Taylor & Francis healthsciences

Mediators of Inflammation, **13**(3), 171–180 (June 2004)

Clinical significance of circulating dendritic cells in patients with systemic lupus erythematosus

E. Robak[1], P. Smolewski[2], A. Woźniacka[1], A. Sysa-Jędrzejowska[1] and T. Robak[2,CA]

[1]Department of Dermatology and Venereology, Medical University of Łódź, 94-513 Łódź, ul. Krzemiemiecka 5, Poland; [2]Department of Hematology, Medical University of Łódź, 93-513 Łódź, ul. Pabianicka 62, Poland

[CA]Corresponding author
Tel: +48 42 689 51 91
Fax: +48 42 689 51 92
E-mail: robaktad@onet.pl, robaktad@csk.am.lodz.pl

Dendritic cells are a complex group of mainly bone-marrow-derived leukocytes that play a role in autoimmune diseases. The total number of circulating dendritic cells (tDC), and their plasmacytoid dendritic cell (pDC) and myeloid dendritic cell (mDC1 and mDC2) subpopulations were assessed using flow cytometry. The number of tDC and their subsets were significantly lower in systemic lupus erythematosus patients than in the control group. The count of tDC and their subsets correlated with the number of T cells. The number of tDC and pDC subpopulation were lower in the patients with lymphopenia and leucopoenia than in the patients without these symptoms. Our data suggest that fluctuations in blood dendritic cell count in systemic lupus erythematosus patients are much more significant in pDC than in mDC, what may be caused by their migration to the sites of inflammation including skin lesions. Positive correlation between dendritic cell number and TCD4$^+$, TCD8$^+$ and CD19$^+$ B cells, testify of their interactions and influence on SLE pathogenesis. The association between dendritic cell number and clinical features seems to be less clear.

Key words: Dendritic cells, Lymphocytes, Natural killer cells, Systemic lupus erythematosus, Disease activity

Introduction

Systemic lupus erythematosus (SLE) is a multiorgan disease of connective tissue, considered a model of autoimmune diseases.[1,2] The disease is characterized by the production of antibodies directed to components of the cell nucleus with a diverse array of clinical manifestations. For many years SLE has been considered mainly as B-cell disease resulting from altered T-cell and B-cell interactions.[3,4] However, a significant role of dendritic cells (DCs) in the control of tolerance and immunity has been recently proved, as has the hypothesis that SLE may be driven through unabated DC activation.[5]

DCs are a group of bone-marrow-derived, lineage-negative Lin$^-$, HLA-DR$^+$ cells, which are specialized for the uptake, transport, processing and presentation of antigens to T cells.[6] This results in T-cell proliferation and differentiation with respect to cytokine production. Moreover, DCs appear to be the only class of antigen presenting cells that have the capacity to stimulate the expansion of naive T cells and thereby initiate primary immune responses. DCs can also generate regulatory T cells that suppress activated T cells, a function of probable importance in autoimmunity.

Basically, two main types of DC have been identified, the CD11c$^-$ subset called plasmacytoid dendritic cells (pDC), and the CD11c$^+$ subset, called myeloid dendritic cells (mDC).[7,8] Plasmacytoid DCs secrete large amounts of interferon-α (IFN-α), when stimulated with interleukin (IL)-3 and CD40-L prime T cells to secrete IL-4 (Th2 cytokine).[9,10] However, when stimulated by viruses they induce T cells to produce IFN-γ and IL-10. mDC include Langerhans cells and interstitial DCs.[11] Interstitial DCs express high levels of non-specific esterases and can induce the differentiation of naive B cells to plasma cells. Langerhans cells express CD1a and Birbeck granules, while interstitial DCs express coagulation activation factor XIII.[10] More recently, a panel of monoclonal antibodies (mAbs) that identify four presumably novel human peripheral blood DC antigens (BDCA-1, BDCA-2, BDCA-3 and BDCA-4) have been identified.[12–16] The BDCA-1 (CD1$^+$) antigen is specifically expressed on cells that are CD11c^{bright}, CD123^{dim}, and represent a major subset of mDC (mDC1) in human blood.[12,16] BDCA-3 is restricted to a small population of myeloid CD123$^-$, CD11c$^+$ DC (mDC2).[14] In fresh human blood, expression of BDCA-2 and BDCA-4 is restricted to plasmacytoid, CD123$^+$, CD11c$^-$ peripheral blood DCs.[12] So far, there are no data available on quantitative characteristics of these DC subsets and their correlation with clinical and laboratory symptoms in SLE patients.

