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GRANULOCYTE-MONOCYTE COLONY FORMING
CAPACITY OF HUMAN MARROW: A CLINICAL STUDY

C. G. Craddock, Esther F. Hays, Nils R. Forsén
and Dona Rodensky

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GRANULOCYTE-MONOCYTE COLONY FORMING
CAPACITY OF HUMAN MARROW: A CLINICAL STUDY

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The development of techniques for the in vitro culture of marrow in soft medium (1,2) has provided the opportunity to assess this parameter of marrow function in human disease. Formation of distinct colonies containing granulocytic and monocytic cells requires the presence of "colony stimulating factor" (CSF). CSF has been the subject of intensive study, and interesting variations have been observed in the activity of this material in serum and urine in various disease states and experimental manipulations. (3-10) This factor stimulates receptive cells in normal mammalian marrow to undergo proliferation to produce mixed colonies of granulocytic and monocytic cells when the marrow is cultured under certain conditions in soft agar or methyl cellulose. (6)

The present study was designed to provide a constant CSF stimulus to human marrow cells with the objective of assessing the colony-forming capacity (CFC) of marrow in various clinical situations. The stimulus employed has been pooled whole serum from C57 Bl mice injected with gram negative endotoxin 4 hours prior to bleeding (hereafter denoted EMS). Such treatment of mice has been shown to elicit a high titer of CSF in association with neutropenia and subsequent granulocytosis provoked by endotoxin administration. (10,11) CSF, like erythropoietin, is effective across species barriers. Others have employed various tissue cells, including granulocytes, as feeder layers or as cells secreting CSF into suspending medium. (1,2,6,10) Preliminary experiments indicated that in our hands serum from endotoxin treated mice provided the most active and constant readily available stimulus for colony formation by human bone marrow cell cultured in vitro.

MATERIALS AND METHODS

Material for culture was obtained from patients undergoing bone marrow examination as part of their diagnostic study. After 1 ml. was aspirated in a separate syringe for the usual cytological processing the needle was advanced a bit further and an additional 5 ml. of marrow-blood admixture withdrawn into a heparinized syringe. The marrow-blood admixture was then mixed with 20 ml. of tris buffered isotonic ammonium chloride to remove erythrocytes, after the method of Harris and Freireich.⁽¹²⁾ This mixture was quickly centrifuged and the sedimented nucleated cells treated again with the ammonium chloride and then suspended in 3 ml. of the culture medium. Control studies on the same marrow sample using the same number of nucleated marrow cells obtained by sedimentation without the ammonium chloride technique yielded identical colony counts. It is evident that this procedure (Fig. 1a and Fig. 1b) does not impair colony growth and simplifies the system by eliminating erythrocytes. The medium was McCoy's with 15% fetal calf serum and 50u/ml. of penicillin-streptomycin* per 100 ml. of medium. Cell aggregates were thoroughly disrupted by repeated pipetting into a Pasteur pipette and the nucleated cells concentration determined. A portion of the cell suspension sufficient to provide 5×10^5 nucleated cells was added to 9 ml. of the culture medium and 1 ml. of agar. From that point the procedure followed that described for mouse marrow culture.⁽⁴⁾

Endotoxin treatment of C57 Bl mice 4 hours before exsanguination with 5 ul. E. Coli endotoxin (Difco) intravenously elicits a serum titer of CSF of remarkable constancy when tested against C57 Bl, C₃H or AKR mouse marrow cells. (Fig. 1a). Pooled EMS employed in these experiments with human and mouse marrow was standardized, using a specimen of human urinary CSF

*Grand Island Biological Company

graciously supplied by Drs. E. R. Stanley and Donald Metcalf of the Eliza Hall Institute, Melbourne, Australia.

