Production of Erythrocytes that Contain Fetal Hemoglobin in Anemia

TRANSIENT IN VIVO CHANGES

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ABSTRACT Serial microscopic immunodiffusion assays of F cells, i.e., erythrocytes that contain fetal hemoglobin (HbF), in four individuals recovering from anemia demonstrate initial increases in the percentage of circulating reticulocytes that contain HbF (F reticulocytes) and subsequent increases in the percentage of mature erythrocytes that contain HbF (F erythrocytes). In one individual responding to therapy for iron-deficiency anemia, the average percentage of F reticulocytes increased from 4.8±1.1 to 16.0±2.8% (mean±SD), while the mean level of F erythrocytes increased from 3.5±0.7 to 7.2±0.6%. Two normal children with transient erythroblastopenia exhibited F reticulocyte percentages of 71.3±6.7 and 41.5±1.5%, respectively, when erythropoiesis resumed. With recovery these values fell to finally measured values of 33.7±4.7 and 12.6±1.1%, respectively. In an adolescent with sickle cell anemia, F-reticulocyte percentages fluctuated between 0.6±1.1 and 34.0±2.8% and paralleled the rise and fall of total reticulocytes associated with therapy for a nasopharyngeal carcinoma.

Such findings suggest that first, the production of F cells and non-F cells are separately regulated. Second, F-cell production is preferentially stimulated during escape from erythropoietic suppression and selectively depressed at the start of suppression. Third, during escape from erythropoietic suppression, F-cell production in vivo resembles that reported for in vitro cultures of erythroid stem cells. Fourth, individuals with sickle cell anemia, like individuals without hemoglobinopathies, can change their relative level of F-cell production.

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INTRODUCTION

The switch during infancy from fetal hemoglobin (HbF)[1] to adult hemoglobin (HbA) is never fully completed. Residual HbF is produced throughout life within a minority of erythrocytes that contain HbF (F cells). Although the number of situations in which F cells are elevated is comparatively limited (1), analyses of erythroid-cell cultures suggest that all individuals retain the capacity to increase their F-cell level. For example, in normal adults, erythrocyte precursors, which synthesize HbF, are selectively propagated in vitro as erythropoietin (EPO) concentration is raised (2, 3). Because it is unclear whether equivalent changes can occur in vivo in all individuals, it is also unclear whether events that occur in cell culture are faithful reflections of physiological processes.

In this report, a recently described assay for reticulocytes that are also F cells, i.e., F reticulocytes (4), is used to assess the in vivo dynamics of F-cell production in four individuals escaping from or entering erythropoietic suppression. During the escape phase, the observed enhancement of F-cell production resembles that reported for erythroid-cell cultures. With suppression, F-cell production is preferentially decreased.

METHODS

Samples were obtained in accordance with the principles established by The Johns Hopkins University Medical School Committee on Clinical Investigation. Venous blood was collected in glass tubes that contained citrated EDTA, stored at

[1] *Abbreviations used in this paper*: EPO, erythropoietin; F cells, erythrocytes that contain fetal hemoglobin; HbA, adult hemoglobin; HbF, fetal hemoglobin; SCA, sickle cell anemia.

4°C, and assayed within 24 h. Hematocrit and reticulocyte counts were obtained by standard techniques (5). Erythrocyte counts were enumerated in a Coulter counter model S (Coulter Electronics Inc., Hialeah, Fla.).

Isolation of purified antibodies, assay and enumeration of F reticulocytes and F erythrocytes by microscopic immunodiffusion, and estimation of whole hemolysate HbF levels by macroscopic immunodiffusion were performed as recently detailed (4). In brief, erythrocytes suspended in 50–100-μM thick agarose gels that contained anti-HbF were lysed with Triton-X100 (Rohm & Haas Co., Philadelphia, Pa.) and F cells thereafter recognized in microscopic darkfield by pericellular HbF-anti-HbF immunoprecipitates. F reticulocytes and reticulocytes unreactive with anti-HbF were easily identified in gels to which new methylene blue was added. The percentage of F reticulocytes was calculated by the equation, 100 × (F reticulocytes/total reticulocytes scored). The overall percentage of F cells was calculated by enumerating the number of erythrocytes reactive with anti-HbF in fields where the total number of erythrocytes had been determined before lysis. The percentage of F erythrocytes was calculated from the expression, 100 × ([fraction of F cells] − [fraction reticulocytes × fraction F reticulocytes]/[1-fraction reticulocyte]). At least 250 reticulocytes and 1,000 erythrocytes were scored in each assay. The F-cell values for each sample are expressed as the mean±SD of scores obtained in several regions in each of several gels.

