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Lithium chloride potentiates tumor necrosis factor-mediated cytotoxicity *in vitro* and *in vivo*

(cytokine/cytolysis/tumorigenesis/cancer therapy)

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ABSTRACT **Tumor necrosis factor (TNF) is cytotoxic for several transformed cell lines *in vitro*. In the presence of LiCl, the murine fibrosarcoma cell lines L929 and WEHI 164 clone 13 became >10 times more sensitive to TNF-mediated cytotoxicity. The human tumor cell lines BT20 and HeLa D98/AH2 were also responsive to the cytotoxicity-enhancing effect of LiCl. Other monovalent or divalent cations did not affect TNF-mediated cytotoxicity. The potentiating effect of LiCl on TNF cytotoxicity was largely independent of transcription, and LiCl could be added to the cells as early as 2 hr before or as late as 4 hr after TNF without loss of effectiveness. The mechanism by which LiCl increases the cytotoxic response seems to differ from the sensitizing effect of actinomycin D or interferon γ, since the latter treatments overcame TNF resistance of several cell lines, whereas LiCl did not. Evidence is presented that LiCl acts, either directly or indirectly, via the TNF-activated phospholipase A_2 pathway. In nude mice, a combination of TNF and LiCl led to hemorrhagic necrosis and growth inhibition of L929 tumors, whereas little effect was observed when TNF was administered alone. HeLa D98/AH2 tumors also were sensitive to the potentiating effect of LiCl *in vivo*. We conclude that LiCl enhances the effectiveness of TNF *in vitro* and *in vivo*, results that may have therapeutic implications.**

Tumor necrosis factor (TNF) is a cytokine that was originally identified in the sera of mice that had been primed with bacillus Calmette–Guérin and challenged with endotoxin. When injected into mice bearing methylcholanthrene-induced sarcomas, TNF causes hemorrhagic necrosis of the tumor (1). *In vitro*, TNF exerts cytostatic and cytotoxic activity against a wide range of human and murine tumor cell lines, although it has little or no antiproliferative activity on nontransformed cell lines (2, 3). However, not all tumor cells are sensitive to TNF-mediated cytotoxicity. The molecular mechanism for this difference in response still remains largely unknown. Recently, a serine-type protease was shown to be involved in TNF-mediated cytotoxicity (4). Furthermore, there is ample evidence for an activation of a phospholipase A_2 (PLA_2) activity (5, 6). In addition to its cytotoxic effect on transformed cells, TNF mediates a variety of other biological activities on various cell types, both *in vivo* and *in vitro* (7).

In our efforts to understand the mechanism of action of TNF on malignant cells, we also evaluated a possible involvement of phospholipase C activity. In these experiments, we tested whether LiCl, which is known to inhibit inositol-1-phosphatase (8), would interfere with specific tumor cell killing by TNF. Surprisingly, we found instead that LiCl potentiated TNF-mediated cytotoxicity *in vitro* almost to a similar extent as has been shown before for actinomycin D (ActD) and cycloheximide (2) and, more physiologically, for interferon (IFN) (3, 9). Moreover, this LiCl-specific TNF potentiation can be extended to the *in vivo* antitumor action of TNF. Elsewhere, we report that TNF cytotoxicity is correlated with the activation of a PLA_2, and this effect is enhanced by LiCl. There is, however, no evidence for a role of the inositol phosphate system in TNF-mediated cytotoxicity.

