Increased HbF in Sickle Cell Anemia is Determined by a Factor Linked to the βS Gene From One Parent

By Paul F. Milner, Johanna Döbler Leibfarth, Janet Ford, Betty P. Barton, Hernan E. Grenett, and Fred A. Garver

Members of 7 large families, containing 20 patients with sickle cell anemia (SS), were studied using immunofluorescence to count F cells and a radioimmunoassay to measure small amounts of HbF. In five of these families, one of the sickle cell trait (AS) parents had a much higher HbF and F-cell count than the other; in one family, both parents had a marked increase in HbF and F cells; in the remaining family, HbF and F cells were at borderline values in both parents. Seven of 14 AS siblings, but only 1 of 8 normal hemoglobin (AA) siblings, also had HbF and F-cell counts above the “normal” range. It seems that a factor for increased F cells, linked to the βS gene of one parent, is segregating in these families and is responsible for the greatly increased HbF and F cells in the SS subjects. HbF per F cell in AS parents and siblings was the same as that of normal AA subjects, whereas in the SS offspring it was greatly increased, suggesting that it was the result of marrow hyperplasia associated with their hemolytic anemia. The similarity of this “increased F-cell gene” to heterocellular hereditary persistence of fetal hemoglobin (HPFH), Swiss type, is discussed, and it is suggested that it may control the persistent synthesis of HbF in sickle cell anemia by its presence in early infancy.

In adults, fetal hemoglobin (α2γ2, HbF) persists normally in a small proportion of erythrocytes called F cells.1-3 Synthesis of γ-chains in these cells is probably completed very early in their maturation,4 and HbF constitutes only about 10%–30% of the hemoglobin, or a mean of about 4.4 ± 0.3 pg HbF/F cell.5-8 Population data indicate that, among normal individuals, F-cell frequency has a skewed distribution, with a range of about 0.5%–7.5% and a mode of about 2.5%.3,7 About 3% of Europeans have more than 8% F cells,1 and family studies have indicated that this is inherited as a heterozygous trait for a dominant gene.3 There is also good evidence that the gene is linked to the γδβ-gene cluster and that there is linkage disequilibrium with the βS gene.3,8,9 so that, among subjects with sickle cell trait, there is a higher prevalence of increased F cells than among subjects with a normal hemoglobin genotype.8,10

Among patients with sickle cell anemia (SS), the proportion of HbF in a hemolysate varies from less than 1% to over 20%, and reticulocytes containing HbF (F retics) vary from 2% to 55%.6,11 The levels remain constant over time.8 Although about a quarter of SS subjects have normal amounts of HbF per F cell, in the majority it is increased to about a mean of 8 pg HbF/F cell,11 and in these subjects, there is preferential survival of F cells in the peripheral blood6-11 because of the well-known inhibitory effect on HbS polymerization of additions of HbF. Thus, each or all of these modulations—increased numbers of F cells, increased HbF/F cell, and preferential survival of F cells—may contribute to the ultimate level of HbF in any SS patient. Obviously, if few F cells were produced, preferential survival will only increase the HbF by a modest amount, and this alone can account for the HbF level in most black SS patients. If the F cells contain more HbF, their improved preferential survival might account for further increases in HbF found, and if a greatly increased number of F cells have this property, their survival will provide, not only very high HbF levels, but a lessening of the hemolytic rate and an overall increase in red cell count and total hemoglobin.

Dover et al.12 have noted that SS siblings frequently have similar HbF levels and that F reticulocytes in these patients correlate with numbers of F cells in their parents. Serjeant et al.13 found that 14/16 SS patients with more than 10% HbF had one parent with more than 0.4% HbF, their upper limit of normal, and these workers14 have also observed that the rate of decline of HbF in SS infants over the first 6 yr of life is related to the maximum HbF level in either parent. The data we present here indicate that, in families where SS patients have more than about 10% of HbF, this tendency appears to be inherited as a dominant characteristic linked to the βS gene of one parent.

