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Presence of a Myeloproliferative Factor in Patients with Polycythemia Vera and Agnogenic Myeloid Metaplasia

I. Expansion of the Erythropoietin-Responsive Stem Cell Compartment[1] (38331)

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(Introduced by W. A. Robinson)

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The existence of a humoral factor that stimulates the multipotential stem cell has been strongly suggested by a variety of radiation recovery experiments. Boggs, Cartwright, and Wintrobe (1), Boggs *et al.* (2), and Marsh *et al.* (3) reported enhancement of endogenous spleen colony formation by foreign plasma. However, they were unable to exclude an internal shift of stem cells or change in stem-cell radiosensitivity as a possible explanation. Hanna *et al.* (4) and Nettesheim *et al.* (5) demonstrated that isologous 19S serum alpha macroglobulin promoted regeneration of hematopoietic stem cells, when given shortly after X-irradiation. Similar conclusions were reported by Berenblum, Burger, and Knysznski (6) and Burger, Knysznski, and Berenblum (7), who isolated a 19S-alpha-2 globulin from human serum that enhanced DNA synthesis of bone marrow cells from sublethally radiated mice. Knospe *et al.* (8, 9) found that injection of a noncellular extract prepared from a variety of organs, including spleen, liver, skin, and lung enhanced hematopoietic stem-cell recovery after radiation injury.

Polycythemia vera (PV) is characterized by hyperplasia of all three myeloid cell types, and agnogenic myeloid metaplasia (AMM) by the appearance of myeloid tissue in extramedullary sites. The trilineage myeloid metaplasia characteristic of AMM and frequently observed in PV represents a reversion of hematopoiesis to areas normally hematopoietic in the phylogeny and embryology of mammalian blood formation (10). Recently, we (10) postulated that these two diseases may be the result of an abnormal humoral stimulus acting on an intrinsically normal stem cell compartment. The presence of a 'myelostimulatory'' humoral factor in PV and AMM has been suggested previously by several workers. Linman *et al.* (11) reported an ether-soluble heat-stable factor in plasma of patients with PV that induced thrombocytosis, leukocytosis, and an erythromicrocytic response in normal rats. Reisner (12), using an *in vitro* culture of normal bone marrow, found that PV serum stimulated erythropoiesis and the serum from one patient with AMM stimulated fibroblast formation. In both reports, problems of technique clouded the separation of the ''myelostimulatory'' factor from the effects of erythropoietin (EP) and made the interpretation of the results controversial.

In recent years, evidence has accumulated that EP has a primary effect on the cell derived from the multipotential stem cell and already committed to the erythroid compartment. Gurney and Hofstra (13) described an assay for this cell, the EP-responsive stem cell, by measuring the degree of response to a standard dose of EP. In the present studies, the existence of a factor acting on the multipotential stem cell and, thereby, increasing the number of EP-responsive stem cells was evaluated in patients with the myeloproliferative syndromes by a modification of the EP-responsive stem cell assay. These studies suggest that serum from patients with PV and AMM does have the ability to increase the EP-responsive stem cell compartment. Failure to find a similar factor in chronic granulocytic leukemia (CGL) suggests a different cellular

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control mechanism in granulocytic leukemia from polycythemia vera and myeloid metaplasia.

Materials and Methods. Serum or plasma was obtained in the fasting state and frozen for 2–4 wk prior to assay. CFl female mice of age 10–12 wk were hypertransfused with two intraperitoneal (ip) injections of 1 ml of packed red cells from isogeneic donors. One milliliter of the serum to be evaluated for "myelostimulatory" factor was injected ip on each of Days 1, 3, and 4 after hypertransfusion. On Day 5 0.25 units of EP (FR No. 1, Connaught Laboratories, University of Toronto, Canada) in 0.5 ml saline was injected subcutaneously. $^{59}FeCl_3$ (0.5 μCi) in 0.5 ml of saline was injected ip on Day 7 and ^{59}Fe uptake into red cells determined on Day 10. The ^{59}Fe uptake was calculated as a percentage of the total dose, with the use of a formula for blood volume as 7% of the body weight.

