Effect of lithium on granulopoiesis in culture

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Lithium carbonate therapy is associated with polymorphonuclear leukocytosis. In vitro studies have shown that lithium ions stimulate formation of granulocytic colonies. In a study undertaken to determine how lithium acts, colony-forming cells uncontaminated by monocytes (which elaborate colony-stimulating factor [CSF] in vitro) were obtained by means of a two-step cell separation procedure. The effects of lithium on colony formation were then studied in (a) cultures stimulated by humoral CSF, (b) cultures in which monocytes were relied upon to synthesize CSF de novo and (c) unstimulated cultures.

Lithium enhanced the action of CSF but did not stimulate colony formation in the absence of CSF. In monocyte-stimulated cultures, colony formation increased with lithium concentrations up to 1 mmol/L but this increase paralleled that in CSF-stimulated cultures and therefore was not due to increased CSF production by monocytes. At higher concentrations of lithium, colony formation decreased in the monocyte-stimulated cultures but increased in the CSF-stimulated cultures. A lithium concentration of 4 mmol/L gave the greatest enhancing effect on colony formation in CSF-stimulated cultures and a concentration greater than 1 mmol/L inhibited de novo synthesis of CSF by monocytes.

Le traitement au carbonate de lithium est associé à une leucocytose polymorphonucléaire. Des études in vitro ont montré que les ions lithium stimulent la formation de colonies de granulocytes. Dans une étude visant à déterminer de quelle façon le lithium agit, des cellules formant des colonies débarrassées des monocytes (qui élaborent, in vitro, un facteur stimulant les colonies [FSC]) ont été obtenues par une technique de séparation cellulaire en deux étapes. Les effets du lithium sur la formation des colonies ont alors été étudiés sur (a) des cultures stimulées par du FSC humoral, (b) des cultures où la synthèse du FSC était assurée par des monocytes et (c) des cultures non stimulées.

Le lithium a augmenté l'action du FSC mais n'a pas stimulé la formation de colonies en l'absence de FSC. Dans les cultures stimulées par les monocytes, la formation de colonies a augmenté avec l'addition de lithium jusqu'à 1 mmol/L, mais cette augmentation a été parallèle à celle qui a été observée dans les cultures stimulées par le FSC et elle n'était donc pas due à un accroissement de la production de FSC par les monocytes. À des concentrations de lithium supérieures, la formation de colonies a diminué dans les cultures stimulées par les monocytes mais a augmenté dans les cultures stimulées par le FSC. Une concentration de lithium de 4 mmol/L a donné l'effet facilitateur maximum sur la formation de colonies en culture stimulée par le FSC mais une concentration supérieure à 1 mmol/L a inhibé la synthèse de novo du FSC par les monocytes.

Lithium ions stimulate granulopoiesis in vivo[1,2] and in vitro.[3,4] Polymorphonuclear leukocytosis occurs in patients treated for mania with lithium carbonate[1,2] and granulocyte colony formation is enhanced in semisolid cultures containing lithium ions.[3,4] Theoretically the in vitro findings could be explained if lithium (a) acts directly on the colony-forming cell (CFC), (b) increases production of colony-stimulating factor (CSF) manufactured by monocytes normally present in the population of cultured marrow cells or (c) potentiates the action of CSF. In the study reported below we used cell separation procedures to investigate these three possibilities, and found that lithium potentiates the action of CSF but does not increase its production or stimulate CFCs to proliferate in its absence.

Methods

Preparation of cultures

The semisolid agar culture system was used. Each culture consisted of a 1-mL agar layer containing 1.5% agar in CMRL 1066 medium (Grand Island Biological Company, Grand Island, New York) supplemented with 30% heat-inactivated normal serum (heated at 58°C for 150 minutes). Each experiment was conducted with target CFCs obtained from the nonadherent light-density fraction of a single aspirate of normal human bone marrow. By means of a two-step cell separation procedure (a) neutrophils were removed by equilibrium density-gradient centrifugation in bovine serum albumin (specific gravity, 1.070 g/mL)[5] and (b) monocytes were removed by adherence separation.[6] It was necessary to remove monocytes to prevent spontaneous colony formation due to endogenous production of CSF. The marrow cell fraction thus obtained was enriched with CFCs and when cultured (10^5 cells per millilitre) did not form colonies unless stimulated to do so by an added cellular or humoral source of CSF.

