

Relationship between peripheral blood dendritic cells and cytokines involved in the pathogenesis of systemic lupus erythematosus

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ABSTRACT. Recent studies indicate that dendritic cells (DC) and several cytokines are implicated in the induction of autoimmune diseases. In this study we investigated the relationship between the total number of DC (tDC), and their plasmacytoid (pDC) and myeloid (mDC) subpopulations, with serum concentrations of interferons (IFN- α and IFN- γ) and selected cytokines (TNF- α , IL-4, IL-6), in patients with systemic lupus erythematosus (SLE) and healthy persons. Subpopulations of DC were determined by the following antigen expression profiles: BDCA-1+/CD11c+/HLA-DR+ (for mDC) and BDCA-2 +/CD123+/HLA-DR+ (for pDC), using flow cytometry. Serum levels of interferons and cytokines were assessed by an enzyme – linked immunosorbent assay (ELISA). The study was performed in 36 SLE patients and 19 healthy volunteers. The mean number of tDC was lower in SLE patients ($13.9 \pm 6.4/\mu\text{L}$) than in healthy persons ($24.1 \pm 12.6/\mu\text{L}$) ($P < 0.001$). The number of pDC was also significantly lower in SLE ($6.6 \pm 3.6/\mu\text{L}$) than in the control group ($12.0 \pm 8.3/\mu\text{L}$) ($P < 0.02$). Moreover, the mean pDC count was lower in active than in inactive disease ($5.5 \pm 3.6/\mu\text{L}$ vs $7.6 \pm 3.4/\mu\text{L}$; $P < 0.04$). The mean serum levels of IFN- α and IFN- γ were significantly higher in SLE patients (63.8 pg/mL and 6.6 pg/mL, respectively) than in the control group (2.7 pg/mL and 0.5 pg/mL, respectively) ($P < 0.008$ and $P < 0.001$, respectively). Serum levels of TNF- α and IL-6 were also higher in SLE patients (mean 7.3 pg/mL and 18.4 pg/mL, respectively) than in healthy controls (4.2 pg/mL and 0.5 pg/mL, respectively) ($P < 0.02$ and $P < 0.001$, respectively). The mean serum IL-4 concentrations were similar in SLE and healthy persons (0.2 pg/mL and 0.31 pg/mL, respectively; $P = 0.119$). A negative correlation was found between pDC number and the serum level of IFN- α ($\rho = -0.386$, $P = 0.02$) and between mDC and IFN- γ ($\rho = -0.377$, $P = 0.024$). In conclusion, the correlation between peripheral blood DC subsets and serum levels of IFN- α and IFN- γ suggests a possible relationship between these cytokines in the pathogenesis of SLE.

Keywords: SLE, dendritic cells, interferon- α , interferon- γ , TNF, IL-4, IL-6

INTRODUCTION

Systemic lupus erythematosus (SLE) is a prototypical autoimmune disease of unknown origin, characterized by different clinical manifestations [1, 2]. SLE can present with a wide range of immunological abnormalities such as the production of a variety of autoantibodies, the presence of circulating immune complexes and a reduction in complement levels [3, 4]. The disease is characterized by disturbances in the immune system that involve B cells, T cells, and cells of the monocytic lineage, resulting in polyclonal B cell activation and increased numbers of antibody-producing cells [1]. Recently, a more significant role of dendritic cells (DC) in the control of tolerance and immunity has been proved, and the hypothesis that SLE

may be driven through unabated DC activation is being considered [5, 6].

Dendritic cells are a group of bone marrow-derived, lineage-negative (Lin-), HLA-DR-positive cells, that are specialized for the uptake, transport, processing and presentation of antigens to T cells [7]. Basically, two main types of DC have been identified, the CD11c-subset called plasmacytoid DC (pDC) and the CD11c⁺ subset, called myeloid DC (mDC) [8, 9].

Dendritic cells have been implicated in the induction of autoimmune diseases, and have been identified in lesions associated with several autoimmune inflammatory diseases [10]. In particular, serum from SLE patients can induce DC from monocytes, which are able to capture

apoptotic cells and present their antigen to autologous T cells [11]. This observation may indicate that SLE blood creates an environment that induces DC differentiation. Recently, considerable alterations to particular subsets have been found in SLE patients, including significant reduction of DC, especially pDC in the patients treated with steroids [12, 13]. Plasmacytoid DC secrete large amounts of interferon- α (IFN- α). It has also been shown that the DC-inducing property of SLE serum is mediated by this protein [5]. On the other hand, these cells are the major source of IFN- α in the blood [12, 14]. Moreover, pDC stimulated with IL-3 and CD40-L, prime T cells to secrete Th2 cytokines, mainly IL-4 [6, 15]. However, when these cells are stimulated with viruses, they induce T cells to produce IFN- γ and IL-10.