RESULTS

Iron-deficiency anemia. A 19-yr-old black male, with a diaphragmatic hernia, esophagitis with gastrointestinal bleeding, and secondary iron deficiency was treated with oral ferrous sulfate, 1,200 mg/day. Preliminary evaluation revealed: microcytic, hypochromic anemia; serum iron, 16 μg/dl; iron binding capacity, 485 μg/dl; absent Fe stores in a bone marrow aspirate; and, by electrophoresis, Hb AC. On day 4 of therapy, before obvious reticulocytosis, the percentage of F reticulocytes and F erythrocytes were each well within the normal adult range (0.1–9.6%) (6). 2 wk after initiation of iron therapy the percentage of F reticulocytes had risen threefold to 16.0±2.8% (Table I). This rise was predictive of a later threefold increase in the absolute number of F erythrocytes (day 62, 361.9 × $10^3/\mu$l).

Transient erythroblastopenia of childhood. Two white females, aged 6 mo and 2 yr, presented with anemia and absent peripheral blood reticulocytes. Their hematocrits had previously been normal. At time of presentation, no erythroid precursors were evident in bone marrow aspirates. Erythrocyte indices, erythrocyte morphology on peripheral blood smear, percentage HbF in whole hemolysate, levels of erythrocyte enzymes (glucose-6-phosphate dehydrogenase, hexokinase, and pyruvate kinase), serum haptoglobin, and serum hemopexin were normal. Both children recovered spontaneously. Striking elevations in the percentage of F reticulocytes, 71.3±6.7 and 41.5±1.5%, respectively in the two children, accompanied the first appearance of reticulocytes in the peripheral blood (Tables II and III). Corresponding values for F reticulocytes in a normal child <1 yr old were 12.4±2.3 and 8.4±0.4%, at the age of 5 mo and 8 mo, respectively (4). As expected from the markedly elevated but subsequently declining F-reticulocyte levels, the number of F erythrocytes slowly increased during the recovery period, then closely paralleled the declining F-reticulocyte levels.

TABLE I
Serial Assays of F Reticulocytes and F Erythrocytes in an 18-Yr-Old Male Treated for Iron-Deficiency Anemia

Day*	Total eryth ×10^6	Total retic ×10^3	F retic‡ ×10^3	F eryth§ ×10^3	Hct	Total retic	F retic‡	F eryth§
	cells/μl whole blood						%	
1	—	—	—	—	21	—	—	—
4‖	3.71	59.3	2.9	127.8	22	1.6	4.8±1.1	3.5±0.7
8	4.25	204.0	18.6	186.1	23	4.8	9.1±1.9	4.6±1.1
9	4.02	184.9	16.6	164.9	24	4.6	9.0±2.6	4.3±1.3
10	—	—	—	—	27	6.0	9.8±1.3	4.1±1.3
12‖	4.30	292.4	34.5	212.4	29	6.8	11.8±2.5	5.3±0.7
15	4.52	325.4	52.1	302.0	31	7.2	16.0±2.8	7.2±0.6
62‖	6.70	120.6	5.8	361.9	46	1.8	4.5±2.9	5.5±2.0

Eryth, erythrocytes; Hct, hematocrit; retic, reticulocytes.
* Days after initiation of therapy with ferrous sulfate.
‡ F reticulocytes = reticulocytes that contain HbF/total reticulocytes, × 100.
§ F erythrocytes = nonreticulocytes that contain HbF/total erythrocytes − total reticulocytes, × 100.
‖ Percentage of HbF in washed erythrocyte lysate was 0.5±0.1, 1.4±0.1, and 0.9±0.2% on days 4, 12, and 62, respectively.