MATERIALS AND METHODS

Cell Lines. L929 murine fibrosarcoma cells (Rega Institute, Leuven, Belgium) and L929(r)2, a TNF-resistant cell clone of the TNF-sensitive L929 cell line (unpublished work), were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated (30 min at 56°C) newborn calf serum. WEHI 164 clone 13 murine fibrosarcoma cells (T. Espevik, University of Trondheim, Norway) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS). EMT6 murine mammary tumor cells (R. F. Kallman, Stanford University, Stanford, CA) were cultured in Waymouth medium supplemented with 10% FBS and 1 mM sodium pyruvate. B16Bl6 murine melanoma cells (M. Mareel, Universitair Ziekenhuis, Ghent, Belgium; by courtesy of I. Fidler, Houston, TX) were cultured in DMEM supplemented with 10% FBS, minimum essential medium (MEM) nonessential amino acids, and 1 mM sodium pyruvate. Murine embryo fibroblast-like (MEF) cells were prepared (10) and were maintained in DMEM with 10% FBS. HT29 human colon adenocarcinoma cells (American Type Culture Collection, Rockville, MD) and HeLa H21 cervix carcinoma cells (R. Kamen, Imperial Cancer Research Fund, London) were maintained in DMEM with 10% newborn calf serum. MCF7 human breast carcinoma cells (M. Mareel; by courtesy of P. Briand, Copenhagen, Denmark) were cultured in MEM with 5% FBS, MEM nonessential amino acids, and bovine insulin (6 ng/ml). BT20 human breast carcinoma cells (ATCC) were grown in MEM with 10% FBS and MEM nonessential amino acids. HOS human osteosarcoma cells (ATCC) were cultured in DMEM with 10% FBS and MEM nonessential amino acids. The human cervix carcinoma cells ME180 (ATCC) and HeLa D98/AH2 (Imperial Cancer Research Fund) were grown in DMEM with 10% FBS. All cells were mycoplasma-free as judged from a DNA–fluorochrome assay (11).

Monokine Preparations. Recombinant human and murine TNF were produced by *Escherichia coli* and purified to at least 99% homogeneity (12, 13). The preparations used had a specific activity of 2–3 × 10^7 and 4.7 × 10^7 units (u)/mg of protein, respectively (units as defined in ref. 2) and contained less than 13 ng and 4 ng of endotoxin per mg of protein, respectively. The preparations were stored in aliquots in phosphate-buffered saline (PBS, free of Ca^{2+} and Mg^{2+}) at −70°C.

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Abbreviations: Δ_4Ach, arachidonic acid; ActD, actinomycin D; IFN-γ, interferon γ; PBS, phosphate-buffered saline; PLA_2, phospholipase A_2; TNF, tumor necrosis factor; u, unit(s).

Recombinant human lymphotoxin was purified from the culture medium of a Chinese hamster ovary cell transformant (provided by J. Van der Heyden, Biogent, Ghent, Belgium). The preparation had a specific activity of 2.5×10^6 u/mg of protein.

Recombinant murine IFN-γ was purified from the culture medium of a Chinese hamster ovary cell transformant, as described for human IFN-γ (14). The pure product had a specific activity of about 10^7 u/mg and was stored in PBS with 0.1% gelatin at −70°C. Recombinant human IFN-γ, prepared and purified as described (14, 15), had a specific activity of 10^8 u/mg and was stored in PBS with 0.5% gelatin at −70°C.

***In Vitro* TNF Cytolysis Assay.** TNF cytotoxicity was assayed in the presence of ActD (2). Target cells were seeded in microtiter plates at 4×10^4 cells per well. One day later, serial dilutions of TNF were added, together with ActD at a final concentration of 1 μg/ml. LiCl (E. Merck, Darmstadt, FRG) and other products were tested by applying various concentrations to the cells at the same time as TNF. Toxicity in the absence of TNF was also measured for each concentration of product tested. After 18 hr, the remaining cells were fixed and stained with crystal violet, and the retained dye was measured as described (4).

***In Vitro* TNF Cytostasis Assay.** Twenty-four hours before treatment, cells were plated in microtiter plates at $5–10 \times 10^3$ cells per well. Then serial dilutions of TNF, IFN-γ, or a combination of both were added. LiCl and other substances were tested by applying various concentrations to the cells 2 hr before TNF. After incubation for 72 hr at 37°C, the cells were assayed as described above.

