Lithium Enhancement of Megakaryocytopoiesis in Culture: Mediation via Accessory Marrow Cells

By Christian Chatelain, Samuel A. Burstein, and Laurence A. Harker

To examine the effect of lithium (Li) on early megakaryocytopoiesis, murine marrow megakaryocytic (CFU-M) and granulocyte-macrophage (CFU-C) progenitors were assayed in vitro with and without addition of lithium chloride (LiCl) to culture. At 2 mM LiCl, the numbers of CFU-M- and CFU-C-derived colonies were increased to 146% ± 8% and 128% ± 6% of controls, respectively (p < 0.005). Enumeration of megakaryocytes per colony showed a 78% increase of colonies (p < 0.05) containing from 6 to 22 cells, suggesting an increased proliferative capacity of CFU-M in the presence of LiCl. Conditioned media from spleen cells cultured in the presence of both pokeweed mitogen (PWM-SCM) and 2 mM Li increased the numbers of CFU-M and CFU-C to 157% ± 8% and 183% ± 8%, respectively (p < 0.025), compared to control cultures stimulated by PWM-SCM alone. Since the production of active colony-stimulating activities (CSA) from mitogen-stimulated conditioned media requires T lymphocytes, we hypothesized that the enhancement of the growth of early hematopoietic progenitors in marrow cultures was due to a Li-induced CSA production by accessory marrow cells, rather than a direct effect of Li on stem cells. To test this, cyclosporin-A (CyA), a T-lymphocyte function inhibitor known to suppress CSA production in PWM-SCM, was added to marrow cultures in the presence of 2 mM Li. CyA (3 μg/ml) abrogated the Li-induced enhancement of CFU-M and CFU-C growth, but had no effect on colony formation when added alone. The data suggest that the Li-induced enhancement of early megakaryocytopoiesis and granulocytopoiesis is due to local production of CSA(s) by an accessory cell population and requires the integrity of T-lymphocyte function.

Neutrophilic Leukocytosis following the administration of lithium (Li) salts has been recognized in man and experimental animals for over 30 yr. Although most reports of the hematopoietic effects of Li have been concerned with the granulocytic lineage, there are also several reports describing the effects of Li salts on the platelet count. Psychiatric patients receiving Li therapy may develop thrombocytosis, although this is generally uncommon. Patients with aplastic anemia have been reported to benefit from Li therapy. Preliminary studies in experimental mice suggest that Li administration induces thrombocytosis.

While the mechanism of action of Li is unknown, experiments performed in culture have shown that Li increases the detectable numbers of murine pluripotent stem cells (CFU-S), human, canine, and murine granulocyte-macrophage progenitors (CFU-C), and murine megakaryocytes. Li has been proposed to affect stem cells directly. Alternatively, other in vitro studies are consistent with the possibility that Li increases the level of colony-stimulating activity (CSA), thereby increasing the numbers of detectable CFU-C and other progenitors secondarily.

We therefore performed the following experiments to investigate the mechanism of action of Li on murine megakaryocytopoiesis in particular and on hematopoiesis in general.

Materials and Methods

Mice

Male C57BL/6 mice, specific pathogen free and 6–8 wk old (Jackson Laboratories, Bar Harbor, ME), were used for all experiments.

Assays for CFU-M and CFU-C

The numbers of megakaryocytic (CFU-M) and granulocyte-macrophage (CFU-C) progenitor cells in mouse bone marrow were determined in agar using a modification of the method of Metcalf et al. Pokeweed mitogen-stimulated spleen cell conditioned medium (PWM-SCM) was prepared with 5% human plasma and added at a final concentration of 20% in all experiments. Colonies were enumerated on day 5 of culture according to previously defined criteria. Quadruplicate plates were enumerated for each experimental point.

Enumeration of Colony Cells

The number of cells in each individual megakaryocytic colony was determined. Colonies were then distributed into classes according to the logarithm (base 2) of their cell number. This distribution expresses the mean number of doublings of progenitor cells in a clonal assay system.