MATERIALS AND METHODS

Hematologic investigations were carried out by standard methods using a Coulter Model S cell counter. Hemoglobin phenotypes were determined by cellulose acetate electrophoresis, and fractions quantified by DEAE-cellulose chromatography.
Family Studies

Blood from patients, parents, and siblings was subjected to a multiple blood group analysis for red cell surface antigens AB, CDE, Jk⁺Jk⁻, Fy/Fyb, MNSs, and P. Glucose-6-phosphate dehydrogenase (G6PD) isoenzymes were determined by cellulose acetate electrophoresis using commercial reagents (Helena Laboratories, Beaumont, TX). Families in which there was doubt about full sibship were excluded from this analysis.

HbF Measurements

HbF was measured in parents and siblings of SS patients by the alkali denaturation method (AD) of Betke et al.¹⁵ and by a radioimmunoassay (RIA).²⁰ At low levels of HbF, results by the two methods were similar, the HbFₐ₈ being much more reproducible at values below 1% than the HbFₐ₈. In SS patients, the HbF was measured by alkali denaturation²¹ and by a microchromatographic technique (MC)²⁷ using commercially prepared microcolumns (Isolab Inc., Akron, OH). At the high levels of HbF found in some SS patients, the HbFₘ₅ gave values about 20% higher than the HbFₐ₈, but were considered more accurate because they correlated with HbF measured by other chromatographic procedures²⁸ and by values obtained by elution from cellulose acetate strips and high-pressure liquid chromatography (unpublished observations).

F-Cell Measurements

The technique used has been previously described²⁹ and was modified as follows. Anti-HbF, prepared in rabbits and exhaustively absorbed with HbA, as previously described,²⁶ was batch diluted, divided into small aliquots, and frozen. Blood smears were made across frosted circles on immunologic glass slides, allowed to dry for 1 hr, and the excess, outside the frosted circles, wiped away. The smears were fixed in acetone:mechanol (9:1, v/v) for 2 min, washed in phosphate-buffered saline (PBS, pH 7.2, 0.01 M phosphate), allowed to dry, and the anti-HbF serum applied to the smeared areas for 30 min at 37°C. After washing thoroughly with PBS, the smears were stained for 30 min at 37°C with fluorescein-isothiocyanate-conjugated goat anti-rabbit globulin (Calbiochem-Behring Corp., San Diego, CA), and, following a final wash, were mounted in glycerol. The smears were examined under phase-contrast and by ultraviolet light using a Leitz Dialux 20-EB fluorescence microscope equipped with a Ploem's incident illumination system and a 100-W mercury vapor lamp. A Miller square graticule placed in one eyepiece was used to define the fields in which fluorescent cells were counted. When the fluorescent cells were greater than about 75%,
Table 1. Hematologic Results in 20 SS Patients and Their AS Parents

