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Granulopoietic Activity of Urine and Cells from Patients with Chronic Granulocytic Leukemia (38134)

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Granulocyte colony formation *in vitro* can be stimulated by a variety of cells and body fluids (1–5). The colony stimulating factor (CSF) found in human urine has been shown to be a glycoprotein with a mol wt of about 40,000 which migrates electrophoretically as an alpha globulin (6). The major sources of this material remain unknown but it is clear that peripheral white blood cells (WBC) are at least one large source in humans (7).

Wide variation in the levels of CSF in urine have been noted in various disease states (2, 3, 8, 9). In patients with aplastic and hypoplastic anemias with agranulocytosis, low urinary levels of CSF have been found and infusion of large numbers of leucocytes leads to a marked rise in the excretion of CSF in the urine (9, 10). In a single patient with chronic cyclic neutropenia urinary CSF has been shown to rise and fall concomitantly with the peripheral granulocyte count (11).

These and other findings have led our laboratory to propose a positive feed-back mechanism in the regulation of granulocyte production (7, 11). In the proposed scheme mature granulocytes secrete factors which stimulate new production of this cell line *in vivo* similar to that seen *in vitro*. Other authors have postulated that the monocyte may be the major cellular source of CSF (12–14). Of particular interest with this scheme in mind have been studies on humans with various forms of leukemia.

Levels of urinary CSF in patients with acute leukemia have been reported (3, 4, 8, 15). In patients with acute granulocytic leukemia, CSF levels have been found to be largely low or absent without definite correlation between the amount found and the peripheral WBC count (3). In this group of patients levels may rise during treatment, often becoming normal to very high during remission, only to fall to subnormal levels with relapse. In patients with acute stem cell or acute lymphocytic leukemia, high levels of urinary CSF have been found (3). In 4 patients with acute lymphocytic leukemia studied by Mack *et al.*, the urinary CSF levels were variable and did not appear to correlate with colony growth of peripheral blood cells, total WBC count, or clinical course (15). There did, however, appear to be a correlation between absolute numbers of mature granulocytes in the peripheral blood and urinary colony stimulating activity (CSA) levels with the highest CSA levels found in patients with the highest mature granulocyte counts.

Urinary CSA levels in 8 patients with chronic granulocytic leukemia have previously been reported (3). In the majority of these, high levels were noted. No mention was, however, made of the relationship between CSA and clinical course, WBC or differential. In 1 patient with untreated chronic granulocytic leukemia (CGL) and spontaneous WBC cycling, the levels of serum and urinary CSA have been found to cycle at similar, though out of phase, intervals (16).

The following studies were done to determine whether in a large group of patients with

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CGL, correlations could be found between total WBC count, differential, and urinary CSA values in an attempt to define both the cellular source and possible regulatory defect in CGL. In addition, the CSA of peripheral leucocytes from 3 patients with CGL have been studied using these cells as feeder layers for normal human bone marrow colony growth *in vitro*.

Materials and Methods. The patients studied were seen between March, 1968 and May, 1973 at the University of Colorado Medical Center and Denver Children's Hospital. The diagnosis of chronic granulocytic leukemia was made on the basis of peripheral blood smear, bone marrow biopsy through the posterior iliac crest, leucocyte alkaline phosphatase staining of the peripheral blood, and chromosomal analysis. In all of the studies reported the patients had not received treatment or transfusions in the preceding 48 hr unless otherwise noted.

The method for testing urine and serum for CSA has been reported in detail elsewhere (3). In brief, 0.15 ml of dialyzed, sedimented, filter sterilized urine or 0.1 ml of untreated serum was placed in triplicate 35 mm petri dishes to which was added 1 ml of a mixture of McCoy's 5A medium and 0.3% agar containing 75,000 nucleated bone marrow cells from the femurs of C57/bl mice. After mixing of medium and urine or serum, the plates were allowed to gel and then incubated at 37° with 100% humidity in an atmosphere of 7.5% CO_2 in air. All of the assays were done on a single day. After 7 days incubation colony counts were done as an index of CSA. A complete blood count with differential was done on the day of urine collection.

The cells of 3 patients were studied for their ability to stimulate colony formation by normal human bone marrow cells. The technical details of human bone marrow colony growth in this system have also been described elsewhere (17). Peripheral blood cells from the patients to be used as feeder layers were collected in heparinized tubes and allowed to sediment by gravity for 1–2 hr. 1×10^6 nucleated cells were then mixed in 1 ml of McCoy's 5A medium in 0.5% agar and allowed to gel. Feeder layers were prepared using cells washed free of plasma and with remaining autologous plasma. Bone marrow cells from normal human volunteers to be used as the target cell source were mixed in a concentration of 2×10^5 nucleated cells/ml in McCoy's 5A medium in 0.3% agar and overlaid on the previous prepared feeder layers in 1 ml aliquots. Control plates consisted of feeder layers prepared from washed and unwashed normal human WBC and medium-agar underlays without feeder layer cells. When using human bone marrow the incubation conditions were the same but colony counts were done at day 14 rather than day 7 of incubation.

Only colonies containing 20 or more cells were counted. For histologic studies colonies were removed from the agar with a finely drawn Pasteur pipette and stained with aceto-orcein as previously described (17).

Results. Table I shows the clinical parameters, urine and serum CSA values in the 18 patients studied. Urinary CSA ranged from 22 to 396 with a mean of 82 colonies for the entire group. The normal range in our laboratory is from 0 to 40. Serum values ranged from 5 to 61 in CGL patients with a mean of 32. Values for normal humans in our laboratory are 0–40. No correlation was found between the total peripheral WBC count and urinary or serum CSA values. Likewise, no correlation was found between the absolute number of mature or immature granulocytes or monocytes and CSA values.

