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The IFN-regulated gene signature is elevated in SCLE and DLE and correlates with CLASI score

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Abstract

Background—There is increased expression of type I interferon (IFN)-regulated proteins in the blood and target tissues of patients with cutaneous lupus erythematosus (CLE) and systemic lupus erythematosus (SLE). Patients with SLE have increased IFN-regulated gene expression pointing towards a possible underlying genetic defect.

Objectives—We measured expression levels of five type I IFN-regulated genes that are highly expressed in SLE in the peripheral blood of patients with CLE and correlated expression levels with cutaneous disease activity.

Methods—Peripheral blood was obtained from 10 healthy controls and 30 patients with CLE, including 8 with concomitant SLE. Total RNA was extracted and reverse transcribed into complimentary DNA. Gene expression levels were measured by real time PCR. Gene expression was normalized to GAPDH, standardized to healthy controls and then summed to calculate an IFN score for each patient. Disease activity was assessed with the Cutaneous Lupus Area and Severity Index (CLASI).

Results—Patients with subacute cutaneous lupus erythematosus (SCLE) and discoid lupus erythematosus (DLE) had elevated IFN scores compared to healthy controls regardless of concomitant SLE ($p < 0.01$ with SLE and $p < 0.05$ without SLE). There was no difference between patients with tumid lupus erythematosus (TLE) and healthy controls. The IFN score correlated with CLASI scores (Spearman's Rho (r) = 0.55, $p = 0.0017$).

Conclusions—Patients with SCLE and DLE have an IFN signature, as seen in SLE. The level of gene expression correlates with cutaneous disease activity. These findings support a shared pathogenesis between SLE and some subtypes of CLE.

INTRODUCTION

Patients with cutaneous lupus erythematosus (CLE) have evidence of increased type I interferon (IFN) signaling and there is increased expression of type I IFN-regulated transcripts and proteins in lesional skin^{1–3}. Type I IFNs are implicated in the pathogenesis of both CLE and systemic lupus erythematosus (SLE). In SLE there is increased expression of type I IFN-regulated genes in the peripheral blood, pointing towards an underlying genetic defect⁴. Growing evidence suggests that CLE shares genetic abnormalities with SLE. For example a polymorphism in IFN regulatory factor 5 (IRF5) is seen in SLE, subacute cutaneous lupus erythematosus (SCLE) and discoid lupus erythematosus (DLE)⁵. Also

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mutations in TREX1, a DNA exonuclease mutated in some cases of SLE and Aicardi-Goutieres syndrome, have been reported in a familial form of chilblain lupus, suggesting a common mutation in these autoimmune diseases characterized by type I IFN signaling⁶.

Here we look at the expression of IFN-regulated genes in the peripheral blood of patients with CLE, with and without concomitant SLE, and correlate expression levels with disease activity. We compared common clinical subtypes of CLE, including SCLE, DLE and tumid lupus erythematosus (TLE), to better understand possible genetic associations with SLE.

MATERIALS AND METHODS

Patients and controls

The study group included 30 patients with CLE, including twelve with SCLE, fourteen with DLE and four with TLE⁷. Eight subjects met criteria for SLE (defined by American College of Rheumatology criteria) and will be referred to also as CLE patients with concomitant SLE or SLE/CLE. Peripheral blood from ten healthy volunteers without autoimmune disease was used as controls. Sample and data collection was performed in accordance with protocols approved by the University of Pennsylvania Institutional Review Board.

Sample collection and RNA processing

Peripheral blood mononuclear cells were isolated from peripheral blood and frozen in RNALater (Qiagen, Valencia, CA) at -80°C . RNA was isolated using RNeasy Mini Kit (Qiagen) and the lysate was homogenized using Qiaschredder minicolumn (Qiagen) according to manufacturers' protocol. RNA was reverse transcribed into cDNA using Invitrogen First-Strand Synthesis Kit (Invitrogen, Carlsbad, CA) according to manufacturers' protocol. RNA samples were stored at -80°C and cDNA samples were stored at -20°C .

IFN-regulated gene expression measurement

We measured five type I IFN-regulated genes shown previously to correlate with disease activity in 48 SLE patients⁴. The genes studied were lymphocyte antigen 6 complex (LY6E), 2',5'-oligoadenylate synthetase 1, 40/46kd (OAS1), 2',5'-oligoadenylate synthetase-like (OASL), interferon-alpha-inducible protein, clone IFI-15K (ISG15), and myxovirus resistance 1 (MXI). Gene expression was measured with Taqman gene expression assays (Applied Biosystems, Foster City, CA). Assay identification numbers for LY6E, OAS1, OASL, ISG15, and MX1 were Hs00158942_m1, Hs00242943_m1, Hs00388714_m1, Hs00192713_m1, and Hs00182073_m1. Experiments were performed in triplicate in 96 well plates using the Applied Biosystems 7000 Sequence Detection System. Reactions were performed in a 25- μl reaction volume in Taqman Universal PCR Master Mix (Applied Biosystems). Fluorescent signal detection used ROX as the internal passive reference dye. Cycling times and temperatures were as follows: initial denaturation for 10 minutes at 95°C , followed by 40 cycles of denaturation at 95°C for 15 seconds, and combined primer annealing and extension at 60°C for 1 minute. Data were displayed using ABI Prism SDS 7000 software, version 1.0 (Applied Biosystems). Human GAPDH was used to normalize cellular RNA amounts (assay identification number Hs99999905_m1, Applied Biosystems).