Using mouse marrow cells at a concentration of 5×10^4 per ml. the pooled EMS showed the approximate equivalence of one ul. to 1.33 units of the Stanley-Metcalf human material. (Fig. 1a). Marrow specimens were routinely cultured in duplicate with zero, 20 ul. and 40 ul. of EMS. In the case of human marrow prepared as described, 5×10^5 nucleated marrow cells were cultured in duplicate plates with 0, 20 ul. and 40 ul. of EMS. The equivalence of 1.3 units of Stanley-Metcalf material to one ul. of EMS was found in the human marrow system. (Fig. 1b). Variation between duplicate samples at each point was less than 10%. Mouse marrow colonies were counted at 4 and 7 days; human marrow colonies were counted at 7 and 10 days. The maximum numbers of granulocytic colonies formed from human marrow usually occurred at 7 days. Thereafter, the colonies diminished in cellularity and were comprised chiefly of monocytes. In both instances colonies were counted which formed loosely spherical constellations of cells in the agar, with approximately 500 to 5000 granulocytes and/or monocytes per colony as verified by removal of selected colonies from the agar and examination under the microscope after staining. Small aggregates of poorly defined cells, observed in cultures of leukemic marrow, and plaque-like collections of cells were not counted in these studies. In certain other reports such cell collections have been counted, which probably accounts for the great variability of AML marrow in this system as reported by different investigators. (8,9,12,14,16) Since it is not clear that this represents a response to CSF analogous to that of normal marrow, we have restricted colony counts to those which meet the gross and microscopic criteria described.

RESULTS

It was found, as might be expected, that the total numbers of colonies from "normal" human marrow varied greatly depending upon several technical factors, the most important being the amount of sinusoidal blood diluting the aspirated marrow sample. Peripheral or sinusoidal blood contains a smaller fraction of cells with colony forming capacity than does marrow. (13)

There was considerable variation in the amount of colony formation per 10^5 human cells with added CSF and there was invariably some colony formation without EMS, as shown in Fig. 2. This variation was roughly related to the concentration of nucleated cells in the marrow specimen after preparation for culture, the more dilute specimens usually providing a lower colony count after culture.

There was a variable increment in colony formation when 20 ul. of EMS was added, as shown in Fig. 2. The further increment with 40 ul. of EMS was more constant with a mean value of 1.75 ($\pm$ 0.227 S.D.) times the 20 ul. point.

The relative constancy of this relationship was further attested by observation on serial marrow samples from hematologically normal subjects with rheumatoid disease undergoing repeated marrow aspiration as part of another study, as shown in Table I. It will be noted that the total number of colonies with or without added EMS varies, there being less colony formation when the marrow specimen is more diluted with blood as indicated by the volume of the marrow suspension required to provide 5×10^5 nucleated cells. However, the colony count at the 40 ul. EMS concentration exceeds that at the 20 ul. EMS concentration by a ratio which varies with narrow limits, indicating that the response to EMS occurs similarly regardless of the numbers of cells with colony-forming capacity plated and regardless of the numbers of cells plated which would form colonies without added CSF in the form of EMS. This ratio is denoted as the CSF response ratio and was used as the major criterion of marrow granulocyte-monocyte colony forming capacity in this study.

The CSF response ratio of 1.75 ($\pm$ 0.227 S.D.) is derived from 43 marrow aspirates on 37 subjects who displayed no obvious abnormality of granulocyte count or marrow morphology. This group included patients with anemia or thrombocytopenia due to various causes (iron deficiency, folate deficiency, acquired, autoimmune hemolytic disease, idiopathic thrombocytopenic purpura, rheumatoid arthritis, acute and chronic renal insufficiency, carcinoma without obvious marrow involvement, Hodgkin's disease without obvious marrow involvement and "stress" erythrocytosis. None of the patients was receiving myelotoxic agents or corticosteroids. The actual amount of colony formation by these patients is shown in Fig. 2.

There were seven patients with untreated, active Hodgkin's disease included in the total group of 37 patients forming the control group of subjects without obvious disturbance of marrow granulopoiesis (Table II). It will be noted that *in vitro* colony formation was active in all these subjects and the CSF response ratios exceeded the mean for the entire group (Table I) in all instances. The CSF response ratio in this group of Hodgkin's disease patients was 2.02 ($\pm$ 0.136 S.D.) compared to 1.704 ($\pm$ 0.208 S.D.) for patients without Hodgkin's disease shown in Fig. 2, a difference that is significant ($p < 0.005$). It may be that the response to CSF in this subgroup of patients with Hodgkin's disease but without marrow involvement more closely approximates the normal than do the other patients included in this "normal" group. On the other hand, the finding may indicate increased in vitro response to CSF in subjects with Hodgkin's disease.

