The Bone Marrow Colony Stimulating Factor (CSF): Relation of Serum CSF to Urine CSF[1] (37682)

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Colony stimulating factor (CSF), a proposed *in vivo* hemopoietic regulator, is essential for the *in vitro* survival and proliferation of hemopoietic precursor cells which have the potential of forming granulocytic and/or macrophage colonies (1).

Elevations have been observed in serum and urine CSF levels in patients and animals with infections and leukemia (2–4) and Chan (5) speculated that urinary CSF might represent filtered serum CSF. Subsequent experimental results were consistent with this contention and renal excretion was proposed as the major fate of serum CSF (5). The possible role of the kidney as a source of CSF was considered of lesser importance (6–10).

In the present study evidence is presented from mice injected with endotoxin that casts doubt on the assumed role of the kidney in clearing plasma CSF to the urine.

Materials and Methods. Animals. Adult C_{57}BL mice were used in these studies.

Stimuli. Animals received *S. typhimurium* endotoxin (kindly supplied by Dr. C. Jenkins, University of Adelaide) 5 μg in 0.2 ml saline iv or 0.2 ml saline iv or no injection.

Operative procedures. Under Nembutal anesthesia (1.5 mg Nembutal in 0.25 ml saline ip) groups of mice were subjected to bilateral nephrectomy or sham bilateral nephrectomy.

Collection of sera and urine. Mice were anesthetized with ether and bled from the axilla. After clot retraction, sera were removed then dialyzed (3 days, 3 changes of water 4°) centrifuged 27,000*g*, 30 min 4° and stored at −20° prior to further treatment or assay.

Urine was obtained both by gently squeezing the lower abdomen of individual mice and collecting the urine into beakers containing sodium azide (NaN_3, final concentration 0.02%) and by sustained collection from mice housed in converted 2 liter polypropylene bottles that drained into azide-containing tubes. In the case of 0–24 hr postendotoxin-injected mice the initial zero time collection of expressed urine was discarded. After determining volume, the urines were dialyzed (3 days, 3 changes of water 4°) centrifuged 27,000*g*, 30 min 4°, then stored at −20° prior to further treatment.

Zone sedimentation. After partial purification of the previously dialyzed serum and urine preparations by "block elution" chromatography on calcium phosphate gel (7) extracts were lyophilized, then dissolved (20 mg protein/ml) and equilibrated with zone sedimentation buffer (0.1 *M* Tris HCl, pH 7.5). Zone sedimentation of 0.1 ml of these extracts containing trace amounts of labeled bovine serum albumin (^{125}I-BSA) and pancreatic ribonuclease (^{125}I-RNAse) were performed in 5–20% sucrose density gradients (4.6 ml). Centrifugation was at 118,000*g* for 36 hr at 4° in the SW39 rotor of a Beckman L4 ultracentrifuge. Fractions were collected by upward displacement with a 40% sucrose solution and the apparent sedimentation coefficient ($S_{20,w}$) of the CSF was determined by comparison with the behavior of the ^{125}I-BSA marker ($S_{20,w} = 4.4$) (11).

Assay for CSF. Material to be assayed

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was sterilized by passage through 0.45 μm Millipore membranes, then pipetted into duplicate 35 mm plastic petri dishes (Falcon Plastics, Los Angeles). Whenever possible serial dilutions of each serum or extract were assayed to ensure stimulation of colony numbers within the linear region of the dose–response curve.

The composition of both the bone marrow collecting fluid and the agar medium have been previously described (1). Bone marrow cells from the pooled femurs of two 2–3-mo-old $C_{57}BL$ mice were suspended in bone marrow collecting fluid. Cells were added to the agar medium to a concentration of 75,000 nucleated cells/ml and using an automatic syringe, a 1 ml sample of this suspension was added to each dish. The contents of the dishes were then mixed and allowed to gel at room temperature. After 7 days' incubation at 37° in fully humidified 10% CO_2 in air colony counts were performed on the unstained cultures at × 25 using a dissecting microscope with indirect illumination.

