Refractory Dysmyelopoietic Anemia and Acute Leukemia

By David S. Rosenthal and William C. Moloney

One hundred and seventeen consecutive patients with refractory dysmyelopoietic anemia (RDA) were followed and studied over 6 yr. All RDA cases had at least two marrow cell lines involved with dysplasia and usually all three. Morphologically, the series could be divided into 55 cases that had primarily "erythroid" hyperplasia and 62 cases with primarily "myeloid" dysplasia. There was a significantly higher number of acute leukemias in the myeloid group, 21/62 (33.9%), than in the erythroid group, 7/55 (12.7%). Of the 117 cases, 64 had marrow culture studies performed using implanted diffusion chambers (DC) in an irradiated rat host. There was a highly statistically significant correlation between the development of leukemia and abnormal growth in DC during the RDA stage.

DURING THE PAST DECADE, there has been an increasing recognition of a bone marrow disorder characterized by refractory anemia and often associated with leukopenia and thrombocytopenia. While the etiology and pathogenesis are unknown, excellent descriptions of the clinical and hematologic aspects of this disease have been published by a number of authors.[1-3] However, serious difficulties in diagnosis and management arise, especially due to the variability of the bone marrow cytology. In this article we present clinical and laboratory findings on 117 cases of refractory dysmyelopoietic anemia (RDA) followed prospectively over the past 6 yr. Results of studies on marrow cells from 64 patients cultured in diffusion chambers are also reported. During this investigation, 28 of the 117 patients with RDA developed acute leukemia. Problems associated with the diagnosis and management of these cases are further discussed.

MATERIALS AND METHODS

One hundred and seventeen patients with refractory dismyelopoietic anemia were followed in the Hematology Division of the Brigham & Women's Hospital from 1976 to 1982. The diagnosis of RDA was based on clinical findings and marrow morphology. Patients were followed periodically with physical examinations, routine blood studies, and bone marrow aspirations, which were carried out repeatedly in many instances; marrow biopsies were obtained when the aspirate was hypocellular. Peripheral blood and marrow smears were stained with Wright-Giemsa, and histochemical methods included myeloperoxidase, alkaline phosphatase, and specific and nonspecific esterases. Serum and urine lysozyme activity was determined in patients with monocytosis. Chromosome analysis, employing standard methods, was carried out in 43/117 cases. Freshly obtained marrow cells from 64/117 patients were cultured in diffusion chambers (DC) using a modified Benestad method.[4,5] Sprague-Dawley rats, weighing between 150 and 200 g, were treated with total body irradiation 24 hr before DC were inserted into the abdominal cavity of the anesthetized animals. Radiation factors were: 760 rad from a ^{127}Ce source, 663 Kv, dose rate 150 rad/min. The DC consisted of 13-mm leucite rings (Millipore Corp., Bedford, MA) with 0.20-μ porosity polycarbonate filter (Nucleopore Corp., Los Angeles, CA) attached to each side. Chambers were tested for leaks by injecting air into DC suspended in sterile distilled water. DC were sterilized at 80°C dry heat for 24 hr. Marrow cells were collected in heparinized saline and suspensions made to allow injection of 0.1 ml (1×10^6 total cells) to each chamber. Apertures in the leucite rings were sealed and 4–6 separate DC were inserted into the peritoneal cavity of ether-anesthetized irradiated rats. DC were transferred weekly to freshly irradiated hosts. Chambers were removed on days 1, 3, 7, 14, and 21 and the contents placed in 1.5 ml solution of 0.5 pronase and 5% Ficoll in RPMI medium and agitated for 15 min. Cells were harvested by puncturing the membrane with a sterile siliconized Pasteur pipette, and the cell-containing fluid transferred to a sterile tube. Cell suspensions were washed repeatedly with RPMI medium, and cell counts were obtained using a Coulter counter. Cell suspensions were then centrifuged, the supernatant removed, and the cells resuspended in fresh rat sera. Coverslip preparations were drawn, air-dried, and stained with Wright-Giemsa. Differential counts were carried out and histochemical methods employed as above to determine cell derivation.

