Developmental Aspects of Splenic Function in Sickle Cell Diseases

By Howard A. Pearson, Sue McIntosh, A. Kim Ritchey, Jeffrey S. Lobel, Yolanda Rooks, and Dorothy Johnston

In order to study developmental aspects of splenic function and dysfunction in children with Hb SS disease and other Hb S syndromes, red cells were examined by interference phase-contrast microscopy. In the youngest children studied (<1 yr) the number of red blood cells bearing “pocks” was low (<1%), the same as in normal eusplenic controls. In most children over 8 yr of age, >12% of the red blood cells were pocked, in similarity to what is observed in surgically asplenic individuals. Between 1 and 8 yr of age, many Hb SS children had intermediate numbers of pocked red blood cells, and that suggested hyposplenism. Young children with Hb AS, S-β thalassemia, and S-HPFH were found to have normal splenic function by this technique. Children with Hb SC disease had a moderate degree of splenic dysfunction. This technique correlates with Howell-Jolly body enumeration and 99mTc spleen scans, but it is semiquantitative, and it appears to differentiate the transfusion-reversible “functional” hyposplenism of childhood and the anatomic asplenia secondary to autosplenectomy of later life. Interference phase microscopic enumeration of red cell pocks is a useful and accurate way to assess the onset of splenic dysfunction, which identifies the time of increased susceptibility to overwhelming bacterial infections.

The spleen undergoes a definite sequence of changes in patients with sickle cell anemia (Hb SS disease). During the first few months of life the spleen has normal size and function. As Hb F is replaced by Hb S in the red cells, with resultant onset of a hemolytic process, the spleen becomes palpably enlarged in many patients. Paradoxically, the functioning of the enlarged organ is defective, as indicated by its inability to remove Howell-Jolly (H-J) bodies or radiocolloids from the blood and by its inability to prevent severe bacterial infections. This early “functional” hyposplenism can be temporarily reversed by red blood cell transfusions. During later childhood, progressive vasoocclusion irreversibly infarcts the spleen to a siderofibrotic nubbin, a process designated autosplenectomy.

Recently a noninvasive nonisotopic method has been introduced for evaluation of splenic function by examination of the circulating red cells using interference phase-contrast microscopy (Nomarski optics). With this technique it can be shown that about 20% of the red cells of asplenic persons contain one or more surface indentations or “pocks,” whereas fewer than 1% of the circulating red cells of normal individuals are pocked. The same changes have been noted in patients with Hb SS disease. We have studied red cells of infants and children with Hb SS disease and other sickle hemoglobinopathies using this technique in order to define
the frequency and developmental aspects of splenic dysfunction. Our data suggest that the loss of splenic function in Hb SS disease is a gradual progressive process, whereas splenic function is largely preserved during childhood in other sickle hemoglobinopathies.

Interference phase-contrast microscopy provides a rapid noninvasive method for semiquantitatively assessing splenic function and defining the time of onset of susceptibility to severe bacterial infection in patients with Hb SS disease.

MATERIALS AND METHODS

Red blood cells of 35 normal children 0.5 to 20 yr of age who had no recognized hematologic, hepatic, or splenic disease were studied as eusplenic controls. Twenty-five patients who had been electively splenectomized 1–5 yr previously for hematologic indications such as hereditary spherocytosis or thalassemia major or as a staging procedure for Hodgkin’s disease were studied and were considered anatomically asplenic controls. Patients with these hematologic or oncologic diseases do not have increased numbers of pocked red blood cells prior to splenectomy.

Twenty-eight patients with Hb SS disease ranged from 3 mo to 22 yr of age. A number of these children were diagnosed at birth by electrophoresis of cord blood hemoglobin in our neonatal screening program. None were on chronic transfusion programs, and transfusions are infrequently used in our clinic. Children with sickle cell trait (Hb AS), sickle β (S–β) thalassemia, Hb S hereditary persistence of fetal hemoglobin (HPFH), and Hb SC disease were also studied. Diagnosis was established by cellulose acetate and acid agar gel electrophoresis. Hb F was quantitated by the 1-min alkali denaturation method of Singer et al. and red cell distribution of Hb F was determined by acid elution. Family studies were done in most cases and specifically in those in which HPFH was diagnosed.

A drop of capillary or fresh venous blood was mixed with 0.5 ml of a buffered, pH 7.4, 3% glutaraldehyde solution and examined as a wet preparation with the oil-immersion objective (×1000) of a Zeiss interference phase-contrast microscope with Nomarski optics. Two thousand consecutive red cells were examined for the presence of one or more rounded surface indentations or pocks (Fig. 1). The

Fig. 1. Two pocked red cells from a patient with Hb SS disease. The cell on the right has a large pock and a small pock. The cell on the left has three pocks. Interference phase-contrast microscopy (×1000).
number of pocked red blood cells was expressed as a percentage of the total. Repeated tests on the same specimen showed a variability of ± 1%.

