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Expression Patterns of Fetal Hemoglobin in Sick Cell Erythrocytes Are Both Patient- and Treatment-Specific During Childhood

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Abstract

Background—Treatment-associated fetal hemoglobin (HbF) expression patterns in children with sickle cell disease (SCD) have not been fully described. The objective of this study was to compare HbF expression profiles (HbF and F-cells) in the peripheral blood of pediatric SCD patients receiving hydroxyurea (HU), chronic transfusions (Tx) or no chronic therapy (Ctrl).

Procedure—Peripheral blood samples were collected from SCD patients between 1 month and 21 years of age and immunostained with anti-HbF and anti-HbA antibodies. Erythrocytes containing HbF (F-cells) were enumerated with this dual staining method. HbF was measured using chromatography (HPLC).

Results—Blood from 44 Ctrl patients 4 years of age was compared with that from older children (50 Ctrl, 17 HU, 17 Tx). Among the older children, the percentage of both HbF and F-cells in the Tx group was significantly decreased compared to the control (HbF $5.4 \pm 4.2\%$ vs. $11.0 \pm 7.2\%$, $P = 0.003$; F-cells $30.2 \pm 16.3\%$ vs. $43.8 \pm 20.4\%$, $P = 0.0071$). While the distribution of F-cells was significantly increased in the HU group ($56.3 \pm 17.1\%$ vs. $43.8 \pm 20.4\%$, $P = 0.016$), the increase in HbF was less robust ($14.7 \pm 6.4\%$ vs. $11.0 \pm 7.2\%$, $P = 0.051$). Positive correlations of HbF and F-cell distributions were noted in all groups ($P < 0.0001$ for all groups). In serial samples from individual patients, relatively static patterns of HbF and F-cell distribution were noted.

Conclusion—Pediatric SCD patients possess distinct patterns of HbF switching and silencing in peripheral blood erythrocytes. Thereafter, erythrocyte HbF expression level and distribution are maintained with both patient- and treatment-specific patterns that may be useful for predicting the need or response to HbF-modulating therapy.

Keywords

F-cells; fetal hemoglobin; sickle cell disease

INTRODUCTION

Sickle cell disease (HbSS, SCD) is caused by the substitution of glutamine for valine at the sixth codon of the beta globin gene ($\beta^{6\text{Glu}\rightarrow\text{Val}}$). Upon deoxygenation, sickle hemoglobin (HbS) polymerizes, leading to hemolysis and vaso-occlusion. Clinical manifestations of SCD vary with age and include painful crises, acute chest syndrome, and stroke. Dactylitis is an early clinical sign of SCD that is manifested during infancy when fetal hemoglobin levels decline [1,2]. Children under age five are also at risk for splenic sequestration and overwhelming bacterial sepsis [3,4]. Comparably, school age children are at high risk of stroke [5]. As a result of life long hemolysis and vaso-occlusion, adults suffer end organ damage, including renal failure and pulmonary hypertension [4]. Hemoglobin F ($\alpha_2\gamma_2$; HbF) mitigates the clinical manifestations of SCD by improving hemoglobin solubility and decreasing hemolysis [6,7]. Red blood cells with high levels of HbF (F-cells) survive up to three times longer than sickle cells with low levels of HbF [8]. The preventative effects of HbF on HbS polymerization appear to be concentration dependent; HbF fractions of 20% have been demonstrated to effectively prevent hemolysis in clinical studies as well as in experimental models [1,9-11]. Among infants without hemoglobinopathies, HbF levels in circulating blood fall to <2% within the first year of life [12]. The decline in HbF is less dramatic among infants with SCD [1].