DCs have been implicated in the induction of autoimmune diseases and have been identified in lesions associated with several autoimmune inflammatory diseases.[17] Recently, several evidences have

ISSN 0962-9351 print/ISSN 1466-1861 online/04/30171-10
DOI: 10.1080/09511920410001713538

been obtained that interplay between DC subsets might have an important role in SLE pathogenesis.[5,10] In particular, serum from SLE patients can induce DCs from monocytes, which are able to capture apoptotic cells and present their antigen to autologous T cells.[18] This observation may indicate that SLE blood creates environment inducing DC differentiation. It has been shown that the DC-inducing property of SLE serum is mediated by IFN-α.[5] On the other hand, pDC are the major cellular source of IFN-α in the blood.[19,20] Moreover, considerable alterations of DCs and their particular subsets have been found in SLE patients, including reduction of mDC by an average of 80%.[20] In pediatric SLE patients undergoing steroid therapy, DC numbers were over 70% lower than in the control group.[21] SLE patients who were not treated with steroids demonstrated a decline number of pDC by approximately 30%. Recently, Shodell *et al*. have shown that circulating human DCs are highly sensitive to corticosteroid treatment.[22]

In the present study we assessed the total number of circulating DCs and their plasmacytoid and myeloid subpopulations defined by a novel panel of mAbs directed against BDCA antigens. We also correlated the number of DCs with peripheral blood immunocompetent cells that are involved in SLE pathogenesis. $TCD4^+$, $TCD8^+$, B cell ($CD19^+$) and natural killer (NK) cell ($CD56^+$) counts. Moreover, peripheral blood DC subsets were assessed in relation to several clinical and laboratory parameters as well as SLE activity.

Patients and methods

Patients

The study group consisted of 53 patients with SLE (49 females and four males), and 33 sex-matched and age-matched healthy volunteers. The mean age of the SLE patients was 44 years (range, 22–66 years). The mean age of the control group was 42 years (range, 18–65 years). The control group consisted of 30 women and three men. The diagnosis of SLE was based on the revised criteria of the American College of Rheumatology.[23] The mean duration of the disease was 64 months (range, 8 months–20 years). Eighteen patients had never been treated with steroids or any other immunosuppressive agents. Thirty-five patients were treated with prednisone at a dose of 5–20 mg/day during the study, including three patients treated with prednisone and azatioprine at a daily dose of 50–100 mg.

Disease activity was scored according to the method described by Liang *et al.*[24] Each patient was examined on two separate occasions, 2–4 weeks apart. The system of Systemic Lupus Activity Measure includes 24 clinical manifestations and eight laboratory parameters. The maximum score in this system is 84 points. We assumed a score of 0–15 points for inactive disease and a score over 15 points for active disease.[25] By this definition, the active stage of the disease was found in 22 patients while 31 patients were in remission. Each patient underwent a thorough physical examination performed by one of the authors (E.R.). The patients with SLE and healthy volunteers showed no clinical signs of infection or neoplastic disease and received no other medications for at least 4 weeks prior to blood donation. All patients samples were collected between 08:00 and 14:00 h in the course of venipuncture for routine laboratory investigations. This project was performed in accordance with the Helsinki Declaration. Informed consent was obtained from all patients participating in the study. The project was also approved by the Local Ethic Committee.

Cell isolation

DCs were detected in peripheral blood mononuclear cells (PBMC) population. Whole heparinized blood samples were mixed with Hanks reagent (1:1), and then centrifuged in the gradient of Ficoll-Paque (2400 *g* for 20 min), then double-washed and resuspended in phosphate-buffered saline (PBS) buffer (Sigma, St. Louis, MO, USA). Freshly isolated PBMC were subjected to the immunophenotype examination.

Immunophenotyping

The immunophenotype of DCs was determined using the panel of mAbs directed against several DC-defined antigens. There were FITC-conjugated BDCA-1, BDCA-2, and BDCA-3 (Miltenyi Biotec, Bergish Gladbach, Germany), PE-conjugated CD11c, CD32 (Caltag Laboratories, Burlingame, CA, USA) and CD123 (Miltenyi Biotec), as well as TC-conjugated HLA-DR (Caltag Laboratories). In a preliminary studies, combinations of another mAbs, against CD14, CD19, and CD64 antigen, as well as CD80 and CD86 costimulatory molecules (all Caltag Laboratories) were also tested. As a control, respective mouse IgG_1 mAbs MG101, MG104, MG106 were used (Caltag Laboratories). Samples were prepared at the concentration of 300,000 cells/100 μl of PBS, in final volume of 250 μl per sample. Antibodies were added in the concentration of 1 μg/100 μl and incubated for 10 min at 4°C, in the dark. Then, the immunophenotype was assessed by triple-color cytometry. Cell fluorescence was measured by flow cytometer (FACScan; Becton-Dickinson, San Jose, CA, USA) and analyzed with green (FL1), orange (FL2) or red (FL3) standard emission filters. In each sample 100,000 events were acquired. Based on SC versus FS distribution, DCs have been gated from a whole PBMC population.

Defining main types of blood DCs and calculating the absolute DC number

Based on preliminary studies, the characteristic pattern of antigen expression of three main subsets of DC were discriminated. There were two myeloid subtypes of DCs: BDCA-1^+/$CD11c^+$/HLA-DR^+ (mDC1) and BDCA-3^+/$CD32^-$/HLA-DR^+ (mDC2), as well as pDC, characterized by the BDCA-2^+/$CD123^+$/ HLA-DR^+ expression profile. Determining the percentage of both myeloid subpopulations with mAbs against CD14 and CD19 antigens for lineage negative selection (BDCA-1^+/$CD19^-$ for mDC1 and BDCA-3^+/$CD14^-$ for mDC2, respectively) gave consistent, comparable results. The method of DC subtype discrimination by flow cytometry is shown and described in Fig. 1, using BDCA-2^+/$CD123^+$ cell subset detection as an example. For absolute DC count assessment, in every patient and healthy volunteer the number of PBMC per microliter was routinely measured. Then, based on the percent of particular DC subset in the whole PMBC population as determined by flow cytometry (Fig. 1), the DC count was calculated and presented as a number of cells per microliter.