Table II shows the feeder layer activity of washed and unwashed peripheral WBC from 3 patients compared with peripheral WBC feeder layers from a normal human volunteer. Washed peripheral WBC cells from all 3 patients with CGL stimulated 2–4 times as many colonies as those from the normal human volunteer. When autologous plasma from the patients was allowed to remain in the culture system, however, the values were similar to those observed using normal peripheral blood feeder layers. This effect of washing was not observed with normal humans WBC feeder layers. Whether this apparent enhancement of colony growth was the result of removal of an inhibitor present in the patient's plasma could not be determined but is suggested by the data presented.

Discussion. The present studies have confirmed the previously reported finding of elevated levels of urine and serum CSA in patients with CGL. Surprising in the present

TABLE I. Clinical Parameters, Urinary and Serum Colony Stimulating Activity (CSA) Values in 18 Patients with Chronic Granulocytic Leukemia.

(Patient)	(Age)	(Sex)	(HCT)	(WBC)	(NEUT)	(Band)	(Lymph)	(Mono)	Differential (Baso)	(Eos)	(Meta)	(Myelo)	(Pro)	(Blast)	Previous therapy	Urine CSA[a]	Serum CSA
M.Q.	22	M	34	6,000	65	5	19	4	3	4	0	0	0	0	Myleran	54	
R.H.	40	M	36	7,000	77	0	18	3	2	0	0	0	0	0		67	
C.Y.	33	M	49	12,800	64	16	16	2	1	1	0	0	0	0	Myleran	49	
D.B.	59	M	22	15,200	29	18	4	1	0	0	8	9	6	25		177	54
R.H.	31	F	30	16,000	50	15	20	5	5	5	0	0	0	0	Myleran	56	
S.B.	74	M	28	37,000	0	39	4	0	0	1	18	8	10	20		24	
I.C.	26	F	20	40,000	48	15	20	0	12	2	0	3	0	0	6 MP	150	5
R.B.	78	M	35	50,600	67	11	11	0	4	0	2	5	0	0		79	
G.S.	25	F	34	59,000	50	10	5	5	0	0	10	10	0	10		49	
P.C.	35	M	41	75,000	15	15	8	0	0	4	10	20	8	20		22	10
W.T.	49	M	37	96,000	42	43	3	0	0	0	4	2	0	6		80	53
M.M.	34	M	41	208,000	35	10	1	2	4	10	12	21	0	5	Myleran	16	18
R.G.	40	F	30	222,000	12	21	1	1	16	19	9	4	10	7		36	
E.B.	49	F	26	260,000	32	22	13	4	0	1	3	7	8	10		130	
J.S.	17	M	40	282,000	12	28	2	0	0	0	14	30	6	8		11	31
M.V.	22	M	23	371,000	34	5	5	8	17	9	11	11	0	0		396	20
M.B.	69	M	39	400,000	18	43	1	0	0	10	12	5	7	4		157	61
D.H.	29	M	27	494,000	54	2	2	3	2	5	12	10	10	0		22	

[a] Mean colony count of 3 plates.

TABLE II. Stimulation of Colony Formation from Normal Human Bone Marrow by Feeder Layers of Peripheral WBC from Patients with Chronic Granulocytic Leukemia Compared with Normal Human WBC.

Source of feeder layer cells	Diagnosis	No. of colonies stimulated	
		Unwashed	Washed
None	—	—	—
M.M.	Normal	41	38
C.S.	CGL	23	**171**
L.F.	CGL	35	**165**
W.T.	CGL	35	**67**

studies, however, was the lack of correlation between peripheral WBC levels and CSA values. If, as previously postulated, one of the major sources of CSA in humans is peripheral WBC such a correlation between one or another cell type and CSA values might have been expected. Previous studies on patients with solid tumors and leukemoid reactions have shown a direct correlation between peripheral neutrophil counts and urine CSA values (18). Likewise, in one previously reported patient with CGL in whom spontaneous cycling of the peripheral WBC occurred, it was found that serum and urine CSA values also cycled in direct relation to the peripheral WBC (16).

Several explanations may be offered for the present findings. This may represent innate variability of the disease, may indicate that WBC are not the only source of CSA or perhaps be the result of variability in levels of factors inhibiting CSA. The latter is the most intriguing and likely possibility. Such inhibitors have been described previously (4, 6, 8), but their exact nature and specificity have not been determined. This possibility is also suggested by the studies on CSA of peripheral WBC from the 3 patients with CGL which indicated that incorporation of the CGL's plasma into the culture system markedly inhibited the CSA of feeder layer cells. Since these patients were not being treated at the time these studies were done, chemotherapeutic agents cannot be implicated as causing this effect. Likewise, numerous studies in our laboratory have indicated that the level of heparin used to collect cells, and remaining in the plasma, is not toxic to the culture system. It should also be noted that when normal human WBC are used as feeder layers that little or no difference is noted when plasma is present or absent.

Further studies are now underway to determine whether the variability in CSA values found in the present studies can be explained on the basis of presence of inhibitory factors. Hopefully, resolution of this question may give valuable clues into the nature and pathophysiology of the disease chronic granulocytic leukemia in human beings.

Summary. Urine and serum from 18 patients with chronic granulocytic leukemia have been tested for ability to stimulate granulocyte bone marrow colony growth *in vitro.* The colony stimulating activity (CSA) in urine of these patients varied from very high to very low with mean levels greater than that of normal humans. There was no correlation between CSA and peripheral white blood count or clinical course of the disease.

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