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Human Leukocyte Interferon-Mediated Granulopoietic Differentiation Arrest and Its Abrogation by Lithium Carbonate

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Interferon has been shown to inhibit erythropoietic and granulopoietic differentiation. Since lithium carbonate (Li) elevates granulocyte levels in a variety of neutropenic disorders, we investigated the effect of Li on human leukocyte interferon (HLIF)-mediated inhibition of granulopoietic differentiation. Using an agar culture technique for cloning granulocyte-macrophage progenitor cells (GM-CFC), we demonstrated that Li blocks HLIF-induced granulopoietic differentiation arrest in a dose-dependent manner. Results of removal of T lymphocytes from marrow cells suggest that this Li effect is not mediated through marrow T lymphocytes.

Key words: human leukocyte interferon (HLIF); lithium carbonate (Li), granulocyte-macrophage progenitor cells (GM-CFC), colony-stimulating factor (CSF), human placenta-conditioned medium (HPCM), granulopoietic differentiation

INTRODUCTION

Lithium (Li) has been shown to ameliorate chemotherapy-induced myelosuppression in man and cyclic neutropenia in man and dog and to raise peripheral granulocyte levels in Felty's syndrome and aplastic anemia [1-5].

Recently, a preparation of human leukocyte interferon (HLIF) has been shown to block erythropoietic and granulopoietic differentiation in vitro [6-8]. That multiple varieties of cytopenic disorders have been shown to benefit from Li therapy indicates that it may enhance myelopoiesis through its varied actions at different stages of myelopoietic regulation. This fact prompted us to investigate the effect of Li on HLIF-induced granulopoietic differentiation block. The data presented in this paper

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suggest that Li can abrogate the HLIF-induced granulopoietic differentiation block in a dose-dependent manner and that this effect is not mediated through T lymphocytes (TL) present in the marrow.

MATERIALS AND METHODS

Collection of Specimens

Normal marrow, a source of granulocyte-macrophage progenitor cells (GM-CFC), was collected from normal human volunteers by posterior iliac puncture under local anesthesia in 15-ml plastic tubes (Falcon Plastics, Oxnard, Calif; #3033) containing 0.3 ml of preservative-free heparin (1000 U/ml) and 0.7 ml of phosphate-buffered saline (PBS).

Cell-Separation Procedures

To obtain light-density mononuclear cells, the marrow specimen was centrifuged at 400g for 35 minutes over Ficoll-Hypaque (density 1.077 gm/ml) columns in 15-ml plastic tubes.

Adherent mononuclear cell removal. The light-density marrow cells were washed twice with PBS and once with α-MEM (minimum essential medium) plus 15% fetal calf serum (FCS), and then suspended in α-MEM plus 15% FCS at a concentration of 2×10^6/ml. The cells were then subjected to active plastic adherence in 60-mm diameter plastic Petri dishes (Falcon Plastics, Oxnard, Calif) for 2 hours at 37°C in a 5% CO_2 atmosphere in air. Subsequently, the nonadherent cells were aspirated, suspended in α-MEM plus 15% FCS and used as target cells.

T lymphocyte removal. To remove TL, we used a modification of the method described by Weiner et al [9]. Briefly, the light-density nonadherent marrow cells (10^6/ml) were incubated with a 0.25% neuraminidase-treated erythrocyte (NE) suspension in 50-ml conical plastic tubes at 37°C for 15 minutes. The mixture was then centrifuged at 200g at room temperature for 5 minutes and chilled at 4°C for 1 hour. The cell mixture was resuspended and then NE rosettes were pelleted by centrifugation over a Ficoll-Hypaque column (density 1.080mg/ml). The TL-depleted interface cells were harvested and used as target cells. These cells contained $<0.5\%$ TL compared with 28.0% in the light-density, nonadherent marrow cells.

Use of Lithium Carbonate and Human Leukocyte Interferon Preparation

Lithium carbonate (Fisher Scientific Co, Pittsburg, Pa) was dissolved in α-MEM as a stock solution containing 1,000 mEq/liter Li. To obtain the required concentrations of Li in the culture dishes, the stock solution was diluted serially in α-MEM plus 15% FCS in such a way that not more than 0.1 ml of the Li solution had to be added to achieve the desired concentrations. The HLIF preparation was obtained from Dr. Cantell in 0.5-ml vials containing 3×10^6 international reference units (IRU) of interferon and 4 mg of protein with no preservative chemicals. For use, a whole vial was thawed and stored at 4°C in a stock solution in α-MEM at a concentration of 10^5 IRU/ml. Prior to use, the stock solution was serially diluted to the required concentrations and incorporated in the target cell suspension.