In Vitro Experiments Employing Li

To examine the effect of Li on megakaryocytopoiesis and granulocyte-macrophage colony formation, 12 μl of various concentrations of lithium chloride (LiCl) diluted in phosphate-buffered saline (PBS) were added to cultures, while an equivalent volume of PBS was added to control plates.
The effect of LiCl on the production of megakaryocytic and granulocyte-macrophage colony-stimulating activity (meg-CSA and GM-CSA, respectively) was determined as follows: 1% (v/v) of various concentrations of LiCl was added to spleen cell cultures in the presence or absence of a 1:15 dilution of pokeweed mitogen. Seven days after the initiation of culture, supernatants of both control and Li-treated spleen cell cultures were prepared and dialyzed in the same container against PBS until Li ion was undetectable by flame photometry (0.00 mM at 24 hr). The dialyzed conditioned medium was then sterilized by filtration through a 0.22 μM Millipore filter.

**Experiments Employing Cyclosporin-A (CyA)**

CyA, a T-lymphocyte function inhibitor known to abrogate the production of CSA from PWM-stimulated spleen cells, was prepared as described previously. The effect of CyA on the production of active CSAs was determined by adding 3 μg/ml to spleen cultures. The supernatants were prepared and dialyzed as described above.

To examine the effect of CyA on the Li-induced enhancement of colony formation, 3 μg/ml were added per plate, while control plates were treated with the CyA diluent.

**Statistical Methods**

Statistical comparisons were performed employing t statistics for two means.

**RESULTS**

**Influence of Li Directly Added to Cultures**

The numbers of CFU-M- and CFU-C-derived colonies that formed in the presence of Li are shown in Fig. 1. They were significantly increased at Li concentrations between 0.1 and 4 mM, with a maximal effect at 2 mM (146% ± 8% and 128% ± 6% of controls, respectively; *p* < 0.005). At 10 mM, inhibition was observed that was greater for CFU-C (−89% ± 2%) than for CFU-M (−22% ± 10%) growth.

The distribution of the number of megakaryocytes per colony is shown in Fig. 2. In the presence of 2 mM LiCl, an increased number of colonies containing 6–22 cells was detected (*p* < 0.05), while colonies comprised of fewer than 6 cells did not differ from control cultures (*p* > 0.1).

**Influence of Li on the Production of Colony-Stimulating Activity**

The effect of Li on meg-CSA and GM-CSA production is shown in Fig. 3. CFU-M-derived colony numbers were maximally increased by conditioned medium generated by spleen cells previously incubated with 2 mM LiCl (157% ± 8%; *p* < 0.025). This effect declined at higher Li concentrations. Similarly, the numbers of granulocyte-macrophage colonies progressively increased in the presence of Li-stimulated conditioned medium (183% ± 8%; *p* < 0.025), with no decline noted at the highest concentration of Li employed (4 mM). Addition of Li to spleen cells in the absence of PWM induced minimal CSA production (4 ± 1 and 7 ± 1 CFU-M- and CFU-C-derived colonies per 7.5 × 10⁴ cells, respectively).

**Effect of CyA**

CyA (3 μg/ml), when added to spleen cell cultures in the presence of PWM, abrogated the production of CSA. Moreover, CyA abrogated the production of CSA in the presence of both PWM and 2 mM LiCl (data not shown). As reported previously, CyA added directly to marrow cell cultures did not influence the numbers of CFU-M- and CFU-C-derived colonies. However, the enhanced growth of CFU-M and CFU-C induced by 2 mM LiCl was abrogated by CyA (Table 1).

**DISCUSSION**

Leukocytosis and neutrophilia have been found repeatedly in psychiatric patients receiving Li thera-
This phenomenon has also been reported in experimental dogs. Although administration of Li to mice had variable effects on the peripheral neutrophil count, bone marrow neutrophils and proliferating myeloid cells were consistently increased. As reflected by autologous labeling with diisopropylfluorophosphate and serum vitamin B12 concentrations, Li has been shown to increase both the rate of production and the pool size of granulocytes.

Li salts increase the numbers of CFU-C in mice, dogs, and man. Several workers have suggested that Li acts directly on the CFU-C, possibly by increasing its responsiveness to CSA. However, GM-CSA production by murine lung tissue was reported to be increased in the presence of Li. In man, an increase in production of urinary CSA was demonstrated in patients with Felty’s syndrome receiving Li.

