PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 694-700 (1975)

Colony Stimulating Factor Levels in Human Serum and Urine Following Chemotherapy[1] (38612)

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A factor has been described in the serum and urine of normal humans which will stimulate the formation of granulocytic and mononuclear cell colonies from normal human and mouse bone marrow cells *in vitro* (1–4). This factor has been characterized as a glycoprotein with a mol. wt of about 45,000 which migrates electrophoretically as an alpha globulin and is stable at a wide pH range (5–7). It has been suggested that this factor may represent a true granulopoietic substance stimulating division and maturation of the granulocyte cell line *in vivo* as well as *in vitro* (4, 8–10).

Serum and urine levels of colony stimulating factor (CSF) have been found to be elevated in a wide variety of disease states including acute lymphocytic, stem cell and chronic granulocytic leukemia (4), infections (1, 11), in patients undergoing operative procedures (12), and in patients with marked granulocytosis secondary to solid tumors (13). Inhibitors have also been described in normal human and mouse sera and have been partially characterized as lipoprotein substances which block CSF but are not toxic for colony forming cells (14, 15). Inhibitory substances have also been described from products of normal and neoplastic hematopoietic cells (16–21).

The cellular source of CSF and the described inhibitors have not been fully elucidated. In man, CSF, in addition to serum and urine, has been found to be associated with a wide variety of tissues including spleen (22), human embryo kidney cells (23), and peripheral white blood cells (WBC) (24–27). The major source of CSF for human bone marrow colony growth has been hematopoietic cells from either peripheral blood or spleen and this has led to the suggestion that granulocyte production in man may be in part regulated through a positive feed-back system (28–31).

The following studies were undertaken to determine the base line serum and urine levels of CSF in patients with Hodgkin's disease and non-Hodgkin's lymphomas and further to determine the changes in CSF levels following chemotherapy. In these studies patients with lymphomas were treated with standard multi-drug chemotherapy and CSF levels measured in the serum and urine. It has been shown that marked rises in CSF levels occur after chemotherapy suggesting that cellular breakdown may signal, or perhaps be the source, of increased CSF production. In addition a newly described CSF inhibitory factor has been found in the urine of two patients with lymphatic neoplasms.

Methods and Materials. All of the patients studied were seen at the Clinical Research Center Ward, University of Colorado Medical Center, between April, 1972 and April, 1973. A total of 10 patients were studied; six had a diagnosis of Hodgkin's disease and four had lymphosarcoma of various histologic types (Table I). None of these patients were transfused during these studies. No instances of overt clinical infection were noted. This was further evidenced by the fact that none of these patients received antibiotic treatment.

Urinary collections were made in sterile bottles refrigerated at 4° during the indicated time intervals. Fifty ml aliquots were removed, dialyzed for 72 hr against distilled water, centrifuged at 9500 rpm for 15 min, and the sediment discarded. The supernatant portions to be used for testing were

[1] Supported by grants from the National Institutes of Health, National Cancer Institute (1R01CA11305-4 and CA05058-10), the American Cancer Society Grant No. ET-4C, and General Clinical Research Centers Program of the Division of Research Resources of the National Institutes of Health (RR-51).

TABLE I. DISTRIBUTION OF CASES BY AGE, TYPE OF LYMPHOMA, AND URINARY COLONY STIMULATING ACTIVITY (CSA).

Patient	Age	Bone Marrow	Diagnosis	CSA Value	
				Control	Peak Post-treatment
J. F.	55	Mild granulocytic hyperplasia	Hodgkin's disease, Stage IVB	6	48
M. H.	31	normal	Hodgkin's disease, Stage IVB	20	180
A. K.	27	lymphomatous involvement	lymphosarcoma	0	2
M. M.	46	lymphomatous involvement	lymphosarcoma	12	18
C. M.	46	normal	Hodgkin's disease, Stage IIIB	22	135
J. S.	24	normal	lymphosarcoma	34	74
M. F.	46	granulocytic hyperplasia	reticulum cell sarcoma	16	130
J. T.	47	normal	Hodgkin's disease, Stage IVB	54	102
C. C.	47	normal	Hodgkin's disease, Stage IVB	2	76
S. S.	45	Hodgkin's involvement	Hodgkin's disease, Stage IVB	28	260