Since HbF protects against SCD-related morbidity and mortality, considerable effort has been made to understand its expression in SCD patients [8]. The Cooperative Study of Sickle Cell Disease (CSSCD) followed more than 3,000 patients ranging from newborns to adults over a 10-year period [13]. Based on this data, normal values for HbF and other hematological values were established for SCD children <5 years of age. Average HbF values ranged from 40.4% in newborn infants enrolled in the CSSCD to 9% in participating 5-year-olds [2]. A more recent cohort study of French infants with SCD measured distribution of HbF [1]. In this study, almost 100% of red blood cells were F-cells in newborns, but this percentage declined to 40% by 2 years of age [1]. Studies in adults with SCD found HbF and F-cells to be comparatively lower, averaging 5% and 33%, respectively [14]. While the HbF patterns in infants with SCD were previously described in detail [1], reports of age-related HbF expression patterns in older children are less robust [15,16]. In addition, comparison of SCD children receiving supportive care versus regular blood transfusions has not been previously reported. Clinically, the absolute level of HbF as well as its distribution among circulating red blood cells are important for reducing or preventing the pathological sequelae of HbS polymerization. We describe the age-related expression patterns of HbF in the peripheral blood of SCD patients from infancy through adolescence and compare HbF expression among those patients receiving supportive care, hydroxyurea, or regular transfusions.

METHODS

Clinical Samples

Patients with HbSS (SCD) between 1 month and 21 years of age and followed in the Children's National Medical Center (CNMC) Sickle Cell Program were eligible to participate in this observational study. Patients treated with daily hydroxyurea (HU) or regular blood transfusions (Tx) for at least 4 months were identified for comparison with control SCD patients who had received neither HU nor regular Tx (Ctrl). The clinical protocol was approved according to the human subjects research guidelines and review of the Institutional Review Boards of CNMC and the National Institutes of Health (NIH). Consent and assent were obtained. Non-hemolyzed discarded peripheral blood samples were acquired and analyzed. Serial samples were collected at clinical visits. In 15 of 17 (88%) Tx

patients, the analyzed blood samples were collected between 25 and 35 days after the last transfusion. In the event that a patient had two or more data points available, the earliest sample was included in this report.

Immunostaining for HbF and HbA

Samples were washed with 0.1% bovine serum albumin in phosphate buffered saline and then fixed with 0.05% glutaraldehyde solution. Cells were washed again and then permeabilized with 1% triton-X (Caltag Laboratories, Burlingame, CA). Immunostaining was performed with antibodies directed against HbF (HbF-PE; Caltag Laboratories) and transfused cells were quantitated by immunostaining with antibodies directed against HbA (HbA-FITC; Perkin Elmer, Norton, OH). Dual staining with both antibodies distinguished transfused and non-transfused F-cells (F-cells, Fig. 1). A minimum of 10,000 gated erythrocytes in all samples was analyzed using a FACS Aria flow cytometer (Becton Dickinson, San Jose, CA). Samples were analyzed within 7 days of collection, and control studies demonstrated equivalent staining patterns on samples analyzed on day 2 versus day 7 after collection (data not shown). Figure 1 illustrates representative flow dot plots of each control and treatment group. Non-transfused F-cells were calculated using the following formula:

$$\frac{F + \text{sickle cells (HbF+HbA-)}}{F + \text{sickle cells (HbF+HbA-)} + F - \text{sickle cells (HbF - HbA-)}}$$

HPLC Analysis

Cellular lysates of all blood samples were analyzed for HbF, HbS, and HbA by high-pressure liquid chromatography (HPLC) using the VARIANT Beta-thalassemia short program from Bio Rad Laboratories (Bio Rad, Munich, Germany). For quantitation of the host's HbF in Tx subjects' blood, the HbF in healthy adult donor blood was estimated to be 1% of the HbA in the transfused blood [17]. Based upon this estimate, the autologously produced fraction of HbF was calculated from HPLC analyses as follows:

$$\frac{\text{Area under HbF curve} - 0.01 (\text{Area under HbF curve})}{[\text{Area under HbF curve} - 0.01 (\text{Area under HbA curve})]} + \text{Area under HbS curve}$$

Statistical Analyses

Unpaired two tailed *t*-tests as well as correlations were performed using ySTAT statistical software (Ming Telecomputing, Inc., Lincoln, MA). *P*-values of <0.05 were considered significant.