TABLE II

Serial Assays of F Reticulocytes and F Erythrocytes in a 6-Mo-Old Female with Transient Erythroblastopenia of Childhood

Day*	Total eryth $\times 10^6$	Total retic $\times 10^3$	F retic‡ $\times 10^3$	F eryth§ $\times 10^3$	Hct	Total retic	F retic‡	F eryth§
	cells/μl whole blood						%	
0‖	2.79	—	—	1,548.5	20	0.0	—	55.5±9.4
4	—	—	—	—	21	0.0	—	44.7±6.6
11	2.81	33.7	24.0	1,068.9	20	1.2	71.3±6.7	38.5±5.3
12	2.88	34.6	26.6	1,115.4	20	1.2	77.2±7.3	39.2±5.0
14	3.10	229.4	148.7	1,125.3	22	7.4	64.8±2.2	39.2±7.1
15	3.39	833.9	571.2	927.9	25	24.6	68.5±5.5	36.3±7.7
28‖	3.81	350.5	158.4	2,182.9	34	9.2	45.2±8.3	63.1±8.9
92‖	3.98	79.6	26.8	1,649.9	34	2.0	33.7±4.2	42.3±3.8

Eryth, erythrocytes; Hct, hematocrit; retic, reticulocytes.

* Days after diagnosis.

‡ F reticulocytes = reticulocytes that contain HbF/total reticulocytes, × 100.

§ F erythrocytes = nonreticulocytes that contain HbF/total erythrocytes − total reticulocytes, × 100.

‖ Percentage of HbF in washed erythrocyte lysate was 11.5±1.2, 17.3±0.5, and 10.9±0.5% on days 0, 28, and 92, respectively.

Sickle cell anemia (SCA). A 14-yr-old black male with SCA was found to have a surgically unresectable nasopharyngeal carcinoma involving the right maxillary sinus. Preliminary evaluation revealed hematocrit 28%, reticulocyte count 9.0%, normal erythrocyte indices, Hb S 87.3, HbF 9.8, and HbA_2 2.9%. As described elsewhere,[2] analysis of the child's family showed no evidence of thalassemia or hereditary persistence of HbF. The patient was intermittently transfused with packed erythrocytes, his tumor was locally irradiated, and he subsequently received periodic administration of methotrexate, vincristine, doxorubicin, and cyclophosphamide. This therapy was associated with alternating suppression and recovery of erythropoiesis as evident from the intermittent rise and fall in the percentage of total reticulocytes in peripheral blood (Fig. 1).

Two observations emerged from serial assays of the

[2] Dover, G. J., S. H. Boyer, S. Chavache, and K. Heintzelman. 1978. Individual variation in the production and survival of F cells in sickle cell disease. *N. Engl. J. Med.* **299**: 1428–1435.

TABLE III

Serial Assays of F Reticulocytes, F Erythrocytes in a 2.5 Yr-Old Female with Transient Erythroblastopenia of Childhood

Day*	Total eryth $\times 10^6$	Total retic $\times 10^3$	F retic‡ $\times 10^3$	F eryth§ $\times 10^3$	Hct	Total retic	F retic‡	F eryth§
	cells/μl whole blood						%	
0	2.37	0	0	154.1	20	0.0	—	6.5±1.9
6	—	—	—	—	16	0.0	—	13.4±2.4
14‖	2.10	39.9	16.6	315.2	17	1.9	41.5±1.5	15.3±2.5
20‖	2.44	156.2	74.3	456.8	22	6.4	47.6±4.7	20.0±3.7
55‖	4.43	70.9	7.9	1,190.0	40	1.6	11.1±1.3	27.3±2.1
187‖	4.40	61.6	5.5	668.1	36	1.4	12.6±1.1	15.4±2.1

Eryth, erythrocytes; Hct, hematocrit; retic, reticulocytes.

* Days after diagnosis.

‡ F reticulocytes = reticulocytes that contain HbF/total reticulocytes, × 100.

§ F erythrocytes = nonreticulocytes that contain HbF/total erythrocytes − total reticulocytes, × 100.