| Kindred E | Fa. 46 0.2 2.0 | F1-1 M 34 | 11.5 ± 0.4 | 3.20 ± 0.16 | 34.1 ± 1.6 | 104.0 ± 3.1 | 35.1 ± 1.0 | 33.6 ± 0.7 | 6.0 ± 1.5 | 2.4 | 15.9 ± 1.6 | 74 |
| Kindred F | Fa. 41 0.9 7.8 | F1-2 M 32 | 10.5 ± 0.5 | 2.68 ± 0.11 | 27.7 ± 0.8 | 112.3 ± 5.1 | 39.0 ± 2.0 | 35.0 ± 0.8 | 9.2 ± 3.2 | 2.7 | 14.7 ± 1.9 | 72 |
| Kindred G | Fa. 40 0.3 2.6 | F1-3 M 24 | 9.8 | 2.84 | 26.2 | 99.8 | 35.7 | 35.8 | 7.9 | 2.6 | 20.6 | 88 |
| Kindred H | Fa. 39 0.4 3.5 | F1-4 M 22 | 10.4 ± 0.6 | 2.70 ± 0.20 | 29.0 ± 2.3 | 103.0 ± 0.8 | 37.1 ± 1.7 | 35.9 ± 1.9 | 8.6 ± 2.0 | 2.7 | 22.6 ± 1.2 | 100 |
| Kindred I | Fa. 38 0.4 3.5 | F1-5 F 21 | 10.5 ± 0.4 | 3.35 ± 0.30 | 30.2 ± 2.4 | 85.7 ± 3.6 | 30.0 ± 1.3 | 34.9 ± 1.6 | 7.6 ± 1.6 | 3.3 | 25.7 ± 1.9 | 96 |
| Kindred J | Fa. 37 0.2 2.6 | F1-6 M 19 | 9.1 ± 0.1 | 2.57 ± 0.11 | 26.0 ± 1.0 | 100.6 ± 2.9 | 35.3 ± 0.9 | 34.9 ± 1.0 | 9.3 ± 3.3 | 2.8 | 14.0 ± 2.8 | 65 |
| Kindred K | Fa. 36 0.1 0.7 | F1-7 F 20 | 10.4 ± 0.1 | 2.92 ± 0.07 | 30.0 ± 0.6 | 102.0 ± 1.5 | 35.6 ± 0.8 | 34.7 ± 0.5 | 6.7 ± 2.7 | 2.1 | 16.6 ± 0.3 | 76 |
| Kindred L | Fa. 35 0.3 12.5 | F1-8 M 20 | 9.4 ± 0.7 | 3.09 ± 0.32 | 28.7 ± 3.0 | 92.0 ± 2.7 | 31.5 ± 1.7 | 34.4 ± 1.0 | 8.8 ± 3.1 | 2.5 | 13.5 ± 3.9 | 62 |
| Kindred M | Fa. 34 0.3 12.5 | F1-9 F 20 | 9.4 ± 0.7 | 3.09 ± 0.32 | 28.7 ± 3.0 | 92.0 ± 2.7 | 31.5 ± 1.7 | 34.4 ± 1.0 | 8.8 ± 3.1 | 2.5 | 13.5 ± 3.9 | 62 |
| Kindred N | Fa. 33 0.3 12.5 | F1-10 M 20 | 9.4 ± 0.7 | 3.09 ± 0.32 | 28.7 ± 3.0 | 92.0 ± 2.7 | 31.5 ± 1.7 | 34.4 ± 1.0 | 8.8 ± 3.1 | 2.5 | 13.5 ± 3.9 | 62 |
| Kindred O | Fa. 32 0.3 12.5 | F1-11 F 20 | 9.4 ± 0.7 | 3.09 ± 0.32 | 28.7 ± 3.0 | 92.0 ± 2.7 | 31.5 ± 1.7 | 34.4 ± 1.0 | 8.8 ± 3.1 | 2.5 | 13.5 ± 3.9 | 62 |
| Kindred P | Fa. 31 0.3 12.5 | F1-12 M 20 | 9.4 ± 0.7 | 3.09 ± 0.32 | 28.7 ± 3.0 | 92.0 ± 2.7 | 31.5 ± 1.7 | 34.4 ± 1.0 | 8.8 ± 3.1 | 2.5 | 13.5 ± 3.9 | 62 |
| Kindred Q | Fa. 30 0.3 12.5 | F1-13 F 20 | 9.4 ± 0.7 | 3.09 ± 0.32 | 28.7 ± 3.0 | 92.0 ± 2.7 | 31.5 ± 1.7 | 34.4 ± 1.0 | 8.8 ± 3.1 | 2.5 | 13.5 ± 3.9 | 62 |
| Kindred R | Fa. 29 0.3 12.5 | F1-14 M 20 | 9.4 ± 0.7 | 3.09 ± 0.32 | 28.7 ± 3.0 | 92.0 ± 2.7 | 31.5 ± 1.7 | 34.4 ± 1.0 | 8.8 ± 3.1 | 2.5 | 13.5 ± 3.9 | 62 |
| Kindred S | Fa. 28 0.3 12.5 | F1-15 F 20 | 9.4 ± 0.7 | 3.09 ± 0.32 | 28.7 ± 3.0 | 92.0 ± 2.7 | 31.5 ± 1.7 | 34.4 ± 1.0 | 8.8 ± 3.1 | 2.5 | 13.5 ± 3.9 | 62 |
| Kindred T | Fa. 27 0.3 12.5 | F1-16 M 20 | 9.4 ± 0.7 | 3.09 ± 0.32 | 28.7 ± 3.0 | 92.0 ± 2.7 | 31.5 ± 1.7 | 34.4 ± 1.0 | 8.8 ± 3.1 | 2.5 | 13.5 ± 3.9 | 62 |
| Kindred U | Fa. 26 0.3 12.5 | F1-17 F 20 | 9.4 ± 0.7 | 3.09 ± 0.32 | 28.7 ± 3.0 | 92.0 ± 2.7 | 31.5 ± 1.7 | 34.4 ± 1.0 | 8.8 ± 3.1 | 2.5 | 13.5 ± 3.9 | 62 |
| Kindred V | Fa. 25 0.3 12.5 | F1-18 M 20 | 9.4 ± 0.7 | 3.09 ± 0.32 | 28.7 ± 3.0 | 92.0 ± 2.7 | 31.5 ± 1.7 | 34.4 ± 1.0 | 8.8 ± 3.1 | 2.5 | 13.5 ± 3.9 | 62 |
| Kindred W | Fa. 24 0.3 12.5 | F1-19 F 20 | 9.4 ± 0.7 | 3.09 ± 0.32 | 28.7 ± 3.0 | 92.0 ± 2.7 | 31.5 ± 1.7 | 34.4 ± 1.0 | 8.8 ± 3.1 | 2.5 | 13.5 ± 3.9 | 62 |
| Kindred X | Fa. 23 0.3 12.5 | F1-20 M 20 | 9.4 ± 0.7 | 3.09 ± 0.32 | 28.7 ± 3.0 | 92.0 ± 2.7 | 31.5 ± 1.7 | 34.4 ± 1.0 | 8.8 ± 3.1 | 2.5 | 13.5 ± 3.9 | 62 |
| Kindred Y | Fa. 22 0.3 12.5 | F1-21 F 20 | 9.4 ± 0.7 | 3.09 ± 0.32 | 28.7 ± 3.0 | 92.0 ± 2.7 | 31.5 ± 1.7 | 34.4 ± 1.0 | 8.8 ± 3.1 | 2.5 | 13.5 ± 3.9 | 62 |
| Kindred Z | Fa. 21 0.3 12.5 | F1-22 M 20 | 9.4 ± 0.7 | 3.09 ± 0.32 | 28.7 ± 3.0 | 92.0 ± 2.7 | 31.5 ± 1.7 | 34.4 ± 1.0 | 8.8 ± 3.1 | 2.5 | 13.5 ± 3.9 | 62 |
fading fluorescence was a problem during the count, so nonfluorescent cells were counted using both light sources. At least 2,000 cells were scanned in each smear. Reproducibility was better than ±10%. All the counts were done by the same observer. The appearance of the smears is shown in Fig. 1. The range of F cells counted in smears from 60 AS subjects between the ages of 15 and 30 are shown in Fig. 2. Blood smears were also examined after acid elution, but these were not used for F-cell enumeration.