TNF-Induced Arachidonic Acid (Δ_4Ach) Release. L929 cells were plated in culture medium (1 ml per 10-cm^2 well) and incubated at 37°C until subconfluency. Then 0.5 μCi of [^{3}H]Δ_4Ach (230 Ci/mmol; Amersham; 1 Ci = 37 GBq) was added per well. After overnight incubation, nonincorporated Δ_4Ach was removed by three washes with conditioned medium (from a 3-day culture of a superconfluent monolayer of L929 cells). The cells were refed with 2 ml of conditioned medium supplemented with 5 mg of fatty acid-free bovine serum albumin (Sigma) per ml. After 2 hr, LiCl (10 mM), recombinant human TNF (5000 u/ml), or their combination was added. Aliquots (350 μl) of supernatant were removed at appropriate times and centrifuged at 16,000 × *g* in a microcentrifuge, after which 300 μl of the supernatant was taken for liquid scintillation counting. Chromatographic analysis confirmed that most of the radioactivity released was in the form of Δ_4Ach, besides a small amount of a metabolite, presumably prostaglandin E2 (5).

Experimental Animals. Female nude mice, 6–8 weeks old, were used (Swiss *nu/nu*; Iffa Credo, Saint Germain-sur-l'Arbresle, France).

RESULTS

LiCl Enhances TNF-Mediated *in Vitro* Cytotoxicity for TNF-Sensitive Cells. Two murine cell lines (WEHI 164 clone 13 and L929) and five human cell lines (HeLa D98/AH2, BT20, ME180, MCF7, and HeLa H21) were used to study the effect of LiCl on the cytolytic/cytostatic activity of TNF. In a cytostasis assay, all these cell lines were sensitive to TNF as such, although sensitivity was more pronounced with the murine cells (data not shown). LiCl gave a dose-dependent enhancement of TNF cytotoxicity for the two murine cell lines and for three of the five human cell lines (partly shown in Fig. 1 for human TNF) but did not influence TNF sensitivity of MCF7 and HeLa H21. At the concentrations used, LiCl did not affect cell viability in the absence of TNF (see also below). At a TNF concentration resulting in 50% cell killing, the order of responsiveness to the potentiating effect of LiCl was WEHI 164 clone 13 = L929 > HeLa D98/AH2

