Lymphocyte Blast Transformation and Peripheral Lymphocyte Percentages in Patients with Sickle Cell Disease

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ABSTRACT

Twenty patients with sickle cell disease (14 black females and 6 black males, mean age 31.5 ± 9.3) were studied by quantitating peripheral T and B lymphocyte percentages and measuring lymphocyte blast transformation (LBT) in response to phytohemagglutinin-P, concanavalin-A and pokeweed mitogen. Compared to normal black controls (19 black females and 1 black male, mean age 32.0 ± 9.2 years) sickle cell patients had decreased T lymphocytes (50.2 percent ± 6.2 compared to 66.2 percent ± 1.7) and increased B lymphocytes (17.0 percent ± 3.4 compared to 7.7 percent ± 1.1). Sickle patients exhibited decreased LBT to all three mitogens.

Introduction

Sickle cell anemia is the most common hereditary hematologic disorder known to man. The homozygous form of the disease, hemoglobin SS, occurs in approximately one out of every 300 Black Americans. Hemoglobin AS or sickle cell trait, the heterozygous condition, occurs in approximately one out of ten. This hemoglobinopathy affects primarily Black Africans and Americans as well as some Latins and is worldwide in distribution.10

The abnormality of sickle cell disease resides in the globin portion of the hemoglobin molecule. Normal adult hemoglobin, HbA, has two alpha and beta chains. In sickle disease, an abnormal hemoglobin results from a genetically determined amino acid substitution in one of the polypeptide chains. Hemoglobin S differs from a normal A by one amino acid substitution in the beta chain; a valine in place of glutamic acid at the sixth position. Hemoglobin S, in the homozygous state, is characterized by significant morbidity. The valine substitution is associated with abnormal stacking of hemoglobin S molecules under a variety of biochemical and physiologic circumstances.19

Clinically, the sickle cell patient may have severe hemolytic anemia, vascular occlusions involving the spleen, kidneys, lungs, retina and central nervous system.
and bones and a variety of other abnormalities secondary to the disease or therapy. Refractory ulcers of the lower leg and episodes of severe back and abdominal pain are frequent.\(^1\) There may also be problems in the reproductive system.\(^8\)

Patients with sickle cell disease have an increased susceptibility to bacterial infections. Streptococcal pneumonia occurs with increased frequency in children with sickle cell disease and is an important consideration. Osteomyelitis is also a significant problem. Bacterial infection accounts for 30 to 40 percent of the mortality in patients with sickle cell anemia.\(^1,7,9\)

There is an increased incidence of glucose-6-phosphate dehydrogenase (G-6-PD) deficiency in the sickle anemia patient. It is possible that this deficiency has an effect on macrophage function and contributes to the increased incidence of infection.\(^1,6\)

There is little doubt that patients with SS disease have abnormal host defense mechanisms. Studies related to abnormalities of granulocyte and macrophage function are available.\(^9\) No data exist which evaluate the function or status of lymphocytes in patients with SS. The purpose of this study was to evaluate lymphocytes in patients with sickle cell disease by (1) measuring lymphocyte blast transformation in response to three commonly used mitogens, phytohemagglutinin-P (PHA-P), concanavalin-A (CON-A) and pokeweed mitogen (PWM) and (2) quantitating peripheral B and T lymphocyte percentages.

**Materials and Methods**

The control group consisted of black, healthy volunteers. The sickle cell group consisted of patients seen at the Hematology Clinic of the Medical University of South Carolina. All sickle cell patients were homozygous as determined by cellulose acetate electrophoresis. No patient was under treatment for or had evidence of an infection at time of the study. No patient in this study suffered from G-6-PD deficiency.