Six patients with persistent mild to moderate neutropenia of unknown cause with diminished marrow content of granulocytic precursors were studied (individual data not shown). The CSF response ratio in this group was 1.385 ($\pm$ 0.121 S.D.).

Other patients with low (<1.5) CSF response ratios included one with a marked erythropoietic response to autoimmune hemolytic disease, one with acute renal failure (acute tubular necrosis) and two patients with collagen vascular disease after prolonged thoracic duct lymph drainage. Both of these latter patients displayed a CSF response ratio within the normal range prior to and during thoracic duct drainage (Table I). Two patients recovering from drug induced neutropenia (one due to phenothiazine, the other propyl thouracil) were assessed at a time when the marrow showed hyperplasia and immaturity in the granulocytic series although the blood granulocyte counts were still low. The CSF response ratios were the highest observed in this study (2.6 and 2.8).

Six patients with various forms of "myeloproliferative syndrome" were tested; not all of these patients were neutropenic, but all showed some abnormality of colony formation in vitro. (Table III). Richly cellular marrow samples were obtained in all patients, so that the failure of colony formation in three was not attributable to hemodilution. The three patients (4, 5, and 6) showing adequate numbers of colonies in vitro, nonetheless showed low CSF response ratios.

Two patients with chronic lymphocytic leukemia were assessed. Neither patient was receiving therapy. One patient showed entirely normal blood values except for an absolute lymphocyte count of $5000/\text{mm}^3$. The other was anemic, neutropenic and thrombocytopenic. The marrow cellularity of each patient was predominantly (over 80%) small lymphocytes. The marrow of neither patient showed detectable colony formation. One patient with lymphocytic lymphosarcoma and focal marrow involvement showed normal growth and CSF response ratio. Another patient with this disease but with over 50% of marrow cells comprised of lymphosarcoma cells showed sparse colony formation.

Three patients with metastatic adenocarcinoma but normal blood leukocyte counts were studied. One had no detectable marrow involvement and showed an entirely normal response. Another had minimal involvement with a few metastatic cells; this patient showed a CSF response ratio of 2.5. The third patient's marrow was almost entirely replaced by tumor and showed no growth.

The marrow of one patient with chronic granulocytic leukemia (Ph¹ chromosome positive) was assessed on three occasions; the patient was on small doses of myeleran at the time of the first two studies and was off myeleran on the third occasion. The CSF response ratios were 1.84, 1.53 and 2.5 respectively. The total number of colonies formed with the first sample was the highest observed in this series.

12 patients with myeloblastic leukemia were assessed, some at different stages of their disease course. (Table IV). All cases in this series with one exception (8 Ke) showed Auer rods in leukemic cells. Cases were studied without treatment being given at the time or within one week prior to the marrow sampling. Colony formation was absent or minimal in all instances when the disease was in an uncontrolled state. There was no correlation between the extent of marrow blastosis and colony formation in these patients with uncontrolled disease. Two patients (4Car and 2Be) with 8% and 20% marrow myeloblasts respectively and a preponderance of abnormal promyelocytes and myelocytes showed no colony formation before any treatment was begun. In patient 12 Pi repeated cultures over a one year period failed to show colony formation regardless of the degree of marrow blastosis. This patient had a subacute form of the disease, usually with 12-20% of more mature granulocytic forms in the marrow. His course was one of chronic neutropenia, blastosis and anemia, usually with adequate platelets. Similar results were obtained on patient 7 Ge who ran a similar

course. Neither patient benefitted from therapy. In 6 of 8 instances, where cultures were made from patients achieving complete or partial remission, colony formation occurred. However, four of these specimens were from the same subject (Ke). Case 8 Ke had a normal CSF response ratio on one occasion and in one other patient this parameter was normal; in all others the CSF response ratio was low. All of these patients with the exception of Ke were on some form of maintenance therapy at the time of the study, but had not received cytotoxic agents for 7 days prior to the study. It is not known how much the diminished CSF response was caused by treatment. It is of interest that patient Ke has achieved the longest sustained remission in this group and is presently hematologically normal without maintenance therapy for over 1 year. This patient lacked Auer rods while in relapse and the accuracy of the diagnosis might be questioned.