RESULTS

Clinical

There were more males than females in this group of patients (78:39). Median age was 69, with a range of 40–86 yr. Onset was usually insidious, and the most common complaints were weakness and fatigue due to anemia. While thrombocytopenia was of common occurrence, only a few patients presented initially with purpura or abnormal bleeding. Except for splenomegaly in 5%, there were no significant physical findings at initial presentation. Diagnosis was frequently incidental, following routine blood studies or during the course of an unrelated illness, such as rheumatoid and osteoarthritis, cardiac and pulmonary disorders, peptic ulcer, thrombophlebitis, and various other diseases. Although the accurate dating of the onset of RDA was often impossible because of the presence of a symptom-free stage, the median duration of survival was esti-

From the Hematology Division, Brigham & Women's Hospital, and the Department of Medicine, Harvard Medical School, Boston, MA.

Supported in part by the Nehemias Gorin Foundation.

Submitted April 1, 1983; accepted August 6, 1983.

Address reprint requests to Dr. David S. Rosenthal, Department of Hematology, Brigham & Women's Hospital, 75 Francis Street, Boston, MA 02115.

0006-4971/84/6302-0010$01.00

mated at 36 mo, with a range of 4 mo to 15 yr. To date, 54 patients are alive, 48 have died, and 15 are lost to follow-up. Of the 48 deaths, 26 were due to development of acute leukemia; the remaining deaths were attributed to cardiovascular disorders, pneumonia, or infectious complications.

Laboratory Findings

Peripheral blood. At initial presentation, anemia of varying degrees of severity occurred in all but 12 cases. The median hematocrit was 29.3%. Abnormalities of red cell morphology were common, especially macrocytosis and poikilocytosis. In some cases, particularly those associated with marked erythroid hyperplasia, small numbers of nucleated red blood cells were present. The median leukocyte count was 4,000/cu mm, and morphological changes often include the acquired Pelger-Hüet anomaly. In a small percent of cases, a few myelocytes, promyelocytes, and rarely myeloblasts were present intermittently. Absolute monocytosis occurred in 22/117 patients (19%), and in these, the median monocyte count was 1,674/cu mm, with a range of 836–3,560. In 8 cases, monocytosis was the initial abnormal laboratory finding. In 3 of the 8 cases, acute leukemia, associated with a striking rise in serum lysozyme, developed 2–5 yr after initial observation. Thrombocytopenia was present at onset in 54% of cases. The decrease in platelets was usually mild to moderate, with a range of 7,000–598,000 and a median of 148,000/cu mm.

Bone marrow. The diagnosis of RDA was based on characteristic findings in the marrow. Of 117 cases, 62 had "myeloid" abnormalities that predominated and 55 had primarily "erythroid" changes (Table 1). In the "myeloid" cases, there was striking myeloid hyperplasia and dysplasia with myeloid:erythroid (M:E) ratios varying from 3:1 to 20:1. Myeloblasts were rarely increased significantly, but dysplastic promyelocytes and myelocytes often made up 20%–30% of the marrow population. Promyelocytes frequently contained nuclei with "punched out" nucleoli and overabundant coarse granules, while myelocytes and metamyelocytes often showed poorly granulated cytoplasm. Segmented neutrophils were usually decreased, and nuclear abnormalities, including Pelger-Hüet cells, were frequently noted. Increased numbers of dysplastic eosinophils and basophils occurred in a small percent of cases. Dysplastic changes were also present in red cell precursors and megakaryocytes. Although, in the "myeloid" cases, ringed sideroblasts were absent, siderocytes and increased iron stores and iron-containing macrophages were not uncommon. Red cell abnormalities included presence of proerythroblasts, increased numbers of basophilic erythroblasts, and cells with megaloblastic features. Of particular interest were dysplastic megakaryocytes, especially cells containing one to several round nuclei, referred to as "dwarf" or "pawn ball" megakaryocytes.[6] Among the 62 "myeloid" cases, 3 had hypocellular marrow aspirates and biopsies. The spotty areas of cellularity revealed dysplastic erythroid, myeloid, and megakaryocytic elements. In patients with monocytosis, the cells in the peripheral blood were usually morphologically and histochemically normal; however, recognition of these cells in the marrow was difficult.

