Studies on Colony-Stimulating Factor (CSF): Role of the Kidney in Clearing Serum CSF[1] (34871)

S. H. CHAN

Cancer Research Unit, Walter and Eliza Hall Institute, Royal Melbourne Hospital, Melbourne, Australia

Certain cells in the mouse bone marrow proliferate and form discrete colonies in semi-solid agar *in vitro*, when colony-stimulating factor (CSF) is added (5, 6). CSF is detectable in normal and leukemic mouse sera (6), human sera (8, 9), and human urine (2). Developing *in vitro* colonies are initially granulocytic in nature but a second population of macrophages can subsequently appear, and most fully developed colonies stimulated by urine and serum usually are composed of macrophages (6, 7, 10).

CSF levels are periodically elevated in the serum and urine of patients and animals with acute infections and leukemia (6, 8, 12) but to interpret the significance of these changes it is necessary to establish the origin of CSF in the urine. Does it represent CSF filtered by the kidney from the serum or CSF elaborated by kidney cells? This question is pertinent since kidney cells have been shown to elaborate, or release, CSF in liquid cultures *in vitro* (7).

Materials and Methods. Bone marrow cultures. The agar culture system and the methods for assaying CSF levels have been described in detail previously (6, 10). Sera for assay were added to 35-mm plastic petri dishes (Falcon Plastics, Los Angeles) in doses of 0.05 or 0.1 ml. Bone marrow plugs from a single femur shaft of each of two 2- to 3-month-old C57BL mice were collected in 4 ml of E1010 (equal volumes of double strength Eagle's medium and distilled water). Dispersed cell suspensions were prepared, and the number of nucleated cells was counted. Equal volumes of 0.6% Bacto-agar in distilled water (boiled for 2 min and held at 37°) and double-strength modified Eagle's medium were mixed. Eagle's medium used was supplemented by the addition of L-asparagine (final concentration 20 μg/ml) and DEAE-dextran (final concentration 75 μg/ml). Sufficient nucleated bone marrow cells were added to the agar-medium mixture to give a final cell concentration of 75,000 cells per ml. One-milliliter aliquots of the final mixture were pipetted into each culture dish using 5-ml pipettes or a Cornwall automatic syringe. The cells in agar-medium were thoroughly mixed with the sera which had been previously added and allowed to gel at room temperature. After gelling, dishes were incubated at 37° in a humidified incubator with a continuous flow of 10% CO_2 in air.

Mouse sera. Eight- to twelve- month-old C57BL mice of both sexes were used. Using nembutal anesthesia groups of mice were subjected to the following operative procedures: bilateral nephrectomy, sham nephrectomy, bilateral ureter tying (just below the renal pelvis), and sham ureter tying. Other control groups were left unoperated. The groups were killed at various time points after operation ranging from 0–48 hr. The numbers of mice used in the unoperated, nephrectomized, sham-nephrectomized, ureter tied, and sham ureter tied were 30, 110, 75, 65, and 50 respectively. Blood was collected from the axillary vessels under ether anesthesia, allowed to clot at room temperature for 1 hr, and sera removed. The sera were stored at −20° and thawed at room temperature before use.

Colony counting and examination. Colony counts were performed after 7 days of incu-

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bation using a dissecting microscope at × 25. For microscopic examination, colonies were removed together with a drop of agar using a finely drawn Pasteur pipette, placed on microscope slides, and stained with 0.4% orcein in 60% acetic acid.

Since linear relationships have been shown between serum CSF levels and the number of colonies forming from a standard number of bone marrow cells (6, 10), serum CSF levels were expressed as the number of colonies stimulated to develop by 0.1 or 0.05 ml of serum.