RESULTS

Seven families containing 20 SS patients with HbFMC above 10% were studied in detail because we could be reasonably certain, on the evidence of blood group antigens and G6PD isoenzyme patterns, that the offspring were all full siblings. These families were intact at the time of the study, and the 20 patients have been followed as outpatients for several years. Their hematologic details are given in Table 1.

Hematologic values obtained by Coulter Counter, blood smear appearances, F-cell counts, Hb electrophoresis results, and the percentage of HbF in lysates were available for every family member studied. The family pedigrees, hemoglobin phenotypes, F-cell counts, and percent HbF are shown in Fig. 3. It was apparent that, where F cells and HbF were increased, the increases were often greater in AS offspring in the F₁ generation than in either AS parent. The decision to regard an F-cell count in any AA or AS subject as abnormally high was based on the levels reported in the literature,3,7,10 and on our own results in randomly selected subjects between the ages of 15 and 30 yr (Fig. 2), ages comparable to the siblings in the F₁ generation of the families. Among subjects considered to have a gene for increased F cells, the mean F-cell count was 10.6% ± 2.2%, whereas among the other siblings in the F₁ generation, it was 4.7% ± 1.8% (Table 2). In the parental generation, several subjects had F-cell counts only fractionally above 7%, although the mean value among those considered carriers of the increased F-cell gene was 10.8% ± 3.2%.

The most striking finding was that, given our rather arbitrary cut-off value of 7%, one AS parent (in one family both parents) and half the AS offspring had increased F cells, while all the SS offspring (with one exception) had greatly increased F cells and more than 10% HbF. The following brief descriptions of the families (Fig. 3) illustrate this point.