RESULTS

One hundred twenty-eight patients were enrolled [17 Tx patients, 17 HU patients, and 94 control SCD patients treated with supportive care (44 SCD controls 4 years of age; 50 SCD controls >4 years of age)]. Table I lists demographic, treatment, and hematological data for the four groups. SCD controls 4 years of age or younger were separated from comparison with the treatment groups in order to age match the control group to the two treatment groups. Patient ages were equivalent in the older SCD control, HU, and Tx groups [mean age 10.9 years, 12.2 years (*P*= 0.17 vs. older SCD control), and 10.9 years (*P*= 0.99 vs. older SCD control), respectively]. Each group had a similar distribution of males and females. Tx patients had received regular transfusions every 3–4 weeks for an average of 32.3 months, and HU patients had been treated for a comparable time (mean 35.1 months, *P*

= 0.77). The mean HU dose was 23.6 mg/kg/day. Hemoglobin levels were equivalent in the HU and older control groups while the Tx group had a significantly higher hemoglobin when compared to the older controls ($P = 0.04$). SCD controls <4 years of age had a significantly lower absolute reticulocyte count (ARC) when compared to the older SCD controls (226.1 ± 106.4 vs. 350.0 ± 140.7 , $P = 0.00002$). HU-treated patients had a significantly lower ($P = 0.0009$) white blood cell count and absolute neutrophil count ($P = 0.024$) and significantly higher ($P = 0.0001$) mean corpuscular volume (MCV) when compared with the older SCD controls.

HbF, F-cells, and F/F-cell analyses for the SCD control group aged 4 years and younger are illustrated in Figure 2. Absolute HbF levels declined sharply during the first year of life. HbF levels averaged 44.3% in the first year of life (range 17.2–95.6%). The decrease in HbF levels continued after the first year of life, albeit more gradually. Average HbF levels in SCD patients between the ages of 1 and 4 years were 23.5% (range 1.5–38.5%). Compared with the absolute levels of HbF, the decrease in the distribution of HbF was more gradual and linear. The range in percentage of F-cells was quite broad during the first year (ranging from 57.6% to 98.2% at 1 year of age). In older children, the percentage of F-cells continued to decrease and the distribution remained broad. Remarkably, only one subject demonstrated the low level of F-cells (<10%) previously reported in infants with HbAA [12]. The F/F-cell levels demonstrated a pattern similar to HbF levels in the first 4 years of life.

The HbF, F-cells, and F/F-cell levels were further examined in the older SCD patients according to the treatment regimen (Fig. 3). In the older SCD control group, HbF and F-cells declined in a linear fashion over time (HbF range 1.8–36%; F-cells range 8.7–99.7%). HbF levels in the Tx group were significantly lower than the older SCD control group ($P = 0.003$), while HbF levels were not significantly elevated in the HU group ($P = 0.051$). Patients receiving HU had a significantly higher ($P = 0.016$) level of F-cells compared to the older control group. Alternatively, patients treated on a chronic transfusion regimen had a significantly lower percentage ($P = 0.007$). No decline in F-cells was appreciated in the Tx group over time. The Tx group had a significantly lower amount of F/F-cell than the older SCD control group ($P = 3.26E - 75$), while the F/F-cell was not significantly different between the HU and control group. The older SCD control and Tx group had a slight decrease in the F/F-cell over time, which was not seen in the HU group.

In children over 4 years old, HbF and F-cell percentages were correlated (Fig. 4). The amount of HbF was positively correlated to the percentage of F-cells in each group studied here [older SCD control ($r = 0.96$, $P = 5.34E - 27$), HU ($r = 0.95$, $P = 5.73E - 9$), and Tx ($r = 0.95$, $P = 6.19E - 9$)]. While the distribution of HbF and F-cells was broad in the control group, the relationship between these two parameters was nearly linear. By comparison, values for the HU and Tx patients were similarly correlated to those in the control group, but the distributions were right- and left-shifted relative to each other.