Table 1. Classification by Bone Marrow Morphology and Status of 117 Patients With RDA Followed Prospectively

Category	Total	Alive	Dead	LTFU*	Leukemia
Erythroid	55	29	19	7	7
Sideroblastic	50	28	15	7	7
Nonsideroblastic	5	1	4	0	0
Myeloid	62	25	29	8	21 ($p < 0.01$)
Myeloid	50	17	26	7	18
Monomyeloid	9	6	3	0	3
Hypoplastic	3	2	0	1	0
Total	117	54	48	15	28†

*Lost to follow-up.
†Two alive.

Among the 55 "erythroid" cases, the M:E was reversed (1:3 to 1:10), and in all but 5 patients, iron stains showed increased iron stores with numerous ringed sideroblasts. In the remaining 5 cases, many early and dysplastic red cell precursors were present, often appearing in sheets and clusters. Characteristic granulocytic and megakaryocytic abnormalities, as described above, were also present. While binucleated late erythroblasts were relatively common, the bizarre multinucleated erythroblasts and myeloblasts characteristic of acute erythroleukemia (DiGuglielmo's) were not found.

Since 1982, some investigators prefer the use of the FAB classification for the myelodysplastic syndromes.[7] Practically all current cases would best fit into the RAEB type (refractory anemia with excess blasts), with defects in all three cell lines. Only a few of the erythroid cases would be subtyped RA (refractory anemia) or RA-S (RA plus sideroblasts).

Other Laboratory Tests

Serum B_{12} and folate levels, as well as neutrophil alkaline phosphatase activity, were quite variable in these RDA cases and were not of diagnostic or prognostic significance. Cytogenetic studies without complete banding were performed in 47/117 patients. Significant aneuploidy was found in 9 cases (19%) and consisted primarily of an addition or deletion of a C chromosome. In one case of sideroblastic RDA, a large

acrocentric marker chromosome was present in 100% of metaphases.

Studies on Control and RDA Marrow Cultures in DC (Table 2)

When marrow cells were cultured from 8 normal young adults and 48 older individuals undergoing hip replacement for arthritis, myeloid cells matured within 3–7 days and failed to proliferate. In the great majority of cases, a lymphocytosis occurred from days 7 to 14; subsequently, cellularity decreased and the remaining cells consisted of macrophages, small numbers of adult lymphocytes, and plasma cells.

Studies were carried out on 64 cases of RDA (31 with "myeloid" and 33 with "erythroid" RDA). In 25/33 and 21/31, myeloid cells matured normally and were replaced by macrophages or following maturation of myeloid cells; a lymphocytosis developed similar to that noted in normal individuals (Table 2). There were 18 cases (10 in myeloid and 8 in erythroid RDA) in which myeloid cells proliferated abnormally from 7 to 21 days; maturation to adult neutrophils was present in 10, whereas in 8, small numbers of promyelocytes and myeloblasts persisted from 14 to 21 days. Among these 18 cases, 10 later developed acute myeloblastic leukemia (AML): 3 of 8 cases of "erythroid" and 7 of 10 "myeloid" RDA. Among the 46 cases with normal growth of myeloid cells, only 1 later developed AML. However, in this case, the marrow cells were obtained 11 mo prior to diagnosis of AML. Applying a chi-squared analysis using Yate's correction, there is a highly statistically significant correlation between the development of leukemia and abnormal growth in DC ($p < 0.001$).

Observations were also carried out on marrow cells obtained from patients in the early stages of AML and on cells from cases of de novo untreated AML (Table 1). Among 31 cases of RDA-AML (15 prospective and 16 retrospective), 5 showed normal growth and 26 proliferated abnormally. Of the latter, only 1 maintained growth of promyelocytic and myeloblasts without further maturation. In 18, differentiation occurred from myeloblasts to promyelocytes, and in 7, maturation to myelocytes, metamyelocytes, and dysplastic adult neutrophils was observed. In contrast, when cells from 32 cases of de novo AML were cultured, none showed normal maturation.