Results. Zone sedimentation studies, serum and urinary CSF. Normal mouse serum CSF (4.5–7.0S) (12) has been shown to be 2 to 3 times the size of normal human urinary CSF (approx 3.4S) (13, 14). As it was not known whether this size discrepancy was due to the species difference or to a basic structural difference between serum and urinary CSF, zone sedimentation studies were performed on normal mouse urine (a low specific activity CSF source) as well as on urine collected between 0–24 hr after the injection of endotoxin and on serum collected 6 and 17 hr after endotoxin injection—the latter three containing CSF of much higher specific activity than the corresponding material from normal mice.

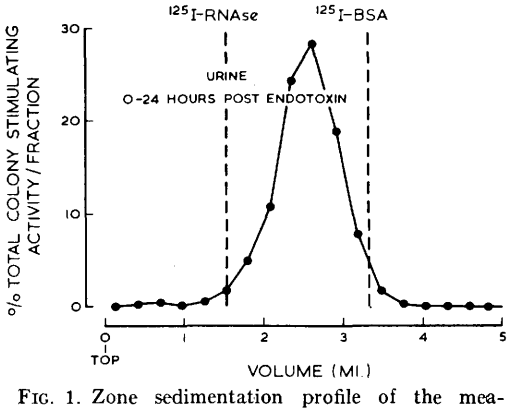

FIG. 1. Zone sedimentation profile of the measurable 0–24 hr postendotoxin urine CSF of a pool from ten mice. Broken vertical lines represent the positions of the marker protein peaks.

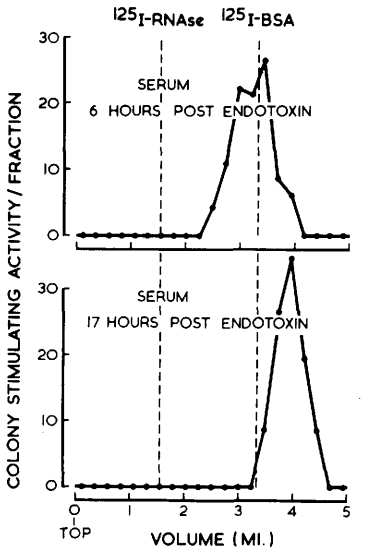

FIG. 2. Zone sedimentation profiles of the measurable serum CSF of pools from mice killed 6 and 17 hr after 5 μg endotoxin iv. Broken vertical lines represent the positions of the marker protein peaks.

The postendotoxin urine pool was found to be sufficiently active to stimulate 3786 colonies/mg protein whereas normal mouse urine would only stimulate 231 colonies/mg protein. From Fig. 1 it can be seen that the postendotoxin urinary CSF, of which 74% was recovered, had an apparent sedimentation coefficient ($S_{20,w}$) of approximately 3.3S. CSF from normal mouse urine sedimented in the same region but with only a 28% recovery of activity. These results are in close agreement with those obtained by Stanley and Metcalf (13, 14) for human urinary CSF. By contrast, sera from mice injected 6 and 17 hr previously with endotoxin were shown to contain CSF's which had apparent sedimentation coefficients of about 4.2S and 5.0S, respectively (Fig. 2). A value of be-between 4.5 and 7.0S was previously obtained by Stanley, Robinson and Ada (12) for normal mouse serum CSF.

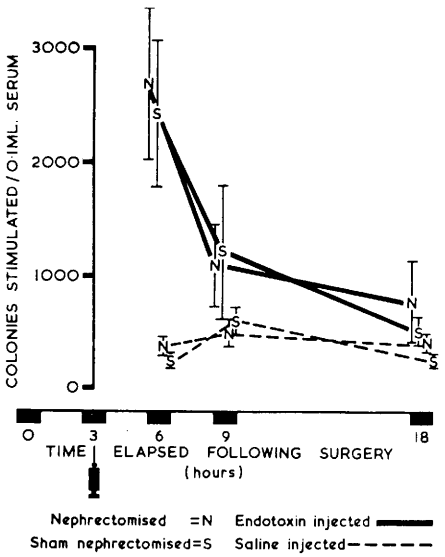

FIG. 3. Effect of bilateral nephrectomy on the clearance of serum CSF from mice injected 3 hr postsurgery with either 5 μg endotoxin iv or isotonic saline iv. Each point represents the mean of separate CSF determinations on serum from 5 animals $\pm$ standard deviation.