Cortisone studies. Groups of 6- to 12-months-old C57BL mice unoperated, bilaterally nephrectomized, bilaterally ureter tied, or sham operated were injected subcutaneously with 2 mg of cortisone acetate (Merck, Rahway, N. J.) in 0.4 ml normal saline at the time of operation. Control groups were injected with 0.4 ml normal saline. All mice were killed at 6 hr after operation, and serum CSF levels were assayed. The numbers of mice used were: normal injected with cortisone, 10; normal injected with normal saline, 10; nephrectomy with cortisone, 26; nephrectomy with normal saline, 24; ureter tie with cortisone, 18; ureter tie with normal saline, 18; sham-operated with cortisone, 26; sham-operated with normal saline, 28.

Results. Nephrectomy. After bilateral nephrectomy, serum CSF levels rose reaching a peak at 18 hr followed by an apparent fall (Fig. 1). Sera from mice 24 hr after either bilateral nephrectomy or sham nephrectomy were pooled and portions dialyzed for 3 days at 4° in distilled water with two changes of water. CSF levels in predialyzed sera from nephrectomized mice rose from 46 ± 4 to 129 ± 6 colonies per 0.1 ml after dialysis. There was a slight rise in the sham-operated serum CSF levels from 52 ± 10 to 76 ± 12 colonies/0.1 ml dialyzed.

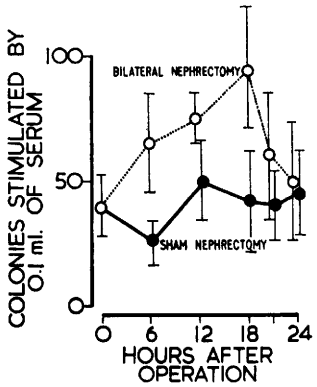

FIG. 1. Serum CSF levels in C57BL mice after bilateral nephrectomy and sham nephrectomy. Each point represents the average of 10–30 animals. Vertical bars are standard deviations.

The C57BL mice used in the present experiments became clinically ill after nephrectomy and 24 hr after nephrectomy the mortality rate was 30–40%. Sham-operated mice remained well and active. Further evidence of the stressing effects of nephrectomy was the fall in thymus weight after operation. By 18 hr after operation the thymus weight in the nephrectomized mice was 24 ± 3 mg and in sham-nephrectomized mice 38 ± 3 mg.

The recoverable blood volumes and hematocrits were similar in both groups throughout the observation period. At 18 hr after operation the recoverable blood volume and hematocrit in the nephrectomized mice were 0.9 ml and 42%; in sham-nephrectomized mice 1.0 ml and 45%.

As another control for operative stress, groups of C57BL mice were partially hepatectomized. Using nembutal anesthesia, 50–70% of the liver was removed. Similar groups were sham hepatectomized. The groups were killed at various time points, from 18 hr to 10 days, using a total of 20 partially hepatectomized mice and 20 sham-operated mice.

The serum CSF level of the partially hepatectomized group was not statistically different from that of the control at all time points.

Ureter tying. CSF levels in the sera of the bilateral ureter-tied mice rose after 12 hr and remained high until 30 hr after which levels fell as in the nephrectomized mice (Fig. 2).

The ureter-tied mice also became clinically ill but this did not become apparent until after 24 hr post-operatively, and the mortality rate was 20% by 2 days.

Morphology of colonies. Under the dissecting microscope the colonies in agar were classifiable into three types, (1) small com-

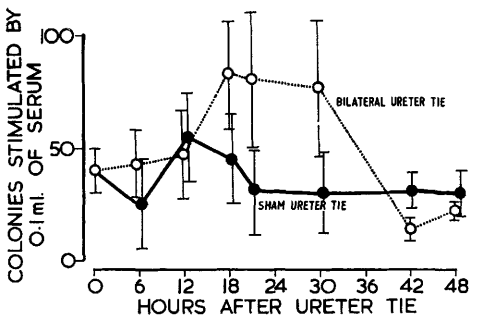

FIG. 2. Serum CSF levels in C57BL mice after bilateral ureter tie and sham ureter tie. Each point represents the average of 5–20 animals. Vertical bars are standard deviations.

pact colonies with tightly clumped cells; (2) large loose colonies with uniformly dispersed cells; and (3) colonies with a central tight core and a loose peripheral mantle of cells. On microscopic examination colonies of type 1 contained pure populations of granulocytic cells ranging from myeloblasts to polymorphonuclear leukocytes. Colonies of type 2 contained pure populations of macrophage cells while colonies of type 3 contained a mixed population of granulocytes and macrophages.