Development of Acute Leukemia During Study

Of 117 patients, 28 developed acute nonlymphocytic leukemia; 26 were males and 2 females. Median age was 69, with a range of 57–83 yr. Only two are alive; one a case of untreated indolent acute erythroleukemia, the other surviving 15 mo following several unsuccessful courses of chemotherapy. Clinical and laboratory findings in the preleukemic phase were similar to other cases of RDA. Of the 62 "myeloid" cases, 21 (33.9%) developed leukemia, whereas only 7 of 55 (12.7%) "erythroid" cases did so—a difference that is statistically significant at $p < 0.01$ (Table 1). In addition, 3 of the 21 "myeloid" cases had monocytosis with monomyeloid marrow dysplasia and 1 was hypoplastic. In 11 of the 28 RDA-AML cases, DC studies were carried out within 1–3 mo in 3 and within 8–11 mo in 8 cases prior to development of AML. No abnormal myeloid proliferation occurred in 2 cases studied 8 and 10 mo prior to conversion to AML. Dysplastic myelocytes and promyelocytes grew in 9 cases from day 5 to 14; however, macrophages replaced the cultures in all but 2, and in these, dysplastic promyelocytes persisted.

Clinically, transition to AML was usually associated with increasing anemia, thrombocytopenia, and leukopenia. In a number of cases, AML was first recognized in the peripheral blood and bone marrow prior to significant changes in the clinical condition. Leukemia was atypical, and only 10/28 developed an elevated white count with florid leukemia at time of transformation. The morphological cell types of leukemia were myeloid in 20, monomyeloid in 4, erythromyelocytic in 3, and monocytic in 1.

Only one case of 19 treated with various forms of chemotherapy had a complete remission, which lasted 9 mo (Table 3). After the onset of acute leukemia, the

Table 2. Results of Marrow DC Cultures in Controls RDA, RDA-AML, and De Novo AML

Disease	No.	Normal Growth	Abnormal Growth	
Controls	56	56		0
RDA				
Erythroid	33	25 (0)	$p < 0.001$	8 (3)
Myeloid	31	21 (1)		10 (7)
RDA-AML	31	5		26
De Novo AML	32	0		32
Total	183	107		76

Number in parentheses is that developing leukemia during study.

Table 3. Results of Therapy in 19 Cases of RDA-AML

Therapy	CR	Failure	Total
CAT	1	7	8
COAP	0	1	1
COD	0	5	5
Low-dose Ara-C	0	4	4
6-MP	0	1	1
Total	1 (5%)	18	19

CAT, cytosine arabinoside, 6-thioguanine; COAP, cyclophosphamide, oncovin, cytosine arabinoside, prednisone; COD, cytosine arabinoside, daunorubicin; Ara-C, cytosine arabinoside; 6-MP, 6-mercaptopurine.

median survival was 3 mo. Two patients, described above, are alive with disease.

DISCUSSION

Clinical and laboratory findings in this series were similar to those reported by Dreyfus,[2] Linman and Bagby,[1] Pierre,[8] and Kass.[9] While RDA may appear to be a heterogeneous group of disorders, it may, in reality, be a single entity. It is apparent that monocytosis and monomyelocytic dysplasia are frequently associated with RDA and should be considered manifestations of the disease. Moreover, the presence of ring sideroblasts does not constitute a separate form of RDA, but signifies a more obvious defect of iron metabolism in red cell precursors.

In addition to the frequently described morphological abnormalities, marrow cells of all three lines of RDA patients exhibit a variety of enzymatic and functional defects, as noted by Schmalze,[10] Breton-Gorius,[11] and Dicke et al.[12] In the red cell precursors, these include abnormal hemoglobin synthesis, membrane defects, disturbances of iron metabolism, and megaloblastic features, including nuclear-cytoplasmic asynchrony. Granulocytes may lack bacteriocidal and other functions and enzyme activities are often abnormal; in this series, 50% of those tested had greatly decreased neutrophil alkaline phosphatase activity. In some myeloperoxidase activity was completely absent. However, more commonly, excessive myeloperoxidase activity was noted, especially in dysplastic myelocytes and promyelocytes.