In the present study, we assessed the total number of peripheral blood DC and their myeloid and plasmacytoid subpopulations, in patients with active and inactive SLE. We also measured the levels of interferons (IFN- α and IFN- γ) as well as selected cytokines (TFN- α , IL-4, IL-6), and correlated them with the DC number.

PATIENTS AND METHODS

The study group consisted of 36 patients with SLE (33 females and 3 males), and sex and age – matched healthy volunteers comprised the control group. The diagnosis of SLE was based on the revised criteria of the American College of Rheumatology [16]. The mean duration of the disease was 52 months (range 2 months to 21 years). Ten patients had never been treated with immunosuppressive agents. Twenty six were treated with prednisone at a dose of 5-20 mg/day during the study, including three patients treated with prednisone and azathioprine at a daily dose 50-100 mg.

Disease activity was evaluated during visits to outpatients clinic according to the SLAM method described by Liang *et al.* [17]. The median score in our group of patients was 15 (range 4-25). We assumed the score 0-15 points for inactive disease, and a score over 15 points for active disease [18, 19]. In the group of patients, 17 had active and 19 inactive disease. SLEDAI-2K scores were also calculated [20]. The median score according this activity criterion was 17.5 points (range – 5-30). The clinical and laboratory characteristics of the patients are presented in *Table 1*. The patients with SLE and the healthy volunteers showed no clinical signs of infection or neoplastic disease and had received no other medication for at least four weeks prior blood donation. Informed consent was obtained from all patients and the normal individuals participating in the study, which was approved by the local ethics committee.

Defining peripheral blood DC subtypes and calculating the absolute DC counts

Dendritic cells were detected in the peripheral blood mononuclear cell (PBMC) population. Whole heparinised blood samples were mixed with Hank's reagent (1:1), and then centrifuged in a Ficoll-Paque gradient (2 400 g for 20 min), then double washed and re-suspended in PBS buffer (Sigma, Aldrich). The immunophenotype of DC was determined using the panel of monoclonal antibodies (MoAbs) directed against DC-defined antigens. There

Table 1
Clinical and laboratory characteristics of patients with systemic lupus erythematosus

Symptoms	Number of patients	%
Total	36	100.0
Active/inactive	17/19	47.2/52.8
Treated/untreated	26/10	72.2/27.8
Arthritis	34	94.4
Skin symptoms	28	77.8
Renal disorder (creatinine > 1.3 mg/dL)	2	5.6
Neurological symptoms	24	66.7
Anti-ds DNA antibodies	5	13.9
Anemia (Hb < 12 g/dL)	18	50.0
Leukopenia (WBC < 3.5 × 10 ⁹ /L)	7	19.4
Thrombocytopenia (platelets < 150 × 10 ⁹ /L)	12	33.3

were: FITC-conjugated BDCA-1 and BDCA-2 (Miltenyi, Biotec, Bergish Gladbach, Germany), PE – conjugated anti-CD11c (Caltag Laboratories, Burlingame, CA, USA) and anti-CD123 or anti-CD19 (Miltenyi, Biotec) and TC-conjugated HLA-DR (Caltag Laboratories). As respective isotype controls, the following mouse IgG1 MoAbs: MG 101, MG104 and MG 106 (all Caltag Laboratories) were used. Samples were prepared at the concentration of 300 000 cells/100 μ L of PBS in a final volume of 250 μ L per sample. Antibodies were added at the concentration of 1 μ g/100 μ L and incubated for 10 min, at 4 °C at the dark. Then, the immunophenotype was assessed by triple-colour cytometry. Cell fluorescence was measured using a flow cytometer (FACScan, Becton-Dickinson, San Jose, CA, USA) and analysed with green (FL1), orange (FL2) or red (FL3) standard emission filters. From each sample 100 000 events were acquired. Based on SC *versus* FS distribution

DC were gated from the whole PBMC population. Based on preliminary studies, the nature of the DC was determined: myeloid subtype (BDCA-1+/CD11c+/HLA-DR+) and plasmacytoid subtype characterized by BDCA-2+/CD123+/HLA-DR+ expression profile. The percentage of mDC detected using MoAb against the CD19 antigen for lineage-negative selection (BDCA-1+/CD19-) was comparable to that assessed by the BDCA-1+/CD11c+/HLA-DR+ immunophenotype. For the absolute DC count assessment, in every patient and healthy volunteer, a number of PBMC per μ L was routinely measured. Then, based on the percentage of the particular DC subset in the whole PMBC population as determined by flow cytometry, the DC count was calculated and presented as the number of cells per μ L.