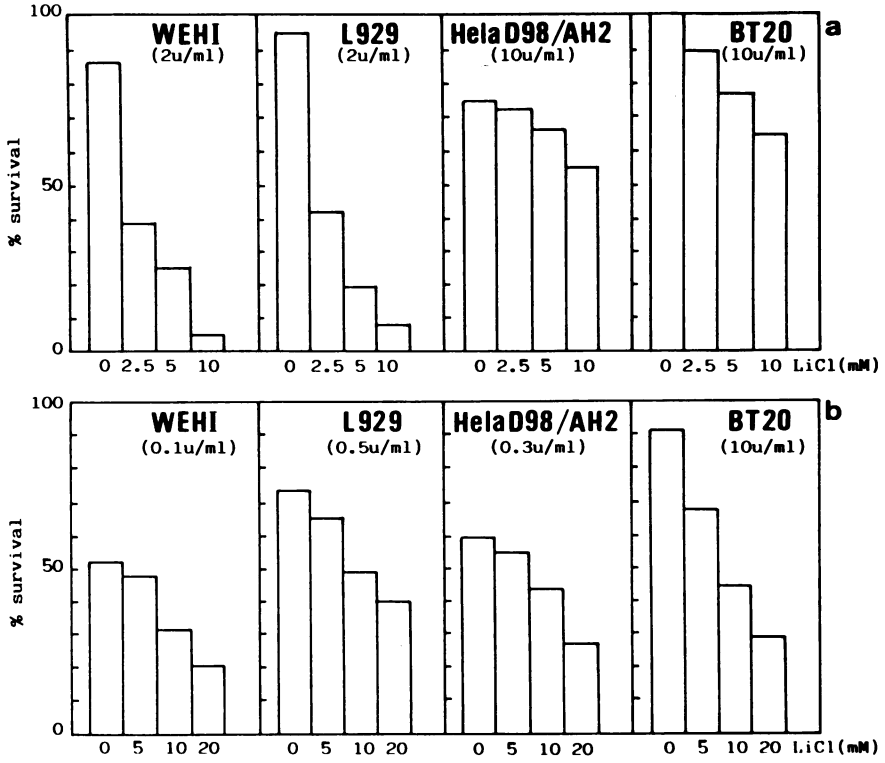

FIG. 1. Potentiation by LiCl of TNF-mediated cytotoxicity for various malignant cell lines. Cells (indicated at top of each panel) were tested in either a 72-hr cytostasis (*a*) or an 18-hr cytolysis (*b*) assay as described in *Materials and Methods*. Percent survival is shown for increasing LiCl concentration at a constant TNF concentration (indicated in each panel). For each LiCl concentration, percent survival is the cell-staining value obtained after treatment with TNF plus LiCl, expressed as a percentage of the cell-staining value obtained in untreated cultures. LiCl alone, at the concentrations used, did not affect cell viability (see also Fig. 2). Data shown are from a representative experiment.

= BT20 > ME180. This potentiation seemed to be largely transcription-independent, since the effect was still observed in the presence of ActD (Fig. 1*b*). In the presence of LiCl, killing of the murine cells was almost complete at human TNF concentrations that had nearly no effect on their own (Fig. 2). Similar results were obtained with murine TNF or human lymphotoxin (data not shown). When tested on L929 cells in an 18-hr assay without ActD, 10 mM LiCl had a similar effect on TNF-mediated cytotoxicity as ActD at 1 μg/ml. However, LiCl presented the considerable advantage of being noncytotoxic, which is obviously not the case for ActD.

LiCl Does Not Affect TNF-Mediated *in Vitro* Cytotoxicity for Cells Sensitive to the Combination of TNF Plus IFN-γ or TNF Plus ActD. The murine cell lines L929(r)2, MEF, B16Bl6, and EMT6 and the human cell lines HOS and HT29 are not sensitive to TNF as such; however, they become susceptible to TNF upon supplementation with IFN-γ or ActD (refs. 3 and 10; unpublished data). None of these cells became sensitive to the combination of TNF and LiCl. LiCl also did not affect the sensitization of these cells to TNF by ActD or IFN-γ (data not shown).

Comparison with Other Monovalent or Divalent Cations. We compared the effect of LiCl with that of the chloride salts of some other monovalent or divalent cations on TNF-mediated cytotoxicity for L929 cells. Comparable, nontoxic concentrations of NaCl, CsCl, $MgCl_2$, or $CaCl_2$ did not affect the TNF sensitivity of these cells (Table 1). We conclude that the effect of LiCl on TNF-mediated cytotoxicity is highly specific.

Kinetics of TNF-Mediated Cytotoxicity in the Absence or Presence of LiCl. Kinetics of the TNF-induced cytotoxicity with and without LiCl were compared. Confluent L929 cells were treated with TNF at 5000 u/ml as described in Fig. 3. Without LiCl, cell death was observed after 7 hr of TNF treatment and 50% mortality was obtained after 9 hr; in the presence of LiCl, however, the start of cell lysis was observed 2 hr earlier and 50% killing was obtained by 6 hr. These observations were confirmed by light microscopy and [^{3}H]uridine release (data not shown). When cells were treated with TNF at 100 u/ml with or without 10 mM LiCl, similar differences between TNF- and TNF/LiCl-treated cells were observed, except that cell lysis began about 2 hr later. To determine whether the effect of LiCl occurred at an early stage of TNF action, 10 mM LiCl was added to confluent L929 cells at various times before or after addition of TNF at 500 u/ml. After 14 hr of TNF treatment, the experiment was stopped and the cells were stained. Adding LiCl either 2 hr before TNF, or simultaneously with TNF, or up to 4 hr after TNF, did not affect the final result. Although LiCl was progressively less effective when given later than 4 hr after TNF, some influence was still detectable when LiCl was given as late as 7 hr after TNF addition (data not shown).