‖ Percentage of HbF in washed erythrocyte lysate was 3.4±0.7, 7.2±0.1, 9.7±0.2, and 3.1±0.1% on days 14, 20, 55, and 187, respectively.

percentages of F reticulocytes performed between days 130 and 250 of therapy (Fig. 1). First, F reticulocytes varied between 0.6±1.1 and 34.0±2.8%. This finding contrasts with the stable F-reticulocyte levels found in individuals with uncomplicated SCA followed for intervals up to 18 mo.[2] Only 3 out of 15 such individuals exhibited significantly different F-reticulocyte values in serial samples. In them, unlike the pattern seen in Fig. 1, the magnitude of change in serial values was never more than 2.5-fold.[2] Second, although the relationship between the percentages of F reticulocytes and total reticulocytes is imperfect, levels for the two variables tended to change in parallel. Thus on the 16 d where F reticulocyte and total reticulocyte levels were each measured, the two variables were significantly correlated (Kendall's coefficient, $\tau = 0.6$, $P = 0.005$). This correlation suggests that F-cell production is preferentially decelerated with the advent of erythropoietic suppression and preferentially accelerated during escape from suppression.

DISCUSSION

In previous reports increased numbers of F cells were observed in leukemia (7), during recovery from aplastic anemia (4), and during successful response to bone marrow transplantation (8). The present cases expand these observations and suggest that increased F-cell production is a general phenomenon associated with accelerated erythropoiesis. In addition, assays of a child with SCA (Fig. 1) illustrate that F-cell production is reduced to a greater degree than non-F-cell production when erythropoiesis is suppressed. Thus F-cell production seems to be regulated separately from non-F-cell production.

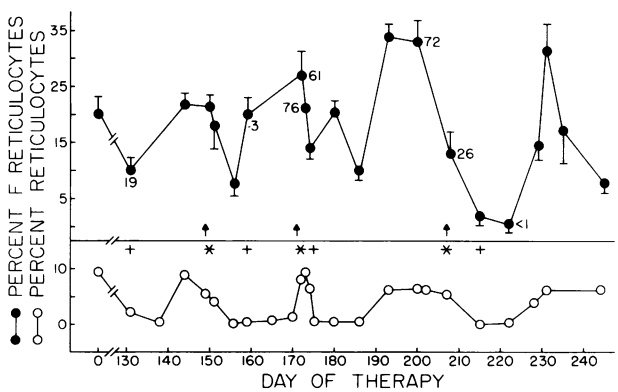

FIGURE 1 Serial assays of percentages F reticulocytes and total reticulocyte in a 14-yr-old male with SCA. As indicated by symbols, the patient was treated with transfusions of 2 U packed erythrocytes (↑); vincristine (2.0 mg i.v.) plus methotrexate (7.5 g/m² of body surface area) with folinic acid rescue (*); and doxorubicin (50 mg/m² i.v.) plus cyclophosphamide (700 mg/m²) (+). Numerals adjacent to F-reticulocyte levels indicate the absolute number of F reticulocytes × $10^3/\mu l$.

The observation that F-cell production increases with response to erythropoietic stimulation provides an important in vivo correlate for recent in vitro experiments. In the presence of supraphysiologic levels of EPO, abundant HbF is synthesized in colonies cultured from erythroid precursors isolated from bone marrow (2) or peripheral blood (2, 3). The average number of erythroid colonies that contain HbF at 14 to 16 d of culture in 13 normal individuals was 34.1±3.9% (2). This value is quite similar to the average maximal F-reticulocyte level (35.2±12.8%) seen in five subjects, older than 2 yr of age, who were recovering from hypoplastic anemia,[3] and who might be expected to have high levels of EPO (9). Whether the selective depression of F-cell production associated with erythropoietic suppression is, in turn, associated with falling levels of EPO is unclear. In any case, further studies are needed to determine whether changing levels of erythropoietin can alone alter F-cell production or whether other factors are required.

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[3] This number includes the subjects in Tables I and III, Fig. 1, and two adults with aplastic anemia previously reported (4).