sterilized by filtration through 0.45 μm porous membranes and stored at 4° prior to testing. Complete blood counts were done during the times indicated. Serum specimens were collected by venipuncture and were dialyzed for 72 hr against distilled water. The method for determining colony stimulating activity (CSA) in serum and urine had been previously described (4, 32, 33). Urine (0.15 ml) or serum (0.1 ml) to be tested is placed in triplicate 35 mm plastic petri dishes. To each plate is added 1 ml of a 9:1 mixture McCoy's 5A medium (supplemented by 15% fetal calf serum) and boiled 3% agar containing a single cell suspension of 75,000 nucleated bone marrow cells obtained from the femurs of C57/bl mice. After mixture of the medium and urine or sera, the plates are allowed to gel at room temperature and are then incubated at 37° under humidified conditions with a constant flow of 7.5% CO_2 in air. Microscopic colonies are visible by the second to third day of incubation. Colony counts are done on the seventh day of incubation with a dissecting microscope. Aggregates of greater than 50 cells were scored as colonies. Previous studies have shown correlation between the concentration of CSF present in either serum or urine and the number of colonies detected (34). Therefore, by counting the number of colonies an estimate of the CSA of the various samples is obtained.

Each patient had control pretreatment urine and serum specimens collected in the manner described above. Subsequently, specimens of serum and 24-hr urine specimens were collected daily following the onset of chemotherapy. In two patients, fractional 6-hr urine collections were made at intervals during the 48 hr following initiation of chemotherapy and subsequently 24-hr urine collections were made. In these same two patients, hourly sera were drawn for the 6 hr following the intravenous administration of the chemotherapeutic agents. Eight of the 10 patients were treated with a four drug protocol which included cytoxan, 10 mg/kg given intravenously on days 1 and 8 and vincristine, 0.25 mg/kg given intravenously on days 1 and 8. Procarbazine, 2.5 mg/kg and prednisone, 1 mg/kg were administered orally daily. In two patients nitrogen mustard (Mustargen Hydrochloride, Merck Sharp and Dohme) was substituted for the cytoxan in a dose of 6 mg/m^2. In each of the 10 patients studied there was a pretreatment bone marrow core biopsy obtained from the posterior iliac crest.

In two patients in whom it was found that subsequent to chemotherapy there was little CSA in either urine or sera, urine specimens at intervals subsequent to the initiation of chemotherapy were studied for the presence of inhibitors.

In one patient, M. M., urine specimens collected 2 and 4 days after the initiation of chemotherapy which demonstrated no CSA were studied for inhibitors. Likewise, all urine specimens from a second patient, A. K. were studied. Aliquots (0.1 ml) from

each of the specimens were mixed with 0.1 ml of a standard control urine of known CSA and plated in triplicate in the manner described above. In both patients specimens were run through a Sephadex G-150 column and the fractions obtained were tested for CSA and inhibitory potential.

Results. Colonies stimulated by either the urine or sera specimens were similar in size and cellular morphology to those described previously (35). At the third to fourth day of culture, the colonies were primarily composed of granulocytes but by the seventh day they were almost exclusively monocyte–macrophages.

In seven of 10 patients control pretreatment urinary CSA values fell within the normal range (0–40 in our laboratory). Two patients, (M. F., C. C.) had elevated pretreatment urinary CSA levels. Both of these patients had high temperatures. One patient (S. S.) evidenced an elevation of CSA in one of two control specimens which was unexplained. In eight of 10 patients the levels of urinary CSA subsequent to chemotherapy evidenced marked increases compared to pretreatment control levels. The increase in CSA was greatest in the 24–48 hr interval immediately following the intravenous administration of cytoxan or nitrogen mustard and vincristine. In two patients in whom hourly serum specimens were collected after the administration of intravenous chemotherapy, similar marked elevations in CSA occurred. In two patients who evidenced little or no CSA after chemotherapy, urine specimens were studied for the presence of inhibitory activity. The results of these studies are detailed below.

Figures 1A and 1B show the changes in urine and serum CSA values of patient C. M. with IIIB Hodgkin's disease following the administration of chemotherapeutic agents. It can be seen that pretreatment control CSA values in both serum and urine were very low. Following the administration of chemotherapy marked rise in urinary CSA was evident during the first 24 hr. This activity gradually fell to control levels until the second intravenous chemotherapy was administered when another marked increase in CSA was found. During this time the patient's granulocyte count did not vary significantly. An early and significant rise in CSA was likewise, found in the serum of this patient (Fig. 1B) with return to control levels 12 hr later. A subsequent rise in serum CSA was noted at the time of the second intravenous injection of chemotherapy.