Five to seven animals were used for each assay point. With each experiment the ^{59}Fe uptake after three injections of test serum or plasma followed by 0.25 U of EP was compared to: (1) EP control, three 1-ml injections of saline or normal serum followed by an injection of the standard 0.25 U of EP in 0.5 ml saline; and (2) test serum control, three 1-ml injections of the test serum followed by an injection of 0.5 ml saline. The normal serum control used in all these studies was pooled serum from blood of isoantigen type AB obtained from donors in the local blood bank. Mean values of ^{59}Fe incorporation into red cells were determined for each group and the significance of any difference determined by Student's *t* test.

The hematopoietic parameters of each patient at the time of study are summarized in Table I. Patients were in various stages of disease and no attempt was made to separate patients according to early or late changes in the natural history. The majority of patients with PV and CGL had received chemotherapy, but all therapy was stopped for at least 1 wk prior to the study. All patients with PV had hematocrits of 59% or higher at the time of study, and none had been phlebotomized in the month prior to study. Patient M. S. with idiopathic leukocytosis had been followed for 2 yr without determining an underlying etiology. Repeated bone marrow evaluations have not been consistent with a "myeloproliferative" disorder.

Results. Table II demonstrates the ^{59}Fe uptake after 1-ml injections of the test serum or plasma

TABLE I. Hematopoietic Parameters of Patients at the Time of Study.

Patient		Hct (%)	WBC (mm^3)	Platelets (mm^3)
Agnogenic myeloid metaplasia				
1 F.P.	61 M	44	18,600	350,000
2 P.W.-1	52 M	28	50,000	530,000
P.W.-2		53	44,700	420,000
3 A.Ma.	64 F	38	23,400	270,000
4 A.K.	48 F	42	17,600	250,000
Polycythemia vera				
1 A.F.	64 M	68	18,700	320,000
2 H.J.-1	57 F	64	9,300	300,000
H.J.-2		61	11,600	480,000
3 E.D.	60 F	67	6,200	500,000
4 S.R.	75 M	62	12,300	250,000
5 V.R.	58 M	59	12,600	327,000
Chronic granulocytic leukemia				
1 H.L.	50 F	52	19,200	325,000
2 G.L.	55 M	31	15,000	425,000
3 R.Mc.	52 M	43	44,800	530,000
4 D.J.	36 M	42	20,900	270,000
Miscellaneous				
1 M.S.	50 F Idio. leuk.	42	20,450	400,000

TABLE II. ^{59}Fe Uptake of Myeloproliferative Patients.

Injection of 1.0 ml on Days 1, 3, 4	^{59}Fe Uptake ± SEM (%)	
	Injection of 0.5 ml saline on Day 5[a]	Injection of 0.25 U EP in 0.5 ml saline on Day 5
Control		
1 Saline	0.7 ± 0.1	10.8[b] ± 1.2
2 AB serum	1.0 ± 0.2	8.6[b] ± 2.2
Agnogenic myeloid metaplasia		
1 F.P.	1.2 ± 0.2	25.6[c] ± 11.5
2a P.W.-1	4.6 ± 1.2	20.5[c] ± 4.6
b P.W.-2	3.2 ± 1.4	28.2[c] ± 7.4
3 A.Ma.	1.1 ± 0.4	24.6[c] ± 6.1
4 A.K.	0.4 ± 0.1	27.5[c] ± 3.5
Polycythemia vera		
1 A.F.	0.3 ± 0.1	30.5[c] ± 2.1
2a H.J.-1	0.9 ± 0.4	7.5 ± 3.4
b H.J.-2	0.3 ± 0.1	4.8 ± 2.4
3 E.D.	0.7 ± 0.1	28.2[c] ± 10.5
4 S.R.	0.4 ± 0.1	35.4[c] ± 6.2
5 U.R.	0.5 ± 0.1	41.9[c] ± 10.2
Chronic granulocytic leukemia		
1 H.L.	0.6 ± 0.1	10.2 ± 1.1
2 G.L.	3.9 ± 2.0	8.6 ± 1.8
3 R.Mc.	0.6 ± 0.1	5.0 ± 1.0
4 D.J.	0.7 ± 0.2	5.3 ± 1.8
Miscellaneous		
1 M.S.	1.2 ± 0.1	9.4 ± 2.9

[a] This column represents the test serum control described in the text and is the measure of endogenous EP used in this experiment.