Table V provides similar data on five adult patients with lymphoblastic leukemia, including seven separate specimens in case 2 Mor. It would appear that in vitro colony formation may approach normal in these patients even though the marrow is not entirely devoid of leukemic blast cells. When the marrow contains a preponderance of leukemic lymphoblasts there is absent or severely depressed colony formation, but percentages of 20 and 30% of leukemic blasts did not prevent colony formation by residual elements with this capacity. Thus, the CSF response ratios were normal or high in each instance where colony formation occurred. This is in contrast to the generally diminished ratios in the few myeloblastic cases which formed colonies when the marrow was not totally replaced with blast forms.

DISCUSSION

Aspirated marrow from human beings contains variable numbers of cells with colony forming capacity when cultured in soft agar under the conditions described. This colony formation without added CSF probably reflects the presence of cells already stimulated to produce colonies before the marrow is aspirated, the presence of CSF in the marrow-blood culture fluid admixture, or both. Marrow removed directly from the femurs of sacrificed mice contains less blood contamination and usually no cells exhibiting autonomous in vitro colony formation in the absence of added CSF when 10^4 cells per ml. are plated. The necessity to plate human marrow cells in ten times the number of mouse marrow cells to obtain comparable colony formation in vitro reflects, in part, the greater dilution of the aspirated human marrow tissue with blood containing a small percentage of cells with colony forming capacity and the differences between human and mouse marrow with respect to content of mature cells with no inherent colony forming capacity. The requirement to plate a higher concentration of human cells to obtain reproducible colony formation and the formation of colonies in the absence of added CSF renders more difficult the assessment of human cultures. In the present study the increment in granulocyte-monocyte colony formation by human marrow between two concentrations of added CSF was determined and found to be relatively constant in subjects who showed no obvious impairment in granulopoiesis.

These studies indicate that the actual number of cells plated with colony forming capacity is a crucial consideration in any quantitative assessment of colony forming capacity by human marrow. Marrow from patients with chronic lymphocytic leukemia and marrow replacement with lymphocytic lymphosarcoma or carcinomatous tissue, failed to produce colonies when plated at the standard

concentration of nucleated marrow cells. In some of these patients blood granulocyte levels were normal, indicating that overall granulopoiesis was not impaired. Furthermore, when the marrow sample contained a minor percentage of lymphomatous or carcinomatous cells colony formation was normal. The total number of cells plated would have to be related to the number of cells with the potential of forming colonies in order to validly assess the in vitro marrow response in such patients. Since this may not be known in advance it is clear that the incremental response to two or more dose levels of CSF provides a more practical maneuver to assess in vitro CSF responsiveness.

The present data on in vitro colony forming capacity of marrow from subjects with leukemia and certain myeloproliferative disorders were carried out with EMS rather than CSF derived from human sources. This may account for some of the differences between our findings and those of others. Furthermore, the criteria of acceptable colony formation were quite rigid. Small, "hard" aggregates of cells without clearly discernable mature granulocytes or monocytes were not accepted as colonies of true granulocytic and/or monocytic cells. Reports by others on patients with these conditions, particularly myeloblastic leukemia, are conflicting. Most reports agree that when the marrow is predominantly blastic, colony formation is absent or markedly reduced. Some isolated cases, however, have shown excessive numbers of small colonies. (8,9,15) Although this type of cell replication may have great significance, it is uncertain whether such small aggregates would have been counted by the criteria set by our study. It is of critical importance to know whether leukemic blast cells can indeed differentiate to form granulocyte-monocyte colonies in vitro, as the report of Duttera et al would indicate. (17) The present study does not provide any data on that important point.