Immunophenotype analysis of lymphocytes and NK cells

Peripheral blood lymphocyte analysis was performed by standard double-color immunofluorescence measurement by flow cytometry. The details of the procedure are described elsewhere.[26,27]

Other clinical and laboratory features of SLE

The number of other clinical or laboratory SLE features, such as the presence of arthritis, skin lesions, reticuloendothelial system involvement, as well as absolute leukocyte, lymphocyte and platelet counts, serological symptoms, renal involvement, presence of C-reactive protein, antinuclear antibodies and erythrocyte sedimentation ratio were also routinely assessed.

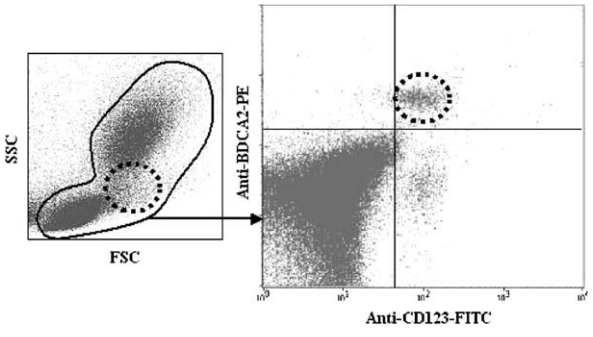

FIG. 1. Detection of DCs by flow cytometry. DCs have been gated from the whole PBMC population based on the SC versus FS distribution (black line). Then, in the gated cells the expression of particular antigens were assessed by triple-color flow cytometry. In the shown case, distinct BDCA-2^+/$CD123^+$/HLA-DR^+ plasmacytoid DCs have been discriminated. According to size (FS) and cellular density (SC), the majority of pDC occurred between lymphocyte and monocyte populations (dashed circle).

Statistical analysis

For the statistical analysis of the data the range of measured variable (minimum–maximum), the mean arithmetic value (x), the median (Me) and the standard deviation (SD) were calculated. The Shapiro–Wilk's test was used to evaluate the distribution. In order to compare mean values, the Mann–Whitney test, the test for two independent samples or the Cochran–Cox test were applied. The correlation between features was evaluated using the Spearman rank coefficient ρ. Comparisons and correlations were considered significant when $p < 0.05$.

Results

The total number of DCs and their subpopulations in SLE patients and healthy donors

The number of circulating dendritic cells (tDC) and their three main subpopulations (pDC, mDC1 and mDC2) in peripheral blood was assessed in 53 SLE patients and 33 healthy donors. The clinical and laboratory characteristics of SLE patients are presented in Table 1.

Table 1. Clinical and laboratory characteristics of patients with SLE

Symptoms	Number of patients	%
Total	53	100.0
Age (years), mean (range)	43.9 (22–66)	
Sex (male/female)	4/49	
Active/inactive	22/31	41.5/58.5
Arthritis	49	92.5
Skin symptoms	44	83.0
Reticuloendothelial system involvement	44	83.0
Renal disorder (creatinine >1.3 mg/dl)	3	5.7
Neurologic symptoms	34	64.2
Antinuclear antibodies, anti-dsDNA antibodies	7	13.2
Anemia (hemoglobin <12 g/dl)	13	24.5
Lymphopenia ($<1.5 \times 10^9$/l)	25	47.2
Leukopenia (white blood cells $<3.5 \times 10^9$/l)	12	22.6
Thrombocytopenia (platelets $<150 \times 10^9$/l)	15	28.3
Raised erythrocyte sedimentation ratio (>25 mm/h)	32	60.4
C-reactive protein (>4.7 mg/l)	6	11.3
Treatment with steroids	35	60.0

Table 2. Analysis of peripheral blood dendritic cells in patients with SLE and healthy donors (cell /μl)

Cell subpopulation	All SLE ($n=53$) (a)	Active SLE ($n=22$) (b)	Inactive SLE ($n=31$) (c)	Control group ($n=33$) (d)	Statistically significant comparison
tDC/μl					
$\bar{x}\pm$SD	13.9±7.1	12.2±6.1	15.1±7.6	21.3±11.3	(a)–(d), $p<0.002$
Me	12.96	12.3	14.2	20.9	(b)–(d), $p<0.003$
Range	2.5–34.4	2.5–24.8	5.3–34.4	2.8–48.3	(c)–(d), $p<0.02$
pDC /μl					(a)–(d), $p<0.002$
$\bar{x}\pm$SD	6.6±3.7	5.5±3.4	7.4±3.7	11.4±7.6	(b)–(c), $p<0.04$
Me	6.0	5.4	6.9	10.0	(c)–(d), $p<0.002$
Range	1.16–18.0	1.2–14.5	2.1–18.0	0.0–29.6	(c)–(d), $p<0.03$
mDC1/μl					
$\bar{x}\pm$SD	6.3±4.5	5.7±3.9	6.7±4.8	7.6±5.8	
Me	5.1	4.52	5.35	6.11	
Range	0.9–21.5	0.9–14.8	1.9–21.5	0.0–21.5	
mDC2/μl					
$\bar{x}\pm$SD	1.0±1.1	1.1±1.6	0.9±0.6	2.3±1.5	(a)–(d), $p<0.001$
Me	0.8	0.7	0.9	2.3	(b)–(d), $p<0.001$
Range	0.1–7.8	0.1–7.8	0.2–2.9	0.0–8.6	(c)–(d), $p<0.001$