Semisolid Agar Culture Procedure

The cultures were prepared as described previously [10]. As a colony-stimulating factor (CSF) source, human placenta-conditioned medium (HPCM) was used at a final concentration of 10% (vol/vol) in the underlayers. All cultures were plated in triplicate and incubated for 8 days at 37°C in a fully humidified atmosphere of 5% CO_2, 12% O_2, and 83% N_2. Cultures were scored for colonies (aggregates of $\geq$ 40 cells) and clusters (aggregates of 4–39 cells) under an Olympus dissecting microscope at $\times$40. The results were reported as the mean of triplicate cultures $\pm$ one standard deviation.

Morphological Examination of Cell Aggregates

Colonies or clusters were stained with Luxol fast blue MBS stain (Hartman-Ladden, Philadelphia, Pa) as described previously [10].

Statistical Analysis

The mean of colony numbers were compared by two-sided sample t-tests.

RESULTS

Human Leukocyte Interferon-Induced Granulopoietic Differentiation Block

Figure 1A shows the results of two experiments investigating the effect of various HLIF concentrations on colony and cluster formation by light-density ($<$ 1.077 gm/ml), nonadherent human marrow cells. As we have reported previously [7] in both experiments, an HLIF dose-dependent decrease in the number of colony-size aggregates and a corresponding increase in the number of small cell aggregates (clusters) were noted. As a result, there was no significant difference in the total number of cell aggregates ($P > .1$).

Since the HLIF preparation we used in these experiments does not stimulate any colony or cluster growth per se, the above findings indicated that HLIF may halt the development of colonies that result from the clonal growth of GM-CFC. By the time the normal GM-CFC clones in semisolid agar culture develop into 40-cell-size aggregates, they show a normal differentiation pattern with increasing percentage of progressively mature granulocytes (40%–80% segmented granulocytes) (personal observation). Therefore, to detect any differentiation block caused by HLIF, we sequentially picked up anywhere from 42 to 54 clusters per observation and examined them morphologically. Table I illustrates the mean of percent differential counts from the two experiments. It is clear from these data that the clusters from the dishes with a progressively increasing concentration of HLIF show a progressive differentiation block compared with the clusters from the control dishes without any HLIF. This finding was also associated with a progressive decline in the average size of the clusters (from 32.6 cells/cluster in the absence of HLIF to 24.0 cells/cluster at 10 IRU/ml and 14.5 cells/ cluster at 1,000 IRU/ml HLIF). These data suggest that HLIF causes granulopoietic differentiation arrest.

Abrogation of HLIF-Induced Granulopoietic Differentiation Arrest by Lithium

Figure 1B shows the effect of various HLIF concentrations on colony formation in the presence of Li. In experiment 1, we tested the effect of 0.5, 1.0, and 2.0 mEq/liter of Li, and in experiment 2, we investigated the effect of 1.0, 2.0, and 4.0

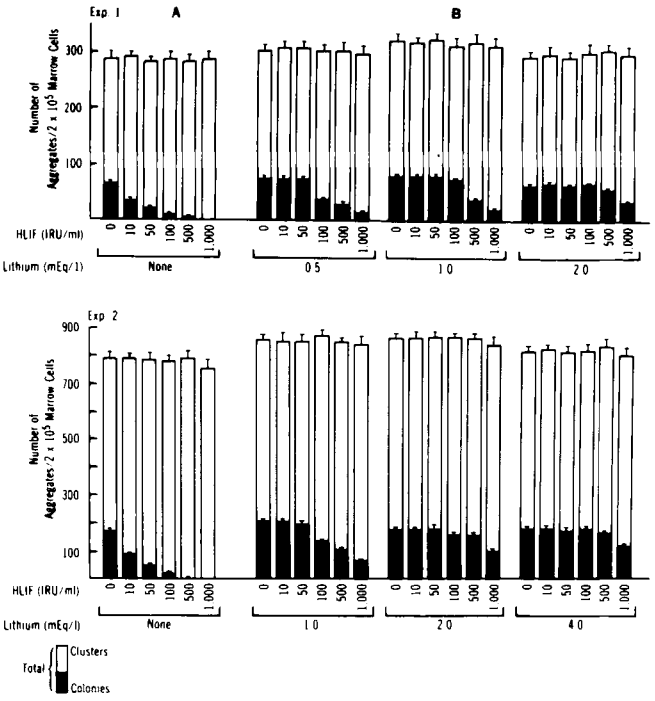

Fig. 1. Effect of HLIF on human GM-CFC clonal growth and its reversal by Li. (A) Increasing concentrations of HLIF caused progressive decline in the development of colony-size cell aggregates and a corresponding rise in the cluster-size cell aggregates. This resulted in almost a constant total plating efficiency regardless of the HLIF concentration. (B) Inclusion of various concentraions of Li in the culture dishes resulted in a partial or complete reversal of HLIF effect depending upon the dose of Li used.

