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A T cell gene expression panel for the diagnosis and monitoring of disease activity in patients with systemic lupus erythematosus

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Abstract Systemic Lupus Erythematosus (SLE) remains a challenging disease to diagnose and follow, as no reliable biomarkers are known to date. We designed a gene expression panel with 40 genes known to play a role in SLE pathogenesis. We found that the combined expression of these genes in SLE T cells can accurately differentiate SLE from healthy individuals and patients with other autoimmune diseases. The accuracy of the test increased further (83%) when only three out of the initial genes (OAS2, CD70 and IL10) were used. A T cell score, calculated from the combined expression levels of these genes, correlated positively with various SLE activity markers in a cross-sectional cohort and in a few patients that were followed prospectively. These data showcase the usefulness of measuring mRNA levels of key molecules in diagnosing and following patients with SLE.

Abbreviations: SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; SLEDAI, systemic lupus erythematosus disease activity index; BILAG, British Isles lupus assessment group; qPCR, real time polymerase chain reaction; CART, classification and regression trees; CMS, comparative marker selection; KNN, k-nearest neighbor algorithm; PPV, positive predicted value; NPV, negative predictive value; ANA, anti-nuclear antibodies; PGA, physician global assessment.

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1. Introduction

Current tools used to diagnose systemic lupus erythematosus (SLE) and to monitor disease activity, including systemic lupus erythematosus disease activity index (SLEDAI) and the British Isles Lupus Assessment Group (BILAG) rely on clinical and laboratory markers and have acknowledged limitations given the clinical complexity of the disease and the frequently superimposed comorbid conditions [1–3].

Most of the markers used by these scoring systems reflect non-specific inflammation in the target tissues. Validated markers that reflect the immunologic aberration of SLE, including complement levels and dsDNA antibodies have not risen to be acclaimed as reliable disease biomarker. It is therefore apparent that reliable and easy to use biomarkers that can objectively diagnose SLE, reflect disease activity and/or predict flares are needed.

Novel approaches for disease diagnosis and classification rely on the use of panels of expression levels of genes related to disease pathogenesis. Recently this approach has been successfully applied to cancer prognosis and commercially available genetic-based risk tests have been produced [4,5]. Similar attempts have been performed in the field of autoimmune diseases. For example, a CD8+ T cell transcription signature was recently found to be associated with disease prognosis in anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis and SLE patients [6]. Furthermore, interferon (IFN)-induced gene signatures have been found to correlate with disease activity in a number of SLE studies [7–9].

Several lines of evidence suggest that T cells play an important role in the pathogenesis of SLE [1,10]. T cells are key modulators of B cell activation and immunoglobulin class switching that are necessary steps for the production of high affinity autoantibodies seen in SLE. SLE T cells also fail to execute appropriately their regulatory functions while at the same time exhibit inappropriate homing to target organs. SLE T cells have an abnormally activated phenotype with pre-aggregated lipid rafts, high calcium flux upon activation, and production of pro-inflammatory cytokines such as IL-17 [10–12]. Abnormally expressed signaling molecules (CD40L, IL10) [10,13,14] have been considered as treatment targets, but their value as disease biomarkers has not been addressed formally.

We sought to evaluate the potential of developing a disease-specific biomarker in SLE based on the expression levels of genes that have been claimed to contribute to the abnormal T cell function. In a preliminary report, we showed that measuring disease specific gene expression using a PCR-based gene array can successfully differentiate SLE patients from control individuals [15]. Using an expanded study sample and prospective collection of samples we show that the expression of specific pathogenesis-linked genes can be used to assist diagnosis and monitor disease activity in individual patients.

2. Patients and methods

2.1. Ethics statement

This study was approved by the Institutional Review Board of BIDMC. Written informed consent was obtained from all participating subjects and all clinical investigation was conducted according to the principles expressed in the Declaration of Helsinki.

2.2. SLE and control samples

Blood samples were obtained from 65 SLE patients members of the Lupus Center at the Rheumatology Division of Beth Israel Deaconess Medical Center (BIDMC). Seven of those patients were followed up longitudinally in two months interval for a period of $\geq$1 year. All participating patients fulfilled the American College of Rheumatology criteria for the diagnosis of SLE. Blood was also obtained from 27 healthy subjects and 16 rheumatoid arthritis (RA) patients.

2.3. Cell extraction and RNA isolation

Density- gradient centrifugation was used to isolate peripheral blood mononuclear cells (PBMCs) from whole blood samples. For T cell isolation, blood was incubated first for 30 min with a rosette T cell purification kit (StemCell technologies, Vancouver, Canada) that contained a tetrameric antibody mixture against CD14, CD16, CD19, CD56 and glyA that attaches non-T cells to erythrocytes. Lymphocyte separation medium (Cellgro, Manassas, VA) was subsequently used to separate these complexes from T cells.

From 3×10^6 cells, RNA, DNA and protein were extracted using Qiagen (Valencia, CA) AllPrep extraction kit. A DNase-I treatment step (Qiagen) was added to the standard protocol to ensure exclusion of genomic DNA from the final product. $OD_{260/280}$ measurements were used as a measure of isolated RNA quality.

Total RNA was reversely transcribed into cDNA using Promega (Madison, WI) reverse transcription system and a mixture of 1:10 oligo $(dT)_{20}$ to random hexamer primers. Reverse transcription was performed in a conventional thermocycler.

2.4. Design of gene array and qPCR

Quantitative real time polymerase chain reaction (qPCR) was performed to measure gene expression levels using the Universal Probe Library (UPL) system from Roche (Indianapolis, IN). Probes only bind to the amplicon of interest and do not release their signal unless the 5´3´ exonuclease activity of the Taq DNA Polymerase unquenches the reporter dye.

Forty gene targets, two reference (CD3ε and GAPDH) and two control assays (human genomic contamination and no template controls) were included in the array (Supplementary Table 1).

All primers and probes were designed using the online UPL Assay Design Center tool from Roche (Indianapolis, IN). Only intron spanning assays were kept as a safeguard against genomic DNA contamination. Amplicon sizes <200 bp were chosen to ensure fast and efficient amplification. A detailed list of all included genes, along with their amplicon lengths, accession numbers, primer and probe sequences can be found in Supplementary Table 2.

For each gene, a reaction mixture was prepared using a final concentration of 200 nM for each primer, 100 nM for probes, 1x LightCycler 480 Probes Master (containing FastStart