The mean arithmetic value, median and range of DC absolute values are presented in Table 2. The mean number of tDC was lower in SLE patients (13.9±7.1/μl) than in the control group (21.3±11.3/μl) ($p<0.002$). A comparable decrease in circulating pDC (BDCA-2^+) and mDC2 (BDCA-3^+) subtypes in SLE patients in relation to the normal control group was observed (6.6±3.7/μl versus 11.4±7.6/μl, $p<0.002$ and 1.0±1.1/μl versus 2.3±1.5/μl, $p<0.001$, respectively). The absolute number of mDC1 (BDCA-1^+) was similar in SLE patients and in healthy donors (Table 2).

Correlation between DC number and circulating T-cell, B-cell or NK subpopulations

The values of T $CD3^+$, T $CD4^+$ and T $CD8^+$ subpopulations, NK cells and B cells are presented in Table 3. No differences in the number of particular subtypes of lymphocytes or NK cells in active and inactive SLE patients were observed. Significant positive correlations were also observed between T $CD4^+$ and tDC, pDC and mDC1 but not with the mDC2 subpopulation (Fig. 2). Positive correlation was also found between T $CD8^+$ cells and tDC, pDC

Table 3. Lymphocyte subpopulations in patients with SLE

Cell subpopulation	All SLE ($n=53$)	Active SLE ($n=22$)	Inactive SLE ($n=31$)
TCD3+cells/μl			
$\bar{x}\pm$SD	1171.9±582.7	1066.9±506.6	1255.8±634.7
Me	1066.5	924.2	1107.2
Range	477.4–3480.0	477.4–2309.5	576.8–3480.0
TCD4+cells/μl			
$\bar{x}\pm$SD	684.1±373.7	601.3±320.3	750.4±405.6
Me	603.4	477.21	660.0
Range	265.7–2001.0	265.7–1394.8	288.9–2001.0
TCD8+cells/μl			
$\bar{x}\pm$SD	482.1±262.3	434.0±240.6	520.6±277.2
Me	402.3	354.5	438.6
Range	159.9–1418.1	159.9–1230.7	218.0–1418.1
NK+cells/μl			
$\bar{x}\pm$SD	43.4±62.3	41.47±66.19	45.1±60.5
Me	11.92	12.08	11.44
Range	0.0–216.5	1.46–216.5	0.0–204.9
Pan B (CD19+) cells/μl			
$\bar{x}\pm$s	150.2±110.7	140.2±112.0	158.1±111.4
Me	121.6	107.0	164.8
Range	5.4–548.1	11.3–355.2	5.4–548.10

p values are not significant between active and inactive SLE for all lymphocyte subpopulations.

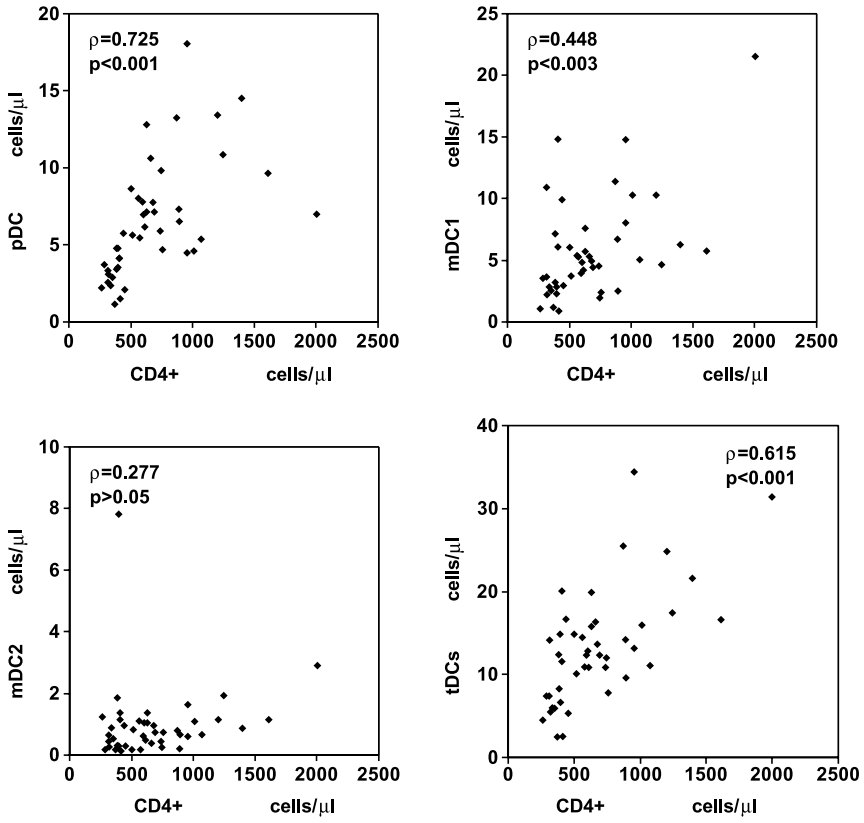

FIG. 2. Correlations between the number of T CD4$^+$ cells and total dendritic cells (tDC) and their pDC and myeloid (mDC1 and mDC2) subpopulations in the peripheral blood of SLE patients.

and mDC1 (Fig. 3). However, the correlation between T CD8$^+$ cells and mDC2 was not statistically significant ($p > 0.05$). In contrast, tDC and pDC correlated with CD19$^+$ B cells but such correlation was not observed with mDC (Fig. 4). Moreover, NK (CD56$^+$) cells did not correlate significantly with DCs neither with a plasmacytoid nor with a myeloid subpopulation (data not shown) ($p > 0.05$ for all comparisons).