In kindred Wi, 9 of the 11 offspring were available and none have died. The four 55 subjects all have a high level of HbF. Of the 4 AS in generation II, two have HbF and F cells clearly above “normal,” and one of these subjects (11-3) has three children: an AS (111-3) and an SC child (111-4), who also have

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**Fig. 3.** Pedigrees of 7 kindreds showing segregation of the “high F-cell gene” (hatched) linked to the β⁺ locus in one parent.
increased HbF and F cells, and an AA child (III-2) who does not. Patient II-2 has not passed her high F-cell tendency to her AS daughter (III-1), who presumably inherits her $\beta^s$ gene from her maternal grandfather. The remaining AA and AS siblings in generation II have normal F-cell counts. In considering the parents, it is clear that the mother has a slightly increased HbF and borderlone F cells. It appears that the tendency to increased F cells is linked to the $\beta^s$ gene from the mother and has been passed to all four SS and two of four AS offspring.

In kindred E, there is a similar situation. The mother’s blood was studied on several occasions, and among a random group of people, her HbF and F cells would fall in the upper part of the normal distribution. The contrast with her husband, however, is striking, and all four of her SS, and two of three AS, offspring available have increased F cells.

In kindred Wa, the two SS and one AS siblings examined have increased F cells, presumably inherited from the father, although his HbF and F cells are barely above the normal range.

In kindred L, both parents have F-cell counts and HbF levels bordering on the upper limit of normal. The eldest son is probably illegitimate, because his Jk blood group is not compatible with that of his father. There are three SS siblings with HbF levels among the highest encountered in black subjects, and they could be homozygous for an F-cell gene inherited from each parent. But, if this is so, II-7 has to be a heterozygote, and II-1 and II-3 are examples of crossovers of the F-cell gene during parental meiosis.

In kindred D, the mother clearly has increased F cells and has passed this to two of three SS and both AS offspring, but not to the AA offspring. The eldest daughter may be an example of a crossover between the $\beta^s$ gene and the F-cell gene, which appears to have missed her, or possibly her F cells do not survive preferentially. This family is extended; the father has an SS brother with 3.0% HbF, who has four AS daughters, all with normal HbF and F cells. One of the mother’s sisters has an SS granddaughter with 17% HbF.

In kindred G, the mother has increased HbF and F cells, and her two SS daughters have a mean HbF of 16.6% and 20.1%, respectively, but two AA and one AS offspring have not inherited this characteristic.

In kindred K, both parents have an increased HbF and F cells. Two SS offspring have only moderately increased HbF. If the F-cell gene is linked to the $\beta^A$ locus of the father, this could explain its absence in the AS offspring who has inherited the $\beta^A$ gene from the mother and the $\beta^s$ gene from the father.

Data from large families of SS subjects with low HbF are limited, but we have examined both parents of 14 such patients and some of their siblings. The results are summarized in Table 2, where they are compared with the families in which a “high F-cell gene” is segregating.

The calculated HbF per F cell in SS patients and their AS relatives is compared in Table 3. F cells from nonanemic AS subjects have a normal content of HbF, no matter whether this is calculated from HbF measured by alkali denaturation or by RIA. The SS patients have a considerably higher content of HbF per

<table>
<thead>
<tr>
<th>Kindred</th>
<th>Total Examined</th>
<th>SS</th>
<th>AS</th>
<th>AA</th>
</tr>
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<tbody>
<tr>
<td>Increased F cells in one parent</td>
<td></td>
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<td></td>
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<tr>
<td>Wi</td>
<td>9</td>
<td>&gt;50% F cells</td>
<td>2/4</td>
<td>0/1</td>
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<td>E</td>
<td>10</td>
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<td>Wa</td>
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<td>1/1</td>
<td>—</td>
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<tr>
<td>L</td>
<td>6</td>
<td>3/3</td>
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<tr>
<td>D</td>
<td>6</td>
<td>2/3</td>
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<td>G</td>
<td>5</td>
<td>2/2</td>
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<tr>
<td>K</td>
<td>3</td>
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<td>—</td>
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<tr>
<td>Total</td>
<td>42</td>
<td>19/20</td>
<td>7/14</td>
<td>1/8</td>
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<td>Mean F cells (% ± SD)</td>
<td>76 ± 14/23.5</td>
<td>10.6 ± 2.2</td>
<td>4.7 ± 1.8</td>
<td>8.0/3.8 ± 2.0</td>
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<tr>
<td>Mean HbF* (% ± SD)</td>
<td>17.4 ± 3.8/6.5</td>
<td>1.68 ± 0.65/0.44</td>
<td>0.14 ± 0.14</td>
<td>0.73/0.36 ± 0.16</td>
</tr>
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Low F cells in both parents

| 11 families | 26 | 0/14 | 0/10 | 0/2 |
| Mean F cells (% ± SD) | 17.2 ± 7.2 | 3.8 ± 1.6 | 3.6 |
| Mean HbF* (% ± SD) | 4.9 ± 1.8 | 0.36 ± 0.10 | 0.28 |

*HbF measured by microcolumn chromatography in SS subjects and by RIA in AS and AA subjects.