Sources of CSF

The cellular source of CSF was provided by monocytes obtained from the buoyant fraction of a suspension of normal human blood leukocytes.[5] Neutrophils had been removed by equilibrium density-gradient centrifugation. Addition to cultures of mononuclear leukocytes (MNL) in a concentration of 2×10^5/mL resulted in colony growth due to de novo synthesis of CSF. De novo synthesis is a protein-dependent process[4,7] that occurs as a linear function of time when monocytes are incubated in medium.[8] It is a relatively slow process since peak concentrations of CSF are reached after 7 days in culture.[8]

Humoral CSF was prepared by incubating normal MNL in human serum at a concentration of 2×10^6/mL for 4 hours at 37°C, then removing the cells by Millipore filtration. This provided a convenient source of CSF. In MNL-conditioned serum CSF does not depend on de novo synthesis. In kinetic studies CSF became detectable within 30 minutes and increased to peak concentrations in 4 hours.[9] This difference is due to the fact that human serum contains a factor (or possibly more than one)[10] that is quickly converted to CSF by monocytes.[9] This factor is thermolabile: it is destroyed by being heated at 56°C for 30 minutes.[10] Therefore it was absent from the heat-inactivated serum used throughout these experiments to supplement the cultures.

Experimental design

Lithium chloride was added to three series of cultures over the concentration range 0 to 64 mmol/L. All cultures contained 10^5 nonadherent light-density marrow cells. Series 1 cultures were stimulated by 5% conditioned serum and series 2 cultures by MNL in a concentration of 2×10^5/mL; series 3 cultures received no stimula-

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tion. Control cultures containing sodium chloride in the same concentration range were set up in parallel to monitor for "nonspecific ionic effects". None were detected.

Cultures were incubated for 7 days at 37°C in a humidified atmosphere containing 5% carbon dioxide. The number of colonies (consisting of more than 20 cells) was counted on triplicate cultures.

Results and further investigation

Cultures stimulated by conditioned serum received a fixed dose of CSF. In these cultures lithium enhanced colony formation in a dose-related fashion up to 4 mmol/L, and thereafter colony formation declined (Fig. 1). Hence, within limits, lithium potentiated the action of CSF.