Lymphocyte blast transformation was performed by a modified technique using heparinized whole blood. After a four day incubation with mitogens, PHA-P (1:2,000 dilution), CON-A (2.5 \(\mu\)g per ml) and PWM (1:50 dilution), 1.0 \(\mu\)Ci per ml of \(^3\)H TdR is added for 16 to 18 hours. Cells are then processed as previously described for counting in a beta scintillation counter.\(^3\)

The determination of T and B lymphocyte percentage was performed by a modified method previously described in detail. Briefly, the T-cell population was measured by formation of “E-rosettes” with sheep red blood cells and B-cells were determined by surface immunofluorescence.\(^2\)

**Results**

The control group of 19 females and 1 male had a mean age of 32.0 ± 9.2 years. Sickle cell patients, 14 females and 6 males, had a mean age of 31.5 ± 9.3.

A decreased response to mitogenic stimulation, as measured by lymphocyte blast transformation, was observed in sickle cell disease (SCD) patients as shown in figure 1. The mean stimulation index \(\frac{\text{mitogen treated}}{\text{untreated}}\) was 36.5 ± 57.6 in SCD compared to 98.2 ± 27.1 in normals using PHA-P. With CON-A, SCD had an S.I. of 34.3 ± 39.0 compared to 93.4 ± 27.5 in normal blacks. PWM response showed a decreased S.I. of 19.7 ± 21.2 in SCD compared to 42.3 ± 21.7 in normals.

The quantitative values of B and T lymphocytes are shown in figure 2. Sickle cell patients had a higher percentage of B lymphocytes (17.0 percent ± 3.4) than normals (7.7 percent ± 1.1) and a decreased percentage of T lymphocytes (50.2 percent ± 6.2 compared to 66.2 percent ± 1.7).
Hematologic parameters are shown in table I. These reflect the expected changes in SCD.

Discussion

Sickle cell patients are known to have an increased susceptibility to infections. Speculations as to mechanism relate the possible G-6-PD deficiency and abnormally function of macrophages. None of our SCD patients had demonstrable laboratory evidence of G-6-PD deficiency.

The sickle cell patients had a decreased percentage of T lymphocytes when compared to the normals. T cell deficiency has been correlated with cell mediated immunity. In a previous study, T and B lymphocytes subpopulations of ambulatory black patients with diabetes mellitus was reported. In general, the T-cells were increased. T lymphocyte subpopulations are decreased in patients with sickle cell disease. The biologic and immunologic significance of this observation is not known.

Lymphocyte blast transformation is a reproducible in vitro method of assessing lymphocyte function. The SCD patients in this study had significantly decreased LBT to all mitogens tested. The mechanism for altered LBT in SCD lymphocytes is not known. Studies with diabetics lymphocytes show impaired activity in the hexose monophosphate shunt and this correlated well with decreased LBT. Why should SCD patients have this abnormal response to mitogens? None of the patients studied here have G-6-PD defi-

<table>
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<th>TABLE I</th>
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<tr>
<td>Hematologic Parameters for 20 Patients with Sickle Cell Disease Compared to 20 Normal Controls</td>
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<tr>
<td>Sickle Cell Patients</td>
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<tr>
<td>White blood cell count (x 10^3/mm^3)</td>
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<td>Red blood cell count (x 10^6/mm^3)</td>
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<td>Hemoglobin (g/dl)</td>
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<td>Mean corpuscular hemoglobin (ng)</td>
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<td>Mean corpuscular hemoglobin concentration (g/dl)</td>
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**FIGURE 1.** Stimulation indices for sickle cell disease (n = 20) and black control (n = 20) lymphocytes when incubated with phytohemagglutinin (PHA-F), concanavalin-A (CON-A) and pokeweed mitogen (PWM).
ciency. Further studies in the metabolic pathways of lymphocytes of SCD patients may be fruitful.

Is there a genetically linked, subtle abnormality in some other metabolic pathway that is yet unrecognized in this patient group? It is possible that the aberrations in B and T subpopulations and LBT responses in the lymphocytes of patients with SCD are additional evidence of the problems of immunocompetence noted in this group. Clinically the increased incidence of infections in SCD can now be correlated, perhaps, with laboratory evidence of abnormal function in granulocytes, macrophages and lymphocytes.

References