Assessment of serum interferons and cytokine concentration

The concentrations of interferons and cytokines were assessed in sera obtained from venous blood samples collected for DC detection. The procedure has been previously described in detail [18, 19]. Briefly, the sera obtained were assayed for IFN- α , IFN- γ , TNF- α , IL-4 and IL-6 concentration. The commercially available enzyme linked assay (ELISA) kits were obtained from Quantikine, R&D Systems Inc (Minneapolis, MN, USA), except IFN- α

Table 2
Analysis of peripheral blood dendritic cells (tDC) and their plasmacytoid (pDC) and myeloid (mDC) subpopulations in patients with SLE and in the healthy control group (cells/ μ L)

Cell subpopulation	All SLE <i>n</i> = 36 (a)	Active SLE <i>n</i> = 17 (b)	Inactive SLE <i>n</i> = 19 (c)	Control group <i>n</i> = 19 (d)	Statistical analysis
tDC cells/ μ L					(a)-(d) <i>P</i> < 0.001
$\bar{x} \pm s$	13.9 \pm 6.4	13.0 \pm 6.3	14.8 \pm 6.5	24.1 \pm 12.6	(b)-(c) <i>P</i> = 0.456
Me	13.4	12.4	14.8	25.3	(b)-(d) <i>P</i> < 0.003
Range	(2.5-27.7)	(2.5-24.8)	(5.3-27.7)	(2.8-48.3)	(c)-(d) <i>P</i> < 0.008
pDC cells/ μ L					(a)-(d) <i>P</i> < 0.02
$\bar{x} \pm s$	6.6 \pm 3.6	5.5 \pm 3.6	7.6 \pm 3.4	12.0 \pm 8.3	(b)-(c) <i>P</i> < 0.04
Me	5.8	4.8	6.9	15.2	(b)-(d) <i>P</i> < 0.02
Range	(1.5-14.5)	(1.5-14.5)	(2.1-13.6)	(0.0-29.6)	(c)-(d) <i>P</i> < 0.05
mDC cells/ μ L					(a)-(d) <i>P</i> = 0.093
$\bar{x} \pm s$	6.2 \pm 4.0	6.2 \pm 4.1	6.3 \pm 4.0	9.4 \pm 6.8	(b)-(c) <i>P</i> = 0.837
Me	5.2	5.02	5.3	8.7	(b)-(d) <i>P</i> = 0.178
Range	(0.9-19.3)	(0.9-14.8)	(2.3-19.3)	(0.0-21.5)	(c)-(d) <i>P</i> = 0.125

which was a product of PBL Biomedical Laboratories, Piscataway, NJ, USA. Standards and samples were assayed as duplicates, and interassay variations were within the range given by the manufacturer. The sensitivity limit for IFN- α was 10 pg/mL, IFN- γ ; 8 pg/mL, TFN- α ; 0.06 pg/mL, IL-4; 0.13 pg/mL and IL-6; 0.7 pg/mL.

Statistics

For the statistical analysis of the data, the range of measured variables (min-max), the mean arithmetic value (\bar{x}), median (Me) and standard deviation (SD) were calculated. The Shapiro-Wilk's test was used to evaluate the distributions. The medians were compared using the Mann-Whitney test and Kruskal-Wallis test. The correlation between features was evaluated using the Spearman rank coefficient ρ . Comparison and correlations were considered significant when *P* < 0.05.

RESULTS

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

The characteristics of the patients are presented in Table 1. The mean, SD, median and range of tDC, pDC and mDC

absolute values are presented in Table 2. The mean number of tDC was lower in SLE patients (13.9 \pm 6.4/ μ L) than in the control group (24.1 \pm 12.6/ μ L) (*P* = 0.456). According to the particular DC subpopulation, the mean pDC count was significantly lower in SLE patients (6.6 \pm 3.6/ μ L) than in healthy persons (12.0 \pm 8.3/ μ L) (*P* < 0.02). The mean number of mDC in SLE (6.2 \pm 4.0/ μ L) was also lower than in the control group (9.4 \pm 6.8/ μ L), but these differences were not statistically significant (*P* = 0.837).

The numbers of tDC and mDC in active and inactive SLE were comparable (Table 2) (*P* > 0.05). The mean number of pDC was significantly lower in active SLE (5.5 \pm 3.6/ μ L) when compared with patients with inactive disease (7.6 \pm 3.4/ μ L, *P* < 0.04). The comparisons of tDC, pDC and mDC in treated and untreated patients are shown in Table 3. The mean numbers of tDC and pDC were significantly lower in treated patients (*P* < 0.004 and *P* < 0.02, respectively).

Serum levels of IFN- α , IFN- γ , TNF α , IL-4 and IL-6

TNF- α and IL-6 were detectable in all SLE patients and in 17 (89.5%) and 19 (100%) healthy donors, respectively. IFN- α and IFN- γ were detectable in 30 (83.3%) and 32 (88.9%) SLE patients, and in four (21.1%) and five

Table 3
Comparison of peripheral blood dendritic cells (tDC) and their plasmacytoid (pDC) and myeloid (mDC) subpopulations in patients with untreated SLE and SLE treated with corticosteroids and/or immunosuppressants (cells/ μ L).

