Enhancement of Interleukin 2 Production in Human and Gibbon T Cells after *In Vitro* Treatment with Lithium (43298)

YIYUAN WU[1] AND XUHAO YANG
Department of Immunology, Cancer Institute, Chinese Academy of Medical Sciences, Beijing, 100021, China

Abstract. **The effect of trace elements lithium, selenium, and zinc on interleukin 2 (IL-2) production by peripheral blood mononuclear cells (PBMC), MLA144, and Jurkat cell lines has been studied. Lithium markedly enhanced IL-2 production by MLA144 and PBMC, but not by Jurkat. Selenium could only enhance IL-2 production by MLA144, whereas in none of these three systems was IL-2 production altered by zinc. The enhancing effect of lithium on IL-2 production showed some differences from that of tetradecanoylphorbol acetate (TPA) in the following aspects: (i) TPA could reverse the inhibitory effect of anti-CD2 monoclonal antibody on IL-2 production, whereas lithium could not; and (ii) lithium was unable to synergistically induce IL-2 production with anti-CD3 monoclonal antibody as TPA did. The effect of lithium on IL-2 production was in the early phase of lymphocyte activation. The addition of cholera toxin or theophylline to phytohemagglutinin-stimulated PBMC culture suppressed IL-2 production. However, IL-2 production could be restored by lithium. There was a corresponding increase in cAMP in cholera toxin or theophylline-treated PBMC, which could be reversed by lithium. Therefore, lithium restores IL-2 production via a decrease in cAMP.** [P.S.E.B.M. 1991, Vol 198]

Lithium is known to cause leukocytosis in normal humans and lithium salts have been used therapeutically in manic-depressive patients and in patients with leukopenia (1). Recent studies also report that interleukin 2 (IL-2) production can be induced by lithium in human peripheral blood mononuclear cells (PBMC) and in some T cell lines (2). However, little is known about the underlying biochemical mechanism of the enhancing effect of lithium on IL-2 production. It has been shown that treatment of activated T cells with tetradecanoylphorbol acetate (TPA) induced augmentation of IL-2 production (3, 4). Recent studies by Drummond (5) show that lithium affects G protein receptor coupling.

The aim of our study was to evaluate the putative participation of adenylate cyclase in the lithium-mediated regulation of IL-2 production. We found that lithium affected production in a different way from TPA and that the ability of lithium to augment IL-2 production was via a decrease in cAMP.

Materials and Methods

Reagents and Monoclonal Antibodies. Lithium chloride, zinc sulfate, and sodium selentite were purchased from the Beijing Chemical Co.; TPA, cholera toxin (CTX), and theophylline (TP) were obtained from the Sigma Chemical Co. Phytohemagglutinin M (PHA-M) was obtained from the Difco Co. Anti-CD2 and anti-CD3 monoclonal antibodies (mAb) were kindly supplied by Dr. Chen (Department of Immunology, Cancer Institute).

Cells. MLA144 and Jurkat cells were grown in RPMI 1640 medium supplemented with 15% heat-inactivated (56°C, 30 min) newborn calf serum. PBMC were separated from the heparinized venous blood of healthy donors by centrifugation on a Ficoll-Hypaque. After washing, cells were resuspended at a density of 1×10^6 cells/ml in RPMI 1640 containing 5% newborn calf serum in 24-well plates. The PBMC were cultured with or without stimulants, such as PHA-M, TPA, or trace elements, under various conditions at 37°C in 5% CO2. The supernatants of culture medium were harvested after a 48-hr culture for IL-2 assay.

IL-2 Assay (6, 7). The IL-2 activity of the culture

[1] To whom requests for reprints should be addressed at Department of Immunology, Cancer Institute, Chinese Academy of Medical Sciences, Beijing, 100021, China.

