Erythropoiesis During Recovery From Iron Deficiency: Normocytes and Macrocytes

By David Bessman

In 26 patients with severe iron deficiency and microcytic anemia (MCV < 70 fl), serial red cell size distribution histograms (erythrograms) were taken before and during iron therapy. Initially all patients had a single population of red cells, all microcytes. With the first reticulocytosis after iron therapy, a new population of cells appeared, larger in volume than the original. In 23 of 26 patients the new population of cells was of normal size (82–96 fl). In 3 of 26, the new population was macrocytic (MCV > 98 fl). Of these 3, 1 had folate deficiency; after folate was given, normocytes were produced. The other 2, both taking phenytoin and al heavy alcohol user, had persistent macrocytosis despite folate administration. Erythrograms allowed quantitative, rapid evaluation of erythropoietic response to iron repletion. Abnormal macrocytic responses could be identified and seemed to occur with some frequency.

CORRECTION OF IRON-DEFICIENCY ANEMIA results in accelerated erythropoiesis with brisk reticulocytosis in 3–8 days and a subsequent increase in red cell mass.1 The newly produced red cells reflect the bone marrow’s response to iron repletion. However, in the first weeks after iron repletion, pretherapy microcytes remain predominant in the peripheral blood. Routine red cell indices, reflecting the aggregate of old and new cells, therefore, are little help in the quantitative evaluation of the new cells. The peripheral smear affords only a subjective impression of the new cells, again complicated by the presence of old cells. Precise identification of the new red cells would allow an early indication of normal or abnormal marrow response. Erythrograms, quantitated distribution curves of red cell size,2,3 allow identification of differentiated subpopulations of red cells. In this study, erythrograms have demonstrated the size of red cells produced in response to iron repletion, and have revealed some abnormal, macrocytic responses.

MATERIALS AND METHODS

Of all patients admitted to Los Angeles County-USC Medical Center between 8/1/75 and 9/1/76, 342 had marked microcytosis, i.e., MCV of less than 70 fl. Of these, 240 had iron deficiency with serum iron saturation less than 15%. Of the 240, 26 met the following requirements: no transfusion or hematinic therapy in the previous 4 mo; no transfusion during the present study; erythrograms available before therapy and at least 1 wk after beginning iron therapy; serum iron, iron binding capacity, folate, and vitamin B₁₂ levels drawn before therapy; hemo-
Other admission values: iron saturation 8%, serum folate 8.6 ng/ml, serum B12 992 pg/ml.

*Therapy: ferrous sulfate, 300 mg per os three times a day beginning 2/23/76, ending 9/28/76.

Table 1. Patient J.L.

<table>
<thead>
<tr>
<th>Fig.</th>
<th>Date</th>
<th>Hemoglobin (g/dl)</th>
<th>MCV (fL)</th>
<th>Neutrophil Lobe Count</th>
<th>Reticulocytes (%)</th>
<th>Therapy*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2/22/76</td>
<td>8.6</td>
<td>63</td>
<td>63</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B</td>
<td>2/29/76</td>
<td>8.9</td>
<td>63</td>
<td>65</td>
<td>—</td>
<td>1.6</td>
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<tr>
<td>C</td>
<td>3/10/76</td>
<td>10.8</td>
<td>63</td>
<td>73</td>
<td>92</td>
<td>2.1</td>
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<tr>
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<td>64</td>
<td>77</td>
<td>93</td>
<td>3.16</td>
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<tr>
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<td>64</td>
<td>81</td>
<td>90</td>
<td>3.15</td>
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<tr>
<td>F</td>
<td>10/2/76</td>
<td>12.3</td>
<td>92</td>
<td>92</td>
<td>3.09</td>
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</table>

Fig. 1. Patient J.L., normocytic response. Solid line, patient; dotted line, normal subject. See Table 1. Ordinate, relative frequency (%); abscissa, cell volume (fL).
RESULTS

In each of the 26 patients the initial blood count showed anemia and microcytosis, iron saturation was reduced, and the initial erythrogram showed a single population of microcytes. A new population of larger cells appeared concurrent with the onset of reticulocytosis following treatment.