Cell subpopulation	Untreated <i>n</i> = 10	Treated <i>n</i> = 26	Statistical analysis
tDC cells/ μ L			
$\bar{x} \pm s$	15.1 \pm 5.7	11.5 \pm 6.6	<i>P</i> < 0.004
Me	14.7	11.1	
Range	(7.8-26.4)	(2.5-27.7)	
pDC cells/ μ L			
$\bar{x} \pm s$	8.2 \pm 3.1	6.1 \pm 3.6	<i>P</i> < 0.02
Me	7.2	5.1	
Range	(4.7-13.6)	(1.5-14.5)	
mDC cells/ μ L			
$\bar{x} \pm s$	6.0 \pm 3.0	6.3 \pm 4.4	<i>P</i> = 0.118
Me	5.1	5.4	
Range	(2.3-11.4)	(0.9-19.3)	

Table 4
Serum level of interferons and cytokines in patients with SLE and in the normal control group
(mean values in pg/mL \pm SD and range in parentheses)

Cytokines	All SLE <i>n</i> = 36 (a)	Active SLE <i>n</i> = 17 (b)	Inactive SLE <i>n</i> = 19 (c)	Control group <i>n</i> = 19 (d)	Statistical analysis
IFN- α					(a)-(d) $P < 0.008$
$\bar{x} \pm s$	63.8 \pm 158.5	80.6 \pm 184.9	48.8 \pm 134.0	2.7 \pm 7.0	(b)-(c) $P = 0.115$
Me	4.6	13.0	0.0	0.0	(b)-(d) $P < 0.003$
Range	(0.0-767.3)	(0.0-767.3)	(0.0-501.6)	(0.0-22.5)	(c)-(d) $P = 0.075$
IFN- γ					(a)-(d) $P < 0.001$
$\bar{x} \pm s$	6.6 \pm 11.4	6.5 \pm 9.0	6.7 \pm 13.4	0.5 \pm 1.2	(b)-(c) $P = 0.453$
Me	1.7	4.3	1.6	0.0	(b)-(d) $P < 0.001$
Range	(0.0-45.1)	(0.0-37.5)	(0.0-45.1)	(0.0-4.9)	(c)-(d) $P < 0.001$
TNF- α					(a)-(d) $P < 0.02$
$\bar{x} \pm s$	7.3 \pm 4.2	7.8 \pm 3.7	6.6 \pm 4.6	4.2 \pm 2.5	(b)-(c) $P = 0.168$
Me	5.9	6.6	4.8	4.7	(b)-(d) $P < 0.004$
Range	(1.7-18.0)	(2.3-15.0)	(1.7-18.0)	(0.0-9.2)	(c)-(d) $P = 0.161$
IL-4					(a)-(d) $P = 0.119$
$\bar{x} \pm s$	0.2 \pm 0.3	0.19 \pm 0.11	0.22 \pm 0.51	0.31 \pm 0.18	(b)-(c) $P = 0.063$
Me	0.1	0.09	0.1	0.28	(b)-(d) $P < 0.01$
Range	(0.0-2.1)	(0.0-0.52)	(0.0-2.13)	(0.0-0.53)	(c)-(d) $P = 0.811$
IL-6					(a)-(d) $P < 0.001$
$\bar{x} \pm s$	18.4 \pm 23.6	20.8 \pm 23.9	16.2 \pm 23.7	0.5 \pm 0.6	(b)-(c) $P = 0.428$
Me	7.8	8.2	4.8	0.3	(b)-(d) $P < 0.001$
Range	(0.3-83.0)	(0.3-83.0)	(0.5-78.7)	(0.0-2.7)	(c)-(d) $P < 0.001$

(26.5%) healthy persons, respectively. IL-4 was measurable in 34 out of 36 (94.4%) SLE patients and 19 (100%) healthy donors. The serum levels of IFN- α , IFN- γ , TNF α , IL-4 and IL-6 in SLE patients and in healthy donors are shown in Table 4.

The serum levels of both IFN- α and IFN- γ were found to be significantly higher in the SLE group (mean 63.8 pg/mL and 6.6 pg/mL, respectively) when compared to the control group (mean 2.7 pg/mL and 0.5 pg/mL, respectively) ($P < 0.008$ and $P < 0.001$, respectively). TNF- α and IL-6 serum levels were also higher in SLE patients (mean 7.3 pg/mL and 18.4 pg/mL, respectively) than in normal persons (mean 4.2 pg/mL and 0.5 pg/mL, respectively) ($P < 0.02$ and $P < 0.001$, respectively). The concentration of IL-4 was similar in SLE patients as a whole group and in the control (mean 0.2 pg/mL and 0.3 pg/mL respectively, $P = 0.119$).

Correlation between tDC, pDC and mDC counts and serum levels of IFN- α , IFN- γ , TNF- α , IL-4 and IL-6

We have found a negative, statistically significant correlation between the number of pDC and the serum level of IFN- α in SLE patients ($\rho = -0.386$, $P = 0.020$) but not in the control group ($\rho = 0.071$, $P = 0.772$) (Figure 1). In contrast, the serum level of IFN- γ correlated negatively with tDC ($\rho = -0.356$, $P = 0.033$) and mDC ($\rho = -0.377$, $P = 0.024$) (Figure 2). However, such correlations have not been found in healthy persons.