In addition to single measurements from each subject, serial measurements of HbF and F-cells were performed on six of the patients (Fig. 5). A summary of demographic, treatment, and hematological data for each patient is provided in Table II. The samples were collected under a variety of clinical states including vaso-occlusive crisis during a several month period. Serial samples were collected at a minimum of 1 week intervals. Data from two representative patients from the control, HU, and Tx groups are shown. The two HU-treated patients had higher HbF (range 20–24.8%) and F-cells (range 68.2–86.9%) than the control and Tx patients. The Tx patients had lower HbF (range 1.3–5.4%) and F-cells (range 20.8–37.8%) than both of the other groups, while the children in the older SCD control group had levels between the children in the two treatment groups (HbF range 6.2–9.0%, F-cell range 32.6–40.7%). As seen in Figures 5, the serial values remained clustered within a relatively

narrow range for each subject when compared to the broader range of values measured among the entire group.

DISCUSSION

This study was performed to describe HbF parameters in children with SCD treated with supportive care, hydroxyurea, or regular transfusions. Flow cytometric analyses of red blood cells stained with both anti-HbF and anti-HbA antibodies allowed accurate quantitation of F-cells even among transfused patients. A gap in HbF expression was demonstrated as two separate erythroid populations in the blood (Fig. 1) similar to the pattern demonstrated among patients without SCD [12]. The gapped HbF expression pattern is consistent with silencing of gamma-globin gene expression during the first year of life [18]. In infants without SCD, fetal hemoglobin silencing is largely a post-natal event and is nearly 75% complete by 6 months of age [12]. Healthy 4-year-olds without hemoglobinopathies were found to have ~15% F-cells [12]. By comparison, we show that the distribution of F-cells in SCD children is 60% by 4 years of age. This pattern is consistent with previous reports of elevated F-cell values between 40% and 80% in 2-year-old patients with SCD [1,19]. Thereafter, HbF expression in SCD children was maintained in peripheral blood erythrocytes at levels higher than reported in healthy children [12,20,21]. From years 1 to 20, a slow decline in HbF and F-cell levels was detected with considerable variation between individual patients. Serial sample analysis revealed that individual patients possessed relatively stable HbF and F-cell levels, even during sickle cell crises. By the age of 20 years, the HbF parameters approached the average values seen in studies of adults with SCD [14].

This is the first study to demonstrate significantly different patterns of HbF in the peripheral blood of pediatric SCD patients according to treatment regimen. The percentage of F-cells was statistically increased in the HU patients, and none of the HU patients had <25% F-cells. Average HbF levels in the HU-treated patients approached statistical significance ($P=0.051$) when compared to the older SCD controls. Conversely, the concentrations of HbF and F-cells in the Tx children were distinctly lower than that measured in the control and HU groups. Since the F-cell quantitations required no correction for transfused blood, the lower levels of HbF may be due to biological factors. One possibility is that children with lower HbF expression during childhood were more likely to be offered chronic transfusions as therapy. Alternatively, transfusion itself may reduce the F-cell levels by reducing the level of stressed erythropoiesis [22]. According to this hypothesis, inhibition of erythropoiesis should be accompanied by inhibition of reticulocytosis; no significant reduction was noted in our patients. A third possibility is that transfusion therapy reduces the clearance and increases the half-life of circulating HbS cells. Further examination of decreased endogenous HbF production among chronically transfused SCD patients will be required to determine its potential importance in determining the response to therapy or managing transfusion therapy regimens among this patient group.

Here we describe several distinct patterns for HbF expression in the blood of children with SCD, but there are several limitations in this study. The results were observational, and there was no control for transfusion compliance or frequency of visits. Additionally, compliance to HU was not monitored. Marrow samples, erythropoiesis, and HbF-containing reticulocytes were not examined. Since the control group is much larger than the two treatment groups, true differences between the treatment groups and the control group may not have been detected because a very large treatment effect would be required. Since pre-treatment values were not available for the HU and Tx patients, prospective and longitudinal studies are needed to further elucidate these relationships.