[b] These values represent the EP controls described in the text.

[c] Values which differ significantly from both EP controls ($P < 0.05$).

followed by saline or 0.25 U of EP. It is apparent that normal AB serum does not differ from saline in its effect upon the ^{59}Fe uptake of either the saline or EP-treated hypertransfused mouse. The serum or plasma from all four patients with AMM and four of five patients with PV markedly enhanced ($P < 0.05$) the response to 0.25 U of EP as compared to the EP controls. A second serum sample from P. W. obtained 3 days after transfusion of the patient to a hematocrit of 53% continued to show enhancement (P. W.-2 in Table II). Furthermore, a second specimen from H. J. (H. J.-2) obtained 2 wk later continued to be without enhancement.

The serum of all four patients with CGL and one patient with idiopathic leukocytosis failed to enhance the response to EP. Significant levels of endogenous EP were found only in anemic patients P. W. and G. L. as measured by the test serum control method used in these experiments.

Discussion. The ability of serum or plasma from patients with PV and AMM to enhance the effect of EP is strikingly apparent. However, the presence of small amounts of endogenous EP in the test sera must be considered as a possible explanation. Gurney, Wackman, and Filmanowicz (14) found that fractionated injections of EP produced a greater response than a single dose. This seems an unlikely explanation in these experiments because eight of the nine serum samples from patients with AMM and PV did not have measurable levels of endogenous EP when evaluated by three injections of serum in the hypertransfused mouse assay. Failure to find EP in the serum of patients with PV confirms previous observations (15, 16). As expected (17), increased EP levels were not found in the three patients with relatively normal hematocrits. Although AMM patient P. W. had an elevated level of endogenous EP the enhanc-

ing effect of the serum was still present after the patient had been transfused to a normal hematocrit (Table II). In addition, the presence of endogenous EP in the serum of one patient with CGL did not enhance the EP response.

Whether the enhancement of the EP response by AMM or PV serum represents an increase in responsiveness of each EP-responsive cell, or an increase in numbers of EP-responsive cells cannot be evaluated by these experiments. The serum factor may alter the rate of proliferation of the stem cell pool and by changing the average length of G_1 increase the EP sensitivity of individual stem cells, a mechanism consistent with the work of Kretchmar, McDonald, and Lange (18). We favor an increase in the number of EP-responsive cells. The major support for this concept rests on the ability of the same serum to increase the number of granulocyte colonies using the *in vitro* agar method (19).

The presence of a serum factor that enhances the EP-responsive stem cells in patients with PV and AMM is consistent with a "myelostimulatory" humoral factor previously postulated as a pathogenetic mechanism in these diseases (10). The failure to find this factor in CGL lends further support to the separation of leukemia from the myeloproliferative disorders. Failure to demonstrate its presence in normal serum may represent insensitivity of the assay system and until chemical characterization allows concentration of normal serum, the role of this factor in normal, baseline hematopoiesis cannot be determined.

Summary. The presence of a factor in patients with the myeloproliferative syndrome that enhances the effect of erythropoietin on the EP-responsive stem cell was evaluated in hypertransfused mice. The serum of four patients with agnogenic myeloid metaplasia and four of five patients with polycythemia vera significantly increased the effect of a standard dose of EP. The serum of four patients with chronic granulocytic leukemia and one patient with idiopathic leukocytosis failed to enhance the response to EP. The enhancing effect could not be ascribed to EP in the injected serum. Evidence is presented that the serum factor either expands the number or increases individual sensitivity of the EP-responsive stem cells. Failure to find this factor in chronic granulocytic leukemia suggests a different control mechanism in leukemia from the other myeloproliferative disorders.

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