The number of DC subsets and SLE activity

The number of tDC in active and inactive SLE was comparable (mean 12.2 ± 6.1/μl and 15.1 ± 7.6/μl, respectively, $p > 0.05$) (Table 2). Similarly, the values of mDC1 and mDC2 did not differ significantly in patients with the disease flare and during remission. Only the pDC number was lower in the active stage (5.5 ± 3.4/μl) than in the inactive stage of the disease (7.4 ± 3.7/μl) ($p < 0.04$).

The correlations between the disease activity score and number of tDC, pDC and mDC were also calculated (Fig. 5). Negative, but statistically not significant, correlations were obtained for all comparisons ($p > 0.05$).

Correlation between DC number and particular features of SLE

The DC number was also analyzed in comparison with particular SLE features (Table 4). When compared with patients with normal or elevated number of lymphocytes or lymphocytes, we observed significantly lower numbers of both tDC and pDC in those with lymphopenia (both $p < 0.001$) and leukopenia ($p < 0.02$ and $p < 0.004$, respectively). Moreover, in patients with lymphopenia, a significantly lower number of mDC, but only mDC1, was also found. We also compared the number of DCs and their subpopulations with other clinical or laboratory SLE features, such as platelet number, arthritis, skin lesions, reticuloendothelial system involvement, serological symptoms, renal involvement, presence of C-reactive protein, antinuclear antibodies and raised erythrocyte sedimentation ratio. The differences were not statistically significant ($p > 0.05$ for all comparisons). The number of total DCs and their plasmacytic and myeloid subpopulations were also similar in the patients treated and untreated with steroids (Table 4).

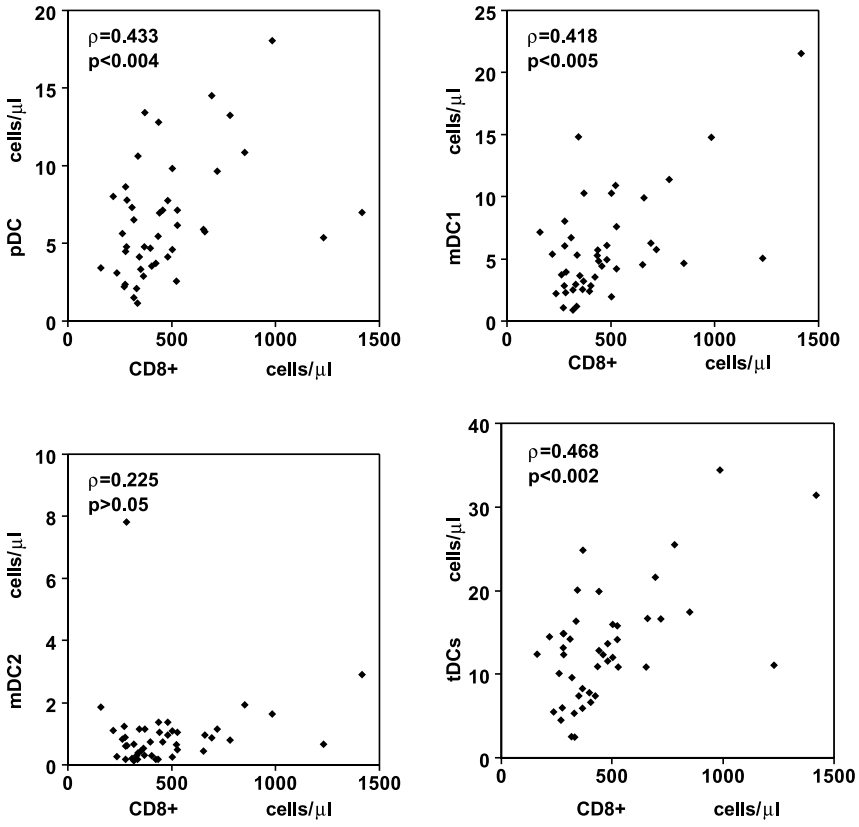

FIG. 3. Correlations between the number of T CD8$^+$ cells and total dendritic cells (tDC) and their pDC and myeloid (mDC1 and mDC2) subpopulations in the peripheral blood of SLE patients.