Despite these limitations, the data strongly suggest that HbF expression depends upon the age and therapeutic regimen of the patient. Indeed, a steady-state level of HbF production and distribution may exist for individual patients. A rapid decline in the HbF per F-cell was determined during the first year of life, but there was an unexpectedly high level of variation in HbF expression among older children. A more thorough understanding about how steady-state levels are achieved or modified by genetics and the environment in individual patients during infancy, childhood, and adolescence may provide an approach to predict disease severity. The ability to predict disease severity should assist with efforts aimed toward the prevention of the long-term sequelae, morbidity, and mortality encountered during adulthood [23]. Ultimately, the ability to predict patient-specific HbF expression patterns should lead to individualized therapeutic plans.

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REFERENCES

1. Maier-Redelsperger M, Noguchi CT, de Montalembert M, et al. Variation in fetal hemoglobin parameters and predicted hemoglobin S polymerization in sickle cell children in the first two years of life: Parisian Prospective Study on Sickle Cell Disease. *Blood*. 1994; 84:3182–3188. [PubMed: 7524767]
2. Brown AK, Sleeper LA, Miller ST, et al. Reference values and hematologic changes from birth to 5 years in patients with sickle cell disease. Cooperative Study of Sickle Cell Disease. *Arch Pediatr Adolesc Med*. 1994; 148:796–804. [PubMed: 7519102]
3. Emond AM, Cloois R, Darvill D, et al. Acute splenic sequestration in homozygous sickle cell disease: Natural history and management. *J Pediatr*. 1985; 107:201–206. [PubMed: 4020541]
4. Steinberg MH. Management of sickle cell disease. *N Engl J Med*. 1999; 340:1021–1030. [PubMed: 10099145]
5. Ohene-Frempong K. Stroke in sickle cell disease: Demographic, clinical and therapeutic considerations. *Semin Hematol*. 1991; 28:213–219. [PubMed: 1887247]
6. Franco RS, Yasin Z, Palascak MB, et al. The effect of fetal hemoglobin on the survival characteristics of sickle cells. *Blood*. 2006; 108:1073–1076. [PubMed: 16861353]
7. Platt OS, Brambilla DJ, Rosse WF, et al. Mortality in sickle cell disease. Life expectancy and risk factors for early death. *N Engl J Med*. 1994; 330:1639–1644. [PubMed: 7993409]
8. Franco RS, Lohmann J, Silberstein EB, et al. Time-dependent changes in the density and hemoglobin F content of biotin-labeled sickle cells. *J Clin Invest*. 1998; 101:2730–2740. [PubMed: 9637707]
9. Stevens MC, Hayes RJ, Vaidya S, et al. Fetal hemoglobin and clinical severity of homozygous sickle cell disease in early childhood. *J Pediatr*. 1981; 98:37–41. [PubMed: 6161241]
10. Powars DR, Weiss JN, Chan LS, et al. Is there a threshold of fetal hemoglobin that ameliorates morbidity in sickle cell anemia? *Blood*. 1984; 63:921–926. [PubMed: 6200161]
11. Noguchi CT, Rodgers GP, Serjeant G, et al. Levels of fetal hemoglobin necessary for treatment of sickle cell disease. *N Engl J Med*. 1988; 318:96–99. [PubMed: 2447498]
12. Oneal PA, Gantt NM, Schwartz JD, et al. Fetal hemoglobin silencing in humans. *Blood*. 2006; 108:2081–2086. [PubMed: 16735596]
13. Gaston M, Rosse WF. The cooperative study of sickle cell disease: Review of study design and objectives. *Am J Pediatr Hematol Oncol*. 1982; 4:197–201. [PubMed: 7114401]

