

# Modulation of Cell-Mediated Immunity by Lithium Chloride

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Immunomodulation of cell-mediated immunity was studied in mice treated with either lithium chloride (LiCl), anti-CD8 monoclonal antibody or their combination. While 6-day LiCl treatment decreased the ability of their splenocytes to induce a local graft-*versus*-host reaction – anti-CD8 abolished this effect. The proliferative response of spleen cells from those three groups of mice to concanavalin A stimulation *in vitro* was significantly increased. The natural killer (NK) cell toxicity of the mice was decreased by over 43% after the 6-day LiCl treatment, but was  $\times 2.5$  higher than the control value after a longer 21-d treatment. These results indicate that the immunomodulatory capacity of lithium is dependent on the type of cell population studied, and on the schedule of administration.

## Introduction

Lithium has been used clinically for over 40 years for the treatment of mania and the prevention of recurrent attacks of manic-depressive illness (Schou, 1992). Its therapeutic effect is attributed mainly to its ability to alter metabolism of biogenic monoamines that have been implicated in the pathophysiology of mood disorders (Barridge *et al.*, 1989). In particular, it has been suggested that lithium exerts an inhibitory effect on the synthesis of secondary transmitters, such as cyclic AMP, cyclic GMP and inositol phosphate, as well as on the expression of autonomic receptors (Masana *et al.*, 1991).

Irrespective of its effect on the central nervous system, lithium provokes a distinctive immunomodulatory activity. In manic-depressive patients, lithium increased the number of leukocytes (Barr and Galbraith, 1983), decreased the frequency of recurrent inflammations (Horrobin, 1981) and alleviated their cellular and humoral immune deficiencies (Greco, 1980). The immunopotentiating

ability of lithium was demonstrated in studies in which the drug was added directly to a culture medium of lymphocytes stimulated with concanavalin A (Con A), phytohemagglutinin P (PHA-P) and Pokeweed mitogen (PWM) (Gelfand *et al.*, 1979). An attempt was made to relate the latter phenomenon to an increased production of interleukin-2 (IL-2) and a decrease in the cyclic-AMP level of lymphocytes, pre-incubated with LiCl (Bray *et al.*, 1981).

We have studied the effect of lithium on cellular-type immunoreactivity. In these studies we have shown that short-term (6 d) lithium administration inhibits both the immunoreactivity of donors' splenocytes in the semiallogenic and xenogenic graft-*versus*-host reaction (GvHR), and of recipients' splenocytes in the semiallogenic host-*versus*-graft reaction (GvHR) (Bubak-Satora *et al.*, 1991). We have also demonstrated that while a longer (21 d) administration of lithium to mice did not affect the reactivity of their donors' splenocytes in the semiallogenic GvHR, it abolished their immunosuppressive effect of cold stress (Bubak-Satora *et al.*, 1994).

In the present study we investigated the effect of short- and long-term lithium chloride administration on the cytotoxic activity of NK cells. Also,

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we studied the ability of splenocytes, collected from animals treated with LiCl for 6 days, to respond to a mitogen in an *in vitro* culture and to evoke an *in vivo* regional GvHR. Anti-CD8 monoclonal antibody served in this study to elucidate the mechanism of an immunomodulatory activity of this lithium salt.

Anti-CD8 monoclonal antibody (MoAb) (against the CD8 antigen present on the surface of suppressor and cytotoxic T lymphocytes), was used in order to find out which subpopulation of lymphocytes is affected by lithium. *In vivo* administration of anti-CD8 MoAb eliminates part of the CD8<sup>+</sup> subpopulations of T lymphocytes. It occurred to us that if lithium activates the CD8<sup>+</sup> (suppressive and cytotoxic) lymphocytes, combination of lithium and anti-CD8<sup>+</sup> will abolish this effect.

## Materials and Methods

### Animals

Inbred C57BL/6 (H-2<sup>b</sup>) mice and (C57BL/6 × CBA)F1 hybrid were raised at the Institute of Pharmacology, Polish Academy of Sciences, Krakow.