Table 2. Segregation of “High F-Cell Gene” Among Siblings

<table>
<thead>
<tr>
<th>Kindred</th>
<th>Total Examined</th>
<th>SS</th>
<th>AS</th>
<th>AA</th>
</tr>
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<tbody>
<tr>
<td>Increased F cells in one parent</td>
<td></td>
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<tr>
<td>Mean HbF per F cell* (± SD)</td>
<td>4.19 ± 1.84 pg</td>
<td>3.76 ± 1.2 pg</td>
<td>3.85 ± 1.68 pg</td>
<td>7.80 ± 0.97 pg</td>
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*MCH × HbF %

F cells %
F cell. This could be an artifact because of the method used for measuring HbF (microcolumn chromatography), but, on the other hand, the calculated mean HbF per F cell is not very different from that reported by other workers when measured directly using the Katsura method.21

DISCUSSION

The mendelian autosomal dominant inheritance of a factor for increased HbF and F cells linked to the β6 locus, which segregates in the families described here, is probably the same as that described in several families reported in the literature9-22-24 where small increases in HbF and F cells in one AS parent have resulted in much larger increases in SS or S/β-thalassemia offspring, or in other families where a patient with homozygous β-thalassemia and an unusually large amount of HbF has been found to have a parent with increased HbF and F cells.25-27 In both SS and homozygous β-thalassemia, increased HbF synthesis is beneficial in prolonging red cell survival. The gene is probably similar to the Swiss type of heterocellular hereditary persistence of fetal hemoglobin (HPFH), which is found in about 2% of Europeans,1,7 but it may be different from that described by Stamatoyannopoulos et al.,10 where an AS father with 7.8% HbF and 30% F cells had an SS daughter with 20% FAD and 100% F cells, and from the nondeletion heterocellular HPFH described in an English kindred by Weatherall et al.,28,29 where homozygotes have 100% F cells in the absence of hemolytic anemia. Swiss type heterocellular HPFH has been documented in a Polish woman who had a transient increase in HbF to 25% associated with acquired autoimmune hemolytic anemia.30

An obvious difficulty in defining this gene is that there may be an age-related diminution in its effect, similar to that found in baboons,31 making it difficult to assess whether a parent has the gene or not. Zago et al.3 had the same experience when studying the segregation of Swiss type HPFH, and recently it has been shown that, in normal people, HbF does decrease with age.32 It is not clear whether the high levels of HbF and F cells reported for the majority of SS patients among the Shiite Arabs33-35 can be accounted for by the same process as described here. The high level of consanguinity and the increased F cells in many AS subjects in that population would suggest that an F-cell gene with a high frequency, dominant in all SS offspring, would result in a high frequency of SS with increased HbF. However, the levels of HbF in Shiite SS and S/β-thalassemia patients are, on the whole, higher than those reported here, and our patients are among those at the extreme upper range of HbF for black SS patients.

Lastly, there remains the question of those SS patients with intermediate levels of F retics and F cells, who are not entirely explained by preferential survival of normal numbers of F cells. Do they also have an F-cell gene, less well expressed and not detectable in their parents by biochemical or morphological methods? The recent investigations of Mason et al.14 suggest that there is a relationship between the rate of disappearance of HbF in SS infants, their HbF at 1 yr of age, and the level of HbF in one parent, whether this be outside or within the normal range.

We looked at the distribution of HbFMC among a population of 120 male and 154 female SS patients over 15 yr of age (Fig. 4). These results are based on the mean of several estimations for each patient over several years. To see what effect total Hb level has on this distribution, we converted each percent HbF to an absolute HbF (g/dl), using the hemoglobin result on that blood sample, and recalculated mean HbF (g/dl) for each patient. The resulting distribution curve is shown in Fig. 4B. While this manipulation accentuates the fact that most of these patients have very little HbF, it does not divide the population into two groups, nor can the distribution be explained by a three-group mode, as would be expected if a recessive gene for heterocellular HPFH was segregating, with partial expression in heterozygotes.