14. Charache S, Terrin ML, Moore RD, et al. Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia. Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. *N Engl J Med.* 1995; 332:1317–1322. [PubMed: 7715639]
15. Marcus SJ, Kinney TR, Schultz WH, et al. Quantitative analysis of erythrocytes containing fetal hemoglobin (F cells) in children with sickle cell disease. *Am J Hematol.* 1997; 54:40–46. [PubMed: 8980259]
16. Strouse JJ, Lanzkron S, Beach MC, et al. Hydroxyurea for sickle cell disease: A systematic review for efficacy and toxicity in children. *Pediatrics.* 2008; 122:1332–1342. [PubMed: 19047254]
17. Hoffman, R.; Benz, EJ.; Shattil, SJ., et al., editors. *Hematology: Basic principles and practice.* 5th edition. Churchill Livingstone Elsevier; Philadelphia: 2008. p. 2442
18. Stamatoyannopoulos G. Control of globin gene expression during development and erythroid differentiation. *Exp Hematol.* 2005; 33:259–271. [PubMed: 15730849]
19. Marcus SJ, Ware RE. Physiologic decline in fetal hemoglobin parameters in infants with sickle cell disease: Implications for pharmacological intervention. *J Pediatr Hematol Oncol.* 1999; 21:407–411. [PubMed: 10524455]
20. Chakalova L, Carter D, Debrand E, et al. Developmental regulation of the beta-globin gene locus. *Prog Mol Subcell Biol.* 2005; 38:183–206. [PubMed: 15881896]
21. Wood WG, Bunch C, Kelly S, et al. Control of haemoglobin switching by a developmental clock? *Nature.* 1985; 313:320–323. [PubMed: 2857475]
22. Bozzini CE, Alippi RM, Barcelo AC, et al. The biology of stress erythropoiesis and erythropoietin production. *Ann NY Acad Sci.* 1994; 718:83–92. [PubMed: 8185254]
23. Pashankar FD, Carbonella J, Bazy-Asaad A, et al. Longitudinal follow up of elevated pulmonary artery pressures in children with sickle cell disease. *Br J Haematol.* 2009; 144:736–741. [PubMed: 19055665]

Fig. 1.

Flow cytometric analyses of HbF and HbA. Representative flow cytometric dot plots from (A) a 1-month-old infant with SCD, (B) a 10.2-year-old child with SCD, (C) a 9.7-year-old SCD patient treated with HU, and (D) a 15.4-year-old SCD patient receiving monthly blood transfusions. HbF fluorescence is shown on the *x*-axis, and HbA fluorescence on the *y*-axis. The lower right quadrant illustrates the non-transfused F-cells (HbF+HbA-). The lower left quadrant illustrates sickle cells with no HbF expression (HbF-HbA-). The upper two quadrants indicate the transfused cells (HbF-HbA+ and HbF+HbA+). The bars indicate the level of fluorescence for the isotypic controls.

Fig. 2.

Non-transfused F-cells, HbF, and F/F-cell in SCD control patients 4 years of age. HbF measured by HPLC in (A), non-transfused F-cells (F-cells) measured by flow cytometry in (B) and calculated F/F-cell shown in (C). Each point represents data from a separate patient.

Fig. 3.

Non-transfused F-cells, HbF, and F/F-cell according to age and treatment group. HbF measured by HPLC (**A,D,G**), F-cells measured by flow cytometry (**B,E,H**), and F/F-cell (**C,F,I**) of SCD patients older than 4 years of age. Data from Ctrl (**A–C**), HU-treated (**D–F**), and Tx SCD patients (**G–I**) are provided. Each point represents data from a separate patient, and trend lines are provided for comparison.

Fig. 4.

Relationships between HbF and F-cells according to treatment. HbF values were compared with F-cells in (A) SCD controls older than 4 years of age, (B) SCD patients treated with HU, (C) SCD patients receiving monthly blood transfusions. Each point represents data from a separate patient, and trend lines are provided for comparison.

Fig. 5.

Serial measurements of HbF parameters. Serial measurements of HbF versus F-cell percentages from six separate SCD patients (see Table II) using blood collected over a 2- to 10-month period were studied for comparison. Samples from Ctrl (**A,B**), HU (**C,D**), and Tx (**E,F**) SCD patients are provided for comparison. Arrows denote the samples collected during sickle cell crises.