Figure 2 shows the changes in urinary CSA of a second patient, M. F., with a reticulum cell sarcoma following initiation of chemotherapy. A marked rise in CSA was found following chemotherapy which grad-

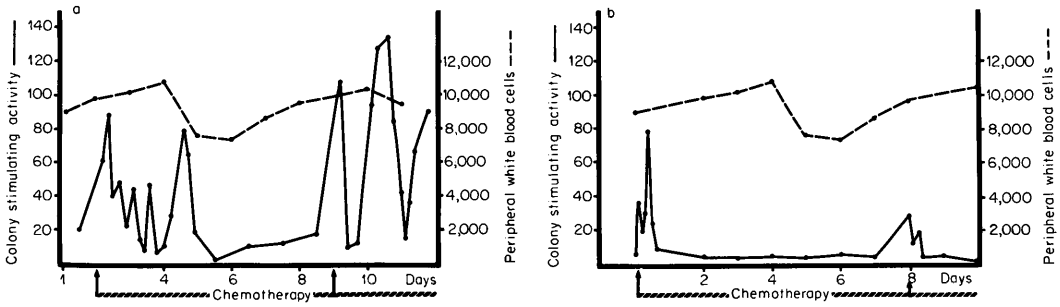

FIG. 1A. Course of patient C. M. with Stage IIIB Hodgkin's disease. The bar along the bottom of the graph represents the time during which chemotherapy was administered. The arrows denote the days on which intravenous drugs were given. Note the low pretreatment urinary colony stimulating activity (CSA), with a prominent rise in CSA during the periods indicated following the administration of intravenous drugs at days 2 and 9. In this and subsequent figures, each point on the CSA curve represents the mean colony count of three plates. B. Course of patient C. M. with Stage IIIB Hodgkin's disease. The bar along the bottom of the graph represents the time during which chemotherapy was administered. The arrows denote the days on which intravenous drugs were given. Note the low pretreatment serum colony stimulating activity (CSA) with a marked rise immediately following the administration of intravenous drugs at days 1 and 8.

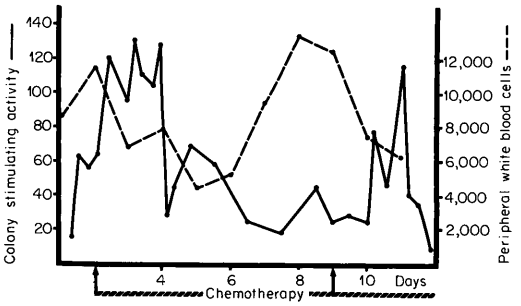

FIG. 2. Course of patient M. F. with reticulum cell sarcoma. The bar along the bottom of the graph represents the time during which chemotherapy was administered. The arrows denote the days on which intravenous drugs were given. Note the low pretreatment urinary colony stimulating activity (CSA) with a rise immediately preceding the initiation of therapy which correlated with the development of fever. The CSA then rises markedly during the periods indicated following the administration of intravenous drugs at days 2 and 9.

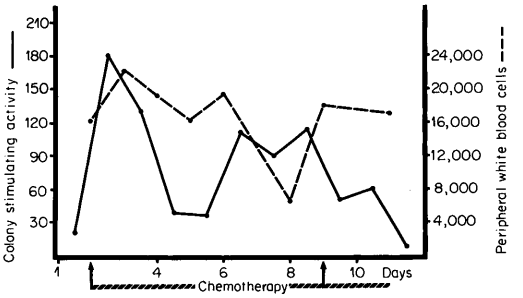

FIG. 3. Course of patient M. H. with stage IVB Hodgkin's disease. The bar along the bottom of the graph represents the time during which chemotherapy was administered. The arrows denote the days on which intravenous drugs were given. Note the low pretreatment urinary colony stimulating activity (CSA) with a prominent rise in CSA during the 48-hr period following the administration of intravenous drugs at day 2.

ually fell toward pretreatment control values until the second administration of nitrogen mustard and vincristine following which there was a second marked rise in CSA values.

Figure 3 depicts the response to chemotherapy in patient M. H. with Hodgkin's disease. Again a marked rise in urinary CSA is seen following the first intravenous administration of chemotherapy. In this patient CSA values increased prior to the second intravenous administration.

Similar findings were noted in five other patients not depicted graphically here. All of these patients with the exception of one, had normal to increased marrow hematopoietic tissue as judged by bone marrow biopsy.