mEq/liter Li. As can be noted, in the absence of HLIF, Li enhanced the HPCM-stimulated colony formation at 0.5 and 1.0 mEq/liter concentrations ($P.02$ and $\leq .005$, respectively) but not at 2.0 and 4.0 mEq/liter concentrations ($P > .1$). At a concentration of 0.5 mEq/liter, Li was able to completely abrogate the diminution in the formation of colony-size aggregates caused by 10 and 50 IRU/ml of HLIF. Higher Li concentrations were able to abrogate the effect of still higher HLIF concentrations; thus, at a concentration of 4.0 mEq/liter (experiment 2), Li was able to completely abrogate the effect of up to 500 IRU/ml of HLIF. This Li-induced reversal of HLIF effect on the formation of colony-size aggregates was also associated with reversal of the differentiation block (Table I).

We performed further experiments to investigate if HLIF-induced granulopoietic differentiation arrest, and its abrogation by Li was mediated through T lymphocytes (TL). As shown in Figure 2A, similar to the results shown in Figure 1A for which adherent-cell-depleted target cells were used, the increasing concentrations of HLIF caused a progressive decline in the colony-size aggregates with almost a corresponding increase in cluster-size aggregates. This effect of HLIF was retained even when the marrow target cells were depleted of E-rosetting lymphocytes (Figure 2B). Simultaneous experiments performed in the presence of 1 mEq/liter Li (Fig. 2A and 2B) revealed that regardless of whether we used nonadherent target cells or nonad-

TABLE I. Effect of Human Leukocyte Interferon on Cell Composition of Aggregates Cloned in Agar Culture

Interferon concentration (IRU/ml)	Lithium (1.0 mEq/liter)	Differential count of cells contained in clusters (%)[a]				
		Myeloblasts	Promyelocytes	Myelocytes	Metamyelocytes	Polymorphs
0	Absent	0.1 ± 0.1	2.5 ± 0.7	13.4 ± 2.6	27.3 ± 3.4	56.7 ± 6.3
10	Absent	0.8 ± 0.3	9.0 ± 2.7	35.7 ± 1.0	29.7 ± 0.8	23.8 ± 1.3
	Present	0.8 ± 0.1	3.2 ± 0.8	15.4 ± 1.6	29.2 ± 0.2	51.2 ± 2.7
100	Absent	3.5 ± 0.4	12.3 ± 4.1	44.7 ± 6.5	27.0 ± 6.1	12.5 ± 5.1
	Present	1.0 ± 0.4	3.0 ± 1.1	15.8 ± 2.9	28.2 ± 3.4	51.9 ± 7.8
1,000	Absent	8.1 ± 1.9	16.4 ± 4.3	43.7 ± 0.5	23.8 ± 4.1	8.0 ± 2.6
	Present	1.9 ± 0.1	6.6 ± 0.2	24.5 ± 3.2	31.0 ± 0.1	35.8 ± 3.1

[a] Mean ± one standard deviation from two separate experiments. In the absence of HLIF, Li did not change the differential counts of day 8 colonies or clusters.

