วารสารเทคนิคการแพทย์ เชียงใหม่
BULLETIN OF CHIANG MAI MEDICAL TECHNOLOGY

Vol. 5 No. 1
January 1972

COLONY-STIMULATING FACTOR (CSF) IN THALASSEMIC URINES : PRELIMINARY REPORT.

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ABSTRACT

The modified method for an in vitro culture of bone marrow cells is described. The colony stimulating factor (CSF) activity in urines of normal and diseased children were detected. Thalassemic urines possess higher CSF activity than normal but still lower than leukemic urines.

INTRODUCTION.

Recently, the methods of hematopoietic cell culture in vitro with which colonies of maturing granulocytes can be grown single cells had been described (1, 2,3,4). In the presence of the stimulating factor colonies of granulocytic and mononuclear cells can be grown from the marrow and speen of animals (1,2), and from human bone marrow (4, 5). Substances which stimulate murine or human marrow growth include various cell feeder layer, urine, serum and conditioned media prepared from tissues (8,9,10). Metcalf and associates (2, 11). noted that bone marrow cells are able to proliferate in agar cultures and form colonies of granulocytes and/or macrophages if stimulated by the colony-stimulating factor (CSF). This factor is found in the serum and urine of normal mice and humans. There is evidence indicates that CSF function in vivo as humoral regulator of granulopoiesis and monocyte formation (7, 12, 13). The excretion of CSF into the urine appears to be a major metabolic fate of this substance. It was noted that serum CSF levels are elevated in both the conventional and germ-free mice with leukemia (14, 15, 16). It was also found that CSF level in

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sera and urine from patients with various types of leukemia were higher than normal (7, 17).

Thalassemia is basically the inborn error of globin polypeptide chain synthesis which affect solely the erythropoietic cells of the marrow. Leukocytosis and granulocytosis are the common findings in these patients but the CSF activity in thalassemic urines has never been reported.

MATERIALS AND METHODS.

I. Collection and preparation of material.

a. Urine CSF (modified from Robinson et al) (6). Urine samples from normal individuals and patients were collected in sterile bottles. Fifty ml. portion of each sample were dialyzed in Visking dialysis tubing (wall thickness 0.001 inch) against 3 daily changes of 1,000 ml. of distilled water at 4° C for 72 hours. Twenty ml. of the dialyzed urine were then centrifuged at 9,000 r.p.m. for 15 minutes. The supernatant fluid was millipore-filtered (with 0.45 micron millipore membrane) then either used immediately or stored at-20° C. until used.

b. Pooled human sera. Sterile pooled sera obtained from the Hospital blood bank.

c. Sterile 6% dextran solution. (Cutter Lab., Berkeley, Cal., USA.). Keep the solution at-4° C.

d. Sterile Hank's solution. Prepared Hank's solution (BBL, Division of Bio Quest, Cockeysville, Md., USA.) pH 7.2 then sterilized by millipore-filtered technic. Keep in the refrigerator.

e. Culture media. The agar media were prepared by dissolving 1.5 gm. of Bacto-Agar (Difco) in 36 ml. of distilled water, boiled and autoclaved for 15 minutes. Bring the temperature of the agar solution to 50° C. (by using 50° C waterbath) then added 4 ml. of Hank's solution, 6 ml. of dialyzed urine and 4 ml. of pooled sera, mixed. Twenty ml. aliquot of the culture media were pipetted into a sterile 35 × 10 mm. plastic Petri dish (Falcon). These culture plates were used immediately or stored at 4° C.

f. Bone marrow cells suspension. Approximately 2-3 ml. of human bone marrow were collected into the sterile heparinized plastic syringe. One-quarter to one-half volume of 6% dextran was added directly into the bone marrow syringe, mixed well, then the syringe was placed end-up in the refrigerator for 60 minutes. The supernatant fluid containing marrow cells was then transfered (either by the pasteur pipette or by squeezing the fluid through the bented needle) into a sterile plastic tube containing equal volume of Hank's solution. After centrifuged at 4° C., 800 - 1,000 r. p. m. for 5 minutes the supernatant fluid was discarded. The sedimented marrow cells were resuspended in Hank's solution and cell count done.