Nevertheless, these data appear to follow a certain pattern with respect to colony forming capacity of marrow from leukemic and myeloproliferative disease subjects. The number of cases is small, as is true for most other reports to date, and these data must be viewed as preliminary. Three separate studies in a patient with Ph¹ chromosome positive CGL showed spontaneous colony formation and a positive response to CSF. A few patients with other myeloproliferative disorders and others with idiopathic neutropenia failed to show adequate numbers of colonies, normal response to CSF, or both. This difference in in vitro responsiveness to CSF of CGL marrow, as contrasted to the other patients with "myeloproliferative" disorders, is consistent with other observations suggesting that CGL is a distinct entity and that these other conditions represent differing disorders of stem cell differentiation and function. (18,19) Clearly, however, confirmation of these preliminary findings is needed before concluding that CGL and other myeloproliferative disorders differ consistently with respect to colony formation in vitro.

Patients with myeloblastic leukemia in whom there was no question about the diagnosis on the basis of Auer rods in leukemic cells, showed no colony formation when the marrow was comprised chiefly of leukemic cells. This was true in all cases even when the percentage of true blast cells was low, the majority being more differentiated but morphologically abnormal promyelocytes or myelocytes (cases 4 Car with 8% blasts and 2 Be with 20% blasts). Others have reported normal colony formation when remission is achieved in such subjects. (9) Our results showed adequate colony formation of 6 of 8 marrow cultures on patients who had achieved complete or partial remission; however, four of these successful cultures were from the same patient. This subject was the only

case included in the AML group with no Auer rods in the leukemic cells. Furthermore, only two studies showed a CSF response ratio which was clearly normal and one of these was in this same subject (Ke) after a sustained remission of six months without therapy. It may be argued that the therapy that these subjects were receiving altered CSF responsiveness, a point that cannot be ignored in the interpretation of these findings. Nevertheless, these data appear to show that subjects with AML, in contrast to ALL, may not show a normal response to CSF in the form of EMS even after achieving remission. Furthermore, colony formation was absent or minimal in four cases where some maturation of the leukemic process beyond the blast stage was clearly evident in the marrow. In two of these cases therapy was not a factor. In all four cases the leukemic process ran more of the course of a subacute hypoproliferative process with features which overlap with variants of aplastic or refractory anemia with pancytopenia. Subjects with this type of disease may be different from AML with a more uniformly blastic-leukemic population and, like certain subjects with refractory anemia which also show diminished in vitro response to CSF, may represent a type of disordered stem cell development and function. (18)

The results in patients with ALL, though limited in number, indicate a quite different response of some marrow specimens to CSF. Unlike AML and myeloproliferative disorders, other than CGL, in many instances (6 of 13) the CSF response ratio was normal or increased. This was true in some specimens even though a significant percentage of the cells plated were believed to be leukemic lymphoblasts. When the leukemic blasts reached 70 to 90%, however, there was no colony formation. The serial studies in patient Mor show this capacity of ALL marrow to form colonies when the patient is in partial remission

or relapse. Not all subjects with ALL in remission show CFC or CSF response, as indicated by 3 Ste. Interestingly, this patient was on high dose prednisone therapy at the time these cultures were made; cortisol has been shown to suppress CFC in cultures of mouse marrow⁽²⁰⁾ and this may have been a factor in the lack of colony formation in this instance. In any case, the data indicate that in some adult patients with ALL CFC and CFS response may be normal even though the marrow contains a significant percentage of leukemic lymphoblasts, similar to our findings in patients with lymphosarcomatous or carcinomatous marrow involvement. This finding is in agreement with observations in this and other laboratories in AKR mice with Gross virus leukemia. This leukemia begins in the thymus and involves the marrow late in the course of the disease. Marrow containing lymphoblastic leukemic cells exhibits normal in vitro colony formation and response to CSF when the number of cells plated is adjusted to a standard number of non-leukemic cells. These data, to be presented in another report, suggest that this animal model may be a useful one to compare with human ALL with respect to this in vitro response.

