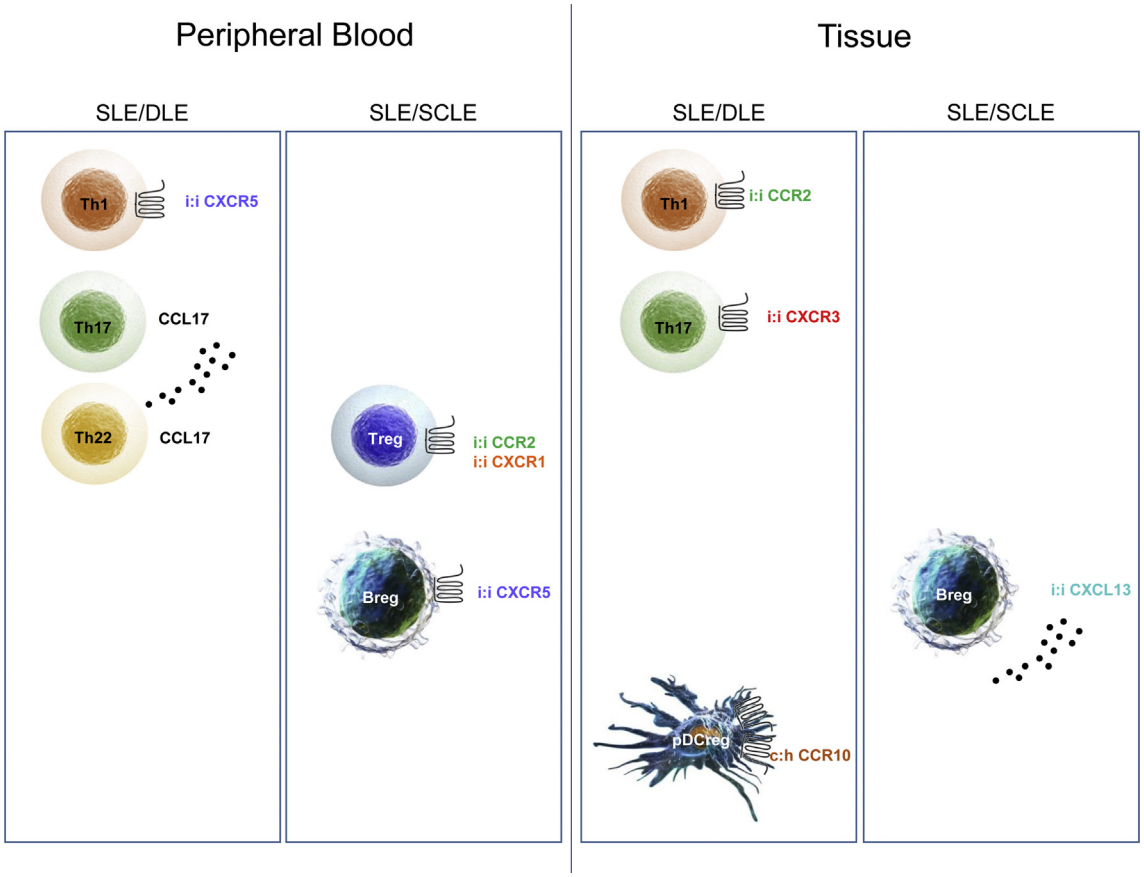

Fig. 5. Hypothetical model of the expression and interaction of the main correlations of chemokines and their receptors with CD4 effector T cells and regulatory cells (A) in the periphery and (B) in the skin tissue of discoid (SLE/DLE) and subacute lupus (SLE/SCLE). c:h, constitutive:basal trafficking/homing; i:i inducible:inflammatory.

3.6.7. IDO and CD123-expressing plasmacytoid dendritic cells and chemokine/chemokine receptor expression

In the periphery, negative correlations were determined in SLE/DLE between CD123 + /CD196 + /IDO + cells and the expression of CXCL10 (Spearman's rho: −0.74, $P = 0.02$) or CCR4 (Spearman's rho: −0.71, $P = 0.03$). A negative correlation was also found in SLE/SCLE between CD123 + /CD196 + /IDO + cells and the expression of CCL2 (Spearman's rho: −0.73, $P = 0.04$). A positive correlation was also determined in SLE between pDCregs and the expression of CCR4 (Spearman's rho: 0.97, $P = 0.005$) (Table 2).

In the tissue, a positive correlation was found in SLE/DLE between the IDO-expressing subset and the expression of CCR10 (Spearman's rho: 0.73, $P = 0.02$). Correlations between CD123 + /IDO + cells and chemokine/chemokine receptor expression in SLE/DLE and SLE were not significant (Table 2).

4. Discussion

CLE is a complex clinical entity whose physiopathology remains to be fully characterized but encompasses interactions between genetics, the environment, cells (keratinocytes, endothelial cells, neutrophils, dendritic cells, T cells and B cells) and cell products. In this sense, chemokines and their receptors are involved in the regulation of infiltration into inflamed cellular sites. Few studies have assessed the complex cell, cytokine and chemokine network in CLE [9]. In a previous study, we showed the participation of Th22, Th17 and Th1 cells as well as pDCregs in DLE and SCLE patients; this response was more intense in DLE than in SCLE [8]. Herein, we now explored the chemokine profiles of these patients and correlated these profiles with tissue-infiltrating and circulating effector CD4 T cell/regulatory cell subpopulations.

