ORIGINAL ARTICLE

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F cells, fetal hemoglobin levels, lymphocyte subsets, and frequency of crises in sickle-cell disease in Kuwait

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Abstract In vitro, fetal hemoglobin (Hb F) inhibits the aggregation of Hb S, and this may be clinically significant. Alterations in B and T lymphocytes seen in sickle-cell disease may be important in the immune deficit in these patients. We examined 16 patients with repeated crises (6 females and 10 males, age 9.3 ± 3.4 years) and 15 patients with infrequent crises (9 females and 6 males, age 7.1 ± 3.8 years) to determine the relationship between F cells, Hb F levels, lymphocyte subsets, and the frequency of crises. The proportion of CD2 and CD3 lymphocytes was significantly lower (P = 0.015 and < 0.0001, respectively) in both groups of patients than controls. CD4 lymphocytes were significantly lower (P = 0.018) and CD19 significantly higher (P = 0.007) than controls. CD45RO levels in both groups of patients were comparable with matched controls but significantly lower (P = 0.002) than adult values. Hb F levels in patients with and without frequent crises were comparable (P = 0.067). However, F cells in patients with infrequent crises were significantly higher than in patients with frequent crises (P < 0.01). Alteration in the lymphocyte subsets did not correlate with the frequency of crisis in these patients.

Key words Sickle-cell disease · Hemoglobin F · Hemoglobin S · F cells · Lymphocyte subsets

Introduction

The variability in the morbidity of homozygous sickle-cell disease (SCD) is well known. However, the complex interactions responsible for this variability are not understood. Watson [1] first speculated that fetal hemoglobin (Hb F) in newborns with SCD would protect them from the clinical manifestations of the disease. Later, some patients with SCD with high Hb F levels were recorded as showing mild disease [2, 3]. Some studies further revealed that some patients in central India and eastern Arabia had high levels of Hb F and showed mild anemia and reduced clinical severity [4, 5]. However, even with a given genotype, high levels of Hb F do not uniformly confer protection and result in mild disease or reduced clinical severity [6]. The inconsistent effect of high levels of Hb F was further reported in SCD patients in India among carriers of the Arab–Indian haplotype [7]. It was shown that groups of patients carrying this haplotype and comparable levels of Hb F and F cells had different clinical severity. In a study of African Americans, it was suggested that Hb F levels of 8–13% may ameliorate the clinical features in SCD. Other studies suggested that Hb F should be at least 20% before protection is conferred [8], indicating a threshold effect. However, much lower Hb F levels have been demonstrated to have ameliorating effects on pain rates [9].

The possible ameliorating effect of Hb F levels in SCD is further complicated by the inconsistency in interpretation of the clinical manifestations and severity of clinical features in patients. In a recent multicenter study on the effect of hydroxyurea in SCD, treatment with this drug, which is known to lead to increased synthesis of Hb F in most patients with SCD, led to a decrease in painful crisis rates [10]. However, because of the genetic variability found in SCD, unstimulated Hb F levels within a population and between populations may not have a consistent

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effect on the severity of the clinical features of the disease.

Alterations in the B and T lymphocyte populations in patients with SCD have been reported [11–14]. However, the clinical significance of these changes is not yet known. As the clinical manifestations of SCD are highly variable, alterations in lymphocyte subsets may be of clinical significance if these differences correlate with the frequency of crisis or other clinical findings in these patients.

To examine how altered lymphocyte subsets, F cells, and Hb F levels may influence vaso-occlusive painful episodes in SCD, patients were segregated into two groups. One group consisted of patients with frequent painful crises. Patients in this group had shown a pattern of three or more vaso-occlusive painful crises per year in the preceding 3 years and continued to show the same pattern of crises during the course of this study. None of these patients was receiving hydroxyurea. The second group consisted of largely asymptomatic patients and those with fewer than three painful crises per year [10]. A comparison of F cells, Hb F levels, and lymphocyte subsets in these two groups of patients is presented.

Materials and methods

Thirty-one patients with homozygous SCD were included in this study. The patients were divided into those with frequent vaso-occlusive painful crisis (FC) and those with infrequent crisis (InFC). Sixteen patients in the FC group showed three or more vaso-occlusive painful crises per year and required hospital treatment during each crisis. The 15 patients in the InFC group showed less than three vaso-occlusive painful crises per year, with most of the group being asymptomatic. Details on the patient and control groups, including age, gender, and leukocyte and lymphocyte counts, are shown in Table 1.

Specimen collection and processing

Blood specimens were taken into tubes containing ethylene diamine tetraacetic acid (EDTA) and processed as described below. Complete blood-cell counts, reticulocytes, and differential counts were determined using a Coulter MAXM cell counter. Lymphocyte subsets were determined in whole blood without density gradient separation of these cells, as it has been suggested that artifactual differences may arise as a consequence of the separation procedure [15].

