CONCISE REPORT

The Cellular Basis for Different Fetal Hemoglobin Levels Among Sickle Cell Individuals With Two, Three, and Four Alpha-Globin Genes

By G.J. Dover, V.T. Chang, S.H. Boyer, G.R. Serjeant, S. Antonarakis, and D.R. Higgs

Fetal hemoglobin (HbF) levels vary widely among individuals with sickle cell anemia (SS). Previous studies have suggested that HbF levels in SS individuals with alphathalassemia (two or three functional alpha-globin genes) are lower than HbF levels in SS individuals with four normal alpha-globin genes. Using immunocytochemical techniques, we studied F cell production as measured by %F reticulocytes, the amount of HbF per F cell, and the preferential survival of F cells versus non-F cells in 51 subjects with four alpha genes, 32 subjects with three alpha genes, and 18 subjects with two alpha genes. Comparison between alpha-globin gene groups was performed for the total sample as well as for a subset of 82 individuals who had replicate samples and a further subset of 39 age-matched individuals. %HbF levels were 6.8, 4.9, and 4.5 percent for the total four-, three-, and two-alpha-globin-gene groups, respectively. The percentage of F reticulocytes, percentage HbF per F cell, and the enrichment ratio (% F cell/% F reticulocytes) did not change significantly with alpha-globin gene number. Moreover, no correlation existed between alpha-globin gene number and the absolute number of F cells in any group studied. However, there was a strong inverse correlation (r = −0.407, P = .0001) between non-F cell levels (1.7 ± 2.2 ± 5, 3.0 ± 1.0 x 10\(^{12}/L\)) and decreasing alpha-globin gene number. These data suggest that falling HbF levels among SS individuals with lessened numbers of alpha-globin genes reflect prolonged survival of non-F cells and are not due to intrinsic differences in F cell production or in the amount of HbF per F cell. The improved survival of non-F cells in SS alpha-thalassemia is presumed to be due to the lower MCHC observed in such individuals.

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From the Department of Pediatrics and Medicine, The Johns Hopkins University School of Medicine; The Howard Hughes Medical Institute, Laboratory for Human Biochemical Genetics, Baltimore; and the Medical Research Council Laboratories, University of West Indies, Kingston, Jamaica.

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Address reprint requests to G.J. Dover, Johns Hopkins University School of Medicine, Traylor Bldg 907, 720 Rutland Ave, Baltimore, MD 21205.

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All samples of blood were obtained from venous blood collected in EDTA. Peripheral blood reticulocyte counts were obtained using conventional new methylene blue staining, and red cell indices were performed using an electronic cell counter (Coulter ZBI 6). Alpha-globin gene number was determined or re-determined, in cases of two-alpha-globin gene subjects, by DNA restriction endonuclease analysis utilizing DNA obtained from peripheral blood leukocytes. Percentage of F reticulocytes was assayed by radial immunoprecipitate reactions. F cell counts were performed either by the above procedure or by analysis of fixed red cells with a monoclonal anti-HbF antibody. Percentage of HbF was determined by alkaline denaturation techniques.

Table 1 summarizes the hematologic data for the overall group. No significant correlations were found between alpha-globin gene number and F enrichment ratios, F reticulocytes, or the percentage of HbF per F cell. The remaining hematologic parameters were highly correlated with alpha-globin gene groups: Picograms of HbF per F cell, percentage of F cells, percentage of HbF, MCHC, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and percentage of reticulocytes; all decreased significantly with decreasing alpha-globin gene number. In contrast, hemoglobin levels and red cell number increased with decreasing alpha-globin gene numbers.

Table 2 provides a comparison of the absolute numbers of F cells and non-F cells in SS patients partitioned according to alpha-globin gene number. Although the percentage of F reticulocytes (Table 1) is not correlated with alpha-globin gene number, the absolute numbers of F reticulocytes (Table 2) significantly decrease as the alpha-globin gene number falls. This decrease is due to the significant decrease in the total reticulocyte percentages noted in Table 1. Non-F reticulocyte levels seem to fall with decreasing alpha-globin genes but not significantly so. Although absolute F cell numbers were not correlated with alpha-globin gene numbers, the absolute number of non-F cells per liter was negatively correlated with decreasing alpha-globin gene. The upshot is that alpha-thalassemia is associated with prolonged survival of non-F cells. The outcome is the same when analysis like that in Table 2 is applied to either mean values from 82 subjects who had repeat tests or to 39 age-matched subjects.

### RESULTS

Table 1 summarizes the hematologic data for the overall group. No significant correlations were found between alpha-globin gene number and F enrichment ratios, F reticulocytes, or the percentage of HbF per F cell. The remaining hematologic parameters were highly correlated with alpha-globin gene groups: Picograms of HbF per F cell, percentage of F cells, percentage of HbF, MCHC, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and percentage of reticulocytes; all decreased significantly with decreasing alpha-globin gene number. In contrast, hemoglobin levels and red cell number increased with decreasing alpha-globin gene numbers.

### DISCUSSION

The intent of this paper was to analyze the origins of differences in HbF levels among individuals with SS disease who have different numbers of alpha-globin genes. Like some previous investigators, we found a significant inverse correlation between the percentages of HbF and the number of alpha-globin genes. In order to determine the origins of these differences, we considered the fact that HbF is discontinuously distributed among red cells in these individuals.