Table 1. Specificity of LiCl effect on TNF-mediated cytotoxicity for L929 cells

Added salt	% survival*	
	TNF at 3 u/ml	TNF at 12 u/ml
None	85	41
$CaCl_2$	92	46
$MgCl_2$	86	39
NaCl	80	40
CsCl	80	38
LiCl	22	11

Cells were incubated with the indicated salt (2.5 mM) and tested in a 72-hr cytostasis assay.
*As defined in the legend to Fig. 1.

Possible *in Vitro* Mechanisms of LiCl Action. In our system, an effect of LiCl on the cation balance (16) can be excluded, since under the experimental conditions used (≥100 mM Na^+), the inhibitory effect of LiCl on Na^+ transport does not occur. Besides, amiloride (Sigma), which inhibits the Na^+/H^+ antiport (17), did not affect TNF-mediated cytotoxicity at a concentration of 25 μM.

LiCl inhibits inositol-1-phosphatase and leads to a depletion of inositol (8). A direct role of this activity in the LiCl potentiation of the TNF cytotoxicity is unlikely in view of the following observations. The effect of LiCl on TNF cytotoxicity was not reversed with 20 mM extracellular inositol (data not shown), which reverses the LiCl-induced reduction of phosphatidylinositol formation in cholinergically stimulated parotid glands (18). NaF also inhibits inositol-1-phosphatase (19) but protected against TNF-mediated cytotoxicity at a concentration of 500 μM (data not shown). These results, as well as the observation that TNF-mediated cytotoxicity and its potentiation by LiCl was unaffected in medium lacking inositol and glucose (data not shown), seem to exclude a role

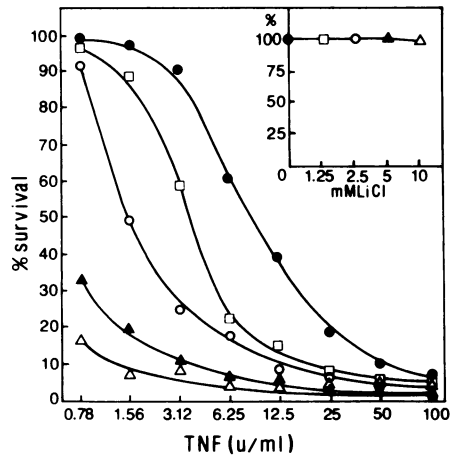

FIG. 2. Dose-dependent effect of LiCl on TNF-mediated cytotoxicity for L929 cells in a 72-hr cytostasis assay. Percent survival (as defined in Fig. 1) is plotted against TNF concentration for LiCl concentrations of 0 mM (●), 1.25 mM (□), 2.5 mM (○), 5 mM (▲), and 10 mM (△). (*Inset*) Effect of LiCl on cell survival in the absence of TNF. Data are from a representative experiment.

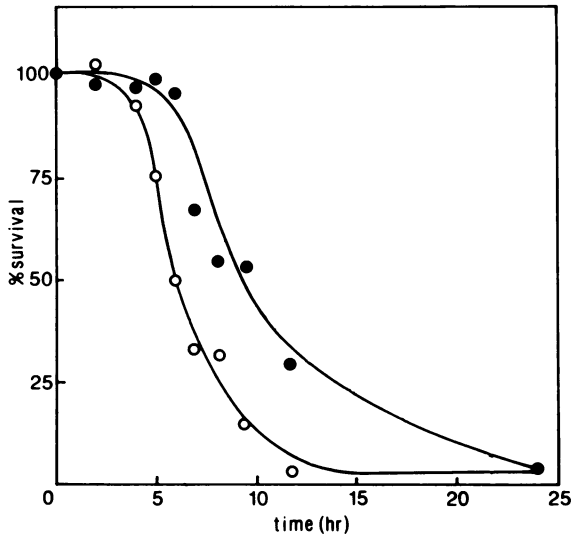

FIG. 3. Time course of L929 killing after treatment with TNF alone or TNF plus LiCl. Confluent L929 cells in 1.7-cm^2 wells were treated with TNF at 5000 u/ml, with (○) or without (●) 10 mM LiCl. At various times, cells were fixed and stained with crystal violet. Percent survival is defined as in Fig. 1. Each symbol represents the mean of three replicate determinations.