The effect of bilateral nephrectomy on the clearance of postendotoxin serum CSF. Sixty 4-mo-old male C_{57}BL mice were divided into two equal groups. All the animals in one group were bilaterally nephrectomized, all in the other were sham operated. Three hours after surgery half of the animals in each group were given intravenous endotoxin, the other half saline. At 6, 9, and 18 hr after surgery, 5 animals from each subgroup were exsanguinated, the sera were individually dialyzed and assayed. As shown in Fig. 3, bilateral nephrectomy did not significantly affect the fall with time in the colony stimulating activity of serum after the initial endotoxin induced rise in activity.

Discussion. The larger sizes of normal, 6 hr postendotoxin and 17 hr postendotoxin serum CSF compared with the approximately 3.3S CSF of normal and postendotoxin mouse urine provide compelling evidence that simple renal filtration is not a fate of these serum CSF's. Furthermore, the finding that bilateral nephrectomy did not prolong the decline phase of the postendotoxin serum CSF response strongly suggests that the fate of this CSF is either largely or totally independent of the kidney. However, as normal serum CSF (12) differs from postendotoxin serum CSF in respect to size (8) it is possible that its fate could be different.

Chan observed CSF levels to be twice as high in nephrectomized mice as in sham nephrectomized mice 18 hr after surgery (5). This observation, confirmed by the present study might alternatively indicate increased production and release of CSF in response to the injurious effects of accumulating toxins. Cultures stimulated by sera from bilateral nephrectomized mice had a higher percentage of granulocytic and mixed colonies than did cultures stimulated by sera from sham operated mice (5), a finding consistent with the appearance of a new form of CSF (8, 15). In otherwise normal animals cortisone injection was shown to reduce serum levels to one-third and to increase urinary CSF loss fourfold by 6 hr (5). However, combined bilateral nephrectomy or ureter tie plus cortisone resulted in no significant change in serum CSF levels by 6 hr which clearly showed that cortisone can influence serum CSF levels by mechanisms unrelated to renal function.

In separate studies, Chan (personal communication) found 50% of a dose of intravenously administered human urinary CSF could subsequently be antigenically recognized in the urine of recipient mice. This finding is incompatible with the present results unless due to species difference or small size (3.4S approximately) (14) human urinary CSF which has a serum half-life in the mouse of less than 3 hr (16) is handled differently from normal mouse serum CSF. A difference in handling seems probable as the calculated half-life of normal mouse serum CSF would need to lie between 15 and 75 hr according to freedom of exchange between serum and interstitial fluid compartments and assuming complete clearance by the kidney into the urine without loss of biological potency or masking by residual inhibitors. That certain antisera raised against human urinary CSF neutralize human serum CSF in addition to mouse urine and serum CSF (17) indicate similarities exist between these molecules.

Concerning the nature of urinary CSF it remains impossible to either distinguish between the following or assess their relative contributions: (a) Urinary CSF may be serum CSF that has been partially degraded by the kidney or other tissues with or without alteration in biological potency during or prior to renal excretion. (b) Urinary CSF may represent a quantitatively minor serum CSF species that is excreted unchanged into the urine. (c) Urinary CSF may be a product of the urinary system. Relevant to this final possibility is the finding that medium conditioned by postnatal kidney cells is rich in CSF (9, 10) and renal extracts (6, 7), particularly those from endotoxin-injected-animals (8) are a good CSF source.

The finding that in man measurable urinary CSF levels frequently do not parallel measurable serum CSF levels (18) and that human urinary CSF is a relatively ineffective stimulus of human bone marrow colony growth (M. A. S. Moore and E. R. Stanley, personal communication) suggest that here also the situation is complex.

Summary. C_{57}BL mouse urine CSF was found to be of lower molecular weight than normal or postendotoxin mouse serum CSF. In addition the fall in activity of postendotoxin serum CSF was found to be unaltered by bilateral nephrectomy. Because of these findings, a reevaluation of previous data was made. It was concluded that the kidney may play little or no part in the clearance of serum CSF. The origin of urinary CSF was not established.

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