We analyzed the three major variables that contribute to the percentage of HbF levels found in peripheral blood. The first of these, i.e., the percentage of F reticulocytes, was not different among the three alpha-globin groups studied (Ta-
HbF LEVELS IN SS DISEASE—ALPHA THALASSEMIA

Table 2. Absolute Number of F Cells and Non-F Cells in SS Individuals and Varying Numbers of Alpha-Globin Gene Loci (Mean ± SD)

<table>
<thead>
<tr>
<th>Alpha-Globin Genes</th>
<th>F reticulocytes* (x 10¹¹/L)</th>
<th>F cells (x 10¹¹/L)</th>
<th>Non-F reticulocytes* (x 10¹¹/L)</th>
<th>Non-F cells (x 10¹¹/L)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>.056 ± .032</td>
<td>.051 ± .035</td>
<td>.043 ± .030</td>
<td>.231 ± .023</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>.93 ± .50</td>
<td>.84 ± .57</td>
<td>.96 ± .57</td>
<td>.129 ± NS</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>.23 ± .13</td>
<td>.21 ± .09</td>
<td>.20 ± .12</td>
<td>.106 ± NS</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.70 ± .17</td>
<td>2.20 ± .49</td>
<td>2.95 ± 1.0</td>
<td>-.407 ± .001</td>
<td></td>
</tr>
</tbody>
</table>

*Absolute number of F or non-F reticulocytes = (RBC) x (reticulocyte percent) x (percentage of F or non-F reticulocytes).
†Absolute number of F or non-F cells = (RBC) x (percentage of F cells or non-F cells).

ble 1). However, the absolute number of F reticulocytes decreased significantly with falling alpha-globin gene number, the level in two-alpha-globin-gene individuals being 77% of that in four-alpha-globin-gene individuals (Table 2). In the absence of significant between-group differences in the percentage of F reticulocytes (Table 1), we presume that the fall in their absolute number is due to a concomitant and significant decrease in overall reticulocyte production (Table 1). These changes would not be expected to have an appreciable effect on Hbf levels, since F reticulocytes, in themselves, represent only a few percent of all Hbf-bearing cells. While there were significant differences in the second variable affecting Hbf production, ie, picograms of Hbf per F cell, among the alpha-globin gene categories, there was a parallel fall in the MCH of F cells. Thus no change developed in the actual percentage of Hbf per F cell.

The third variable that affects Hbf levels is the difference in survival of F cells versus non-F cells in the peripheral blood. This difference in preferential survival was measured two ways. As assessed by comparison of enrichment ratios (% F cells/% F reticulocytes), a decline in enrichment ratios with decreasing alpha-globin gene number (Table 1) was observed. However, the magnitude of this change was not statistically significant. The difficulty with such analysis of enrichment ratios is that it is perturbable by known variation in each of the two estimates, F reticulocytes and F cells, that contribute to it. Consequently, such estimates are less precise than the measure of absolute numbers of non-F cells which utilize only one Hbf-centered variable. Here, a highly significant inverse correlation in the number of non-F cells per liter between groups of individuals with decreasing alpha-globin gene number was observed (Table 2). Since non-F reticulocytes did not change between groups, the higher concentration of non-F cells in the two- and three-alpha-globin-gene groups indicate that non-F cells survive longer in SS individuals with alpha-thalassemia in comparison to SS individuals with four alpha-globin genes.

Why do non-F cells last longer in SS individuals with alpha-thalassemia? We suppose that it is the indirect outcome of a lessened MCHC associated with alpha-thalassemia (Table 1).1-3 Sickle hemoglobin (Hbs) polymerization is highly dependent upon Hbs concentration within the red cell.4 Recently, Brittenham demonstrated that intracellular Hbs polymer levels, calculated on the basis of MCHC and Hbs concentration, accounted for at least 80% of the variation in hemoglobin levels among different SS syndromes.5 Alpha-thalassemia leads to decreased MCHC in all cells6 and to a decreased proportion of dense irreversibly sickled cells.6 The dense cell fraction (MCHC > 37 g/dL) has two hallmarks: it contains very few F cells and exhibits shorter survival than less dense cells.6 A decrease in the proportion of dense cells should therefore lead to a relative decrease in the difference between F cell survival and non-F cell survival. The fact is that the majority of non-F cells in SS individuals with two-alpha-globin genes have MCHC that are near normal.4 Consequently, in this setting, one would expect that non-F cells with near-normal MCHC would last longer than they do when MCHC is elevated. Thus, in these respects, our data indicating prolonged survival of non-F cells in SS individuals with two and three alpha-globin genes are consistent with previous reports.

In summary, differences in Hbf levels among SS individuals with different numbers of alpha-globin genes are not due to differences in F cell production or percentage of Hbf per F cell. The difference is due to the relatively prolonged survival of non-F cells in alpha-thalassemic SS individuals compared to non-F cells in SS individuals with four alpha genes. We speculate that previously demonstrated declines in MCHC among SS alpha-thalassemic individuals account for this prolonged survival.

REFERENCES

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The cellular basis for different fetal hemoglobin levels among sickle cell individuals with two, three, and four alpha-globin genes

GJ Dover, VT Chang, SH Boyer, GR Serjeant, S Antonarakis and DR Higgs