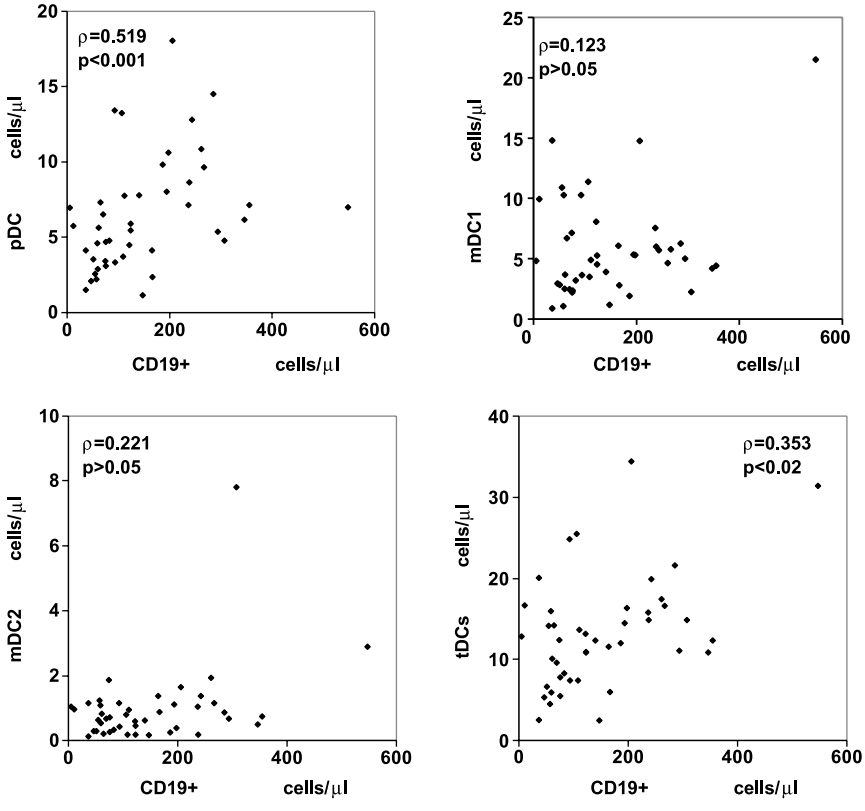

FIG. 4. Correlations between the number of B CD19$^+$ cells with total dendritic cells (tDC) and their pDC and myeloid (mDC1 and mDC2) subpopulations in the peripheral blood of SLE patients.

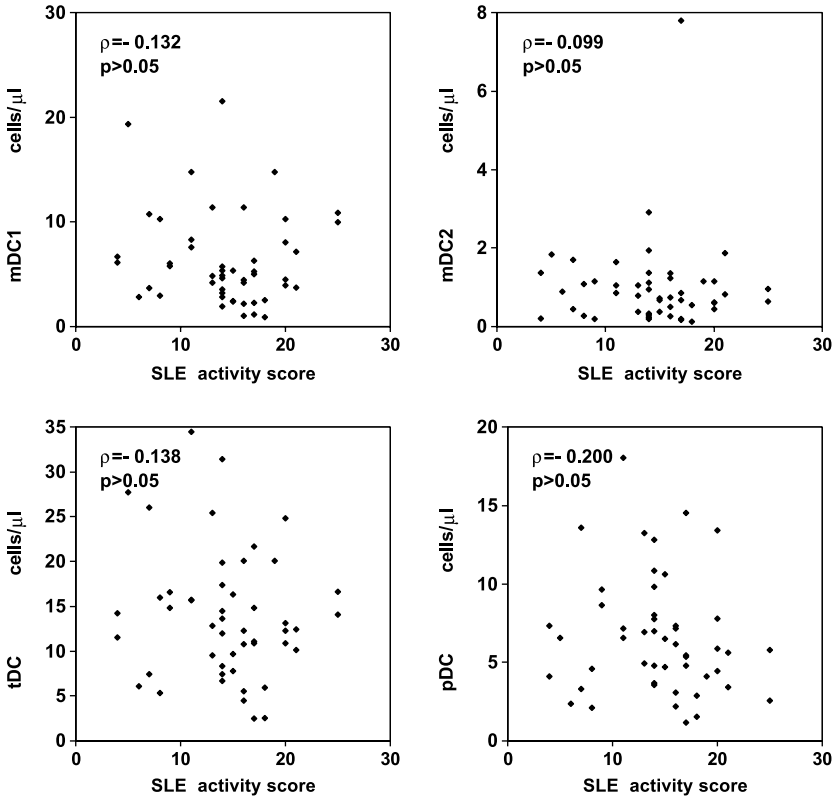

FIG. 5. Correlations between the disease activity score and number of total dendritic cells (tDC), pDC and mDC subpopulations in peripheral blood of SLE patients.

Discussion

In this study, the quantitative evaluation of peripheral blood DCs and their particular subsets in patients with SLE was performed in comparison with healthy volunteers as a control. We showed that tDC, pDC and mDC2 populations were significantly lower in SLE patients than in healthy subjects. These observations are partially consistent with previous reports.[21,28] Scheinecker *et al*. showed that the DC count in patients with SLE was 50% lower than in the control, regarding the number of $CD11^+$ cells (mDC) even 80% lower.[20] However, unlike those results, our study showed a number of $CD11^+$ mDC in SLE patients similar to the healthy control. The reduction in total DC count was clearly dependent on the decrease in pDC and mDC2, the small subset of myeloid $BDCA3^+$ DCs. Most recently, a similar finding was reported also by Blomberg *et al*.[14]