In vitro, UV-irradiated keratinocytes release lymphocyte chemoattractants such as CCL5, CCL22, CCL27, and CXCL28 [10,11]. Most skin-infiltrating lymphocytes in patients suffering from inflammatory and autoimmune skin conditions express CCR10, while epidermal basal keratinocytes express CCL27. The latter is a TNF-α- and IL-1β-induced chemokine that plays a pivotal role during the recruitment of circulating CCR10-positive memory T cells into the skin [11]. In this work, we observed increased immunoreactivity for CCL27 and its receptor CCR10 in patients with CLE versus healthy controls, with this immunoreactivity being highest in the DLE subset.

Some authors have reported the predominance of the Th1 response in cutaneous lupus. Type I IFNs produced by plasmacytoid dendritic cells recruit Th1 lymphocytes to lesions by upregulating the production of CXCL10 by keratinocytes and other skin cells [9] as well as recruiting CXCR3-positive lymphocytes (Th1, Th17, Th2, and CD8 + T cells) into the skin with a concomitant decrease in the peripheral blood [12–14]. Moreover, IFN-γ induces the expression of CXCL9, CXCL10, CXCL11 and their receptor CXCR3 in Th1 cells [13,15]. In CLE tissue, CXCL10 is most prominent at the interface between the epidermis and dermis, whereas CXCR3-positive lymphocytes invade the epidermis [16]. Furthermore, a study showed that higher mRNA expression of CXCL9 and CXCL10 correlates with IFN-γ expression in different subsets in CLE and that the expression is higher in SCLE than in lupus tumidus and discoid lupus [3]. Additionally, local balances between effector or regulatory T cells and CXCL10 have been proposed [13,17].

Herein, we also corroborated increased expression of CXCL10 in all the SLE groups (with or without CLE) compared with the healthy donor group, but this expression was highest in the DLE group. The expression of CXCR3 was similar in the SLE groups (with or without CLE).

Moreover, a Th1-associated chemokine receptor profile was

observed by Freutel and colleagues, who showed that the CCR5+/CCR3 + T helper cell ratio in patients with CLE correlated with disease activity [4]. Similarly, patients with SLE and cutaneous involvement had higher serum levels of CXCL16 than SLE patients without skin features. This chemokine is upregulated by Th1 cytokines, including IFN-γ, TNF-α, and IL-18 [18]. In addition, in this study, we demonstrated that CCR2 and CXCR5 expression is an important determinant of Th1 cell differentiation and migration into the skin of SLE/DLE patients, as was previously shown by Kim et al. and Morita et al. [19,20]. Likewise, it has been demonstrated that CCR2 contributes to defining T cell polarization to enrich major subsets of Th1 versus Th2 cells in inflamed tissues [19], which reinforces the absence of correlations between Th2 cells and CCL2/CCR2. On the other hand, CXCR5 + Th1 cells lack the capacity to induce naïve B cells to produce immunoglobulins via IL-21 [20]. In our patients, whether CXCR5+/CD4 + T cells represent a circulating pool of memory Tfh cells that can be differentiated into Th1, Th2, and Th17 cell subsets with different capacities to regulate B cell responses, as previously reported by Morita et al., remains unclear [20].

Th17 cells produce IL-17, which stimulates T cells; increases the production of autoantibodies, inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-8, IL-17, and IL-22) and chemokines (CCL2, CCL7, CCL20, CXCL1 and CXCL5); and induces neutrophil recruitment through chemokine regulation [21]. As other authors have shown, we found a high number of peripheral and tissue-infiltrating IL-17A-expressing CD4 T cells in the SLE/DLE and SLE/SCLE patients [22,23]. As Reiss et al. showed, we found that the chemoattraction of circulating CD4+/CD161+/IL-17A + cells to the skin in SLE/DLE patients preferentially depends on the expression of CCL17 [24]. We also determined that the migration of IL-17A-expressing CD4 T cells into the skin in SLE/DLE patients but not in SLE/SCLE patients is dependent on the expression of CXCR3 but is independent of CCL2.

On the other hand, a higher CD4+/IL-22 + cell percentage in CLE tissue in comparison to healthy tissue as well as negative correlations of tissue and peripheral CD4+/CD161-/IL-22 + cell percentages with the CLASI score have been recognized [8]. In line with these findings, a study identified that SLE patients with skin involvement also have higher frequencies of CCR6+/CD4+/IL-22 + cells. Moreover, the IL-22-producing cell frequency is positively correlated with disease severity and negatively correlated with C3 serum levels [25].