TABLE I
Demographic, Treatment and Hematologic Data

	SCD controls (<4 yrs of age) n = 44	SCD controls (>4 yrs of age) n = 50	Hydroxyurea (n = 17)	Transfused (n = 17)
Mean age (range)	1.5 yrs (1 mo to 4 yrs)	10.9 yrs (4.6–20.3 yrs)	12.2 yrs (4.5–20.6 yrs)	10.9 yrs (4.8–18.3 yrs)
Male (%)	21 (47.8)	23 (45.1)	7 (41.2)	8 (47.1)
Average length of therapy (mos) (range)	n/a	n/a	35.1 (4.7–79.1)	32.3 (4.2–125.6)
Mean age at start of therapy (yrs) (range)	n/a	n/a	9.4 (2.5–20.1)	7.9 (2.2–17.9)
Hemoglobin (g/dl)	8.7 $\pm$ 1.5 *	8.0 $\pm$ 1.5	8.4 $\pm$ 1.0	8.7 $\pm$ 1.1 *
Absolute reticulocyte count ($\times 10^9$ /L)	226 $\pm$ 106 **	350 $\pm$ 141	265 $\pm$ 123 *	361 $\pm$ 166
MCV (fL)	78.4 $\pm$ 6.8	80.6 $\pm$ 6.9	91.0 $\pm$ 9.3 **	82.7 $\pm$ 4.2
White blood cell count ($\times 10^3$ /mcL)	12.8 $\pm$ 5.2	15.2 $\pm$ 6.5	10.4 $\pm$ 4.3 **	12.8 $\pm$ 5.0
Absolute neutrophil count ($\times 10^3$ /mcL)	4.8 $\pm$ 3.7 **	7.8 $\pm$ 4.9	5.6 $\pm$ 2.8 *	7.3 $\pm$ 4.0
Platelet count ($\times 10^3$ /mcL)	358 $\pm$ 121 **	428 $\pm$ 137	376 $\pm$ 275	357 $\pm$ 117 *
Non-transfused F-cells (%)	81.2 $\pm$ 19.3 **	43.8 $\pm$ 20.4	56.3 $\pm$ 17.1 *	30.2 $\pm$ 16.3 **
HbF (%)	34.5 $\pm$ 21.3 **	11.0 $\pm$ 7.2	14.7 $\pm$ 6.4	5.4 $\pm$ 4.2 **

yrs, years; mos, months.

* $P < 0.05$ compared to the SCD controls >4 years of age.

** $P < 0.01$ compared to the SCD controls >4 years of age.

TABLE II
Demographic, Treatment, and Hematologic Data for Six Patients With Serial Samples

	SCD control (A) ^a	SCD control (B) ^a	HU (C) ^a	HU (D) ^a	Tx (E) ^a	Tx (F) ^a
Age (yrs)	14	8	14	7	13.5	15
Gender	M	M	F	M	M	F
Length of therapy (mos)	n/a	n/a	17.5	59.1	8.8	23.1
No. of measurements	8	6	4	4	8	7
Length of time samples were collected (mos)	4.2	5.3	3	2.5	10.5	7.2
No. of measurements while in crisis	6	4	0	1	0	0
Average hemoglobin (g/dl)	5.8±0.1	7.9±0.7	9.8±0.3	8.2±1.0	9.1±1.1	10.1±0.5
Average absolute reticulocyte count (×10 ⁹ /L)	378±55	362±22	166±39	175±35	218±49	238±123
Average NT F-cells (range, %)	37.0 (33.5–40.7)	35.3 (32.6–39.2)	72.8 (68.2–76.1)	80.8 (74.7–86.9)	25.3 (20.8–28.8)	29.9 (26.0–37.8)
Average HbF (range, %)	7.9 (7.0–9.0)	6.4 (6.2–7.1)	21.3 (20–23.2)	23.0 (21.4–24.8)	2.9 (1.3–4.4)	4.5 (3.9–5.4)

yrs, years; mos, months.

^aLetters correspond to panels in Figure 5.