Hb F determination

The percentage of Hb F was determined in hemolysates by means of alkali denaturation [16]. In addition, Hb F levels were confirmed using the Helena Hb F QUIPlate immunodiffusion method. In brief, hemolysates were prepared, as outlined in the procedure, by diluting 0.2 ml whole blood with 1.4–2.0 ml purified water. Hemolysates (5 μl) were applied to each well on the immunodiffusion plates, and the plates were incubated at room temperature in a humidified chamber for 24 h. The diameters of the precipitation rings for Hb F standards, controls, and patient specimens were measured. The percentage of Hb F in patients’ blood was determined from a standard curve of percentage Hb F against ring diameter squared. Hb electrophoresis at alkaline pH (8.6) was also used to confirm estimated levels of Hb F.

Detection of F cells by flow cytometry

Hb F-containing erythrocytes (F cells) were analyzed by means of flow cytometry using a modification of the method of Camp-bell et al. [17]. In brief, 10 μl whole blood was washed twice in phosphate-buffered saline (PBS). The cells were centrifuged at 800 g for 5 min. Cells were re-suspended in 1 ml 4% formaldehyde/PBS and kept at room temperature for 1 h. Then, 250 μl 0.05% glutaraldehyde/PBS was added directly to the cells. The cells were mixed and the suspension was left at room temperature for 30 s. The cells were then centrifuged as above and the supernatant removed. The pellet was washed in 2 ml PBS.

After washing, 250 μl of non-fat dry milk/PBS was added to the cells, followed by an incubation at room temperature for 10 min. The cells were centrifuged at 150 g for 5 min and the supernatant removed. The cells were suspended in 500 μl Triton X-100 in PBS/0.1% bovine serum albumin (BSA). A sample (100 μl) of the latter suspension was taken and stained with fluorescein isothiocyanate (FITC)-labeled anti-Hb F monoclonal antibody (mAb; Batches G6177 and G6772 /IRXG 111491 F, Accurate Chemical and Scientific Corp. N.Y.). The antibody/cell suspension was mixed and the suspension was left at room temperature for 30 min at room temperature while sealed from light with aluminum foil. The mixture was centrifuged and the cells washed once with 1 ml PBS. The washed cells were resuspended in 1 ml PBS and analyzed by means of flow cytometry on a Coulter EPICS profile-II flow cytometer. Isotypic controls were treated as outlined, but stained with FITC-labeled IgG not specific for human antigens.

Lymphocyte subsets

Lymphocyte subsets including CD3+, CD4+, CD8+, CD19+, HLA-DR+, CD45RA+, and CD45RO+ cells were determined as previously described [18]. In brief, approximately 0.5 × 10^6/1 cells in whole blood were labeled with 10 μl of the respective fluorescent mAb (Coulter, Hialeah, Fla.). After 30 min of incubation in the dark at room temperature with mAbs, cells were hemolysed and fixed with paraformaldehyde using a Coulter Q-Prep system. Isotypic controls for the mAbs were set up with each investigation. Labeled lymphocytes were investigated on a Coulter EPICS profile-II flow cytometer by selecting the appropriate area on the dot-plot from the forward and side scatter histogram. The percentage of lymphocytes bearing the antigen under investigation was computed in the positive analysis region on single parameter histograms against isotypic controls.

Table 1 Blood cell count, gender, and age distribution in homozygous sickle-cell disease patients with frequent (FC) and infrequent (InFC) crises and in control subjects. Hb hemoglobin

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of cases</th>
<th>Age (years)</th>
<th>Hb (g/dl)</th>
<th>White blood cells (× 10^9/l)</th>
<th>Lymph (× 10^9/l)</th>
<th>Male/female</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbSS (FC)</td>
<td>16</td>
<td>9.3 ± 3.4</td>
<td>9.3 ± 1.2</td>
<td>8.9 ± 3.0</td>
<td>3.1 ± 0.8</td>
<td>10/6</td>
</tr>
<tr>
<td>HbSS (InFC)</td>
<td>15</td>
<td>7.1 ± 3.8</td>
<td>9.5 ± 1.0</td>
<td>7.7 ± 3.2</td>
<td>2.5 ± 0.6</td>
<td>9/6</td>
</tr>
<tr>
<td>HbAA</td>
<td>17</td>
<td>4.5 ± 4.9</td>
<td>12.8 ± 3.8</td>
<td>9.7 ± 1.4</td>
<td>4.3 ± 1.2</td>
<td>8/9</td>
</tr>
</tbody>
</table>