Moreover, we have found a significant, negative correlation between IL-4 serum concentration and the number of tDC, mDC and pDC in healthy persons ($\rho = -0.599$, $P = 0.007$; $\rho = -0.505$, $P = 0.028$; and $\rho = -0.609$, $P = 0.006$, respectively), but not in SLE patients (Figure 3).

There was no significant correlation between the tDC count and serum levels of TNF- α and IL-6 in either SLE patients ($\rho = -0.136$, $P = 0.428$ and $\rho = -0.058$, $P = 0.739$, respectively) or in the healthy control ($\rho = -0.077$, $P = 0.753$ and $\rho = 0.121$, $P = 0.622$, respectively). No correlations were observed between the numbers of pDC and mDC subpopulations, and serum TNF- α and IL-6 concentrations in either examined groups (not shown).

The correlation between mDC, pDC and serum levels of interferons and cytokines was also analysed in patients with active and inactive SLE (Table 5). A negative correlation between the number of mDC and serum IFN- γ level was found in those patients with active SLE ($\rho = -0.057$, $P = 0.02$).

DISCUSSION

In this study, the relationship between the number of peripheral blood DC, and their particular subtypes, and serum concentrations of interferons and cytokines, known as important factors for SLE pathogenesis, was assessed. We proved that the number of both tDC and pDC in SLE patients is significantly lower than in healthy persons. We did not find however, any significant differences in mDC1 or mDC2 numbers between both groups. These results indicate that the decrease in the pDC subtype count is responsible for a low total number of DC in SLE, which has also been observed by other authors [21, 22]. However, some of them also found a decrease of mDC in patients with SLE [22, 23]. This phenomenon is probably due to DC accumulation in tissues. The increased number of DC, particularly the pDC subpopulation, was found in cutaneous lupus erythematosus lesions, but not in non-involved skin [24, 25]. The decrease in peripheral blood DC count