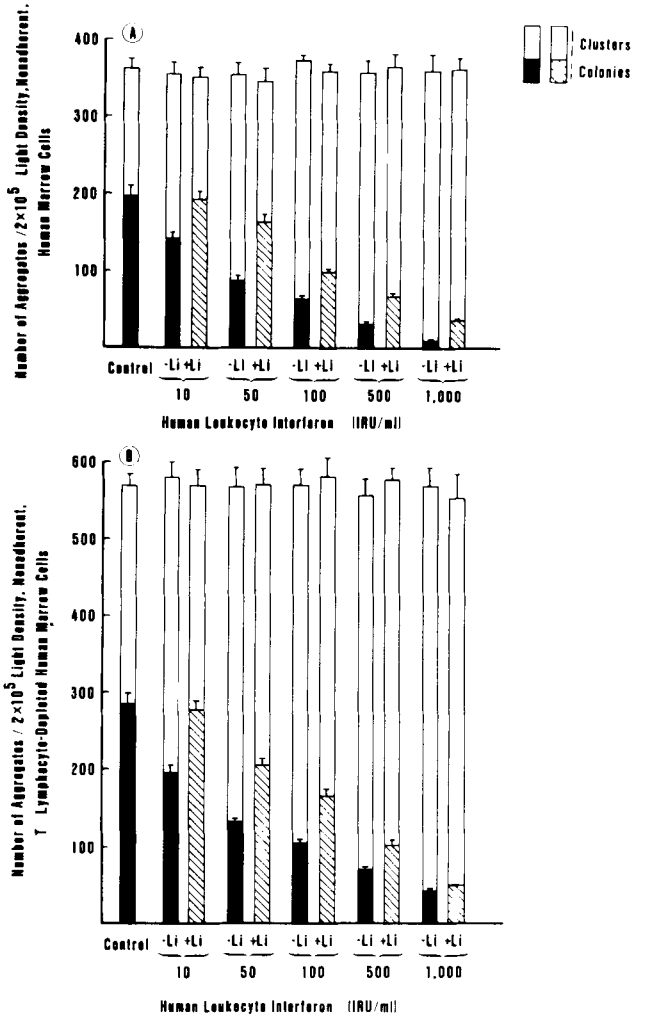

Fig. 2. Effect of TL removal from the target marrow cells on HLIF-mediated granulopoietic differentiation and its reversal by Li (1.0 mEq/liter). (A) It is clear that adherent mononuclear cell depletion did not effect the HLIF-mediated diminution in colonies and a corresponding rise in clusters. (B) The control cultures showed an increase in both colonies and clusters after TL depletion of marrow target cells. This was solely related to the concentration of GM-CFC due to the removal of non-GM-CFC containing TL. However, as shown in (B), TL depletion did not abrogate the HLIF effect on granulopoiesis or its reversal by Li.

herent, TL-depleted target cells, Li was able to abrogate the HLIF-induced diminution in the formation of colony-size aggregates, although this was more pronounced at lower HLIF concentrations.

DISCUSSION

Lithium has been shown to enhance myelopoiesis through various mechanisms. It has been reported to augment CSF elaboration from peripheral blood and marrow mononuclear cells and to enhance CFU-S proliferation and differentiation in mouse marrow suspension culture system [11-13].

We have reported previously that a partially purified preparation of HLIF blocks granulopoietic differentiation [7, 8]. This effect of HLIF does not seem to be due to impurities contained in the HLIF preparation since homogeneous preparations of the natural and cloned HLIF show a similar effect an granulopoietic differentiation [14]. In this paper, we clearly demonstrate that Li can abrogate this differentiation-arresting effect of HLIF if incorporated at the beginning of the cultures. This Li effect seems to depend upon the dose of Li used—higher concentrations are able to reverse completely the effect of relatively lower HLIF concentrations, and partially, that of higher HLIF concentrations. The differentation-arresting effect of HLIF does not seem to be mediated through either marrow-adherent mononuclear cells or TL, since removal of these cell populations from marrow target cells did not result in an abrogation of the HLIF effect compared with the experiments reported previously [7] when we did not remove adherent cells or TL from marrow. We have previously reported that a larger CSF concentration can counteract the effect of relatively smaller concentration of HLIF, and vice versa [8]. However, since Li releases an insignificant amount of CSF from nonadherent fraction of human marrow cells [12], it does not seem likely that Li-induced abrogation of granulopoietic differentiation block caused by HLIF is mediated through CSF. That the TL depletion of marrow target cells did not abrogate the Li reversal of the differentiation-arresting effect of HLIF further suggested that the observed effect of Li are not mediated through marrow TL.

Whether HLIF and Li act directly upon the granulopoietic precursors or through some other cell population is a matter of speculation. However, it is known that interferon activates adenyl cyclase and thus increases cyclic AMP levels [15]. On the other hand, Li inhibits adenyl cyclase activity, which results in diminished intracellular cyclic AMP levels [16]. It is possible that these contrasting cellular effects of HLIF and Li could be responsible for the mediation of the granulopoietic responses we have described in this paper.

Interferon therapy, as an antitumor agent, has generally been associated with neutropenia [17]. Based on the findings described in this paper, it would be of interest to investigate whether simultaneous Li administration in these patients would selectively block the neutropenic effect of interferon therapy.

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