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supernatants was determined by adding serial 2-fold dilutions (100 μl) of culture supernatants to flat-bottomed 96-well tissue culture plates containing 1×10^4 cells/well of the IL-2-dependent cytotoxic T lympho-

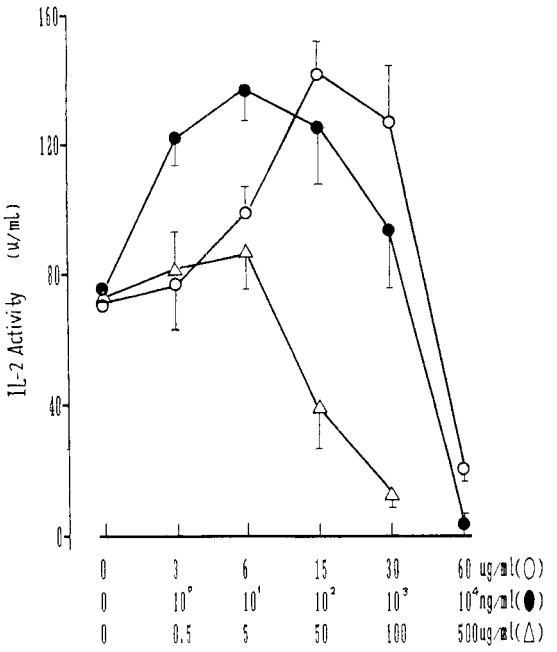

Figure 1. Effect of lithium, selenium, and zinc on IL-2 production by MLA144 cells. Cells (5×10^5/ml) were cultured with various concentrations of lithium (open circles), selenium (closed circles), or zinc (open triangles) for 48 hr, and their supernatants were assayed for IL-2 activity.

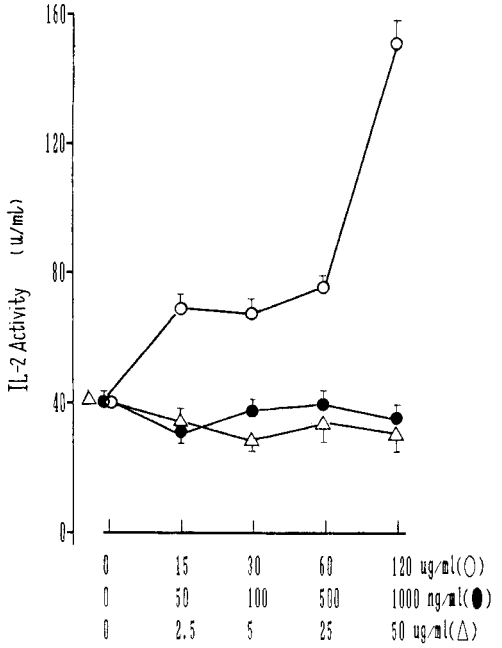

Figure 2. Effect of lithium, selenium, and zinc on IL-2 production by human PBMC. Cells (1×10^6/ml) were cultured with various concentrations of lithium (open circles), selenium (closed circles), or zinc (open triangles) for 48 hr, and their supernatants were assayed for IL-2 activity.

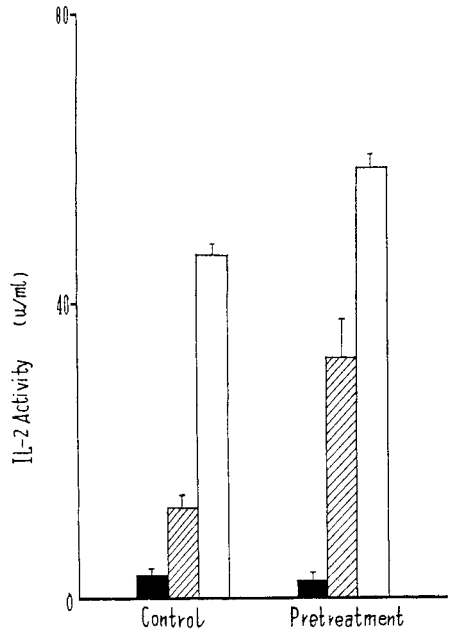

Figure 3. Effect of lithium on IL-2 production by PBMC pretreated with lithium. PBMC (1×10^6/ml) were pretreated (37°C, 30 min) with 60 μg/ml of lithium and were then cultured as described in Figure 2. (Closed bar) PBMC alone; (cross-hatched bar) PBMC + PHA; (open bar) PBMC + PHA + LiCl.