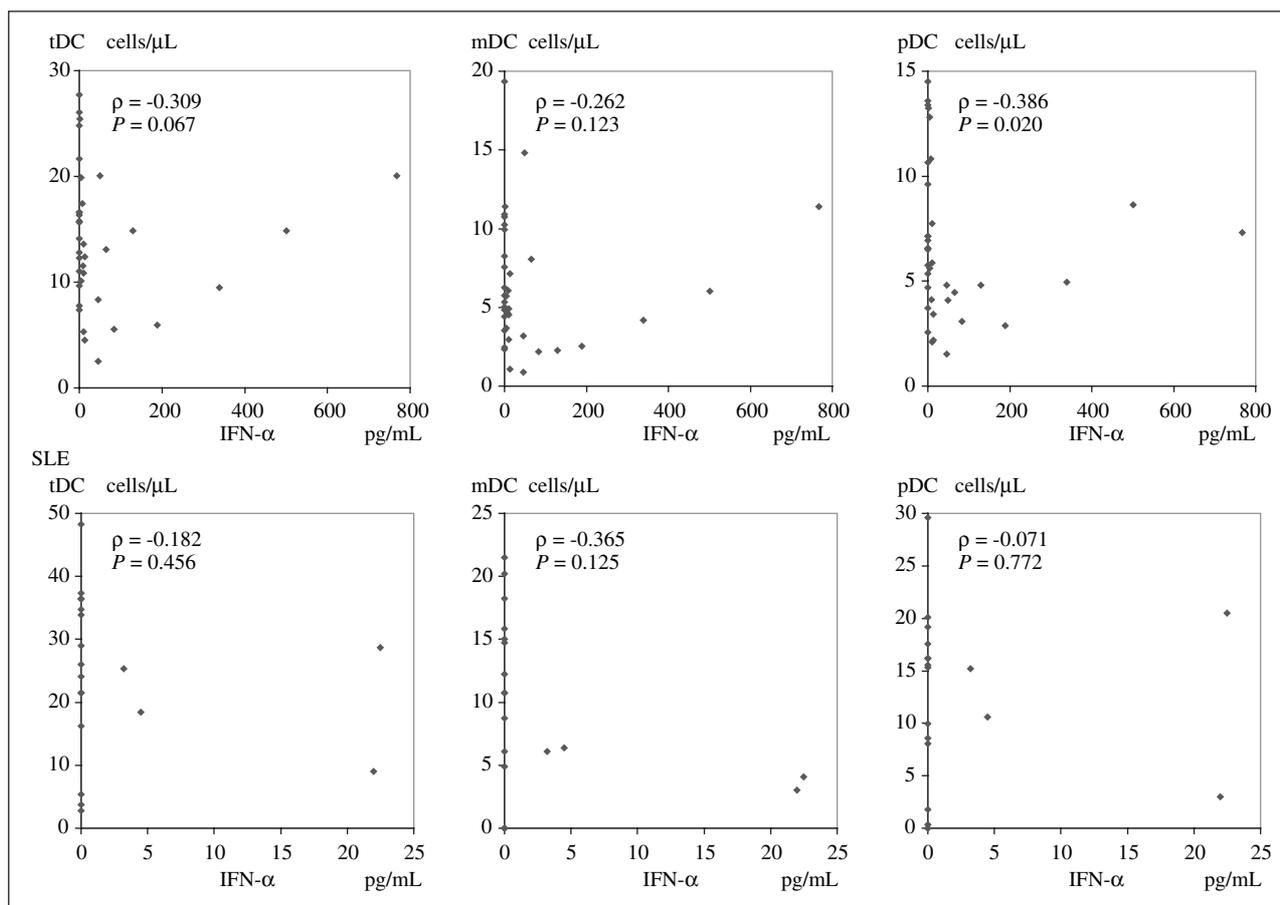


Figure 1

Correlations between the numbers of peripheral blood dendritic cells (DC) and their plasmacytoid (pDC) or myeloid (mDC) subpopulations, and the levels of IFN- α in SLE patients and in the control group.

may also depend on enhanced apoptosis of these cells. The increased apoptosis of SLE lymphocytes in comparison to normal lymphocytes has been observed *in vitro* [26, 27]. The positive correlation found between the absolute peripheral blood lymphocyte count and the number of circulating DC provides additionally support for this hypothesis [23].

We have shown significantly lower tDC and pDC in the SLE patients treated with steroids than in untreated patients. In contrast, mDC numbers were similar in both groups. These results are in line with the observations of Shodell and colleagues who have found that human DC are highly sensitive to corticosteroids [13]. Chronic or acute therapeutic administration of corticosteroids decreases peripheral blood pDC number and function [28]. However, both number and function substantially recovered within three days following cessation of the treatment.

Our study showed that serum concentrations of IFN- α and IFN- γ in SLE patients are higher than in healthy persons. This fact is in agreement with previous reports [5], however, other data presented were ambiguous [29, 30]. The role of IFN- α in the pathogenesis of SLE has been stressed by several authors [5, 21]. IFN- α induces the production of both antinuclear and anti-DNA antibodies [31]. Moreover, the possibility of SLE development in patients who received IFN- α for other reasons has been previously documented [32, 33]. IFN- α is produced by different cell types,

but the main source of this protein are pDC, most probably in SLE patients too [5]. In this respect, the strong inverse correlation found between serum IFN- α concentrations and the pDC count requires additional explanation. However, it seems possible that the main source of IFN- α in SLE patients are pDC, which are localized in the skin or other tissues [24, 25, 34]. In contrast to SLE patients, we did not find any significant correlation between IFN- α levels and pDC number in healthy controls, which may serve as an additional argument for such an interpretation (Figure 1).

In recent years, pDC have been identified as the natural IFN- α -producing cells that respond to diverse microbial agents as well as to DNA or RNA/autoantibody complexes found in the serum of SLE patients [35, 36]. However, in SLE patients, CD123⁺ pDC produce as much IFN- α as healthy donor pDC in response to viral triggering [35]. On the other hand, in contrast to healthy donor, blood from SLE patients also contains CD123⁻ negative cells that produce IFN- α [35]. Moreover, IFN- α present in the serum of SLE patients is able to drive the differentiation of monocytes to DC [37, 38]. Monocytes can also differentiate into DC *in vitro* when they are stimulated with IL-4 or GM-CSF [38]. It is possible, that in autoimmune disorders, these cytokines also stimulate DC production from monocytes *in vivo*.