Spleen scans were performed using 15μCi kg^{-1} Tc-sulfur-colloid per kilogram administered intravenously. The abdomen was scanned with a Picker Dynacamera in several projections for extrahepatic uptake of the radionuclide.

RESULTS

Normal and Asplenic Controls

In normal controls the mean percentage of pocked red blood cells was 0.08 ± 0.003% (range 0.0%-0.8%). In the postsplenectomy asplenic group, they averaged 23.4 ± 9.6% (range 12.2%-33%) (Table 1).

Hb SS Disease

In patients with Hb SS disease the percentage of pocked red blood cells increased with advancing age (Fig. 2). The youngest infants we studied had pocked red blood cell percentages that were intermediate between those found in the eusplenic

<table>
<thead>
<tr>
<th>Table 1. Percentages of Pocked Red Blood Cells</th>
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<td>No. of Patients</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Eusplenic</td>
</tr>
<tr>
<td>Asplenic</td>
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Fig. 2. Percentages of pocked red cells in children with Hb SS disease. The ranges of pocked red cells in normal eusplenic controls and in surgically asplenic individuals are indicated by the lower and upper hatched areas, respectively.

Fig. 3. Percentages of pocked red cells compared with levels of Hb F in children with Hb SS disease.
normal and asplenic controls. After 6–8 yr of age the percentages of pocked red blood cells of most children were in the asplenic range (>12%).

There was a general inverse relationship between the level of Hb F and the percentage of pocked red blood cells (Fig. 3). The youngest infants with the highest Hb F levels also had the lowest percentages of pocked red blood cells. There was a definite increase in the number of pocked red blood cells as the level of Hb F fell consistently below 20%.

Fig. 4. Serial percentages of pocked red cells in 3 infants with Hb SS disease.

Fig. 5. Serial levels of Hb F and percentages of pocked red cells are compared with Tc spleen scans in a child with Hb SS disease.
Three young infants were followed serially in order to determine sequential aspects of splenic function in early life (Fig. 4). The percentages of pocked red blood cells in these children were low when they were originally studied at 3–12 mo of age. With passing time, the numbers of pocked red blood cells in these children’s circulations increased to 5%–10%.

Most of the infants in our newborn cohort had 99mTc spleen scans performed at 3–6 mo of age and again when their levels of Hb F fell below 20%. These scans regularly showed normal splenic size and function initially, but later they showed a lack of splenic uptake of the radiocolloid, thus indicating functional hyposplenia.

Sequential studies in one patient permitted comparison among Hb F levels, spleen scans, and the percentage of pocked red blood cells (Fig. 5). At 8 mo of age, when the child was first studied, Hb F was 28%, pocked red blood cells were 1.0% and the spleen scan was normal. Six months later, at 14 mo of age, the Hb F level had fallen to 22.4%, the percentage of pocked red blood cells had increased to 3.7%, and the scan showed reduced splenic uptake of radiocolloid. One month later the Hb F was 18.0%, the pocked red blood cells were 4.8%, and there was virtually no splenic uptake of radiocolloid.

Six older children, 6–11 yr of age were studied on two or more occasions over a period of 9–12 mo. There was little fluctuation in the numbers of pocked red blood cells (Fig. 6).
Table 2. Pocked Red Blood Cells in S Hemoglobinopathies

<table>
<thead>
<tr>
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<th>Number</th>
<th>Age  (yr)</th>
<th>Pocked Red Cells (mean ± SD)</th>
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<tbody>
<tr>
<td>AS</td>
<td>10</td>
<td>1-15</td>
<td>0.1 ± 0.04%</td>
</tr>
<tr>
<td>S-β thalassemia</td>
<td>4</td>
<td>1-11</td>
<td>0.8 ± 0.05%</td>
</tr>
<tr>
<td>S-HPFH</td>
<td>2</td>
<td>1-2</td>
<td>0.5 ± 0.02%</td>
</tr>
<tr>
<td>SC</td>
<td>5</td>
<td>2-10</td>
<td>3.5 ± 1.9%</td>
</tr>
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Restoration of Splenic Function

We had previously demonstrated a return of the spleen’s ability to concentrate radiocolloid after red blood cell transfusions.4 One infant who was studied sequentially had a low number of pocked red cells at 3 mo of age (Fig. 7). By 9 and 10 mo, splenic hypofunction was indicated by increases in the numbers of pocked red cells to 6.5% and 7.8%. Shortly after this, the child developed sepsis due to *Haemophilus influenzae*, type B. An exchange transfusion with normal blood reduced the proportion of circulating Hb S to 24%. Three days after the exchange transfusion the percentage of pocked red blood cells had decreased to 1.6%, and they remained low for more than 2 mo. Since normal transfused red cells acquire surface pocks a short time after their transfusion into an anatomically asplenic recipient, the persistently low levels of pocked red blood cells for more than 2 mo after transfusion indicate restoration of splenic function.11 Three months later the percentage of pocked red blood cells had increased above their pretransfusion level. At this time the 99mTc scan showed no detectable uptake of radiocolloid.