The decrease in peripheral blood tDC number in SLE patients was not unexpected. Accumulation of DCs, especially pDC, in cutaneous lupus erythematosus lesions has been previously shown in patients with SLE.[17,29] Moreover, in SLE patients accelerated migration of DCs to other sites of inflammation is also possible. An alternative explanation might be that pDC normally protect from autoimmune disease by inducing tolerance, and in their absence T cells and autoimmune T cells are more activated.[10,11]

Another cause of decrease in DC number may be prolonged treatment with prednisone, administered to the majority of SLE patients. Peripheral blood pDC from healthy people were previously found to be very sensitive to steroids.[22] However, in our study the numbers of both tDC and their subpopulations were similar in patients treated and not treated with prednisone (Table 4). Scheinecker *et al*. showed negative correlation between the dose of steroids and the DC count ($r = -0.44$, $p < 0.04$). The lack of treatment-dependent differences in DC number in our patients can be due to treatment with low doses of prednisone (5–20 mg/day). Another possible reason for the decrease in DC number is their enhanced apoptosis. In *in vitro* studies, abnormal apoptosis activation was found in lymphocytes from SLE patients.[30,31]

According to disturbances in circulating lymphocyte interactions observed in SLE patients,[3,4] we correlated the total number of DCs and their subsets with T-cell, B-cell and NK-cell subpopulations. Interestingly, we found a simultaneous positive correlation between the number of tDC, pDC and mDC1, and both $CD4^+$ and $CD8^+$ T-cell count. Additionally, with regard to tDC and pDC, distinct correlation with the number of B cells was also found. These findings strongly suggest complex interactions between those cells, which probably play an important role in the development of SLE. There was no correlation found

Table 4. Differences between the total number of peripheral blood dendritic cells (tDC) and their pDC and myeloid (mDC1, mDC2) subpopulations in patients with and without selected SLE symptoms

SLE symptom		*n*	tDC	pDC	mDC1	mDC2
Lymphopenia	Lymphocytes $<1.5\times10^9$/l	25				
	$\bar{x}\pm$s		9.8±4.7	4.3±2.1	4.6±3.3	1.0±1.5
	Me		9.2	3.9	3.8	0.63
	Range		2.5–20.1	1.2–8.6	0.9–14.8	0.1–7.8
	Lymphocytes $\geq1.5\times10^9$/l	28				
	$\bar{x}\pm$s		17.8±7.0	8.8±3.6	8.0±4.9	1.0±0.6
	Me		15.9	7.3	6.2	0.9
	Range		9.5–34.4	4.5–18.0	1.9–21.5	0.2–2.9
	p value		$p<0.001$	$p<0.001$	$p<0.002$	$p>0.05$
Leukopenia	WBC $<3.5\times10^9$/l	12				
	$\bar{x}\pm$s		9.5±5.2	4.0±2.0	4.9±4.0	0.6±0.5
	Me		10.1	4.10	3.70	0.60
	Range		2.49–20.06	1.16–7.80	0.89–14.81	0.13–1.86
	WBC $\geq3.5\times10^9$/l	41				
	$\bar{x}\pm$s		15.2±7.2	7.4±3.8	6.7±4.6	1.1±1.3
	Me		14.36	6.75	5.33	0.87
	Range		4.49–34.44	2.20–18.04	1.06–21.50	0.19–7.80
	p value		$p<0.02$	$p<0.004$	$p>0.05$	$p>0.05$
Thrombocytopenia	Platelets $<150\times10^9$/l	15				
	$\bar{x}\pm$s		11.6±5.3	6.1±3.4	4.9±3.3	0.7±0.3
	Me		10.82	5.62	4.18	0.64
	Range		4.49–21.64	2.20–14.52	1.06–11.38	0.24–1.36
	Platelets $\geq150\times10^9$/l	38				
	$\bar{x}\pm$s		14.9±7.7	6.8±3.9	6.9±4.8	1.1±1.3
	Me		13.91	6.53	5.53	0.91
	Range		2.49–34.44	1.16–18.04	0.89–21.50	0.13–7.80
	p value		$p>0.05$	$p>0.05$	$p>0.05$	$p>0.05$
Treatment with steroids	Steroids	35				
	$\bar{x}\pm$s		13.6±7.3	6.4±4.0	6.2±4.4	1.0±1.3
	Me		12.81	5.36	5.00	0.80
	Range		2.49–34.44	1.16–18.04	0.89–19.35	0.13–7.80
	No steroids	18				
	$\bar{x}\pm$s		14.5±6.8	7.1±3.0	6.4±4.6	1.0±0.7
	Me		13.61	6.97	5.26	0.88
	Range		6.03–31.37	2.36–13.58	2.35–21.50	0.19–2.91
	p value		$p>0.05$	$p>0.05$	$p>0.05$	$p>0.05$

Values of DCs are in cells/μl.

between DCs and NK cell count. Our data showed negative, but not statistically significant, correlation of the number of tDC and their subsets with activity of SLE assessed by the Systemic Lupus Activity Measure scale. Similar results, although in distinctly smaller groups of SLE patients, were described by Scheinecker *et al*.[21] They showed that the tDC population inversely correlated with the activity of the disease, defined according to the European Consensus Lupus Activity Measurement scale ($r=-0.56$, $p=0.01$). However, unlike our study, they did not correlate the SLE activity with particular DC subpopulations.

