Erythropoiesis During Recovery From Macrocytic Anemia: Macrocytes, Normocytes, and Microcytes

By David Bessman

In seven patients with marked megaloblastic anemia (MCV > 110 fl), red cell size distribution curves (erythrograms) demonstrated the size of red cells produced after therapy. In six, the new red cells were normocytic throughout recovery. In the seventh patient, folate repletion alone produced a new population of microcytes, due to unsuspected iron deficiency; after iron repletion normocytes were produced. Three patients with autoimmune hemolytic anemia had macrocytosis (MCV > 110 fl) without folate or vitamin B_{12} deficiency. During recovery with prednisone therapy, instead of a discrete new normocytic population appearing, the entire population progressively returned to normal size. Normal rather than "stress" reticulocytes, and remodeled stress reticulocytes remaining, may explain this different pattern of recovery. Two patients initially had minor subpopulations of smaller red cells that disappeared soon after therapy. These probably reflected the dyserythropoiesis of severe megaloblastic anemia.

TREATMENT OF MEGALOBLASTIC ANEMIA rapidly produces accelerated effective erythropoiesis with increased peripheral reticulocytosis, followed by an increased red cell mass.[1] Since macrocytes remain in the peripheral blood along with the new cells, routine red cell indices and the peripheral blood smear do not allow quantitative evaluation of the new cells. Serial red cell size distribution curves (erythrograms[2]) of seven patients with severe megaloblastic anemia have clearly revealed the size of the new cells.

Anemia equally macrocytic but not megaloblastic may occur in autoimmune hemolytic anemia (AIHA). "Stress" reticulocytes have been proposed as the cause of this macrocytosis.[3] Erythrograms taken serially before and during the treatment of three patients with AIHA have shown a pattern distinctly different from that of recovery from megaloblastic anemia.

MATERIALS AND METHODS

Between 7-1-75 and 9-1-76, the mean MCV exceeded 110 fl in 138 adult patients at Los Angeles County USC Medical Center. Of these, 10 had received no transfusion or hematinic therapy in the previous 4 mo and no transfusions during the present therapy. Serum iron, iron-binding capacity, folate and vitamin B_{12} levels, and bone marrow examinations were obtained on admission. Erythrograms were obtained before and at least 2 wk after therapy was begun. Hemoglobin electrophoresis demonstrated hemoglobin AA with normal hemoglobin A_2 and F. A diagnosis of vitamin B_{12} or folate deficiency, or of AIHA was established. These 10 patients are the subjects of this report.

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Table 1. Case I

Fig. 1	Date	Hemoglobin (g/dl)	MCV (fl)	Reticulocytes (corrected, %)
A	7/28/75	9.8	138	—
B	8/ 4/75	10.1	144	2.1
C	8/ 9/75	10.4	126	6.4
D	8/11/75	11.0	121	3.2
E	8/18/75	12.3	110	2.2
F	3/24/76	16.7	99	0.7

Routine complete blood counts, reticulocyte counts, and the blood tests noted above were determined as in a previous report.[4] Erythrograms, obtained as described in the previous article,[4] permitted determination of distributional median cell volumes (MCV_D).[2] MCV_D thus measured the average cell volume of each red cell population if more than one was present.

RESULTS

Of the 10 patients, 7 had vitamin deficiencies: 4 of B_{12}, 2 of folate, and 1 of both. Admission serum transferrin saturation was elevated in all 7; after initial vitamin therapy without iron, transferrin saturation was less than 15% in 4 and greater than 20% in 3. The bone marrows in all cases were megaloblastic with increased iron stores on admission. Serial erythrograms and hematologic data are shown for two illustrative cases.

Case 1 (Table 1) initially had a single population of macrocytes (Fig. 1A). Four days after initial therapy, a brisk reticulocytosis began; a new population