Our studies show that both CFU-C- and CFU-M-derived colonies are increased in the presence of Li. One possible mechanism of action is that Li increases the sensitivity of the progenitor cells for CSA, resulting in a higher production of active CSA.
in the recruitment of progenitors into cellular proliferation. Indeed, it has been shown that Li exposure increases the percentage of CFU-C in S-phase.\(^{28}\)

In the present study, the addition of Li to spleen cells in the presence of pokeweed mitogen produced conditioned medium that increased the number of CFU-C- and CFU-M-derived colonies. The conditioned medium produced in the presence of Li was dialyzed free of the ion to remove any possible direct effect when tested on marrow cell cultures. The enhancement of detectable colony numbers suggests that at least one effect of Li is to produce a more potent conditioned medium, perhaps by increasing the concentration of CSA. In this regard, Li has been shown to promote lectin-stimulated lymphocyte proliferation in man.\(^{29}\) The production of human GM-CSA is enhanced by a Li effect on marrow macrophages and T lymphocytes.\(^{18,20}\)

The reported data are consistent with two possible mechanisms of action of Li on CFU-M, in line with previous hypotheses on the enhancement of CFU-C numbers. However, it is possible that the observed effects of Li on marrow cultures are entirely due to the in situ elaboration of CSA from accessory cells in the plated marrow population. To examine this possibility, we employed CyA, a noncytotoxic T-lymphocyte function inhibitor. The mechanism of action of CyA may be the inhibition of interleukin-2 (T-cell growth factor) production from T cells, or the inhibition of interleukin-1 from macrophages.\(^{30}\) Previous experiments performed in our laboratory have shown that CyA abolishes active CSA production by mitogen-stimulated spleen cell cultures, but has no effect on colony formation when added directly to marrow cell cultures in the presence of CSA. Consequently, we reasoned that the addition of CyA to marrow cell cultures would prevent any in situ elaboration of active CSA(s) by T lymphocytes (or other CyA-sensitive accessory cells) within the cultures. The results showed that the addition of CyA totally eliminates the Li-induced enhancement of both CFU-M- and CFU-C-derived colonies. These data also show that the effect of Li can be fully explained by its enhancement of CSA production by accessory cells, probably T lymphocytes.

Individual cells in megakaryocytic colonies are easier to enumerate than cells of other colony types, and our data suggest that at least one extra doubling has occurred. This increase in the size of megakaryocytic colonies in the presence of Li is consistent with the addition of increasing concentrations of meg-CSA. Granulocyte-macrophage colonies were also subjectively larger, and thus, a similar extra doubling may have occurred. These increases in the numbers of cells within colonies may be manifested as the increased numbers of circulating granulocytes noted in in vivo studies and in the enhanced numbers of megakaryocytes observed in long-term marrow cultures exposed to Li.\(^{11}\)

Several in vivo phenomena might be explained on the basis of our hypothesis concerning the mechanism of Li action. The defect in canine cyclic hematopoiesis, which is correctable by Li, may be due to abnormalities of T lymphocytes or accessory cells.\(^{31,32}\) Those patients with aplastic anemia who respond to Li therapy may have defective T lymphocytes, but relatively normal stem cells, at this phase of their disease.

The response of hematopoietic stem cells to Li and the abrogation of Li-induced enhancement by CyA also suggest that drugs, hormones, or other substances that augment stem cell numbers may operate indirectly by means of enhancement of endogenous CSA production.\(^{33}\)

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### REFERENCES


### Table 1. The Influence of CyA on Lithium-Stimulated Colony Growth

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<tr>
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<th>CFU-M</th>
<th>CFU-C</th>
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<tbody>
<tr>
<td>Control</td>
<td>24 ± 1</td>
<td>80 ± 11</td>
</tr>
<tr>
<td>CyA (3 μg/ml)</td>
<td>24 ± 1</td>
<td>78 ± 10</td>
</tr>
<tr>
<td>Li (2 mM LiCl)</td>
<td>32 ± 1</td>
<td>117 ± 13</td>
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<tr>
<td>Li + CyA</td>
<td>24 ± 1</td>
<td>82 ± 13</td>
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*Mean ± 1 SE per 75,000 marrow cells (3 experiments).
†The increase in CFU-M- and CFU-C-derived colonies in the Li group is significant (p < 0.05) compared to all other groups.
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