We evaluated the expression of CCR4 and did not observe any differences among the groups. However, CCL17 was expressed at lower levels in the SLE/DLE and SCLE groups than in the SLE w/o CD and healthy control groups. CCL17 is synthesized by activated keratinocytes, DCs, and endothelial cells in the skin [26,27], while CCR4 is expressed by Langerhans cells, endothelial cells and fibroblasts. The interaction between CCL17 and CCR4 plays a critical role in classical-type activation of macrophages [28]. A previous study by Reiss et al. showed that both CCL27 and CCL17 can support the homing of T cells to the skin [24,29]. As Reiss et al. observed, we found that the chemoattraction of circulating CD4+/CD161-/IL-22 + cells to the skin in SLE/DLE patients preferentially depends on the expression of CCL17. On the other hand, CCR4 is reported to be necessary for CD4 T cell entry into inflamed skin [30], while other works have shown that CD4 and CD8 T cell infiltration does not require CCR4 and instead may depend on CCR10 [24] or CXCR3 and CCR5 [31]. We determined that the recruitment of the circulating CD4+/CD161-/IL-22 + cell subset into the skin of SLE/SCLE patients seems to be independent of CCR4.

B cells act as antigen-presenting cells and autoantibody producers, and these cells are in the peripheral blood and lesioned skin of patients with SLE/DLE [32]. Nonetheless, newly identified IL-10-producing B cells or regulatory B (Breg) cells with an immunosuppressive phenotype support immunological tolerance. Through the production of IL-10, IL-35, and transforming growth factor β (TGF-β), Bregs suppress immunopathology by prohibiting the expansion of pathogenic T cells and other proinflammatory lymphocytes [33]. B cell migration to sites of inflammation is mediated by CXCL13, a B cell-attracting chemokine whose receptor is CXCR5, which is normally expressed on mature B cells and follicular T helper cells. In some clinical studies, the serum levels of this chemokine were increased in patients with SLE and correlated with disease activity and lupus nephritis [34,35]. Herein, we observed that the expression of CXCL13 was higher in the skin of the SLE w/o CD patients than in that of the SLE/SCLE patients. However, its receptor CXCR5 was more highly expressed in the SLE/DLE group than in the SLE/SCLE group. However, we showed that the trafficking of IL-10-producing B cells into the skin of SLE/SCLE patients is dependent on the expression of CXCR5 and chemoattraction by CXCL13.

However, cumulative evidence suggests that chemokines play a role in the pathogenesis of pain and itch [36,37]. CCL2, also called MCP 1, is a major inflammatory chemokine and is specifically responsible for recruiting monocytes and basophils to the site of inflammation in the skin lesions of SLE patients [38]. It binds to CCR1, CCR2, and CCR4 to exert distinct biological functions. Evidence indicates that CCL2/CCR2 signaling is essential for the development of pain and itch [39]. We found higher levels of CCL2/CCR2 in the skin of the patients with SLE and SLE/CLE than in the skin of the healthy donors. By means previously reported by Liu T et al. and Liou J-T et al., we also determined positive correlations in the SLE/SCLE patients between pain and CCL2 or CCR6 expression [36–39].

Additionally, we also found that the migration of not only IFN-γ-producing CD4 T cells in SLE/DLE but also Foxp3-expressing CD4 T cells in SLE/SCLE depends on the expression of CCR2 [19,40].

CXCL8 is a chemotactic leukocyte/neutrophil-recruiting and leukocyte/neutrophil-activating molecule that primarily acts on neutrophils but can also affect other granulocytes and induce phagocytosis. Anti-IL-8 neutralizing antibody administration prevents neutrophil-dependent tissue damage as well as neutrophil infiltration and, consequently, acute inflammation in LPS-induced dermatitis [41].

We found higher expression of CXCR1 in the SLE/DLE, SLE/SCLE and SLE w/o CD groups than in the healthy control group, with the highest expression in the SLE/DLE group. Conversely, there were no significant differences in the expression of CXCL8.

In our SLE/DLE patients, skin damage was related to CXCL8 expression, based on the positive correlation between the chemokine and CLASI damage score. Moreover, we determined that the migration of Foxp3-expressing CD4 T cells in SLE/SCLE patients depends on the expression of CXCR1, while pDCregs in SLE/DLE patients correlate with the expression of CCR10. However, the levels of CXCR1 and CCR10 in the skin of these patients were not sufficient to modulate the tissue damage due to the imbalance between effectors and regulators [41–43].

In conclusion, the immune response is more vigorous in patients with SLE/DLE than in those with SLE/SCLE. However, the correlations between chemokine/receptor expression and subpopulations of effector/regulatory T cells, B cells and pDCs showed differential responses among these cutaneous pathologies (Fig. 5).

5. Author disclosure statement

The authors have no conflict of interest to declare.

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Authors' Contributions

S.M-F., J.F-C. and G.H-M. Study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript,

critical revision of the manuscript for important intellectual content, statistical analysis, obtained funding, technical support. J.Z. Study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis, technical support. D.A-Ll. And J.R-D. Acquisition of data, analysis and interpretation of data, critical revision of the manuscript for important intellectual content, technical support.

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Appendix A. Supplementary material

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