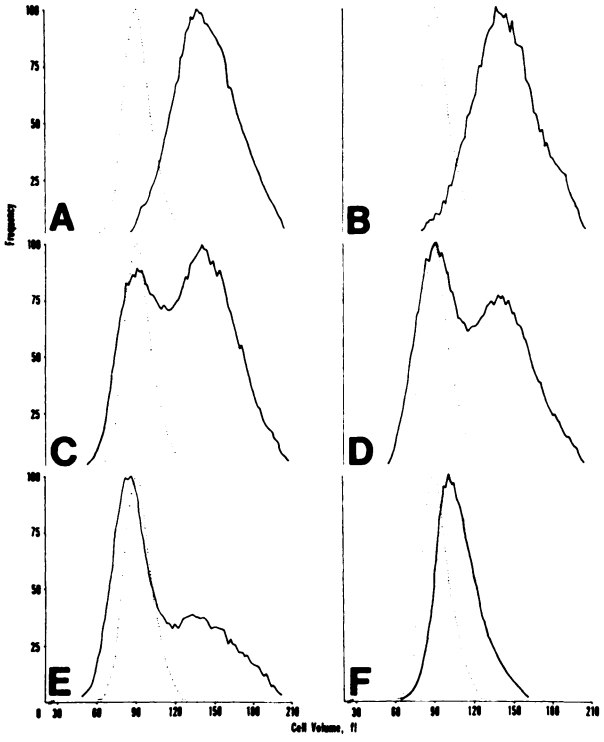

Fig. 1. Recovery from pernicious anemia. Solid line, patient; dotted line, normal subject. Therapy: folate, 5 mg per os daily 7/30/75 to 8/27/75; vitamin B_{12}, 1 mg intramuscularly daily, 7/30/75 to 8/10/75, and 1 mg twice weekly 8/11/75 to 8/28/75; no subsequent therapy. See Table 1.

Table 2. Case 7

Fig. 2	Date	Hemoglobin (g/dl)	MCV (fl)	Reticulocytes (corrected, %)
A	9/29/75	5.1	111	0.8
B	10/ 2/75	5.2	110	2.7
C	10/ 3/75	5.6	107	3.6
D	10/ 5/75	6.8	95	3.0
E	10/ 8/75	7.2	92	5.4
F	10/15/75	9.9	97	2.6

of normocytes appeared (Fig. 1B) and progressively increased in proportion (Figs. 1C-E). The new cells' average size was normal from the first increment. Seven months later the patient again had a single population of cells, on average borderline macrocytic (Fig. 1F). Cases 2-4 had similar serial erythrograms.

Case 7 (Table 2) initially had a single population of macrocytes (Fig. 2A). After folate was given, a second, microcytic population appeared (Fig. 2B) and progressively increased in proportion (Figs. 2C-D). Serum transferrin saturation was then 7%. After iron was added and folate continued, normocytes emerged, intermediate in size between the macrocytes and microcytes (Fig. 2E). As more normocytes were produced, they predominated, obscuring the remnants of the micro- and macrocytic populations (Fig. 2F).

Three patients, cases 8-10, had AIHA and macrocytosis. On admission all had bone marrows with increased erythropoiesis but no megaloblastosis, in-

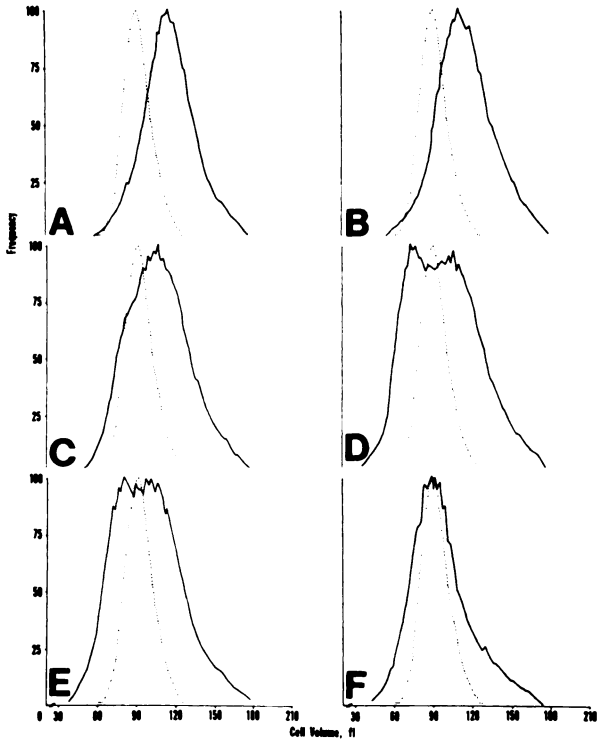

Fig. 2. Recovery from combined folate and iron deficiency. Solid line, patient; dotted line, normal subject. Therapy: folate, 5 mg per os daily 9/30/75 beyond 10/15/75; ferrous sulfate, 300 mg per os three times a day 10/5/75 to beyond 10/15/75. See Table 2.

Table 3. Case 8

Fig. 3	Date	Hemoglobin (g/dl)	MCV (fl)	Reticulocytes (corrected, %)
A	3/22/76	7.4	126	12.2
B	3/25/76	7.6	122	11.2
C	3/29/76	8.8	116	9.3
D	4/ 7/76	10.4	101	7.2

creased reticulocytes, positive direct Coombs' tests, and normal serum vitamin B_{12} levels. In cases 8 and 9 serum folate was normal, and in case 10, borderline. Case 8 (Table 3) is representative. Initially there was a single macrocytic population. (Fig. 3A). After prednisone alone, no second population of cells appeared. As reticulocytosis continued and red cell mass increased, the mean size of the entire population progressively decreased (Figs. 3B–D). Cases 9 and 10 had similar serial erythrograms.

