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Absolute Count of T and B Lymphocyte Subsets is Decreased in Systemic Sclerosis

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Abstract
Background: Previous reports on lymphocyte subpopulations in systemic sclerosis (SSc) are conflicting. Therefore, we aimed to investigate the lymphocyte subsets in SSc patients who were not on immunosuppressive therapy.
Methods: Lymphocyte subsets were assessed in the peripheral blood of SSc patients (n = 29) and healthy controls (n = 29) using the four colour flow cytometry method. Correlation studies were also performed in order to assess the relationship between lymphocyte subsets and clinical parameters.
Results: The absolute count of lymphocytes (P = 0.0042), CD3+ (P = 0.0014), CD4+ (P = 0.0070), CD8+ (P = 0.021), and CD19+ cells (P = 0.024) was significantly decreased in SSc patients when compared to healthy controls. CD4+/CD8+ ratio and the absolute count of CD56+ cells observed in SSc patients did not significantly differ from controls (P=0.165; P = 0.632, respectively). There was no substantial relationship between the lymphocyte subset levels and clinical features (i.e., SSc subtype, autoantibody profiles, organ involvement), except for a significant inverse correlation of CD19+ cells and the modified Rodnan skin score (r = -0.43, P = 0.020).
Conclusion: Our data support previous reports indicating that subsets of T lymphocytes as well as B lymphocytes play a role in the pathogenesis of SSc.

Introduction

Systemic sclerosis (SSc) is a connective tissue disease characterized by excessive extracellular matrix deposition in the skin and other visceral organs. The most widely accepted classification system is that proposed by LeRoy et al. [1], which includes limited cutaneous SSc and diffuse cutaneous SSc, a more rapidly advancing type with more frequent visceral involvement. It was suggested that immune system changes play the major part in the development of vasculopathy and fibrosis. The presence of autoantibodies, such as DNA topoisomerase I and centromere, is a central feature of SSc. Moreover, a wide range of B and T cell abnormalities has been described in SSc. Nevertheless, previous reports on lymphocyte subpopulations in SSc are conflicting [2-13]. We here conducted a prospective study investigating lymphocyte subsets in immunosuppressant-untreated SSc patients and healthy controls.

Material and Methods

This was a prospective study on patients with SSc who were evaluated in the Department of Dermatology of the Ruhr-University Bochum (Germany) from February 2007 to July 2008. SSc was diagnosed according to the classification system proposed by LeRoy et al. [1]. We only included patients who had no history of photopheresis and immunosuppressive treatment (e.g., glucocorticosteroids, methotrexate, cyclophosphamide) for at least 6 months. The patients included in the study, however, had physiotherapy and/or rheological treatment. We saw to it that the control group, which included healthy individuals, did match the gender and age of the patient group. The study was conducted in the light of the declaration of Helsinki and followed a protocol approved by our institutional review board. All the patients and healthy controls were fully informed and gave their consent to participate. A complete work-up was performed in SSc patients including bodyplethysmography, thoracic high-resolution computed tomography, oesophagogastroscopy, abdominal and cardiac ultrasound, and blood collections. Skin involvement was quantified using the modified Rodnan skin score (MRSS) and was assessed clinically by palpation of the skin, as described previously [14].

In order to identify and determine the percentages and absolute counts of lymphocyte subsets in the peripheral blood, flow cytometric methods were used. For this purpose the following antibodies (BD Biosciences, San Jose, CA, USA), directed against mature human lymphocyte subsets were employed: leucocytes (CD45+), T-lymphocytes (CD3+), B-lymphocytes (CD19+), helper/inducer T lymphocytes (CD3+CD4+), suppressor/cytotoxic T lymphocytes (CD3+CD8+) and natural killer lymphocytes (CD3-CD16+ and/or CD56+). The antibodies were conjugated to the following fluorescent dyes: Fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyllprotein (PerCP) and allophycocyanine (APC). Additionally, Trucount tubes™ (BD Biosciences, San Jose, CA, USA) were used to determine absolute counts of lymphocytes. The FACSCalibur™ cytometer (BD Biosciences, San Jose, CA, USA), equipped with 635 nm and 488 nm lasers, together with computer hardware and Multitest-software™ were used to acquire and analyse the lymphocyte subsets.

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Analysis of data was performed using the statistical package MedCalc Software (Mariakerke, Belgium). Normal distribution of data was confirmed by the D'Agostino-Pearson test. Data are expressed as mean ±SD and median and range. Data were analysed using the F-test for equal variances, independent t-test, Chi-square test, and Spearman correlation procedure. We constrained experiment-wise error rates due to multiple comparisons to the standard alpha (P) level of <0.05 by the Bonferroni method.

Results

All in all, we included 29 patients with SSc. Twenty-two patients had limited SSc, seven patients had diffuse SSc. Further clinical data of the patients is detailed in Table 1. There was no significant difference between SSc patients and healthy controls (n = 29) with regard to gender (male/female ratio: 1/28 vs. 4/25; P = 0.29, respectively) and age (mean ± SD: 56.3 ± 12.9 vs. 51.1 ± 13.4; P = 0.13, respectively). As also detailed in Table 2., the absolute count of lymphocytes (P = 0.0042), CD3+ (P = 0.0014), CD4+ (P = 0.0070), CD8+ (P = 0.021), and CD19+ cells (P = 0.024) was significantly decreased in SSc patients when compared to healthy controls. By contrast, CD4+/CD8+ ratio and the absolute count of CD56+ cells observed in SSc patients did not significantly differ from healthy controls (P = 0.165; P = 0.632, respectively). The percentages of lymphocyte subsets investigated did not significantly differ between SSc patients and healthy controls. There was no substantial relationship between the lymphocyte subset levels and clinical features (i.e., SSc subtype, autoantibody profile, organ involvement), except for a significant inverse correlation of CD19+ cells and the MRSS (r = -0.43, P = 0.020).