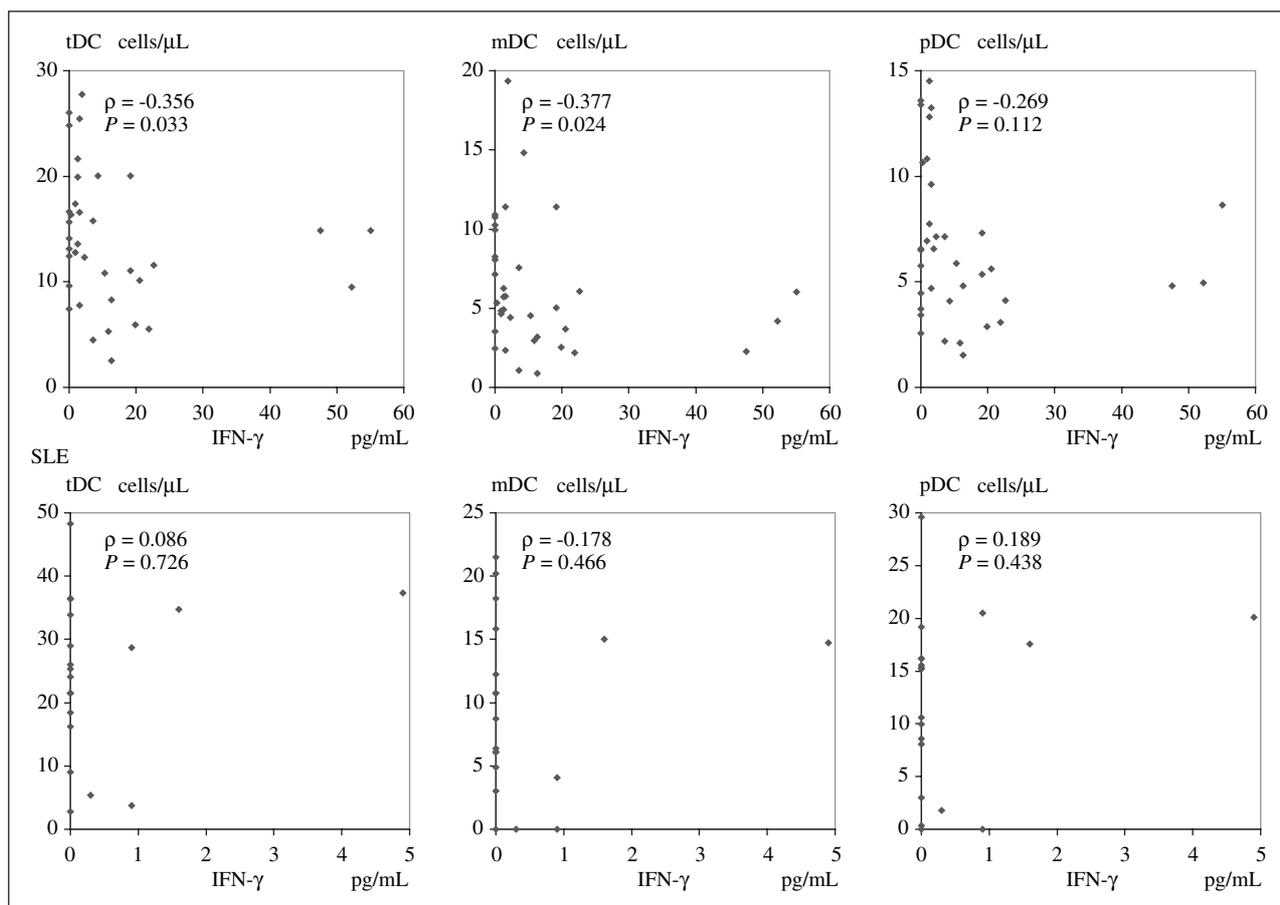


Figure 2

Correlations between the numbers of peripheral blood dendritic cells (DC) and their plasmacytoid (pDC) or myeloid (mDC) subpopulations, and the levels of IFN- γ in SLE patients and in the control group.

The proposed etiopathogenic role of IFN- α in SLE suggests the possibility of the inhibition of its production or action for therapeutic purposes [36]. The action of IFN- α can be inhibited by the use of neutralizing anti-IFN- α antibody, soluble IFN- α receptor or antibody blocking IFN- α receptor. An alternative method is the degradation of the endogenous IFN- α inducers by nucleases or inhibition of IFN- α gene expression. Another method may be ligation of the BDCA-2 receptor with monoclonal antibody, which abolishes the ability of pDC to produce IFN- α [39].

The role of IFN- γ in SLE pathogenesis also seems to be important, however, its potential relationship with DC is less clear than in the case of IFN- α . In this study we found a negative correlation between serum concentrations of IFN- γ and tDC and mDC numbers in SLE patients, but not in healthy control (*Figure 2*). However, this correlation was statistically significant only in patients with active disease ($\rho = -0.557$, $P = 0.02$). It is worth noting that viral triggering leading to IFN- α production also induces generation of T-cells secreting IFN- γ [40]. Similarly to IFN- α , also the treatment with IFN- γ can also induce SLE, supporting its role in the development of this disease [41,

42]. In contrast to IFN- α , IFN- γ is produced mainly by T and NK cells, but not by DC cells [35, 36].

The concentrations of the remaining cytokines examined, i.e. TNF- α , IL-4 and IL-6 did not correlate with the number of DC in SLE patients. However, it should be stressed, that an inverse correlation between IL-4, tDC, pDC and mDC counts was found in the healthy controls. This finding indicates the existence of abnormalities in the balance between IL-4 and DC in this autoimmune disease. In our study, the mean serum concentration of IL-4 in SLE and in the control group did not differ significantly. In SLE patients with the active disease however, serum IL-4 levels were lower than in healthy volunteers. These observations are consistent with results of other authors who showed that the production of IL-4 by peripheral blood mononuclear cells from SLE patients is similar or even lower when compared with healthy persons [44, 45, 46].

In conclusion, among several immunological abnormalities detectable in patients with SLE, we found a negative correlation between the number of peripheral blood DC and serum IFN- α and IFN- γ concentration. This finding may indicate a role for these immune system components in the pathogenesis of SLE.