Two of the patients (cases 5 and 6) initially had predominantly macrocytes but also a group of diverse small cells (Fig. 4). In these two, the routine MCV was less than the MCV_D of the macrocytes by 10 and 12 fl. Of eight cases without small cells initially, MCV was less than MCV_D in six (1, 2, 3, 3, 4, and 5 fl) and greater than MCV_D in two (1 and 3 fl). In cases 5 and 6 the small cells disappeared during therapy as a normocytic recovery progressed similar to that in case 1.

DISCUSSION

Six of seven patients with megaloblastic anemia produced normocytes as soon as therapy was started. Characteristic of the hemolytic anemia of megaloblastosis,[5,6] admission iron saturation was elevated in all, and fell to normal or subnormal with therapy.[6] Though four had low transferrin saturation after

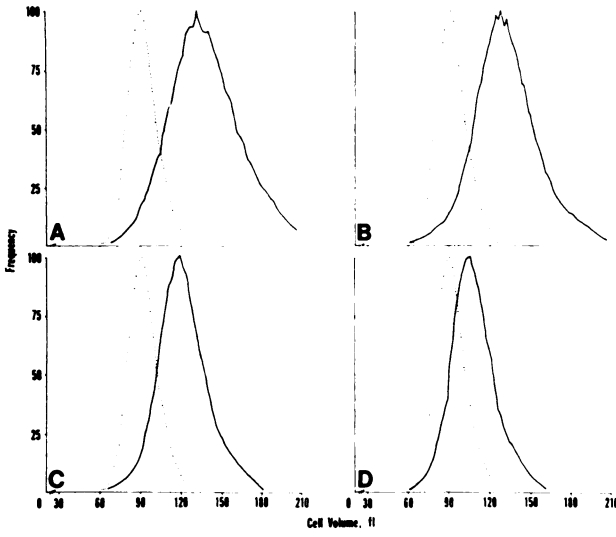

Fig. 3. Recovery from AIHA. Solid line, patient; dotted line, normal subject. Therapy: prednisone, 60 mg daily begun 3/22/76 and tapered during the next 3 wk. See Table 3.

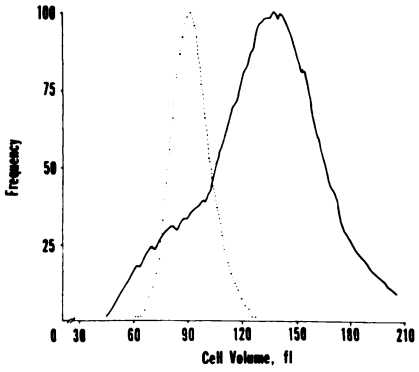

Fig. 4. Subpopulation of small cells coexistent with macrocytes (patient 5). Solid line, patient; dotted line, normal subject.

therapy, only one produced microcytes. Thus, iron saturation was not predictive of normo- or microcytic production.

Initially, two patients with megaloblastic anemia had a minor population of variously sized small cells. Rather than microcytes caused by iron-deficient areas of marrow within a marrow megaloblastic overall,[7] these small cells may have reflected the dyserythropoiesis with microcytes characteristic of severe megaloblastic anemia.[8,9] The small cells disappeared rapidly after therapy without iron. In one patient with small cells, iron saturation was normal after therapy without iron. In the patient with a microcytic response after folate therapy, there were no small cells before therapy (Fig. 2A).

Routine red cell indices average the small red cells with the macrocytes.[9] In the two cases with small cells before treatment, the aggregate MCV was considerably less than the median of the macrocytes alone, while in the eight cases without small cells, the MCV and the median of macrocytes were nearly identical. Though the present series was very small, a population of small cells appeared to lower the routine MCV. Erythrography allowed a truer appreciation of the derangement of cell size.

Patel and Chanarin reported that after treatment of pernicious anemia, the MCV often rose slightly at first, before a progressive fall as recovery continued.[10] This pattern occurred in three of the patients reported here, two with small cells initially and one without small cells. Factors individualizing the rate of fall of the MCV may include: the number and survival of pretherapy macrocytes averaged with the new cells; the rate of appearance of new cells; the size of the new cells (e.g., microcytes because of associated iron deficiency); and the initial presence of small cells that disappeared during therapy.

Serial erythrograms in the three patients with AIHA were remarkably different. After therapy, instead of a discrete new population appearing, the entire cell population seemed to decrease in average volume. The following explanation is proposed: Maximally stimulated marrow, as in AIHA, releases "stress" reticulocytes. Unusually large because of missed division or divisions in the marrow, these cells are "remodeled" to normal size in the spleen[11] and have a short life span. As AIHA subsides after treatment, progressively more normal sized reticulocytes are produced. Remaining stress reticulocytes are pared down and die, and the entire peripheral red cell population remains relatively homogeneous as the MCV progressively falls.

Red cells produced after marrow recovery from microcytic or macrocytic disorders should reflect the marrow's new status. As discussed in this and the preceding article,[4] erythrograms offer an easy, reliable way to assess serially the size of red cells produced during treatment.

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