It is obvious that continued clinical and hematologic observations will neither reveal the pathogenesis of RDA nor disclose the mechanisms of conversion to acute leukemia. A number of investigators have employed a variety of cell culture methods to study bone marrow cells in dysmyelopoietic and preleukemia states.[12-17] In this series, we studied marrow cells cultured in DC. It was pointed out that, in this study, marrow cells from young and old hematologically normal adults failed to proliferate and that a marked lymphocytosis occurred in the majority of cases. This lymphocytosis was probably stimulated by the mitogenic effect of rat globulin present in the DC.[18] Marker studies indicated that the lymphocytes in the cultures were predominantly T cells, however, it was not possible to determine whether they were derived from the marrow or peripheral blood.

Marrow cells cultured from RDA patients retained, for the most part, the capacity to behave like normal cells (46/64). However, when there was abnormal growth in DC, it correlated significantly with the development of acute leukemia. The myeloid cells from early cases of RDA-AML responded variably in culture; some grew normally, while the great majority (26/31) showed abnormal growth patterns characterized by maturation to promyelocytes, and in one instance, persistent growth of myeloblasts and promyelocytes. Cells from de novo AML patients uniformly (32/32) lacked differentiation and continued to proliferate in culture. These observations suggest that the basic problem in RDA, RDA-AML, and de novo AML may be a variable defect of pluripotential stem cell maturation. In the described culture system, the nonleukemic and early leukemic cells apparently retain the ability to respond to cell maturation factors, while this capacity is lacking in de novo AML cells. Unfortunately, these observations do not provide clues as to the etiology of the maturation defect, nor does this technique, although yielding statistically significant results, furnish a practical method of predicting which cases of RDA will convert to AML. Nevertheless, improved cell culture methods should contribute valuable information on growth and maturation of marrow cells in this disorder.

Refractory anemia, occurring in elderly people who frequently have underlying illnesses, makes management problems particularly difficult. In view of the fact that many RDA patients, especially those with acquired sideroblastic anemia, may survive and enjoy reasonably normal lives on transfusion therapy only, it is best to employ conservative measures as long as possible. Moreover, recent developments in the control of iron overloading by the use of desferroxamine have considerably alleviated the problem created by numerous blood transfusions. Even with onset of overt AML, it is obvious that many patients should be treated conservatively. Nonaggressive programs, such as reduced dosages of cytosine arabinoside and 6-thioguanine (CAT) and low-dose cytosine arabinoside, have been ineffective.[19] However, aggressive chemotherapy with daunorubicin and cytosine arabinoside resulted in no remissions and considerable toxicity.[20] It should be pointed out that some investigators have reported better tolerance of chemotherapy and complete remissions with reduced and less frequent doses of daunorubicin and cytosine arabinoside.[21] Our more recent experience tends to support these latter findings.

While the clinical and hematologic features of RDA have been studied extensively, thus far, the etiology and pathogenesis of the disorder remains enigmatic. In this series, there was no evidence of a significant exposure to chemicals, drugs, radiation, or other potential leukemogenic agents. It is important to emphasize that RDA is a disorder of older patients, especially males. This suggests a possible relationship to aging with the loss or impairment of factors or mechanisms

involved with regulation of hematopoiesis. Recent developments in the study of T cell biology and the potential role of T cell subsets on the regulation of hematopoiesis raises interesting possibilities, especially as it is known that aging is associated with loss of peripheral blood suppressor T cells (T5). It is possible that some combination of repeated insults to marrow stem cells from viral, chemical, or ionizing radiation associated with loss of T cell or some other regulating mechanism may ultimately emerge as the cause of this disorder.

ACKNOWLEDGMENT

The authors would like to thank Vincent King for his expert technical assistance.

