CORRESPONDENCE

VARIATION OF HbF AND F-CELL NUMBER WITH THE G-γ Xmn I (C-T) POLYMORPHISM IN NORMAL INDIVIDUALS

To the Editor:

In normal adults the synthesis of fetal hemoglobin (HbF) is reduced to very low levels, usually accounting for less than 0.5% of total hemoglobin. The synthesis is restricted to a subpopulation of erythrocytes defined as F cells¹ and generally there is a good correlation between the amount of HbF and the number of F cells. However, the measurement of HbF by the alkali denaturation procedure² is not consistently reproducible particularly in the very low range, and thus the determination of the number of F cells is the parameter of choice in conditions associated with a modest increase of HbF, such as heterocellular hereditary persistence of fetal hemoglobin (HPFH).³

These low levels of HbF are heterogeneously distributed among the F cells. Using a fluorescence-activated cell sorting (FACS) technique after labeling intracellular HbF with a monoclonal antibody, we observed a 10-fold variation in the amount of fluorescence emitted by cells scored as positive within each sample. Therefore, it is possible that minor variations in the synthesis of HbF, not easily detectable by direct measurement of the HbF itself, may indirectly influence the evaluation of the percentage of F cells by shifting the distribution of positive cells across the threshold for detection.

Among the factors known to influence HbF production are a number of rare mutations in the promoter region of the G-γ and A-γ genes, which are associated, in the heterozygous state, with HbF values of 2% to 20%.⁴ A common sequence variation (C-T) at position -158 upstream of the G-γ gene is also thought to influence HbF production, but its effect on HbF levels is evident only in conditions characterized by severe erythropoietic stress⁵ while it appeared to have no influence in normal individuals.⁶

We decided to re-evaluate its possible influence on the synthesis of HbF in hematologically normal individuals, using F-cell number as a parameter. F-cell number was determined in 138 healthy blood donors by immunofluorescence staining of fixed blood films with a monoclonal anti-γ chain antibody. In a subset of subjects F cells were also assessed by FACS after staining of fixed erythrocytes in suspension with the same antibody.⁷

Erythrocytes were washed in phosphate-buffered saline (PBS) before fixation in 4% formaldehyde in PBS for 20 minutes at room temperature. Red blood cell membranes were permeabilized by treatment with 50% acetone in water (2 minutes), neat acetone (5 minutes), and 50% acetone in water (2 minutes) at -20°C. After washing in PBS, the permeabilized erythrocytes were incubated with the anti-γ chain antibody for 20 minutes at room temperature and, after washing in PBS, with an FITC conjugate antimouse IgG for 20 minutes. The cells were then washed and resuspended in PBS for FACS.

F-cell percentage, as assessed by microscopic scoring of 2,000 cells ranged from 0.3% to 11.4% (Fig 1) with a distribution in good agreement with that reported in the Japanese population.⁸ The previously proposed cut-off point of 4.4% F cells⁹ fitted our distribution well. Of the subjects, 89.2% had F cells below 4.3%, and 10.2% had F-cell percentages scattered between 5.5% and 11.4%. We observed that, while values obtained by microscopy and FACS were generally in good agreement (r = 0.87, P < .001), the correlation appeared to be slightly erratic at very low percentages of F cells scored by microscopy, with consistently higher values obtained by FACS. This is probably due to the heterogeneity in the distribution of HbF within F cells, the FACS procedure being more sensitive in detecting F cells containing very low amounts of HbF.

The sequence at position -158 to the G-γ gene was assessed by Xmn I restriction of a 670-bp amplified DNA sequence from the promoter region of the G-γ gene. Fifteen subjects with increased F cells (high F-cell group, HF) and 16 subjects with F cells lower than 4.4% (low F-cell group, LF) were analyzed. Eight individuals in the LF group have F cell less than 1% (very low F-cell group = VLF). In all of these subjects HbF levels were measured by alkali denaturation.⁹ The G-γ Xmn I polymorphism and HbF values in HF, LF, and VLF groups are reported in Table 1. There is a significant difference (P < .001) in the Xmn I status between the two groups with high and low F cells. Thirteen of 15 individuals in the HF group are homozygous or heterozygous for the presence of the Xmn I site, while in the LF group only 5 of 16 are heterozygous for the presence of the site. Furthermore, while only 2 of 15 subjects in group HF are G-γ Xmn I +/+ , this pattern is found in 11 of 16 and 7 of 8 in groups LF and VLF, respectively.

The association between the presence of F in position -158 to the G-γ gene and an increased production of HbF was originally recognized in conditions characterized by erythropoietic stress such as severe β-thalassemia and sickle cell anemia,¹⁰ while no conclusive evidence for its influence on HbF levels was available in heterozygous β-thalassemia and in normal individuals.¹¹ Our data indicate that the presence of the C-T variation at position -158 in the G-γ gene promoter not only affects the G-γ/A-γ ratio of hematologically normal individuals,¹² but also increases the actual production of HbF in those cells that maintain the potentiality to do so in adult life. However, the effect appears to be modest. This

Table 1. HbF and Status of the -158 G-γ Xmn I Restriction Site in Hematologically Normal Individuals With High (HF), Low (LF), and Very Low (VLF) Numbers of F Cells

<table>
<thead>
<tr>
<th></th>
<th>Xmn I</th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>F Cells (%)</td>
<td>HbF (%)</td>
<td>+/+</td>
<td>+/-</td>
<td>-/-</td>
<td>VLF</td>
</tr>
<tr>
<td>HF</td>
<td>&gt; 4.4</td>
<td>0.79 + 0.27</td>
<td>3</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>LF</td>
<td>&lt; 4.4</td>
<td>0.49 + 0.13</td>
<td>0</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>VLF</td>
<td>&lt; 1</td>
<td>0.34 + 0.09</td>
<td>0</td>
<td>1</td>
<td>7</td>
</tr>
</tbody>
</table>
variation in the number of F cells with the $Xmn\ I$ status is likely to be an indirect effect related to the amount of HbF produced by the individual F cells. If the number of F cells and the amount of HbF actually produced by each F cell are under separate controls, when more HbF is produced per cell a higher number of F cells will reach the threshold for detection; on the other hand, F cells that produce very little HbF may escape detection, especially by microscopic immunofluorescence. The modest effect of the G-$\gamma Xmn\ I$ polymorphism on the synthesis of HbF may be of minor consequence in normal individuals, but certainly not irrelevant in the diagnosis and genetic analysis of heterocellular HPFH, which is thought to be determined by a locus distinct from the $\beta$-globin gene cluster but whose phenotypic expression is clearly modified by factors associated with the cluster.

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REFERENCES

Variation of HbF and F-cell number with the G-gamma Xmn I (C-T) polymorphism in normal individuals [letter]

M Sampietro, SL Thein, M Contreras and L Pazmany

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