These findings in AML and the myeloproliferative disorders, though preliminary, suggest that these states alter myelopoiesis in such a way that colony forming capacity and responsiveness to added CSF is altered in vitro. This is in contrast to the retained responsiveness of cells with CFC from marrow specimens containing tumor cells, ALL blast cells, Gross leukemia cells or CGL tissue. The findings also emphasize the multiplicity of variables to be considered in terms of clinical diagnosis, effect of therapy and techniques of handling the cells and culture system in clinical studies of this type. Some of these variables may account for the discrepancies in reports concerning CFC in hematological diseases.

Nevertheless, it is believed that this procedure and other modifications will have considerable value in the future in the diagnosis, management and investigation of these diseases. The results in the two subjects with drug induced neutropenia and marrow findings confusing with a leukemic state is a good example. In these two cases CFC was increased, in contrast to the subnormal responses in patients with AML in relapse, myeloproliferative disorders or chronic idiopathic neutropenia.

SUMMARY OR ABSTRACT

A modification of the in vitro culture of marrow cells in soft agar to form granulocyte-monocyte colonies has been applied to human marrow using a constant stimulus of colony stimulating factor (CSF) in the form of pooled serum from endotoxin treated C57 Bl mice (EMS). With this system reproducible increments in colony formation were observed, between two concentrations of EMS, in 43 marrow cultures from 37 patients with no obvious disturbance in granulopoiesis. This assay was applied to a number of patients with different types of marrow involvement. Only those colonies which contained mature granulocytes and/or monocytes and had the colonial form of "true" colonies were counted.

Results indicate:

1. Colony-formation in vitro can be validly assessed only if the numbers of marrow cells plated contains cells with colony-forming capacity. The incremental response to two doses of CSF (CSF response ratio) provides a consistent assessment of in vitro colony formation and, we believe, provides more reliable measurement of CFC than colony counts at a single dose level of CSF.
2. The presence of minor percentages of sarcoma cells, carcinoma cells and leukemic lymphoblasts does not impede in vitro colony formation.

3. One patient with Ph¹ positive CGL, studied on three occasions showed active colony formation and normal or increased response to EMS. In contrast patients with other types of myeloproliferative disorders, including myeloblastic leukemia, usually showed some impairment of response even when the marrow appeared normal. Marrow from two patients recovering from drug induced neutropenia showed increased response to CSF. Normal or increased response was observed in some cases with ALL in partial or complete remission, in contrast to the subnormal response in most specimens from patients with AML.

TABLE I

CASE #	DATE	VOLUME OF SUSP. TO GIVE 5×10^5 NUCLEATED CELLS	COLONIES PER 10^5 CELLS (AMT. PER EMS PER PLATE IN UL)			CSF RESPONSE RATIO
		(ml)	(0)	(20)	(40)	
1	7/7/71	0.16	10	42	70.5	1.66
1	8/9/71	0.50	8	34	44	1.73
1	10/22/71	0.13	68	82	141	1.72
1	11/24/71	0.14	62.5	80	140.5	1.75
2	1/18/72	0.20	22	25	55	1.76
2	1/30/72	0.33	32	38	44	1.58
3	7/7/71	0.25	30	60	100	1.67
3	9/2/71	0.5	6	20	30	1.70

Legend:

Response to EMS of marrow from hematologically normal subjects with collagen-vascular disease undergoing thoracic duct drainage. Repeat marrow cultures showing variability in colony numbers, chiefly related to the amount of hemodilution of the marrow aspirated as indicated by the variation in the volume of marrow suspension required to provide 5×10^5 nucleated cells in the culture mixture. Note that the CSF response ratio (colonies at 40 ul EMS/plate over colonies at 20 ul EMS/plate) is relatively constant.

TABLE II

HODGKIN'S DISEASE

CASE #	CLINICAL STAGE	VOLUME OF SUSP. REQUIRED FOR 10^5 /ml. NUC. CELLS	COLONIES PER 10^5 CELLS (AMT. OF EMS PER PLATE IN UL)			CSF RESPONSE RATIO
			(0)	(20)	(40)	
1. (E.C.)	II	0.3	10	80	160	2.0
2. (R.G.)	Ib	0.1	0	8	20	2.0
3. (H.G.)	IVb	0.2	6	20	45	2.25
4. (R.G.)	IVb	0.4	5	25	45	1.8
5. (S.V.)	I	0.1	20	38	75	1.97
6. (D.Z.)	I	0.2	30	45	90	2.0
7. (D.S.)	IV	0.3	4	18	38	2.1

Legend:

Response to EMS of marrow aspirated from patients with Hodgkin's disease but without obvious marrow abnormality. This subgroup is included in the hematologically "normal" group shown in Fig. 2.