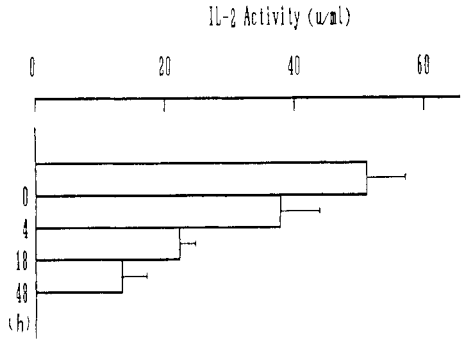

Figure 4. Effect of addition of lithium at different times on IL-2 production. PBMC (1×10^6/ml) were cultured in the presence of 1% PHA and 60 μg/ml lithium for 48 hr.

cyte line 2 (CTLL-2 or T blasts) in a total volume of 200 μl. [^{3}H]thymidine incorporation was determined after 24 hr of culture, and the results are expressed as international units, as measured from a standard curve obtained with the standard reference preparation of rIL-2 (Cetus). All the reagents of the concentrations remaining in the conditioned medium after incubation had negligible direct effect in the murine cytotoxic T lymphocyte line or T blast cell assay.

cAMP Assay. Cyclic AMP was assayed according to the method described by Carrera *et al.* (8), with some modification. Cells (2×10^6) were incubated in 1 ml of serum-free RPMI 1640 for 30 min at 37°C. Subsequently, triplicate 1-ml aliquots were incubated for 1 hr at 37°C with the different reagents. Samples were centrifuged (5 min at $400 \times g$), resuspended in 200 μl

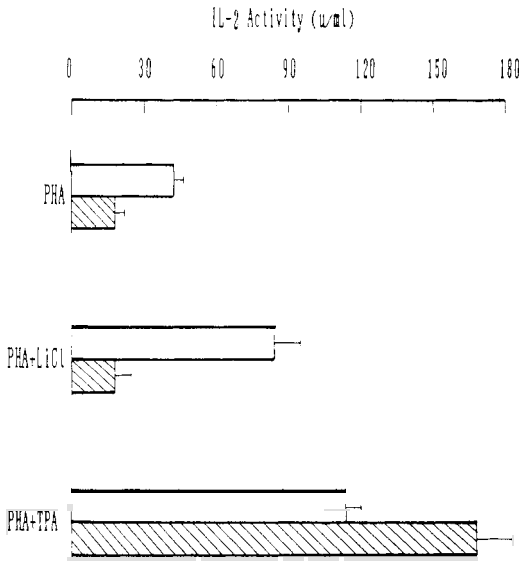

Figure 5. Effect of lithium on the inhibition of IL-2 production by anti-CD2 mAb. PBMC (1×10^6/ml) were cultured in the presence (cross-hatched bar) or absence (open bar) of anti-CD2 mAb with different reagents (PHA (1%), LiCl (60 μg/ml), or TPA (5 ng/ml)) for 48 hr, and IL-2 activity was subsequently assayed by the cytotoxic T lymphocyte line.

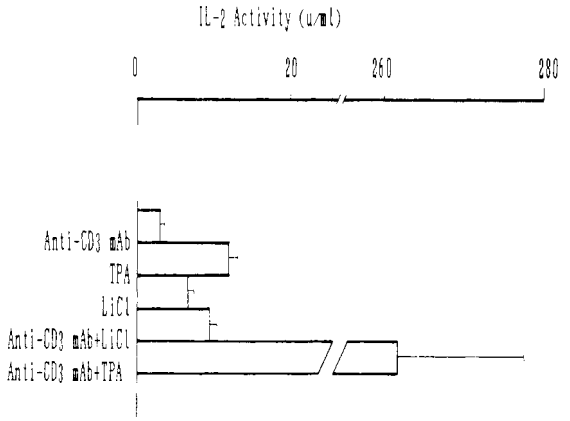

Figure 6. Synergistic effect of anti-CD3 mAb and TPA on IL-2 production by PBMC. PBMC (1×10^6/ml) were cultured with different reagents (anti-CD3 mAb, 1/10 of ascites fluid; TPA, 10 ng/ml; or LiCl, 60 μg/ml) for 48 hr, then IL-2 activity was assayed.