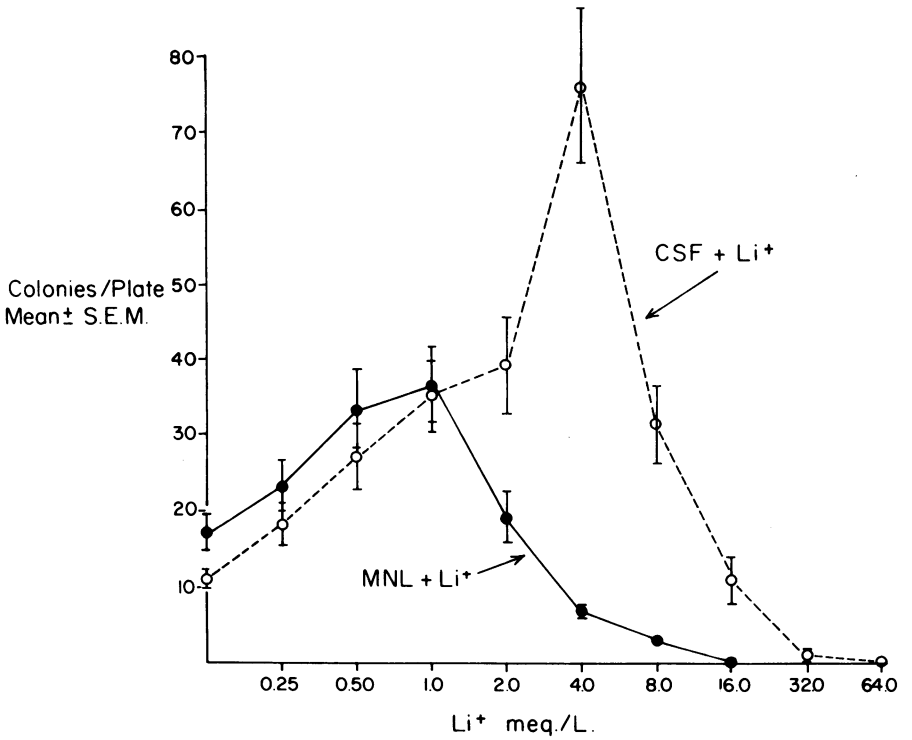

FIG. 1—Effect of lithium concentration on colony formation in cultures stimulated by conditioned serum, which provides colony-stimulating factor (CSF), and by monocytes, which have to synthesize CSF de novo. Curves are parallel up to lithium concentration of 1 mmol/L.

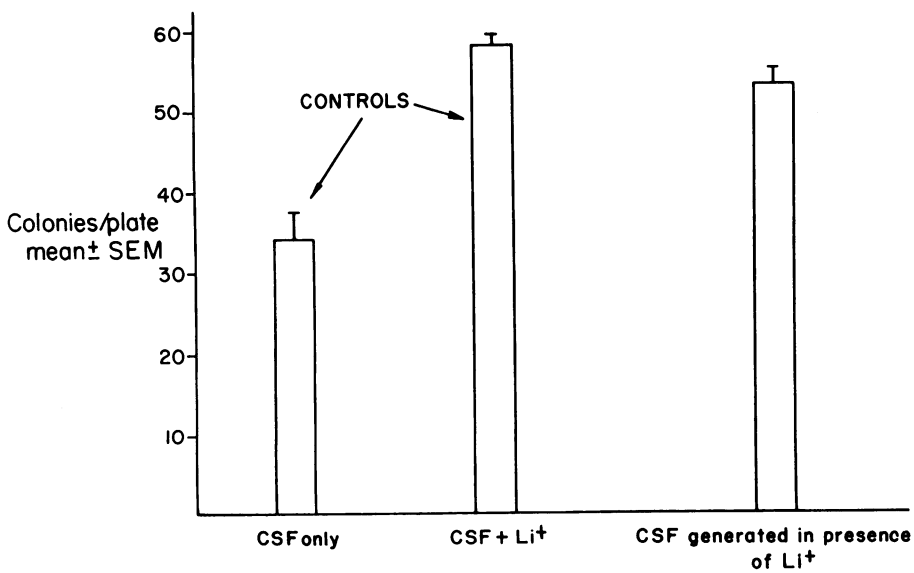

FIG. 2—Production of CSF by monocytes incubated in neutropenic human serum (containing "thermolabile factor") for 4 hours. Control conditioned sera (5%) were prepared (a) without lithium and (b) with lithium (4 mmol/L) added at end of 4-hour incubation period. Presence of lithium during incubation period did not reduce conversion of thermolabile factor to CSF by monocytes. Colony formation did not occur unless CSF was added (not shown).

In MNL-stimulated cultures, which depended on de novo CSF synthesis, colony formation increased with lithium doses only up to a concentration of 1 mmol/L; at higher concentrations colony formation declined (Fig. 1). Lithium did not enhance colony formation in unstimulated cultures, which did, nevertheless, contain small aggregates (clusters) of fewer than 10 cells.