Table 2. LiCl enhances TNF-induced Δ_4Ach release in L929 cells

Treatment	[^{3}H]Δ_4Ach release, cpm*
None	2840 ± 226
LiCl (10 mM)	2940 ± 421
TNF (5000 u/ml)	4020 ± 174
TNF + LiCl	6493 ± 566

*Radioactivity released from 10^6 cells after a 4-hr treatment period. Values represent the mean ± SD for three replicate cultures.

of inositol metabolism in the LiCl enhancement of TNF cytotoxicity.

TNF treatment of L929 cells results in release of Δ_4Ach into the medium (ref. 5; unpublished data). LiCl at 10 mM potentiated the TNF-induced release, whereas LiCl itself did not induce Δ_4Ach release (Table 2). Quinacrine (Sigma), which specifically inhibits PLA_2 under the conditions used (20) and protects against TNF-mediated cytotoxicity (6), also offered partial protection against the potentiating effect of LiCl on TNF-mediated cytotoxicity. At a TNF concentration of 0.7 u/ml, which by itself did not affect cell viability in the cytostasis assay, 2.5 mM LiCl decreased the number of surviving cells to 40%. In the presence of 2.5 μM quinacrine, the percentage of survival was increased to 85%. These results suggest that LiCl acts, either directly or indirectly, via the TNF-induced PLA_2 pathway.

LiCl Enhances the *in Vivo* Antitumor Action of TNF. Subcutaneous injection of 10^6 L929 cells into nude mice yielded fast-growing tumors. The tumors were perilesionally treated (daily s.c. injection near the tumor site, but outside the nodule) with PBS, TNF, LiCl, or TNF plus LiCl for several periods of 5 consecutive days, interrupted by 2 days without treatment. The TNF doses were increased each week, whereas the LiCl dose was kept constant at 1 mg per injection. This treatment resulted in a serum concentration of 0.5 mM LiCl (measured by flame photometry of a diluted serum sample) 0.5 hr after s.c. injection. The prolonged combination treatment for several weeks did not result in side effects. The results of a representative experiment are shown in Fig. 4*a*. Compared with TNF alone, the combination of TNF with LiCl was very effective. LiCl alone had no effect. From the second week on, some tumors necrosed after treatment with either TNF or TNF plus LiCl. In both cases, however, growth inhibition was generally not complete. Nevertheless, mice treated with TNF survived longer than control mice; 40 days after tumor cell injection, survival was 16% for untreated or PBS-treated mice, 33% for LiCl-treated mice, and 67% for TNF-treated mice but was 100% for animals subjected to the combination treatment.

HeLa D98/AH2 cells (3×10^6) were likewise s.c. injected into nude mice, which resulted in slower-growing tumors. The treatment protocol was the same as described above, except for the use of higher TNF concentrations. The results of a representative experiment are shown in Fig. 4*b*. In this experiment, TNF alone had no detectable effect on tumor growth. A combined treatment with LiCl, however, led to growth inhibition, although necrosis was not visible. In the experiment shown, after a prolonged treatment with the combination, two mice (out of six) were completely tumor-free. The mean survival time was also increased. Only 50% of the mice treated with PBS, LiCl, or TNF alone survived for 100 days after tumor cell injection, whereas no mice of the TNF plus LiCl-treated group were dead at that time.

