

Cytotoxic T-cells as Novel Biomarkers for Systemic Lupus Erythematosus Disease Activity

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A. Study Purpose and Rationale

Systemic Lupus Erythematosus (SLE) is a chronic, multi-organ autoimmune disease that is characterized by clinical flares and remission. A major problem in the management of SLE patients is the difficulty for the clinician to predict a relapse or to distinguish between active and quiescent disease. Several indices, such as systemic lupus evaluation disease activity index (SLEDAI) and systemic lupus activity measure (SLAM), have been developed and validated and are useful for assessing SLE disease activity, but these scoring systems rely primarily on subjective measures (1). Investigators have tried to identify additional objective parameters to determine SLE activity, but have investigated primarily products of B-cells, such as anti-double-stranded DNA antibodies and complement levels. These laboratory values, however, do not always correlate with SLE clinical flares. For example, anti-dsDNA antibodies were not detected in more than 40% of clinically active SLE patients (2), whereas up to 15% of clinically-asymptomatic patients had high anti-dsDNA antibody titers (3, 4). Therefore, a reliable marker of SLE activity is still needed.

T-cell activation plays a pivotal role in SLE and is responsible for B-cell activation, cytokine secretion and antibody production (5). The role of cytotoxic T lymphocytes is well known and well documented in various autoimmune diseases, such as type I diabetes, thyroiditis and polymyositis, and has recently been implicated in other autoimmune diseases, such as Behcet's disease, ankylosing spondylitis and multiple sclerosis (6). A few recent studies have focused on the role of cytotoxic T lymphocytes in patients with SLE. A high proportion of activated CD8+ T lymphocytes, expressing perforin and granzyme B, was found in the blood of 61 patients with active SLE (7). CD8+ T lymphocytes were found in the periglomerular area of patients with severe (class III and IV) lupus nephritis and therefore implicated in the pathogenesis and prognosis of these SLE patients (8). Activated CD8+ T lymphocytes, expressing HLA-DR, were found to be elevated in sixty SLE patients with active disease, but it could not be concluded whether these cells could be used as biological markers of SLE disease activity (9).

The aim of this study is to study T-cell activation to see if these cells can be used as biomarkers of clinical SLE disease activity and therefore potential therapeutic targets. The hypothesis is that activated CD8 T cells and the proportion of activated CD8 to CD4 T cells will correlate with clinical SLE flares and will be higher in those with active disease states.

B. Study Design and Statistical Analysis

The study design is a prospective cohort study where patients with quiescent SLE will be recruited and followed over one year. These patients will be observed for evidence of a clinical SLE flare using the validated SELENA-SLEDAI survey. Flare will be defined as

mild/moderate flare and severe flare according to the SELENA trial definitions of flare (1):

“Mild/Moderate Flare”	“Severe Flare”
<ul style="list-style-type: none"> • a change in SLEDAI > or equal to 3 points, or • new/worse skin, stomatitis, serositis, arthritis, fever, or • increased prednisone <0.5mg/kg/d, or • added NSAID/Plaquenil, or • > or equal to increase in physician’s global assessment (0-3 scale) 	<ul style="list-style-type: none"> • change in SLEDAI >12, or • new/worse CNS-SLE, vasculitis, nephritis, myositis, plt <60,000, hemolytic anemia with hb< 7 mg/dl, requiring doubling or > 0.5mg/kg/d prednisone, or • hospitalization for SLE, or • prednisone >0.5mg/kg/d, or • new immunosuppressive, or • increased physician’s global assessment to > 2.5

The primary endpoint will be the comparison of the number of activated CD8 T cells among the subjects in both their quiescent and flare states. Secondary outcome measures will compare activated CD4 T cells and the ratio of activated CD8 to CD4 T cells among these subjects in their two disease states. Another secondary outcome will examine whether there is a change between activated CD4 T cells, activated CD8 T cells and the ratio of the two among patients who remain in a quiescent disease state throughout the study.