The percentage of frequency of the various types of colonies simulated by the sera from mice of the different groups is shown in Table I. 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. The percentage of granulocytic and mixed colonies was increased when the sera of nephrectomized and sham-operated mice was predialyzed.

Cortisone studies. In agreement with earlier studies (11), the injection of 2 mg of cortisone acetate to control, unoperated mice was found to lower serum CSF levels to 25% by 6 hr after injection (Fig. 3). A similar decrease was observed in serum CSF levels in sham-operated mice after cortisone injection. In contrast, no significant decrease of serum CSF levels occurred in the bilaterally nephrectomized or bilaterally ureter-tied mice, when injected with cortisone.

It was noted previously that mice injected with large doses of cortisone developed a diuresis as compared with control mice (11). To investigate whether the fall in serum CSF levels after cortisone might be due to loss of CSF in the urine, groups of 8- to 12-month-

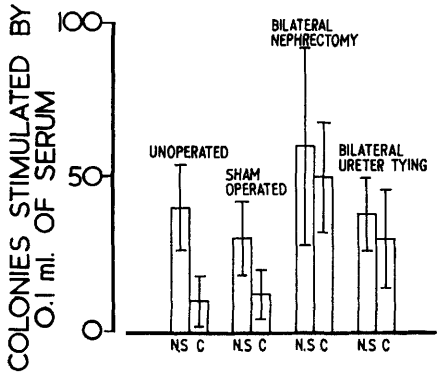

FIG. 3. Serum CSF levels in C57BL mice 6 hr after bilateral nephrectomy, bilateral ureter-tying, and sham operation. Groups were given 2 mg of cortisone acetate (c) in 0.4 ml or an equal volume of normal saline (NS) at the time of operation. There were 10–28 animals in each group. Vertical bars represent standard deviations.

TABLE I. Percent Frequency of Colonies of Different Types Stimulated by Sera from Nephrectomized and Sham-Operated C57BL Mice.

Source and treatment of serum-stimulating colony formation	Total no. of colonies typed	Percentage of types of colonies[a]		
		Granulocytic	Mixed	Macrophage
Bilateral nephrectomy (not dialyzed)	80	10	5	85
Bilateral nephrectomy (dialyzed)	100	25	19	56
Sham-operated (not dialyzed)	122	0	8	92
Sham-operated (dialyzed)	110	7	21	72

[a] Mean data from three separate experiments in which 25–45, unselected, sequential colonies were sampled from each group of cultures.

TABLE II. Serum and Urine CSF Levels after Cortisone Injection in C57BL Mice.

Exp.	Treatment	No. of mice	Mean no. of colonies stimulated by: Serum[a] Undialyzed	Serum[a] Dialyzed	Dialyzed urine[b]	Vol (ml) of urine/ mouse[c]	Calculated total no. of colonies stimulated by urine
1	Cortisone acetate 2 mg	7	6 ± 1	8 ± 2	72 ± 5	1.0	480
	Normal saline	7	24 ± 2	34 ± 2	36 ± 3	0.45	108
2	Cortisone acetate 2 mg	7	12 ± 3	36 ± 4	86 ± 6	1.1	630
	Normal saline	7	46 ± 3	76 ± 2	64 ± 4	0.45	193
3	Cortisone acetate 2 mg	6	16 ± 3	20 ± 2	78 ± 8	1.1	580
	Normal saline	6	42 ± 2	56 ± 4	30 ± 4	0.6	120
4	Cortisone acetate 2 mg	6	8 ± 2	26 ± 6	24 ± 9	1.25	200
	Normal saline	6	34 ± 3	58 ± 3	9 ± 3	0.65	30
5	Cortisone acetate 2 mg	4	5 ± 2	20 ± 5	54 ± 3	1.35	440
	Normal saline	4	39 ± 4	84 ± 5	36 ± 3	0.5	120