### Lithium chloride administration

LiCl (Aldrich Chem., Gillingham, England) was injected IP at a daily dose of 150 mg/kg BW, to C57BL/6 female mice for 6 ( $n = 40$ ) or 21 ( $n = 8$ ) consecutive days. Physiological saline was injected to a control group ( $n = 8$ ).

### Regional GvHR

The local graft-versus-host reaction (popliteal lymph node weight gain assay) was performed in a semiallogenic system. Donor mice were killed by cervical dislocation, and their spleens aseptically removed and gently homogenized. Cells for the *in vivo* study were suspended in balanced Hanks' solution, centrifuged and resuspended in the same medium. Splenocytes of each donor were also used for the mitogen-stimulated proliferation test. Splenocytes ( $5 \times 10^6$ ) from 2-month-old C57BL/6 female mice (in a volume of 0.02 ml of balanced Hanks' solution), were injected SC into the right hind footpad of 6-week-old (C57BL/6 × CBA)F1 female hybrids. Seven days later the right and left popliteal lymph nodes were removed, cleaned of

adhering surrounding tissues and weighed immediately to an accuracy of 0.01 mg. An "enlargement index" (EI) for each mouse was expressed as a weight ratio between the draining (right) lymph node and the contralateral one.

### MoAb treatment

Cytotoxic anti-CD8 (TIB 150) monoclonal antibody was prepared as ascites fluid and was diluted 1:10 with phosphate buffer immediately prior to inoculation. Its cytotoxic titers were higher than 1:10,000. The C57BL/6 mice, that were the donors of the splenocytes in the GvHR, were inoculated IP with 0.1 ml, 5, 3 and one day prior to the GvH test day (designated as "day 0").

### Mitogen-stimulated proliferation

Monodispersed spleen cells ( $2 \times 10^6$  cells/ml) were suspended in Eagle's minimal essential medium (MEM) supplemented with 20 mM L-glutamine, 100 U/ml penicillin G sodium, 100 µg/ml streptomycin sulfate and 10% heat-inactivated fetal bovine serum.  $2 \times 10^5$  cells/well were incubated in a final volume (including the mitogen) of 0.2 ml, were stimulated with 10 µg/ml Con A (Sigma Chem., St. Louis, MO, U.S.A.) and were incubated at 37 °C for 72 h, under an atmosphere of 5% CO<sub>2</sub> in air and 100% humidity. In order to determine cell proliferation, 1 µCi of [<sup>3</sup>H]thymidine was added per well at the end of 56 h of incubation. 16 h later the cells were harvested. Radioactivity of the filters was counted 24 h later in a liquid scintillation cocktail, using a Packard model B3255 liquid scintillation counter. Background count (cells in medium, without mitogen) were subtracted from all other counts.

### Assay of NK activity *in vitro*

Mice were treated with LiCl as described. They were sacrificed by cervical dislocation, their spleens removed and a single-cell suspension was prepared by sieving their homogenates through a cell-dissociation sieve (Sigma Chem., St. Louis, MO, U.S.A.) into a RPMI 1640 medium (GIBCO Ltd., Paisley, Scotland), supplemented with 15 mM HEPES, 2 mM L-glutamine, 100 U/ml penicillin G sodium and 100 µg/ml streptomycin sulfate. After lysis of the erythrocytes, the spleen cells were

washed  $\times 3$  in Hanks' balanced salt solution (HBSS) (GIBCO Ltd., Paisley, Scotland), and resuspended in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (GIBCO Ltd., Paisley, Scotland).