In this study we compared the number of DC subsets not only with SLE activity, but also with several clinical and laboratory symptoms of the disease. However, we found that only lymphocyte and leukocyte counts influenced the number of DCs. Namely, we showed that the tDC and pDC number was significantly lower in patients with lymphopenia or leukopenia than in those with normal lymphocyte or leukocyte count, which clearly underlines the origin of peripheral blood DCs, especially pDC. Interestingly, in patients with lymphopenia a significantly lower number of mDC, but only the mDC1 subpopulation, was also found. Thus, the number of mDC2 was even relatively higher than in those patients with normal or elevated leukocyte/lymphocyte count. Positive correlation between total numbers of lymphocytes and DCs has been also observed by other authors.[21] It should be stressed that in other

lymphopenia-associated disorders (AIDS), as well as in advanced neoplastic diseases, a decrease in peripheral blood DC count was also described.[32,33]

There was no statistically significant relationship to other laboratory or clinical features of SLE, such as antinuclear antibodies level, anemia, thrombocytopenia, as well as the presence of skin, renal or neurological symptoms, which could be due to a small number of patients with particular SLE symptoms.

To our best knowledge, this is the first complex quantitative assessment and correlation of peripheral blood DC subpopulations defined by a recently developed panel of anti-BDCA mAbs, with detailed clinical and laboratory characteristics of SLE patients. According to recent findings, freshly isolated peripheral blood DCs can be divided into three main subsets: pDC and two mDC subpopulations, mDC1 and mDC2. However, for several years peripheral blood DCs have been defined by a variety of markers, which still complicates their repeatable and comparable assessment. pDC are actually characterized as cells expressing specific BDCA-2 and BDCA-4 antigens, but they have also Lin^-, $HLA\text{-}DR^+$, $CD123^+$, $CD11c^-$, $CD4^+$, $CD2^-$ and $CD45RO^+$ characteristics.[12,13]

Previously, Upham *et al.* detected HLA-DR/$CD33^-$-positive cells as a mDC subpopulation.[34] The frequency of $CD33^+$ cells was similar to the $CD11c^+$ cell count. In the present approach using BDCA antigens for DC assessment, the mDC1 are CD1c /$BDCA\text{-}1^+$, as well as Lin^-, $HLA\text{-}DR^+$, $CD11c^{bright}$, $CD123^{dim}$, $CD4^+$, $CD45RO^+$, $CD2^+$, and these cells express myeloid lineage markers (CD13, CD33) as well as Fc receptors (CD32, CD64, Fc RI). The second myeloid population, mDC2, is BDCA-3-positive and expresses markers similar to the mDC1, but they are simultaneously $CD11c^{low}$, $CD123^{dim}$ and $CD2^-$. Unlike CD1c/$BDCA\text{-}1^+$ DCs, they lack expression of Fc receptors like CD32, CD64 or Fc RI.[12,35] In this study, based on that knowledge and several preliminary measurements, we established three sets of parameters that can optimally characterize the main subsets of DCs: $BDCA\text{-}1^+/CD11c^+/HLA\text{-}DR^+$ (for mDC1), $BDCA\text{-}3^+/CD32^-/HLA\text{-}DR^+$ (for mDC2), and $BDCA\text{-}2^+/CD123^+/HLA\text{-}DR^+$ (for pDC).

Our data suggest that fluctuations in blood pDC count in SLE patients are much more significant then in mDC, especially in the main myeloid DC subpopulation, mDC1. We have also found that the pDC count was lower in patients with active SLE compared with non-active disease, whereas numbers of mDC1 and mDC2 were similar in both groups. Moreover, only tDC and pDC, but not mDC subsets, correlated with B-cell count. DCs are considered a crucial cells in regulation of immune response.[36,37] Plasmacytoid DCs are the main peripheral blood cells producing IFN-α.[36,37] They also play a major role in regulation of Th1 and Th2 cell response and participate in T-cell activation and tolerance.[38,39] In this aspect, since an enhanced concentration of IFN-α has an important role in the development of SLE, the decreased number responsible for its production of circulating DCs in patients with active phase of the disease may be difficult to explain. In SLE patients, however, DCs infiltrate skin involved in inflammatory process, and probably also other tissues, and those cells can be the main source of IFN-α.[17,29,40]

In conclusion, this is the first assessment of the absolute number of tDC, pDC and myeloid mDC1 and mDC1 subpopulations in SLE patients, with special emphasis on the correlation with particular clinical and laboratory feature. Our data suggest that fluctuations of DC count in SLE patients' blood are much more significant in pDC than in mDC (especially in the main myeloid DC1 subset). The reduced number of DCs in SLE patients can be caused by their accelerated migration to the sites of inflammation including skin lesions. Positive correlation between DC number and $TCD4^+$, $TCD8^+$ and $CD19^+$ B cells testifies to their interactions and influence on SLE pathogenesis. The association between DC number and clinical features seems to be less clear.

ACKNOWLEDGEMENTS. The authors would like to thank Ms Barbara Cebula for excellent technical assistance, Elzbieta Dziankowska-Stachowiak for performing statistical analysis of the data, and Ms Krystyna Marszalek for secretariat assistance. This work has been supported in part by Grant No. 502-11-783(26) and Grant No. 515-02-03 awarded by the Medical University of Łódź, Poland

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Received 16 February 2004
Accepted 13 April 2004