[a] Mean colony counts of 3–6 replicate plates stimulated by 0.1 ml of serum ± standard deviation.
[b] Mean colony counts of four replicate plates stimulated by 0.15 ml of urine ± standard deviation.
[c] Average volume of urine per mouse collected over 6 hr.

old C57BL mice were injected with 2 mg of cortisone acetate or normal saline. Urine from the two groups was collected during the next 6 hr. After 6 hr the two groups of mice were killed and the sera from each group pooled. A portion of each serum pool and the urines were dialyzed for 3 days at 4° and were assayed for CSF levels.

Table II shows that there was a reduction of serum CSF levels in the cortisone-injected mice compared with the levels in the control group injected with normal saline. On dialysis of the sera there was an increase in the colony-stimulating activity of some of the sera from the cortisone-injected group but levels remained below that of the dialyzed sera of the normal saline-injected group.

The cortisone-injected mice excreted about twice the volume of urine excreted by saline-injected mice. Assays on the CSF content of this urine were performed after dialysis of the urine against water for 3 days (2). Urine from cortisone-injected mice contained 3.5–6 times the amount of CSF present in urine from saline-injected mice.

Calculation of the additional CSF in the urine of cortisone-treated mice indicated that it approximately equalled the reduction in total CSF in the serum of these animals over the 6-hr observation period.

Discussion. The present results indicate that serum CSF levels rise after bilateral nephrectomy or bilateral ureter tie. This strongly suggests that the kidney clears CSF from the serum to the urine. The apparent fall in serum CSF levels after 18 hr in bilateral nephrectomy and after 30 hr in bilateral ureter ligation may have been due to the accumulation of toxic material in the serum. This would inhibit bone marrow cell proliferation and mask CSF levels. After dialysis there was a 3-fold rise in the serum CSF. Despite the evidence that kidney cells can elaborate CSF *in vitro* (3, 7), the rise in serum CSF levels after bilateral nephrectomy suggests that the kidney is probably not a major site of production of CSF. The possibility that the kidney is also a site of destruction of CSF cannot be eliminated, since, after ureter ligation, the kidneys may be damaged.

The injection of cortisone into mice reduces serum CSF levels dramatically in 6 hr (11). The present experiments show that this fall does not occur in bilaterally nephrectomized and bilaterally ureter-tied animals. The urine of cortisone-injected mice was

found to contain much more CSF than that of the control mice injected with normal saline. This suggests that one of the ways in which cortisone reduces serum CSF may be by causing an increased clearance of CSF into the urine.

The number of granulocytic colonies stimulated to develop by mouse serum was found to increase after bilateral nephrectomy. The percentage and total number of granulocytic and mixed colonies also rose when the sera of nephrectomized and sham-operated animals were dialyzed. This suggests that there may be two factors, a granulocyte-stimulating and a macrophage-stimulating factor in normal mouse serum, and that the kidney may be involved in the breakdown of the granulocyte-stimulating factor. Alternatively, since the number of granulocytic colonies increased after dialysis of the stimulating serum, a dialyzable granulocytic inhibitory factor may normally be present in mouse serum. Ichikawa *et al.* (3) showed the development of granulocyte and macrophage colonies *in vitro* using feeder layers and conditioned media from mouse embryo and kidney cells and have reported a dialyzable inhibitor for both granulocytic and macrophage colonies (4), though macrophage colonies were preferentially inhibited.

Summary. Serum CSF levels in C57BL mice rose after bilateral nephrectomy and bilateral ureter tie. These procedures prevented the fall of serum CSF levels caused by cortisone. The urine of cortisone-injected mice contained more CSF than that of saline-injected mice. In mice, most of the CSF in the urine appears to be CSF cleared by the kidney from the serum.

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