YAC-1 mouse lymphoma cells were transferred *in vitro* and served as target (T) cells. They were harvested, centrifuged, resuspended in 0.2 ml of medium and labelled with 100  $\mu\text{Ci}$   $\text{Na}_2^{51}\text{CrO}_4$  (Amersham, Braunschweig, Germany) for 1 h in a 37 °C, 5%  $\text{CO}_2$  in air incubator. After three washes with HBSS medium, the concentration of the labelled cells was adjusted to  $2 \times 10^5/\text{ml}$ . Serial dilutions of the spleen cells (E:T of 200:1 and 100:1) were pipetted to 96-well microplates in triplicates, with control plates containing either the medium (for background count) or 0.2% SDS (for total release). Target cells ( $2 \times 10^4$  in 0.1 ml) were added to all wells, the final volume in each one being 0.2 ml. The plates were then incubated at 37 °C for 4 h in a 5%  $\text{CO}_2$  in air incubator. At the end of incubation the plates were centrifuged at 500 rpm for 5 min at 4 °C, the supernatants removed and 0.1 ml counted in an LKB 1275 Mini-gamma counter. Cytotoxicity was calculated using the following formula:

$$\text{Percentage lysis} = \frac{\text{test CPM} - \text{background CPM}}{\text{total CPM} - \text{background CPM}} \times 100.$$

### Statistical analysis

Each study was performed 4 times. Statistical significance was evaluated by the Mann-Whitney U-test for the *in vivo* experiment and by the Student's "t" test for the *in vitro* experiment.

## Results

### Effect of LiCl on the GvHR *in vivo*

Treatment of donor C57BL/6 mice with LiCl (150 mg/kg BW) for 6 consecutive days caused a significant decrease ( $p < 0.01$ ) of their responsiveness in a local GvHR, as calculated from their enlargement indices. Simultaneous administration of the LiCl and the anti-CD8 MoAb, abolished that decrease in response. Anti-CD8 MoAb by itself did not have any effect in this system (Fig. 1).

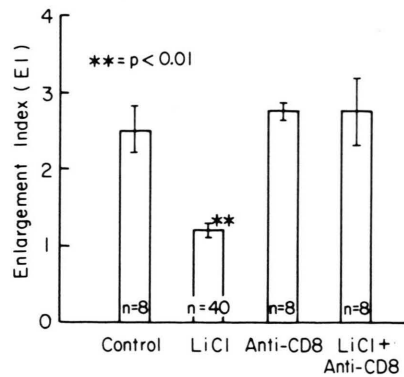


Fig. 1. Responsiveness of splenocytes in a regional GvH reaction, after treatment of C57BL/6 mice (splenocytes' donors) for 6 days with LiCl, anti-CD8 or their combination (mean  $\pm$  SEM). \*\* =  $p < 0.01$  as compared to control.

### Effect of concanavalin A on proliferative response of spleen cells taken from LiCl and/or anti-CD8-treated mice

Spleen cells harvested from the C57BL/6 mice pre-treated for 6 days with either LiCl, anti-CD8 or their combination, exhibited significantly ( $p < 0.01$ ) enhanced proliferative response to Con A stimulation *in vitro*. Con A stimulation was noticed in all three groups of splenocytes, but the combined pre-treatment (Li + anti-CD8) potentiated this stimulation (Fig. 2).

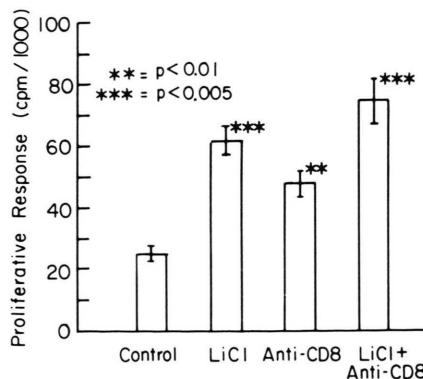


Fig. 2. *In vitro* response to Con-A, of spleen cells taken from C57BL/6 mice pre-treated for six days with LiCl, anti-CD8 or their combination (mean  $\pm$  SEM;  $n = 12$ ). \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$  as compared to control.

### Effect of prolonged treatment with LiCl on NK cell cytotoxicity

C57BL/6 mice were treated with LiCl (150 mg/kg BW) for 6 or 21 consecutive days, and the NK cytotoxicity of their spleen cells was tested *in vitro*. While 6-day treatment resulted in a significant ( $p < 0.05$ ) decrease in cell cytotoxicity, a 21-day treatment increased this toxicity remarkably ( $p < 0.01$ ) (E:T = 200) (Fig. 3). Similar results were obtained with E:T ratio of 100.