Figures 4 and 5 show changes in urinary CSA values of two patients M. M. and A. K. with lymphosarcoma with marked bone marrow involvement. It can be seen that little or no change occurred in CSA values of these two patients following the first intravenous administration of chemotherapy. In one patient, M. M., there was a marked rise following the second injection of the chemotherapeutic agents on day 8, but no rise was seen in the urine of patient A. K. at a similar time point. Figure 6 shows a photomicrograph of the pretreatment bone

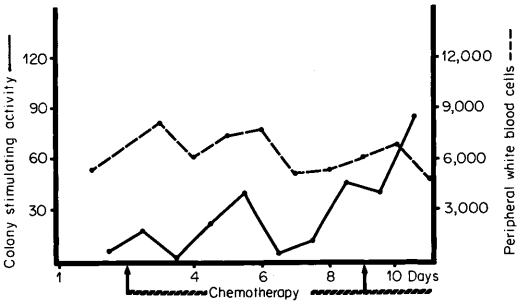

FIG. 4. Course of patient M. M. with lymphosarcoma. The bar along the bottom of the graph represents the time during which chemotherapy was administered. Note the low pretreatment urinary colony stimulating activity (CSA) with initially little increase in CSA after the initiation of chemotherapy until the fifth day when a modest increase is seen. There is a prominent increase in CSA during the 24- to 48-hr period after the second intravenous administration of drugs at day 9.

marrow core specimen from patient A. K. showing marked replacement of normal hematopoietic tissue with lymphoblasts.

Urine specimens collected from patient M. M. during the 24-h interval of the second and fourth days following initiation of chemotherapy when little or no CSA could be demonstrated were studied for the presence of inhibitors. These urine specimens were mixed in increasing dilutions with a standard urine of known colony stimulating activity and the CSA deter-

mined. The results of these studies are depicted in Fig. 7. The addition of urine from the patient to the standard markedly inhibited the CSA of the latter with a linear relationship between the amount of test urine and the degree of inhibition. Fractions of urine from both of the specimens studied obtained from a Sephadex G-150 column, however, demonstrated a marked urinary CSA and it was concluded from these studies that the CSA in the unfractionated urine specimens was masked by the simultaneous presence of an inhibitory factor(s).

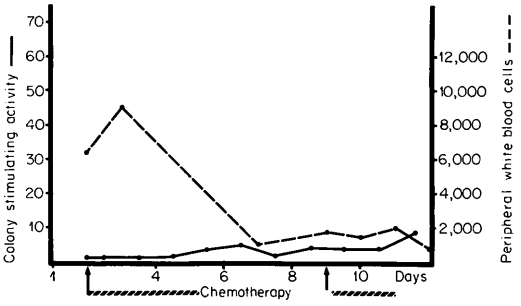

FIG. 5. Course of patient A. K. with lymphosarcoma. The bar along the bottom of the graph represents the time during which chemotherapy was administered. Note the low pretreatment urinary colony stimulating activity (CSA) and the subsequent lack of rise in CSA after the initiation of chemotherapy. The peripheral white blood cell (WBC) count is seen to fall precipitiously by the sixth day and to remain low throughout the course of therapy.

Discussion. Results of the present studies indicate that the majority of the patients with malignant lymphoma receiving standard multi-drug chemotherapy evidence marked elevation of CSA in specimens of urine and serum following treatment. The development of increased CSA values appears to coincide with the administration of intravenous agents and occurs at a time when the greatest cytotoxicity is probably manifest. This correlation suggests that cell death may be an important factor in the events leading to the development of increased CSA values. The effect of these agents is, however, totally nonspecific in terms of cellular targets and no definite conclusion can be reached as to whether this finding gives any evidence as to the possible cellular source of CSA. Data reported by others,