The number of subjects recruited will be one hundred. Among the one hundred subjects recruited, there is an underlying assumption that about thirty of those subjects will experience a flare over the course of one year. This assumption is based on previous research which showed that about 20-40% of SLE patients flare over the course of 1 year (10, 11). 100 subjects were chosen, with an assumption of 30 flares, to detect an effect size of about ½ standard deviation using the power calculation for a paired t-test (<http://www.biomath.info/gcrc/>). This allows for an 80% power of detecting a difference between the subjects with a pre-determined level of significance of alpha being 0.05. There is currently no data in the literature on the standard deviation of the change of activated T cells between patients in their original quiescent state who then develop a flare. Therefore, the number of subjects were chosen to detect an effect size that was a fraction of the standard deviation, in this case about ½ of a SD.

C. Study Procedure

Whole blood will be collected in two 10-ml vacutainer tubes containing EDTA via venipuncture when quiescent SLE patients enroll in the study and when they flare. It will also be collected as part of routine laboratory blood draws before office visits and in the hospital so there will be at least two data points for each person. Peripheral blood mononuclear cells will be isolated from the blood using the standard method of density gradient centrifugation. Flow cytometry will be utilized to obtain the number of CD4 and CD8 T cells. These cells will be analyzed for their cell surface activation markers, specifically for CD69, CD25 and HLA-DR (12).

These blood samples will be taken at the same frequency as routine laboratory work, and therefore, in accordance with standard clinical care. Patients will also be required to fill out SELENA-SLEDAI questionnaires initially and during each clinic and/or hospital visit. These questionnaires are routinely used in clinical practice. The subjects will be followed over the course of one year.

D. Study Drugs

There will be no study drugs used in this protocol.

E. Medical Device

There will be no medical devices used in this protocol.

F. Study Questionnaires

Please see attached SELENA-SLEDAI questionnaire, which is a standard and validated SLE questionnaire, used to determine SLE disease activity.

G. Study Subjects

The patients studied will be those that meet the ACR criteria of SLE (4 or more) who have clinically quiescent disease, as indicated by a score of less than or equal to 6 on the SELENA-SLEDAI index. Patients will be recruited from both the inpatient and outpatient setting at CUMC.

Inclusion Criteria:

- diagnosis of SLE, must meet at least 4 of ACR criteria of SLE
- quiescent disease, defined by SELENA-SLEDAI score of less than or equal to 6
- at least 18 years of age

Exclusion criteria:

- patients less than 18 years of age
- patients unable to give informed consent
- patients with active infections, including HIV
- patients with known active malignancies or currently being treated for malignancy
- patients who are pregnant
- patients receiving cytoxan
- patients receiving high treatment dose prednisone (>0.5mg/kg/day)

H. Recruitment of Subjects

Subjects are to be recruited by their primary rheumatology physicians in the inpatient and outpatient settings, i.e. by the rheumatology consult service in the inpatient setting, and by the rheumatology physician in SLE outpatient clinics. The patient's primary rheumatologist will determine if the patient is suitable for the study and will ascertain from the patient if he/she is willing to discuss the study with the research team. The subjects will be recruited from one institution, namely CUMC.

I. Confidentiality of Study Data

A unique code number will be given to all study subjects and data will be stored in a secure location, accessible only to the investigators.

J. Potential Conflict of Interest

There is no potential conflict of interest in this study.

K. Location of the Study

The study will be carried out only within CPMC in clinical care areas.

L. Potential Risks

There is only a minimum risk of hematoma following phlebotomy and the associated discomfort of phlebotomy.

M. Potential Benefits

The research subjects may or may not benefit from their participation in the study. The data may help understand the mechanisms and biomarkers of SLE, as well as provide new treatment targets, but it is possible that the data will yield no useful results.

N. Alternative Therapies

There are no therapies offered in this trial, and therefore there are no alternative therapies that need to be considered.

O. Compensation to Subjects

There will be no compensation to subjects who participate in this study.

P. Costs to Subjects

The participants will not endure any extra costs as a result of participating in the study.

Q. Minors as Research Subjects

There are no minors allowed to participate in this research study.

R. Radiation or Radioactive Substances

This study does not involve radiation or radioactive substances.

References:

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