Discussion

In the present study, we found that SSc patients, who were not on immunosuppressive treatment, had significantly reduced numbers of lymphocytes and its subsets including CD3+, CD4+, CD8+, and CD19 cells. However, the percentages of lymphocyte subsets investigated were very similar to those observed in healthy controls. In accordance with our results, Ercole et al. [7] recently found that SSc patients had similar percentages of CD3+, CD4+, and CD8+ cells and CD4+/CD8+ cell ratio when compared to matched normal controls. Ingegnoli et al. [8] found that absolute counts of CD4+CD25+ and CD8+ lymphocytes are reduced in SSc patients. On the other hand, Artlett et al. [9] reported that the numbers of

Table 1. Main clinical and laboratory features of 29 patients with systemic sclerosis (SSc).

Gender (male/female)	1/28
Age (mean ± SD)	56.3 ± 12.9
SSc type (limited/diffuse)	22/7
Duration of disease (median, months)	42 (1 – 390)
Raynaud phenomenon (no/yes)	0/29
MRSS (median)	7 (2 – 24)
Renal involvement (no/yes)	26/3
Lung involvement (no/yes)	21/8
Oesophagus involvement (no/yes)	20/9
Antinuclear antibodies on HEp-2 cell line (negative/anti-centromere/ anti-topoisomerase I)	7/16/6

MRSS, modified Rodnan skin score.

Table 2. Peripheral blood lymphocyte subsets in patients with systemic sclerosis (SSc) and healthy controls.

Lymphocytes	SSc (n = 29)	Controls (n = 29)	P-value
Lymphocytes/μl	1676 ± 602	2273 ± 561	0.0042*
CD3+			
absolute/μl	1193 ± 474	1688 ± 424	0.0014*
percentage (%)	71 ± 7	74 ± 5	0.419
CD4+			
absolute/μl	808 ± 279	1115 ± 346	0.0070*
percentage (%)	49 ± 9	49 ± 8	0.747
CD4+/CD8+ ratio	2.3 ± 1.2	2.8 ± 1.6	0.165
CD8+			
absolute/μl	363 ± 256	570 ± 214	0.021*
percentage (%)	21 ± 7	25 ± 8	0.582
CD19+			
absolute/μl	197 ± 89	297 ± 136	0.024*
percentage (%)	12 ± 5	13 ± 4	0.725
CD56+			
absolute/μl	265 ± 153	247 ± 114	0.632
percentage (%)	16 ± 8	11 ± 4	0.0588

*, statistically significant according to independent t-test and Bonferroni-correction

CD4+ and CD8+ cells were found to be significantly higher in SSc patients than in controls. However, Fiocco et al. [6] observed that patients with SSc showed increased CD4+CD26+ and CD4+CD25+ absolute numbers and percentages and decreased CD8+CD29+ percentages compared with controls.

Natural killer cells are large granular lymphocytes easily identified morphologically by the presence of azurophil granules in their cytoplasm and they commonly express certain cell surface markers such as CD56+ which is a homophilic adhesion molecule that belongs to the immunoglobulin superfamily. Natural killer cells mediate antigen presentation and secrete immune modulator cytokines like interferon, colony stimulating factor - these functions suggested the involvement of natural killer cells in the pathophysiology of SSc. Reduction of natural killer cells has been described by Riccieri et al. [11]. Interestingly, Mitsuo et al. [12] observed that the frequency as well as the absolute number of CD161+CD8+ cells, which are closely related to CD8+ natural killer cells, were decreased in the peripheral blood of SSc patients.

Presence of autoantibodies and hypergammaglobulinaemia support the role of humoral immunity. CD19+ is a cell surface marker of B lymphocytes. We observed significantly reduced levels of CD19+ cells which inversely correlated with the clinical score of SSc patients. Sato et al. [13] found that CD19 is overexpressed in naive B cells and, to a greater extent, in memory B cells in the blood SSc patients. This CD19 overexpression was specific to SSc, since SLE patients exhibited significantly down-regulated CD19 expression. Sato et al. [13] suggested that the CD19 overexpression in SSc memory B cells induces both the activated phenotype of memory B cells and augmented IgG production. However, Majone et al. [15] recently reported on increased apoptosis in circulating lymphocyte cultures of anti-RNA polymerase III positive patients with SSc. Indeed, this might be a possible mechanism to explain the reduced lymphocyte counts in SSc patients.

In conclusion, the aforementioned data on lymphocyte subsets in SSc are conflicting very likely due to different methodologies particularly with regard to patient selection, pre-treatments, assays, and sample sizes. Although the relations between the lymphocyte subpopulations investigated was not different from healthy controls, we found a significant reduction of absolute numbers of lymphocytes and CD3+, CD4+, CD8+, and CD19+ cells SSc patients. Interestingly, the absolute number of CD19+ cells appears to inversely correlate with the severity of cutaneous involvement. Hence, the CD19 count may be a potential biomarker for the severity of skin involvement. Taken together, our data support previous reports indicating that subsets of T lymphocytes as well as B lymphocytes play a role in the pathogenesis of SSc.

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