To investigate whether the *in vivo* effect of LiCl was direct or indirect, 1.7×10^6 HeLa H21 cells were s.c. injected into nude mice. *In vitro*, these cells are fairly resistant to TNF, and they cannot be sensitized to TNF by LiCl. The tumors were treated according to the protocol mentioned above. After 4 weeks, neither treatment proved to inhibit tumor growth (data not shown). These results suggest that most of the positive response observed *in vivo* with the TNF-susceptible HeLa D98/AH2 cells was due to a direct action of the combination TNF plus LiCl on the tumor cells.

DISCUSSION

This report shows that both *in vitro* and *in vivo* LiCl can enhance the sensitivity of several transformed cell lines to TNF cytotoxicity. *In vitro*, this effect can be compared with the sensitizing effect of ActD on TNF-mediated cytotoxicity. However, the levels at which LiCl and ActD act are quite different: cells that were resistant to TNF alone but sensitive to TNF in combination with ActD or IFN-γ could not be rendered sensitive to TNF by addition of LiCl. Furthermore, LiCl was not capable of increasing the sensitivity of cells treated with TNF plus ActD or TNF plus IFN-γ. Most important for possible clinical applications is the fact that, whereas ActD is toxic, LiCl at moderate doses causes virtually no side effects.

As the selective TNF cytotoxicity for transformed cells remains poorly understood, the exact mechanism by which LiCl potentiates TNF cytotoxicity cannot be pinpointed either. Several reports have advanced possible processes that

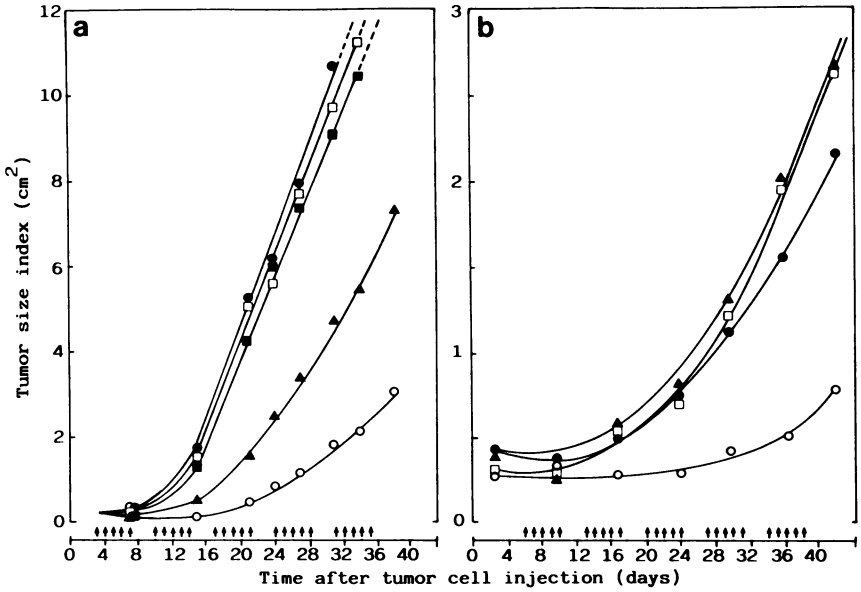

FIG. 4. Effect of treatment with TNF or TNF plus LiCl on the growth of s.c. tumors in nude mice. Mean tumor size index [the product of the largest, perpendicular diameters (21)] is plotted against time (days after tumor cell injection) for L929 tumors (*a*) and for HeLa D98/AH2 tumors (*b*). All mice (six per group) received a 0.1-ml injection of the solvent PBS (●; free of Ca^{2+} and Mg^{2+}), LiCl (□), TNF (▲), or TNF plus LiCl (○) perilesionally on the days indicated by arrows on the *x* axis. One group was not treated (■). The following TNF doses per injection were used: 5 μg (first week), 10 μg (second and third weeks), 15 μg (fourth week), and 20 μg (fifth week) for the L929 tumors; 10 μg (first week), 15 μg (second and third weeks), and 25 μg (fourth and fifth weeks) for the HeLa D98/AH2 tumors. The LiCl dose was 1 mg per injection in all instances.