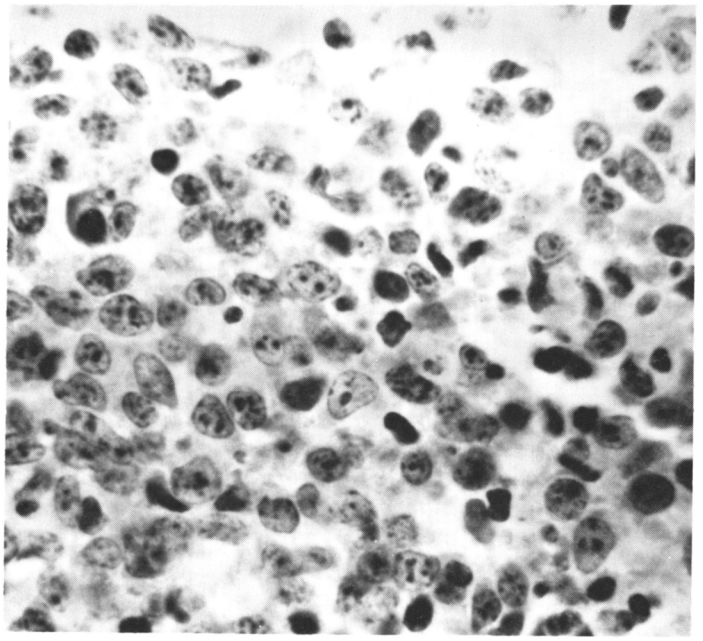

FIG. 6. Pretreatment bone marrow biopsy specimen of patient A. K. who was found to have colony stimulating factor (CSF) inhibitors in urine specimens. Note the marked replacement of normal hematopoietic tissue with lymphoblasts. (mag. 430×).

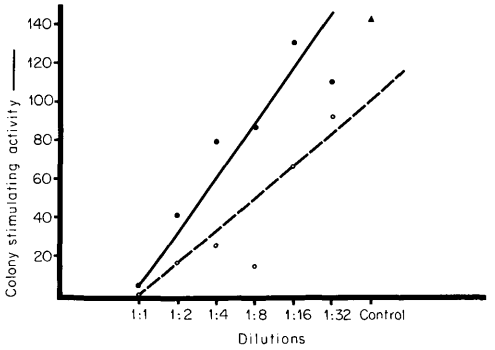

FIG. 7. Demonstration of the presence of colony stimulating factor (CSF) inhibitors in two urine specimens from the patient M. M. Serial dilutions of the urine specimen were mixed with a standard urine of known colony stimulating activity (CSA). The triangle represents the CSA of standard control alone.

however, suggest the apparent cellular source of CSA (12, 22–24, 26, 36). It is also possible that the apparent relationship between intravenous chemotherapeutic administration and increases in CSA may be a result of events other than cell death such as transient bacteremia or occult infection. It is also possible that the administration of these chemotherapeutic agents may turn on the cellular source of CSA in some as yet undetermined way.

As indicated previously the major source of CSA in humans appears to be the monocyte–macrophage system. It is difficult to envision a mechanism whereby administration of chemotherapeutic agent might "turn on" the production of CSA by this cellular system without an intermediary signal such as cellular breakdown. It is tempting to speculate that these cells may be sensing the breakdown of hematopoietic precursor cells in the bone marrow by some, as yet, unrecognized mechanism with subsequent increased production of CSA. Such a mechanism could be envisioned as a means whereby normal hematopoiesis and cellular production is maintained and restored (28, 31).

The finding of an inhibitory material in the urine of two patients with lymphosarcoma is of considerable interest and under further investigation. The finding that this inhibitory material was present only in the urine of patients with marked involvement of the bone marrow by lymphoblasts might suggest that these cells were the source of this material. Further studies using conditioned medium prepared from lymphoblastic tissues and cell mixing experiments to substantiate this suggestion are being carried out at the present time. Elaboration of such an inhibitory material by neoplastic lymphocytes might be suggested as a mechanism whereby these cells gain a selective advantage for proliferation of the malignant cell line over normal hematopoietic tissues.

Perhaps the major importance of the present studies is that they add to the growing body of knowledge that suggest that CSA as measured in serum and urine represents activity of a true granulopoietic regulatory substance. The finding of increased CSA values at a time when granulocytic reserves are being depleted as the result of chemotherapeutic intervention suggests that CSA as measured in these studies may reflect such a factor. Theoretically, one might anticipate and expect that granulopoietic factors would be at their highest levels during such times of granulocyte depletion. Further studies are needed to clearly document the role of CSA in granulocyte production and maturation.

Summary. The colony stimulating activity (CSA) of serum and urine specimens from 10 patients with lymphomas before and after treatment with standard multidrug chemotherapeutic regimens has been studied. Prominent rises in the CSA in the urine of eight of these 10 patients correlating temporally with initiation of therapy was demonstrated when compared to pretreatment control values. Hourly serum specimens were collected following the administration of intravenous chemotherapeutic agents in two of these patients. In one of these two patients there was a prominent and early rise in CSA which returned to control values by the 12th hr after intravenous drug administration. In two patients who developed little or no increase in CSA during therapy, urine specimens were studied for the presence of inhibitors to colony stimulating factor. Aliquots of urine from each of these two patients when mixed

with a standard control urine of known CSA, produced marked inhibition.

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Received September 6, 1974. P.S.E.B.M. 1975, Vol. 148.