There is a lot of evidence from studies in both baboons13,36-38 and humans39,40 that erythropoietic stress produced by anemia induces increased γ-chain synthesis in early erythrocyte precursors. In both baboons and humans,40 some subjects respond with much higher levels of HbF than others, and this appears to be genetically determined. These studies have been done on animals with induced hemolytic or iron-deficiency anemia.37 Adult humans recovering from iron deficiency39,40 or marrow repression caused by cyclophosphamide,38 and on children over 6 mo old recovering from transient erythroblastopenia39,40 or aplastic anemia.5 In sickle cell anemia, hemolysis commences within the first month of life, and the resultant erythropoietic stress would be expected to increase γ-chain synthesis to some extent. In fact, in the majority of SS infants, this response to increasing hemolysis is negligible, and it is mainly because of the relatively longer survival of the few F cells produced that the F-cell count and HbF in peripheral blood is increased. The possession of a factor for persistence of γ-chain synthesis in a greater number of red cell precursors presumably allows for augmentation of this compartment when hemolytic anemia begins early in postnatal life. Persistent reticulocytosis in our patients

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is evidence of continued hemolysis, presumably because, even when F-cell counts are high, the HbF in some cells is insufficient to prevent sickling and premature senescence. These patients are also distinguished by an increased average level of HbF per F cell, which may be the result of an expanded, stressed erythron. Baboons made anemic by hemolysis and then maintained at a hematocrit of about 20% by phlebotomy, and subjected to further erythropoietic stress by hypobaric conditions, maintain high F-cell counts and acquire a higher HbF per F cell.41 To maintain this state, however, they must be kept in a severely anemic or hypoxic condition, with reticulocyte counts of greater than 50%. The mean reticulocyte count in our patients was 9.1% (± SE 4.5%), yet they maintain a mean HbF of 16.9% (± SE 1.0%) and F cells of 74% (± SE 4.0%). This is a very different situation to that in the experimental baboons.

Among AS subjects (Fig. 2), it could be that those with 10%-13% F cells are homozygous for a gene controlling increased F-cell production, in which case those with 7% F cells might be heterozygotes. Among the homozygotes, one of the genes would have to be associated with the chromosome carrying the β^s locus, and half of the AA offspring of such subjects should have increased F cells, as well as half the AS offspring. The data presented here (Fig. 3), and the frequency of increased F cells among AA and AS subjects tested at random, do not support this concept. If the gene for increased F-cell production is in disequilibrium with the β^s locus, however, homozygotes for it would be more common among SS subjects, but both AS parents of such subjects would be expected to have increased F cells, as would all their AS offspring. The present data do not support this, except possibly in family L. It could be that inheritance of two genes for increased
F-cell production in trans is responsible for the very high HbF levels reported in some black and most Shiite Arab SS subjects. The levels of HbF in most of the SS subjects reported here, however, appear to be due to heterozygosity for an increased F-cell gene linked to the β5 locus of one parent.

It has recently been found that treatment of anemic baboons and human β-thalassemia with DNA methylation inhibitor, 5-azacytidine, causes a great increase in α-chain synthesis, which is associated with an increase in F cells. In the anemic baboons, there was an increase in HbF per F cell, and the response was greatest in genetically high F-cell responders to phlebotomy alone. Such animals have been shown to have higher resting HbF levels.

In a sickle cell anemia patient, 5-azacytidine treatment caused F reticulocytes to increase from 10% to 52%. This has been associated with demethylation of DNA associated with γ-globin genes recovered from bone marrow erythroid cells. Heterocellular HPFH of different degrees may be caused by mutations in γ-globin gene associated DNA, which inhibits the methylation of these areas in postnatal life. Linkage to the β5 locus could have come about by natural selection; first, because the ability of the β5 homozygote to make more F cells would have favored survival to reproductive age, and second, because β5 heterozygotes with increased F cells might also have a marginal survival advantage in a malarious environment.

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Increased HbF in sickle cell anemia is determined by a factor linked to the beta S gene from one parent

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