might be influenced by LiCl *in vitro* and *in vivo*, such as interference with cAMP metabolism, inositol phosphate metabolism, Na^+ transport, and GTP binding (8, 22, 23). Li^+ has been shown to affect several cells of the immune system, both *in vitro* and *in vivo*; reported effects include stimulation of degranulation of polymorphonuclear leukocytes *in vitro* (24), as well as correction of polymorphonuclear defects *in vivo* (25), and induction of leukocytosis *in vivo* (26). LiCl enhances mitogen stimulation of both human and hamster lymphocytes *in vitro* (27). Treatment with LiCl increases the natural killing activity of murine lymphocytes *in vitro* and *in vivo* (28). Finally, LiCl has been found to stimulate the release of colony-stimulating factor from bone marrow macrophages (29). None of these findings, however, would suggest that LiCl facilitates direct TNF cytotoxicity for malignant cells *in vitro*.

Inositol-1-phosphatase (8) and other inositol phosphatases (30) are sensitive to LiCl. However, the results presented here and the observation that TNF does not affect the accumulation of inositol phosphates (unpublished data) do not support the notion that the effect of LiCl on TNF cytotoxicity involves these inositol phosphatases. Li^+ interferes with Na^+ transport (23), but only at low NaCl concentrations, whereas in our assays 1.25 mM LiCl still had a pronounced effect in the presence of 120 mM Na^+. A third possibility is an involvement of LiCl in the cAMP metabolism; this is unlikely, however, in view of the lack of a TNF effect on cAMP levels in transformed cell lines (ref. 31; unpublished data).

Guanine nucleotide-binding proteins (G proteins) have been reported to be involved in TNF-mediated cytotoxicity (31). In agreement with these findings, we obtained evidence that TNF-induced PLA_2 activation (5, 6) is mediated by a G protein (32). It has been reported that LiCl can either inhibit (22) or stimulate (33) GTP-binding in particular cell systems. Our results concerning the enhanced Δ_4Ach release after treatment with TNF in combination with LiCl, as compared with TNF alone, as well as the inhibition by PLA_2 inhibitors of the sensitizing effect of LiCl on TNF cytotoxicity, suggest that LiCl may sensitize the TNF effect by stimulating the PLA_2 pathway. A G protein coupled to PLA_2 could be the target of LiCl in our system. An important role of Δ_4Ach metabolization after TNF plus LiCl treatment is unlikely, since Δ_4Ach metabolites appear not to be involved in TNF cytotoxicity (5, 6).

We also have addressed the question whether the *in vitro* and *in vivo* potentiation of the antitumor effect of TNF is a result of the same bioactivity. Tumors formed by cell lines that are not sensitive to the enhancing effect of LiCl *in vitro*, such as HeLa H21, fail to respond *in vivo*. This suggests that the *in vivo* action of TNF plus LiCl on sensitive tumors is mainly direct. Besides, the possibility that mature T lymphocytes, for which many effects of LiCl have been described (27), participate in the observed sensitization can be excluded, since we used nude mice, which lack these cells.

Lithium salts in moderate doses are nontoxic to humans and are widely used in psychiatry for the treatment of manic-depressive disease states (34). In mice, we could inject up to 6 mg of LiCl without any visible side effects. At 30 min after s.c. injection of 1 mg of LiCl per mouse, we obtained serum concentrations of 0.5 mM LiCl, which decreased afterwards. This level is far below the toxic serum Li^+ concentrations in humans, which vary at 1.5 mM or more, whereas the minimal effective dose in treatment of mania and manic depression is 0.3 mM (35). Therefore the *in vivo* potentiation of the antitumor action of TNF, as observed in the present animal studies, can be reached at LiCl concentrations that are well tolerated in humans. This suggests that combined treatment with TNF and LiCl may also result in improved clinical responses in human cancer patients.

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