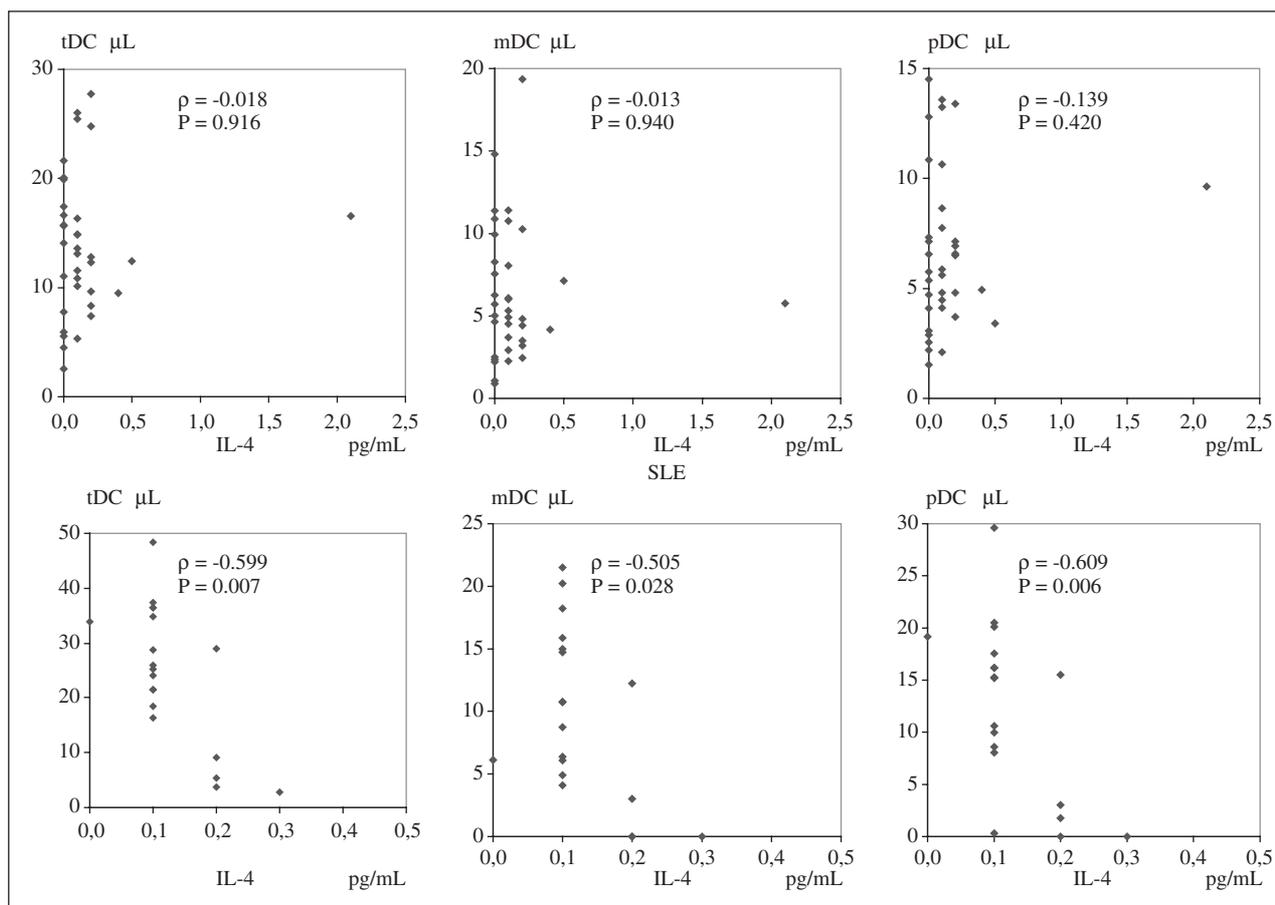


Figure 3

Correlations between the numbers of peripheral blood dendritic cells (DC) and their plasmacytoid (pDC) or myeloid (mDC) subpopulations, and the serum levels of IL-4 in SLE patients and in the control group.

Table 5
Correlations between the numbers of peripheral blood myeloid (mDC) and plasmacytoid (pDC) subpopulations with interferons (IFN) and cytokines in active and inactive SLE

Cytokines	SLE	mDC		pDC	
		ρ	<i>P</i> value	ρ	<i>P</i> value
IFN- α	Active	-0.223	0.388	-0.368	0.146
	Inactive	-0.183	0.453	-0.118	0.631
IFN- γ	Active	-0.557	0.020	-0.138	0.598
	Inactive	-0.031	0.900	-0.268	0.268
TNF- α	Active	-0.104	0.692	0.168	0.520
	Inactive	-0.033	0.892	-0.312	0.193
IL-4	Active	0.030	0.9077	0.333	0.191
	Inactive	-0.161	0.509	-0.246	0.311
IL-6	Active	0.183	0.481	0.077	0.767
	Inactive	-0.167	0.495	-0.053	0.830

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