IFN score calculation

The expression level of the five genes are expressed as a cumulative IFN score. The IFN score was calculated by summing standardized expression levels of each gene for each subject as described by Feng *et al*⁴. Levels were standardized to the level of each IFN-regulated gene in the healthy control group.

CLE disease activity measurement

Cutaneous lupus activity was assessed using the Cutaneous Lupus Area and Severity Index (CLASI) activity score⁸. All CLASI scoring was performed by VPW.

Statistical analysis

Data were analyzed with Graph-Pad Prism, software (version 5.01). Statistical comparisons were made using the Kruskal-Wallis test with Dunn's Multiple Comparison post hoc test or the Mann Whitney U-test. Correlation between groups was evaluated using the Spearman's test. P-values less than 0.05 were considered significant.

RESULTS

Subject characteristics

The majority of study subjects were female (93%). The average age was 48, ranging from 29 to 75 with a median of 47. The healthy control cohort was composed of 70% women with an average age of 33.5, ranging from 26–54 with a median of 28. Table 1 summarizes the clinical characteristics of the 30 CLE patients.

Eight CLE patients met SLE criteria (SLE/CLE) based on photosensitivity (5/8), oral ulcers (4/8), arthritis (4/8), malar rash (2/8), discoid lesions (2/8), proteinuria (1/8) and anti-Ro positivity (1/8). Most had mild SLE disease activity as measured by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). The median SLEDAI score was 6, ranging from 0–15, with an interquartile range of 2.8–8.5. Four subjects had SLEDAI scores of 5 or less, which represents mild disease.

Increased type I IFN-regulated gene expression in SCLE and DLE

Patients with SCLE and DLE had increased type I IFN-regulated gene expression, or IFN signature, compared to healthy controls (Fig 1) ($p < 0.05$, Kruskal Wallis test $p = 0.0016$). Patients with SCLE also had a significantly increased IFN signature compared to TLE patients ($p < 0.05$). There was no difference in IFN score between TLE patients and healthy controls.

We next compared the IFN scores of subjects with SCLE or DLE with and without concomitant SLE. None of the patients with TLE had concomitant SLE. We found elevated IFN scores compared to healthy controls in both groups ($p < 0.01$ and $p < 0.05$, respectively) while there was no statistically significant difference between SCLE and DLE patients with or without SLE (Fig 2, Kruskal-Wallis test $p = 0.0022$).

Eight CLE patients were taking prednisone at the time of the blood draw. There was no difference in IFN score between those on or off prednisone ($p = 0.67$). There were also no associations between high and low IFN score when looking at age, gender, comorbidities, use of hydroxychloroquine or smoking status in our subject pool.

Finally, the IFN score correlated with CLASI activity scores in the 30 patients with CLE (Fig 3, Spearman's Rho (r) = 0.55, $p = 0.0017$). The CLASI activity score also correlated with IFN score when the four patients with TLE were excluded (Spearman's Rho (r) = 0.46, $p = 0.019$) and when the eight patients with SLE were excluded (Spearman's Rho (r) = 0.60, $p = 0.0035$).

DISCUSSION

Here we demonstrate an elevation in type I IFN-regulated gene expression, or IFN signature, in SCLE and DLE (Fig 1). We highlight the finding of an IFN signature in SCLE and DLE

patients without manifestations of SLE (Fig 2). Also we found that the IFN score correlates with cutaneous disease activity (Fig 3) pointing towards a possible biomarker for CLE activity.

In our study, patients with SCLE and DLE with concomitant SLE have a median IFN score of 18 (interquartile range: 7.3–27) and those without SLE manifestations have a median IFN score of 12 (interquartile range: 2.1–28). In a prior study by Feng *et.al.* the median IFN score in those with SLE and an active rash was 20, similar to our findings⁴. The eight patients with SLE in our study included five with DLE and three with SCLE (Fig 1). They had low to moderate SLE activity as measured by SLEDAI scores and fulfilled mostly cutaneous criteria for SLE. The finding of an elevated IFN score in CLE patients with only mild to moderate SLE activity and also in patients with no manifestations of SLE is a novel finding which we report to highlight a possible shared pathogenesis between some types of CLE and SLE.