Splenic Function in Other Sickle Hemoglobinopathies

The percentages of pocked red blood cells in infants and children with sickle cell trait, Hb S-β thalassemia, and Hb S herditary persistence of fetal hemoglobin approached or were within the normal range (Table 2). This indicates relatively normal splenic function in these syndromes, and it is consonant with the clinical observation that overwhelming bacterial infections are unusual in patients with these sickle syndromes.

The percentages of pocked red blood cells in a small group of children with Hb SC disease were above the normal range; however, they were lower than those observed in comparably aged children with Hb SS disease.

DISCUSSION

In 1935 Diggs described sequential histologic changes of the spleen in Hb SS disease.1 During early life the enlarged spleens of children with Hb SS disease demonstrate congestion of the splenic pulp by masses of sickled red cells, as well as hemorrhages surrounding the malphigian corpuscles. By late childhood vascular occlusion and infarction are observed, and the spleen becomes atrophic, fibrotic, and siderotic. An enlarged spleen is distinctly unusual in adults with Hb SS disease in the United States.12

Our studies over the past few years have also demonstrated serial changes of splenic function as indicated by spleen scans with radiocolloid and by the presence
of circulating H-J bodies. The usual noninvasive methods for evaluation of splenic function, such as radiocolloid scans, are nonquantitative and do not differentiate between functional and anatomic splenic dysfunction. Since H-J bodies are infrequent (1 per 1000–5000 red blood cells), it is impossible to assess degrees of functional and anatomic splenic pathology. Determination of the percentage of pocked red blood cells by interference phase-contrast microscopy permits better quantitative measurement of splenic function. In the patient with Hb SS disease during the first months of life the number of pocked red blood cells is low. This indicates that the spleen is functioning normally at this time. \(^{99m}\text{Tc}\) spleen scans are normal, and H-J bodies are not observed in the circulating red blood cells.

Toward the end of the first year of life, as the level of Hb F falls consistently below 20\%, a state of functional hyposplenia occurs. In our series of 20 Hb SS infants diagnosed at birth, the earliest time at which functional hyposplenia was documented was 5 mo, and the latest was 36 mo (mean 13 mo).\(^{13}\) This state of functional hyposplenia is characterized by a few H-J bodies in the circulating red cells and \(^{99m}\text{Tc}\) scans that show little or no uptake of radiocolloid. Up to 12\% of the circulating red cells are pocked. During this time, splenic function can be rapidly restored by transfusions of normal red blood cells. Finally, after 6–8 yr of age, anatomic asplenia secondary to autosplenectomy develops. The spleen is small, and the scan shows no spleenic uptake of radiocolloid. More H-J bodies are noted, and the percentage of pocked red blood cells exceeds 12\%. Transfusions are ineffective in restoring splenic function.

It has been estimated that children with Hb SS disease are some 300 times more likely to develop severe bacterial meningitis than children with a normal hemoglobin genotype from the same population.\(^3\) These infections are important contributors to the very high mortality in early life caused by this disease, which may be as high as 25\%–35\%.\(^{14}\) Many factors contribute to this susceptibility, including opsonic deficiencies and other humoral defects.\(^{15,16}\) Splenic dysfunction is doubtless a crucial factor, and it helps explain the temporal pattern of these infections.\(^{17,18}\) Our studies show that interference phase-contrast microscopic enumeration of red cell pocks is a useful and accurate way to assess the onset of splenic dysfunction.

Immunization with polyvalent pneumococcal and \(H.\) \textit{influenzae} polysaccharide vaccines may offer an effective approach for prevention of serious bacterial infections in hyposplenic persons.\(^{19}\) However, these vaccines are probably ineffective in children less than 2 yr of age. The average age of onset of functional asplenia in Hb SS disease is 13 mo, but it may occur as early as 5 mo.\(^{13}\) Protection of the Hb SS child during the interval between when the spleen becomes inactive and when polysaccharide vaccines are effective remains a therapeutic challenge. Penicillin prophylaxis has been suggested, but a controlled study of this practice is necessary to prove effectiveness.

In our cohort of infants with Hb SS disease we have opted for close observation and immediate access to treatment when febrile episodes occur. Five of our 19 infants with Hb SS disease who have been followed from birth for up to 5 yr of age have had six episodes of sepsis and/or meningitis (four due to \textit{staphylococcus pneumoniae}, two due to \textit{H. influenzae}, type B). We have had no deaths or significant morbidity in the group.
REFERENCES

1. Diggs LW: Siderofibrosis of the spleen in sickle cell anemia. JAMA 104:538-541, 1935
17. Powars DR: Natural history of sickle cell disease—the first ten years. Semin Hematol 12:267-285, 1975
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