II. Bone marrow culture assays.

Approximately 5×10^5 nucleated marrow cells were transfered on to the culture plate to make a thin fluid film convering the media surface. The cell cultures were then incubated in a humidified candle jar placed in an incubator at 37° C. The culture plates were examined daily for the appearance and sizes of colonies. Colony counts were performed at ×25 magnification using a dissecting microscope with direct lighting. All tests were done in triplicate.

RESULTS.

All cell colonies appeared on day 6-7 but were small and difficult to count. The accurate estimation of colony numbers was possible after 10 days. Colony size was fairly uniform in any given culture. It is interesting that :

1. No colony growth observed from bone marrow cells of aplastic anemia and chornic myelogenous leukemia when normal or thalassemic urine were added.

2. There is no difference in colony formation ability of thalassemia patient and normal individuals.

3. Thalassemic urines possess highter CSF activity than normal reflected by the higher colony counts when they were used instead of normal urine.

4. Leukemic urine has the strongest CSF activity. This is in agreement with previous reports.

TABLE I : EFFECT OF CSF IN DIFFERENT TYPES OF URINE

Subjects	Colony counts (per 5×10^5 marrow cells)		
	Normal urine	Thalassemic urine	Leukemic urine
Normal	26	–	–
Normal	25	56	61
Normal	28	23	57
Thalassemia	34	44	43
Thalassemia	13	19	30
Aplastic anemia	–	–	4
CML.	–	–	7

DISCUSSION.

Colony-stimulating factor (CSF) is a serum glycoprotein of molecular weight approximately 45,000 (18, 19). which is excreted in the urine and has the specific activity to stimulate in vitro the proliferation of granulocytes and macrophages. Detection of CSF activity in serum is often masked by the presence of lipoprotein inhibitors which block the in vitro action of CSF. These inhibitors can be precipitated by dialysis of the serum. Normal human sera possess uniformly high inhibitor levels. Previous studies of sera and urines from patients with various types of leukemia indicated that CSF levels were higher than normal in some patients and subsequent studies on urines from such patients have shown fluctuations in CSF putout during the course of the disease. (7, 17). Most recently. Metcalf and associate (23) note the abnormally large amount of CSF were present in about half of the urine specimens from patients with acute leukemia.

Our study indicated that urines of thalassemic patients possess higher CSF activity than normal urine. But caution is needed in interpreting its significance since it is noted that higher urine excretion of CSF does not necessarily a reliable index of serum CSF levels. (23) However, since clearance in the urine is a major metabolic fate of CSF (24) the higher output of CSF in urine does suggest a higher overall level of CSF production in our thalassemic patients.

Results from animal studies indicated that cells capable of repopulating the entire hematopoietic system can be found circulating in the blood stream. It has been debated whether such cells circulate in human until Chervenick and Boggs, (27) and Kurnick and Robinson (28) demonstrated that circulating leukocytes also capable of giving rise to such colonies but are considerably less than those from marrow cells. The morphology of colony cells from blood and bone marrow cells is similar. All colonies appear to begin as large mononuclear cells (5-10 days) with a gradual progression to cells with the morphology of mature granulocytes (20-25 days). Subsequently, many larger phagocytes appeared in the colonies. (3, 4) Colony formation was observed after 6-10 days of incubation and increased to a maximum size of 200-1,000 cells after 18-20 days and then began to undergo destruction. Colony size was fairly uniform in any given culture. The rate of growth observed is considerably slower than colonies arising from mouse bone marrow where initial growth can be observed within 24 to 48 hrs. and maximum growth is observed by 10-12 days.

It is interesting to note that in the in vivo colony assay system in the mouse,

colonies of erythrocytic, megakaryocytic and granulocytic cells appear on the spleen of mice following irradiation, (29,30) while in vitro system only granulocytes and macrophages have been observed. This suggests that the in vitro colonies from a more differentiated stem cell than that giving rise to in vivo colonies. (31) Whether cell colonies arise from a single stem cell or from several is not entirely clear at the moment.

CONCLUSION

Urine from thalassemia patients contain higher colony-stimulating factor (CSF) activity than normal urine. This is probably reflecting the higher CSF activity in their sera and may be partly responsible for granulocytosis observed in these patients.

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