TABLE III

CASE	BLOOD	MARROW	COLONY #	CSF RESPONSE RATIO
1. (F)	Anemia Thrombocytopenia	Erythroid↑	Few	--
2. (P)	Anemia Thrombocytopenia	Erythroid↑ Megakaryocytes↓	Few, slow growing	1.35
3. (M)	Thrombocytosis P.R.V.	Megakaryocytes↑ Erythroid↑	Few, slow growing	1.3
4. (S)	Thrombocytosis	Megakaryocytes↑	Many	1.2
5. (A)	Thrombocytosis Erythrocytosis	Megakaryocytes↑ Erythroid↑	Many	1.31
6. (N)	Sideroblastic Anemia	Erythroid↑	Many	1.18
7. (H)	Anemia Thrombocytopenia	Erythroid↑ Megakaryocytes↓	No colonies	--

Legend:

In vitro colony formation and response to EMS by marrow from patients with various "myeloproliferative" disorders. CSF response ratio was not determined in those instances where the numbers of colonies formed was severely reduced (< 10 per 10^5 cells at 40 ul of EMS per plate). Richly cellular marrow were obtained in all, with a minimum of hemodilution.

TABLE IV

CASE & STATUS	AUER RODS	MARROW BLASTS %	VOL. SUSP. WITH 5x10 ⁵ NUC. CELLS ml.	COLONIES PER 10 ⁵ CELLS (AMT. EMS/PLATE, IN UL).			CSF RESPONSE RATIO*
				(0)	(20)	(40)	
1. Al 7d p AraC, 6-TG ⁺	+	2	2.5	3	4.5	5	--
2. Be Initial Dx	+	20	0.5	0	1	3	--
3. Br Initial Dx 7d p Ara C, 6-TG	+	81 20	0.07 1.0	1 9	0.5 15	3.5 24	-- 1.6
4. Car Initial Dx	+	8	0.4	0	0	3.5	--
5. Ev 12d p Ara C, 6-TG	+	50	0.3	1.5	3	4	--
6. Fr (4/22/71) 12d p AraC (5/27/71) 12d p AraC, #2 (8/5/71) 12d p COAP (8/9/71) 16d p COAP (11/14/71) 14d p COAP, #2	+	19 2(M-1) 85 60 68	1.5 0.14 1.7 1.5 0.1	0 28 0 0 0	0 39 0 0 0	0 59 2 2 0	-- 1.51 -- -- --
7. Ge 14d p AraC, 6-TG 3d p 6MP, Pred.	+	52 15	0.22 0.3	0 0	0 0	1.5 0	-- --
8. Ke (6/1/71) 7d p AraC, 6TG 12d p Pred, 6MP (6/10/71) Wkly AraC (11/23/71) Wkly AraC (6/20/72) None, 6 mos.	-	5 2(M-1) 1(M-1) 1(M-1)	0.51 1.7 0.22 0.20	5 3 26 32	33 16 29 58	50 19 43 102	1.52 1.19 1.50 1.78
9. LaM 14d p AraC, 6-TG	+	75	0.23	0	0	0	--
10. LO 7d p AraC	+	76	0.3	0	0	2.5	--
11. Na Initial Dx	+	80	0.3	0	0	0	--
12. P1 (6/28/71) Initial Dx (7/13/71) 14d p AraC, 6TG (7/23/71) 1 wk. p AraC (8/12/71) 1 wk. p AraC (12/13/71) 1 wk. p COAP (1/6/72) none (3/3/72) none	+	50 40 20 20 50 50 40	0.82 0.25 0.9 2.0 3.0 1.0 0.15	0 0 1.5 0 0 0 0	0 0 1.5 0 0 0 0	0 0 3.5 0 0 0 0	-- -- -- -- -- -- --

Legend:

Marrow from patients with Myeloblastic Leukemia: Response to EMS.