Our results show that lithium potentiates the action of CSF. We could find no evidence to suggest that lithium enhances de novo synthesis of CSF by monocytes, and in fact the evidence presented in Fig. 1 is against this. Up to a dose of 1 mmol/L of lithium the slopes of the dose–response curves were similar in the two types of culture, indicating that any increase in colony formation over this range of lithium concentration could be attributed to an effect of lithium on CFCs. At higher concentrations of lithium, colony formation increased further in CSF-stimulated cultures but declined in MNL-stimulated cultures, indicating that at concentrations greater than 1 mmol/L lithium inhibited de novo synthesis of CSF by monocytes. De novo synthesis of CSF is a two-step process. In the first step thermolabile factor is produced, presumably by lymphocytes,[7] and in the second step it is converted to CSF by monocytes.[10,11]

The effects of lithium on monocyte conversion of serum thermolabile factor to CSF were investigated as follows: CSF was prepared by incubating monocytes in neutropenic serum for 4 hours with lithium in a concentration of 4 mmol/L. The concentration of CSF was assayed subsequently in cultures containing nonadherent light-density marrow cells. Control conditioned sera (5%) were prepared (a) without lithium and (b) with lithium added at the end of the 4-hour incubation period; the second control was necessary to ensure that equal quantities of lithium were carried forward into the assay. From the results shown in Fig. 2 it is clear that lithium did not inhibit the rapid conversion of thermolabile factor to CSF and must therefore act on the first step in the production of CSF, which is dependent on protein synthesis.

Discussion

Our results show that lithium ions potentiate the action of CSF on CFCs. They do not enhance production of

CSF. We are able to state this because we used cell separation techniques to assess the effects of lithium ions in cultures that lacked cells with the capacity to make CSF — monocytes. Unless this is done it is impossible to distinguish between the effects of lithium on CSF production and on CFC, as we were able to do (Fig. 1). We also showed that lithium does not influence the rapid conversion of serum thermolabile factor to CSF (Fig. 2) and therefore must act to decrease the de novo synthesis of this factor, presumably by a process that is dependent on protein synthesis and on lymphocytes.[7] Thermolabile factor can be produced by cells in the nonadherent light-density fraction of marrow[10] and presumably by blood lymphocytes.[7]

Serum concentrations of lithium that are acceptable therapeutically and cause no clinical signs of toxicity (0.5 to 1.0 mmol/L) were optimal in enhancing monocyte-stimulated cultures. Therefore if CSF plays a role in granulopoiesis in vivo, and if monocytes are needed for the production of CSF in vivo, our results could explain the granulocytosis associated with lithium therapy.

Before we can understand how lithium ions potentiate the action of CSF we must have more precise information on how CSF works. Preliminary kinetic studies have suggested that CSF is bound to receptors on the surface of the CFC.[12] Perhaps lithium ions, by their ability to form ligands, influence the affinity of CSF for receptor sites and thus effectively increase the concentration of CSF on the cell surface. However, the mechanisms involved undoubtedly are more complex than this, since lithium is distributed both intracellularly and extracellularly.[13]

In pharmacologically acceptable doses lithium influences a wide variety of intracellular processes,[13] particularly those that involve activation of cyclic nucleotides. It appears that lithium competitively displaces magnesium from adenosine 3′,5′-cyclic monophosphate (cyclic AMP) receptor proteins in the cell cytoplasm[14] and consequently inhibits a variety of magnesium-dependent enzymes.[14,15] This could explain the decrease in de novo production of CSF, since this is dependent on protein synthesis.[7] Why lithium does not inhibit proliferation of CFCs at concentrations between 2 and 4 mmol/L is less clear. Such concentrations would be toxic, and possibly lethal, in vivo. Recent studies on cyclic nucleotide concentrations in synchronized cell populations have indicated variation during different phases of the replicative cell cycles. Concentrations of guanosine 3′,5′-cyclic monophosphate (cyclic GMP), for example, appear to increase during the DNA synthetic phase and mitosis.[16] Possibly lithium, by depressing intracellular concentrations of cyclic AMP, influences those of cyclic GMP and thus triggers DNA synthesis.[17,18]

It is possible only to speculate on how lithium influences granulopoiesis in vivo. Since it does not increase CSF production in vitro it is highly unlikely that it does so in vivo. In fact, the role of CSF as a regulator in vivo has yet to be established.

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BOOKS

This list is an acknowledgement of books received. It does not preclude review at a later date.

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