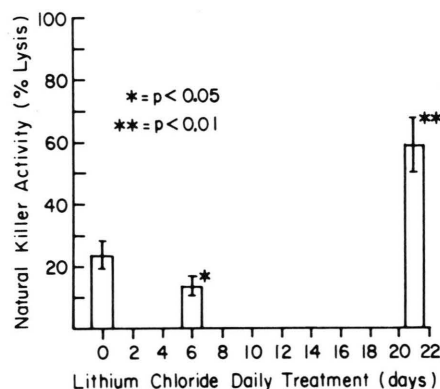


Fig. 3. Effect of prolonged *in vivo* administration of LiCl to C57BL/6 mice, on the cytotoxicity of NK cells derived from their spleens. The toxicity was evaluated against  $^{51}\text{Cr}$ -labelled-YAC-1 target cells (mean  $\pm$  SEM;  $n = 8$ ). \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ .

### Discussion

This study demonstrates that splenocytes of C57BL/6 mice, pre-treated with LiCl for 6 days, have a diminished ability to provoke a local GvHR, and have an increased proliferative ability *in vitro*, following their stimulation with a mitogen. Inhibitory effect of short-term LiCl administration on splenocyte activity has been reported by some of us (Bubak-Satora *et al.*, 1991, 1994). Recent *in vitro* studies demonstrated activation of the donor CD4<sup>+</sup> T-cell, during the first few days after induction of acute and chronic GvH reaction (Via, 1991). The lymph nodes' weight gain was due to enhanced division of the cells. This is consistent with our knowledge that recirculation of host B cells evoke lymph node enlargement (Krzystyniak *et al.*, 1992; Rolstad *et al.*, 1986). The decrease in the lymph node enlargement in the local GvHR may be due to an impaired ability of the T-helper lymphocytes to evoke a reaction in the host cells

and to an increased activity of cytotoxic and suppressive donor lymphocytes which inhibit proliferation of both the donor's and recipient's lymphocytes.

When splenocytes from animals pre-treated with 6 doses of LiCl were cultured *in vitro*, their response to Con A was increased by more than 100%, while concomitant administration of the anti-CD8 monoclonal antibody and lithium to cell donors did not change this effect. This observation suggests that there is not always a correlation between tests that assess cellular immunoreactivity *in vitro* and *in vivo*. In an unpublished study we demonstrated a considerable increase in the expression of muscarinic receptors on lymphocytes isolated from spleens taken from animals pre-treated with six doses of lithium. This expression ability returned to control level after 21 doses of lithium. The enhanced expression of the muscarinic receptors was explained as accompanying the increased proliferation of those lymphocytes (Maslinski, 1989).

Lithium influences the synthesis and function of G-proteins that regulate the synthesis of secondary transmitters (such as cAMP, inositol, 1,4,5-triphosphate, diacylglycerol), rather than on the synthesis and affinity of autonomic receptors on neurons to neurotransmitters (Mork *et al.*, 1992). The increased reactivity to mitogens after a short-term (6 days) of lithium administration may also be connected with the stimulatory effect of lithium on the IL-2 synthesis, observed *in vitro* (Kishter *et al.*, 1985).

Hyporeactivity of NK cells was observed after administration of biological response modifiers like lithium (Savary and Lotzowa, 1978). A possible mechanism for hyporeactivity of NK cells by lithium may be their redistribution among particular tissues and induction of cells and/or agents that exert a suppressive effect on them. After 21 days, a considerable increase in the cytolytic activity of the splenocytes was observed. This biphasic effect is probably controlled by the activity of IL-2 and interferon-gamma on the NK cells (Piccoli *et al.*, 1984). The NK cells are also involved in processes that regulate activity of normal cells, especially cells of the immune system. The immunostimulatory effect of prolonged administration of LiCl on the reactivity of NK cells has practical importance in increasing antineoplastic immunity.

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