REFERENCES

1. Linman JW, Bagby CG: The preleukemic syndrome: Clinical and laboratory features, natural courses and management. Blood Cells 2:11, 1976

2. Dreyfus B: Preleukemic states. Blood Cells 2:33, 1976

3. Heimpel H: Conventional morphological examination of blood and bone marrow cells in the diagnosis of preleukemic syndromes, in Schmalze F, Hellriegel KP (eds): Preleukemia. Berlin, Springer-Verlag, 1979, pp 4–11

4. Benestad HB: Formation of granulocytes and macrophages in diffusion chamber cultures of mouse blood leukocytes. Scand J Haematol 7:279, 1970

5. Greenberger J, Gans B, King V, Muse M, Karpas A, Moloney WC: Diffusion chamber culture in irradiated rats of myeloblasts and promyelocytes from 84 untreated patients with myeloid leukemias of refractory dysmyelopoietic anemia: Comparison to continuous tissue culture line, in Bentvelzen P, et al. (eds): Advances in Comparative Leukemia Research. Amsterdam, Elsevier/North-Holland Biomedical, 1978, pp 299–302

6. Smith WB, Ablin A, Goodman JR, Brecher G: Atypical megakaryocytes in preleukemic phase of acute myeloid leukemia. Blood 42:535, 1973

7. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DAG, Gralnick HR, Sultan C: The French-American-British (FAB) co-operative group. Proposals for the classification of the myelodysplastic syndromes. Br J Haematol 51:189, 1982

8. Pierre RV: Preleukemic states. Semin Hematol 11:73, 1974

9. Kass L: Preleukemic States. Chicago, Charles C Thomas, 1979

10. Schmalze F: Cytochemical investigation on the blood and bone marrow cells in preleukemia. Demonstration of maturational abnormalities, in Schmalze FS, Hellriegel KP (eds): Preleukemia. Berlin, Springer-Verlag, 1977, pp 48–55

11. Breton-Gorius J: Abnormalities of granulocytes and megakaryocytes in preleukemic syndromes, in Schmalze F, Hellriegel KP (eds): Preleukemia. Berlin, Springer-Verlag, 1977, pp 24–53

12. Dicke KA, Spitzer G, Verma DS: The value of in vitro culture in diagnosis and prognosis in preleukemia and oligoblastic leukemia, in Schmalze FS, Hellneyel KP (eds): Preleukemia. Berlin, Springer-Verlag, 1977, pp 118–122

13. Beran M, Hast R: Studies on human preleukemia. II. In vitro colony forming capacity in a regenerative anemia with hypercellular bone marrow. Scand J Haematol 21:139, 1978

14. Milner GR, Testa NG, Geary CG, Dexter TM, Muldal S, MacIver JF, Lajtha LG: Bone marrow culture studies in refractory cytopenia and "smouldering leukemia." Br J Haematol 35:251, 1977

15. Killmamm SA: Preleukemia: Does it exist? Blood Cells 2:33, 1976

16. Moore MAS: Bone marrow cultures in preleukemia. Proceedings 16th International Congress Series. Excerpta Medica 45:99, 1977

17. Hoelzer D, Kurrie E, Schmucker M, Harriss EB: Evidence for differentiation of human blood cells in diffusion chamber culture. Blood 49:729, 1977

18. Chikkappa G, Carsten AL, Chanana AD, Cronkite EP: Culture of normal human blood cells in diffusion chamber system II. Lymphocyte and plasma cell kinetics, in Cronkite EP, Carsten AL (eds): Diffusion Chamber Culture. Berlin, Springer-Verlag, 1980, pp 65–70

19. Moloney WC, Rosenthal DS: Treatment of early acute nonlymphocytic leukemia with low dose cytosine arabinoside, in Neth R, Gallo RC, Graf T, Mannweiler K, Winkler K (eds): Hematology and Blood Transfusions, vol. 26. Modern Trends in Human Leukemia IV. Berlin, Springer-Verlag, 1981, pp. 59–62

20. Moloney WC, Rosenthal DS: Acute leukaemia in adults secondary to other disorders, in Roath S (ed): Topical Reviews in Haematology, vol 1. Bristol, John Wright & Sons, 1980, pp 138–156

21. Bloomfield CD, Preisler H, Cuttner J, Yates J, Rai K, Wiernik P, Berkowitz E, Anderson J: Treatment-induced acute non-lymphocytic leukemia (t-ANLL): Response to cytarabine-anthracycline therapy. Blood 60:152a, 1982