* CSF Response Ratio (colony count at 40 ul EMS/plate over value at 20 ul/plate)
not calculated unless colonies exceeded 10 per plate at higher concentration of
EMS.

+ Drugs abbreviations: Ara C = Cytosine Arabinside ; 6-TG = 6 Thioguanine;
COAP = Southwestern Chemotherapy Group protocol for Cyclophosphamide, Cytosine
arabinside, vincristine and prednisone; Pred = prednisone; 6-MP = 6-mercaptopurine;
Mtx = methotrexate.

TABLE V

MARROW FROM PATIENTS WITH
LYMPHOBLASTIC LEUKEMIA: RESPONSE TO EMS

CASE AND STATUS	MARROW BLASTS %	VOL. SUSP. WITH 5×10^5 NUC. CELLS ml.	COLONIES PER 10^5 CELLS (AMT. EMS/PLATE IN UL).			CSF RESPONSE RATIO
			(0)	(20)	(40)	
1. Le Initial Dx	90	0.1	0	0	0	--
2. Mor (6/15/71) 7d p Mtx	30	0.3	4	10	27	2.7
(6/18/71) 10d p Mtx	80	0.3	0	0	1.5	--
(6/28/71) 7d p VAMP	27	0.4	0	21.5	38	1.73
(6/30/71) 9d p VAMP (fever)	10					
(7/9/71) 6d p VAMP, #2	5	0.5	21.5	44.5	80.0	1.78
(11/12/71) 7d p COP	75	0.73	0	0	2.5	--
(11/30/71) 7d p COP	40	1.9	2.5	4.0	4.0	--
(1/25/72) 7d p L-ASP	5	0.43	20	20	44	2.2
3. Ste 14d p VAMP, on Pred.	5(Ml)	0.33	0	1	2.5	--
Ste 7d p VAMP, on Pred.	15	0.32	0	0	0	--
4. Wal 7d p 6MP, VCR. on Pred.	43	0.57	4	6	14	2.33
5. Wi 7d p VCR, Mtx on Pred.	20	0.24	13	19	36	1.9
Wi 10d p COAP	85	0.09	0.5	1	3	--

Drug abbreviations:

Mtx = Methotrexate parenterally; VAMP = Vincristine x 6-MP x Methotrexate and Prednisone;
COP = Cyclophosphamide, Oncovin (Vincristine) and Prednisone; L-ASP = l-asparaginase

FIG. 1a Colony formation by mouse (10 C₃H, male and female, 4 wk.) bone marrow at various increments of EMS (pooled serum from C57 Bl mice given E. Coli endotoxin intravenously 4 hours before bleeding) and *human urinary CSF supplied by Drs. E. R. Stanley and D. Metcalf of Melbourne, Australia. The human material is designated in terms of unitage as defined by these investigators. Similar linear response to EMS were observed in a variety of normal mouse strains; although the amount of colony formation may vary from strain to strain, the quantity is similar within a given strain provided the mice are of similar age. Vertical brackets represent one standard deviation.

FIG. 1b Colony formation by normal human marrow (from a patient with stage Ia Hodgkin's disease) at various increments of EMS and *human urinary CSF. Note that the numbers of cells plated is 10 times the number of mouse marrow cells plated (Fig. 1a) and that colony formation is observed without added EMS or human urinary CSF. Each point represents the average of four separate plates at each concentration of EMS or human urinary CSF; variation between plates was less than 10%.

FIG. 2 Colony formation by human marrow from "normal" subjects ("normal" with respect to obvious defects in granulopoiesis; see text) with 0, 20 μ l and 40 μ l of EMS per plate. Note that in spite of great variation between individuals with respect to the quantity of colonies formed, that the increment between the 20 μ l and 40 μ l concentrations of EMS is relatively constant. The value at 40 μ l EMS over the value at 20 μ l EMS is the "CSF response ratio."

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