Peripheral blood gene expression in discoid lupus erythematosus and systemic lupus erythematosus patients

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Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease with variations in clinical presentation that can affect the kidney, blood vessels, heart, lungs, joints, CNS, and/or the skin. The most common cutaneous form is discoid lupus erythematosus (DLE), which is characterized by erythematous scaly papules, especially in sun-exposed areas, that become dyspigmented, atrophic, and scarring plaques. SLE and DLE can occur together or independently, and differentiating between these two diagnoses can be ambiguous. Identifying differences in the transriptomes of DLE patients versus SLE patients can aid both in diagnosis of DLE versus SLE and prediction of progression from DLE to SLE.

A number of studies have identified genes related to the type I interferon (IFN) pathway that are up-regulated in peripheral blood mononuclear cells (PBMCs) of SLE patients compared with normal controls [1,2]. Additionally, a recent study of chronic cutaneous lupus patients (a category which includes but is not limited to DLE patients) determined that type I IFN pathway-related genes are also up-regulated in the peripheral blood of these patients compared with normal controls [3]. Other studies have suggested that overexpression of particular cytokine genes could serve as biomarkers of disease progression from DLE to SLE [4]. We sought to determine if the level of type I IFN and cytokine gene expression in peripheral blood differs between DLE and SLE patients.

Project Aim and Hypothesis

To determine differences in whole blood transcriptomes, with particular attention to type I interferon-related genes and cytokine genes, between DLE and SLE patients who were not taking oral medications at the time of blood sample collection. We hypothesize that patients with SLE will have elevated levels of IFN-related and cytokine gene expression due to their more widespread disease compared with patients with DLE.

Methods

Sample collection and preparation:

Blood samples were collected from DLE and SLE patients recruited at the outpatient University of Texas Southwestern Medical Center and Parkland Hospital dermatology clinics. Demographics, number of SLE diagnostic criteria, and skin and systemic disease activity measurements including Cutaneous Lupus Activity and Severity Index (CLASI) activity and damage scores, and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) scores were collected for each patient. Patients were excluded from the study if they were taking oral medications at the time of blood collection. Blood samples from normal patients with no history of autoimmune disease who were not taking immunosuppressive medications at the time were also collected. RNA was directly extracted from whole blood samples and converted to cDNA.

Reverse transcription polymerase chain reaction (RT-PCR):

Ten genes, including five interferon-related genes (MX1, LY6E, OAS1, OASL, and ISG15) and five cytokine genes (CXCL10, TNF-α, IL-6, IL-10, and IL-12), were selected for real time PCR analysis. cDNA of selected genes was amplified with SYBR Green PCR Master Mix using forward and reverse primers. The following conditions were used for real time PCR: 3 mins at 95ºC, then 38 cycles of 20 secs at 95ºC, 1 min at 55ºC, and 30 secs at 72ºC.

Analysis:

Cycle threshold (C_T) values were standardized to the housekeeping gene GAPDH and converted to fold change using the $2^{-\Delta\Delta CT}$ formula. An interferon score was calculated for each patient using the formula in Feng *et al* [5]. A Kruskal-Wallis test was used to analyze whether there was a significant difference between the three groups of patients.

Results

Table 1: Patient Characteristics

	Normal	DLE	SLE
N	12	12	13
Age	39 (22-59)	39 (22-55)	46 (34-82)
Gender	9F, 3M	9F, 3M	13F
Ethnicity	8 AA, 3 C, 1 H	8 AA, 3 C, 1 Mixed	1A, 4AA, 6C, 1H, 1 Mixed
CLASI Activity	N/A	3 (0-12)	3 (0-17)
CLASI Damage	N/A	6 (0-20)	0 (0-24)
SLEDAI	N/A	0 (0-1)	2 (0-6)
# SLE Criteria	N/A	2 (1-3)	5 (4-8)
Malar Rash	N/A	0	8
Discoid Rash	N/A	12	7
Photosensitivity	N/A	8	13
Oral Ulcers	N/A	0	6
Arthritis	N/A	0	7
Serositis	N/A	0	2
Renal Disorder	N/A	0	2
Neurological Disorder	N/A	0	0
Hematological Disorder	N/A	1	12
Immunological Disorder	N/A	0	3
Antinuclear Antibody	N/A	2	13

Figure 1: Type I IFN-related genes are upregulated in DLE and SLE patients compared to normal controls

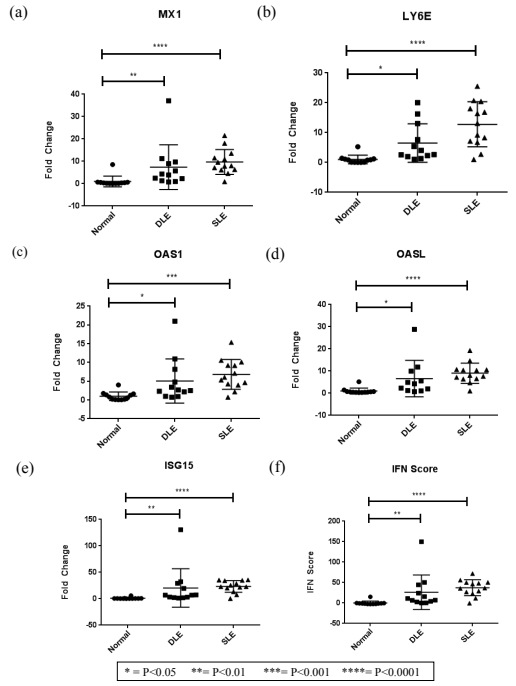
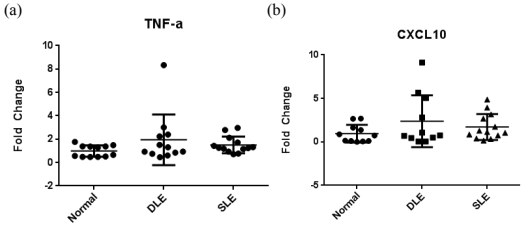


Figure 2: TNF-a and CXCL10 are not upregulated in DLE or SLE patients compared to normal controls



Conclusions

- Type I IFN-related gene expression is elevated in DLE and SLE patients compared to normal controls.
- Our results did not show a difference in type I IFN-related gene expression between DLE and SLE patients.
- DLE is often considered to be a pre-SLE phase. These results suggest that IFN-related genes are already upregulated in this pre-SLE phase.
- 7 out of 13 SLE patients had discoid lesions. No significant differences were found when SLE patients with and without DLE were compared.
- Expression of type I IFN-related genes did not correlate with measures of disease severity (CLASI activity, CLASI damage, or SLEDAI) in DLE and SLE patients.
- Expression of many of the cytokine genes was too low to be measured. CXCL10 and TNF-a were high enough to be measured, but did not show any significant up-regulation in DLE or SLE compared to normal controls.
- Future directions include RT-PCR analyses of cytokine gene expression of stimulated patient PBMCs, and whole blood gene expression of other candidate biomarker genes identified from our whole blood microarray data.

References

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Funding

This research was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health.