In comparison to prior studies of IFN signature and cutaneous manifestations of SLE we only included patients with well characterized lupus specific CLE subtypes. Doing this allowed us to observe an interesting difference in TLE, a form of CLE that is usually not associated with SLE⁹. The lack of an elevated IFN signature in TLE raises an interesting correlate to this clinical finding.

We noticed that the median IFN score was lower in the patients without SLE manifestations and there are more patients in that cohort that have lower IFN scores similar to healthy controls. We looked at multiple variables, including age, gender, comorbidities, use of hydroxychloroquine or prednisone, and smoking status. We found no clear correlation between high and low IFN score except for CLASI activity score. It is possible that an unmeasured variable could explain the differences seen in IFN score. Further study and longitudinal follow up of patients may help elucidate these factors.

The correlation of IFN signature and disease activity is in keeping with studies in dermatomyositis¹⁰. Dermatomyositis and CLE are both autoinflammatory dermatoses characterized histologically by an interface dermatitis and the presence of IFN α -producing plasmacytoid dendritic cells^{10,11}. These similarities suggest a shared mechanism of these clinically distinct entities and also may imply that IFN-regulated genes are common pro-inflammatory markers seen in inflammatory conditions, perhaps specifically in those characterized by interface dermatitis. We find it interesting that TLE patients did not have an elevated IFN signature and hypothesize that IFN score may correlate with the histologic finding of interface dermatitis.

It is important to note that some studies in SLE fail to show an association between type I IFN gene expression and longitudinal changes in disease activity, highlighting the need for further study of IFN-regulated genes as biomarkers of CLE disease activity^{12,13}.

CONCLUSIONS

In conclusion, we report an increase in type I IFN-regulated gene expression in SCLE and DLE regardless of concomitant SLE. These finding support a shared pathogenesis of some subtypes of CLE with SLE. We show that type I IFN gene expression levels correlate with disease activity in CLE, however further study into the changes of IFN score with disease flare and remission are needed.

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What's already known about this topic?

- There are increased type I IFN-regulated proteins in the peripheral blood and target tissues of patients with cutaneous lupus erythematosus (CLE) and systemic lupus erythematosus (SLE). SLE patients have elevated IFN-regulated gene expression suggesting an underlying genetic pathway.

What does this study add?

- Type I IFN-regulated gene expression is elevated in the peripheral blood of SCLE and DLE patients suggesting a genetic link with SLE. IFN-regulated gene expression levels also correlate with cutaneous disease activity.

Figure 1.

Subacute cutaneous lupus erythematosus (SCLE, n=12) and discoid lupus erythematosus (DLE, n=14 patients have significantly elevated interferon (IFN) scores as compared to healthy controls (HC, n=10). SCLE patients also have significantly elevated IFN scores as compared to patients with tumid lupus erythematosus (TLE, n=4) (* $p < 0.05$). Patients with cutaneous lupus erythematosus with concomitant systemic lupus erythematosus (SLE/CLE) are shown as red diamonds. Horizontal line shows median value and interquartile range. An asterix denotes $p < 0.05$. Kruskal-Wallis, $p = 0.0016$.

Figure 2.

SCLE and DLE patients with and without SLE have an elevated interferon (IFN) score compared to healthy controls (HC) ($p < 0.01$ and $p < 0.05$). There was no statistical difference between patients with SLE or DLE with SLE (SLE/CLE) and those without (DLE/SCLE). Horizontal line shows median value and interquartile range. One asterix denotes $p < 0.05$ and two denote $p < 0.01$. Kruskal-Wallis, $p = 0.0022$.

Figure 3. The interferon (IFN) score correlates with Cutaneous Lupus Area and Severity Index (CLASI) activity score (Spearman's Rho (r) = 0.55, p = 0.0017). Patients with CLE and concomitant SLE (SLE/CLE) are shown as red diamonds.

Table 1

CLE Patient Clinical Characteristics

History of SLE	N (%)
Positive	8 (27%)
CLE Subtype	N
DLE	14
SCLE	12
TLE	4
Age	Median (interquartile range)
	47 (38–53)
Sex	N Female (% F)
	28 (93%)
Race	N (%)
Caucasian	24 (80%)
African-American	5 (17%)
Asian	1 (3%)
Comorbidities	N (%)
Sjögren's syndrome	6 (18%)
Rheumatoid arthritis	6 (18%)
Autoimmune hepatitis	1 (<1%)
Medications at time of blood draw	N (%)
Systemic corticosteroids	8 (27%)
hydroxychloroquine	13 (43%)
Smoking history	N (%)
Actively smoking	14 (46%)
Quit smoking	5 (17%)

SLE, systemic lupus erythematosus; N, number; CLE, cutaneous lupus erythematosus; DLE, discoid lupus erythematosus; SCLE, subacute cutaneous lupus erythematosus; TLE, tumid lupus erythematosus; SEM, standard error measurement; and LE, lupus erythematosus.