The MCV of the new population was normal (90 ± 8 fl) in 23 of the 26 patients. Patient J.L. was representative. He was a 67 yr-old white man who had purulent exacerbation of chronic bronchitis and a benign duodenal ulcer that healed successfully without surgery. He was given oral ferrous sulfate. Figure 1 and Table 1 present representative erythrograms, hemoglobin, MCV,

Table 2. Patient L.B.

<table>
<thead>
<tr>
<th>Fig. 2</th>
<th>Date</th>
<th>Hemoglobin (g/dl)</th>
<th>MCV (fl)</th>
<th>Neutrophil Lobe Count</th>
<th>Reticulocytes (%)</th>
<th>Therapy*</th>
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<td>10/19/75</td>
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<td>-</td>
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<td>1.9 Iron</td>
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<td>16.8 Iron + folate</td>
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<td>120</td>
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<td>12.2 Iron + folate</td>
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<td>7.2 Iron + folate</td>
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<tr>
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<td>12.4</td>
<td>65 87</td>
<td>92</td>
<td>3.26</td>
<td>1.3 Iron + folate</td>
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Other admission values: iron saturation 4%, serum folate 2.0 ng/ml, serum B12 1012 pg/ml.

*Therapy: Imferon, 26 ml intravenously, 10/19/75; ferrous sulfate, 300 mg per os three times a day from 10/21/75 to past 12/3/75; folic acid, 5 mg per os three times a day 10/28/75 through 11/5/75, and 5 mg per os daily from 11/6/75 to past 12/3/75.
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and admission iron saturation, serum folate, B₁₂. The admission erythrogram (Fig. 1A) showed a single population of red cells, all microcytes. Five days after iron therapy was begun, a new population of normocytic red cells appeared (Fig. 1B), and during the next weeks became increasingly predominant (Figs. 1C-E). Eight months later all the cells were normocytic (Fig. 1F).

The other 22 cases had similar findings: initial hemoglobin 4.3-10.2 gm/dl (mean ± 2 SEM, 7.4 ± 2.1); initial MCV 46-69 fl (59 ± 7); MCV of new population 82-96 fl (89 ± 4). The average size of the new population exceeded 98 fl in the other 3 patients.

Figure 2 and Table 2 show serial erythrograms and other laboratory data in one of these patients. L.B., a 23-yr-old white woman, had idiopathic thrombocytopenic purpura diagnosed a year before with a satisfactory remission with oral prednisone. She stopped taking this 3 mo before admission and for 2 mo before admission had gingival bleeding and menorrhagia. She was treated initially with prednisone and intravenous iron, and subsequently also with oral folate.

Initially the erythrogram showed a single population of microcytes very similar to that in the previous case (Fig. 2A). In contrast, after iron therapy the new population of cells was macrocytic (Figs. 2B-C); folate deficiency was detected. After folate repletion normocytes began to appear (Figs. 2D-E). A month later the new normocytes greatly outnumbered the surviving microcytes and macrocytes (Fig. 2F). The other two patients with initial macrocytic responses continued to produce macrocytes despite folate administration. Both had a single population of microcytic red cells before therapy. After iron repletion the new population of cells was macrocytic, and macrocytes continued to be produced during continued adequate folate intake. Hematologic data are shown in Table 3. Both had taken and continued to take phenytoin; G.W. also was a heavy alcohol user.

The neutrophil lobe count is shown in the figures for the four cases described above. L.B. had hypersegmented neutrophils before and during iron therapy.
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until soon after folate was begun; neutrophil morphology then became normal. Of 23 patients with normal responses to iron therapy, 4 had elevated lobe counts initially (normal is 3.17 ± 0.25). All became normal after therapy. Two had borderline serum folate levels (3.0–4.9 ng/ml) and 2 had normal levels (≥5 ng/ml). The other 19 patients with normocytic responses, including J.L., and 2 of the patients with macrocytic responses, M.J. and G.W., had normal lobe counts before and after therapy.

Of the 23 patients with normocytic responses, 1 had a low pretherapy serum folate level (2.9 ng/ml), 7 had borderline levels, and 15 had normal levels. Of the 3 with macrocytic responses, L.B. had a low serum folate before therapy, and the other 2 had normal serum folate levels. All 26 patients had normal serum B12 levels and iron saturations between 2% and 11% (5% ± 2%). The 3 with macrocytic responses had iron saturations of 4%, 5%, and 2%. After folate therapy M.J. had a red cell folate level of 1169 ng/ml; these levels were not available for other subjects.

In all patients the MCV increased progressively as new red cells were produced. However, in the 2 wk following iron therapy, the MCV did not reach the size of the new cells in any of the 26 (Figs. 1 and 2; Tables 1–3).

DISCUSSION

In the cases presented, postiron erythropoiesis was quantitatively and sequentially identifiable. A new, markedly larger population of cells appeared concurrently with reticulocytosis. While in most patients the new cells were of normal size, in 3 of 26 cases the postiron response was macrocytic. These 3 cases all had a preexisting abnormality, revealed morphologically once the predominant iron deficiency was corrected.

Macrocytic erythropoiesis may follow iron repletion for four reasons. First, folate deficiency may be unmasked. Patient L.B. initially had a very low serum folate; when iron allowed effective erythropoiesis the resultant new cells were abnormally large. Normocytes were produced after folate and iron was repleted. The changes in neutrophil segmentation confirmed this change in folate balance. Second, similar red cell size changes may occur if vitamin B12 rather than folate deficiency coexists, though we found no such patient. Third, drugs may induce macrocytosis. Despite normal serum folate and B12 and normal red cell folate, patient M.J. continued to produce macrocytes after iron repletion and folate administration. Chronic phenytoin use seemed the likely cause.4,14 Phenytoin and/or alcohol4,15 seemed to be the cause of a similar course in patient G.W. Unlike patient L.B., in these two patients oral folate caused no morphologic changes in red or white cells. Fourth, “stress” reticulocytosis may be elicited at the onset of maximal marrow response.16,17 In these three cases, macrocyte production was sustained after reticulocytosis had subsided, and in each case a likelier cause was present. Nonstress reticulocytes usually are only about 15% larger than the corresponding mature cells,18 so reticulocytes would not appear discretely as macrocytes except when the mature cells would be macrocytes. However, reticulocytes may well be the largest volume cohort in the aggregate of new cells.

In each case of uncomplicated iron deficiency, from the first new cells after
therapy, all cells were normocytes. No cells were intermediate in size between the pretherapy microcytes and the normocytes reflecting full repletion. Therefore, fully normocytic erythropoiesis must begin within 1 or 2 days after the start of iron therapy.

Iron deficiency may frequently be accompanied by reduced serum folate levels. Only 2 patients had low initial serum folate: 1 of 23 with a normocytic response (7 others had borderline values), and 1 of 3 with a macrocytic response. Low or borderline folate is common in hospitalized patients, most of whom have no true folate deficiency. During subnormal folate intake or absorption, serum folate falls several months before red cell folate, so low serum folate does not necessarily mean tissue depletion. Initial serum folate, iron saturation, and neutrophil lobe counts do not seem to predict for macro- or normocytic response. Hypersegmented neutrophils in apparent pure iron deficiency may result from folate deficiency undetectable by serum or red cell folate levels.

The best indicator of marrow response to iron repletion seems to be the initial cohort of new red cells. In 1942 Wintrobe described a patient with microcytosis, iron deficiency, and pernicious anemia. After iron repletion, the red cells produced seemed macrocytic, and the MCV eventually became abnormally large. However, early evaluation of the new cells by the peripheral smear and the red cell indices has been difficult. In our series, even the 3 patients with macrocytic response had normal MCVs for weeks after therapy, since many microcytes remained and were averaged into the MCV. Serial erythrography allowed rapid detection of normal and abnormal responses.

The iron-deficient patients reported here were selected for marked microcytosis (MCV < 70 fL) to show most clearly the changes in red cell size after iron therapy. If macrocytic disorders counterbalance the microcytosis of iron deficiency, they may coexist more frequently in those iron-deficient patients with less pronounced microcytosis. Thus, the present study may have minimized the frequency of coexisting disorders. At the least, iron repletion reveals a concomitant macrocytic disorder sufficiently often to warrant increased clinical awareness.

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