of distilled water, and sequentially frozen and thawed in order to disrupt cells. After centrifugation for 10 min at 100*g*, supernatants were collected and cAMP was quantified using a specific radioassay (Nuclear Research Institute, Beijing, China).

The data for each figure were shown from one representative experiment of three.

Results

Effect of Lithium, Selenium, and Zinc on IL-2 Production. Using PBMC and two IL-2 secreting cell

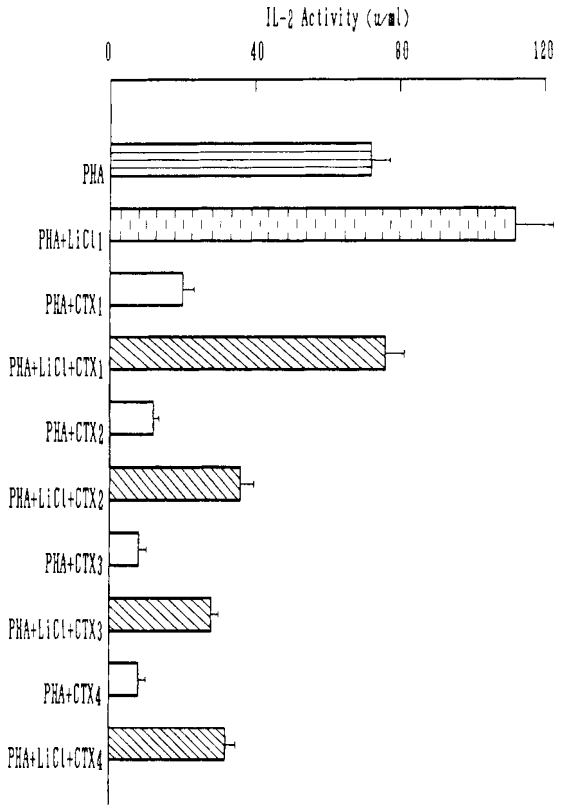

Figure 7. Effect of lithium on the inhibition of IL-2 production by CTX. PBMC (1×10^6/ml) were cultured in the absence or presence of lithium (120 μg/ml). IL-2 assay was performed as described previously. The concentration of CTX was CTX_1 (0.5 ng/ml), CTX_2 (5 ng/ml), CTX3 (50 ng/ml), and CTX_4 (500 ng/ml).

lines, MLA144 and Jurkat, the effect of lithium, selenium, and zinc on IL-2 production was observed. As shown in (Fig. 1), in MLA144, both lithium and selenium markedly enhanced IL-2 production in a dose-dependent manner. The optimal concentration of lithium was 15–30 μg/ml, whereas the enhancing effect was not shown by zinc. However, the results obtained from the PBMC system were different (Fig. 2); the augmentation of IL-2 secretion could only be induced by lithium. The optimal dose was 150 μg/ml. In contrast, neither augmented nor reduced IL-2 production was observed when lithium was used in Jurkat cells (data not shown).

The effect of lithium on IL-2 production occurs in the early phase of lymphocyte activation. Lithium was added in the whole culture for a comparison with PBMC pretreated by lithium; both groups showed the enhancing effect (Fig. 3). As lithium was added at different times to the PBMC culture, it was found that the most significant effect was obtained with the addition of lithium at the beginning of the culture (Fig. 4). The findings suggest that lithium-enhancing IL-2 production occurs in the early phase of T cell activation.

Comparison of the Effect of Lithium on IL-2 production with TPA. It has already been shown that

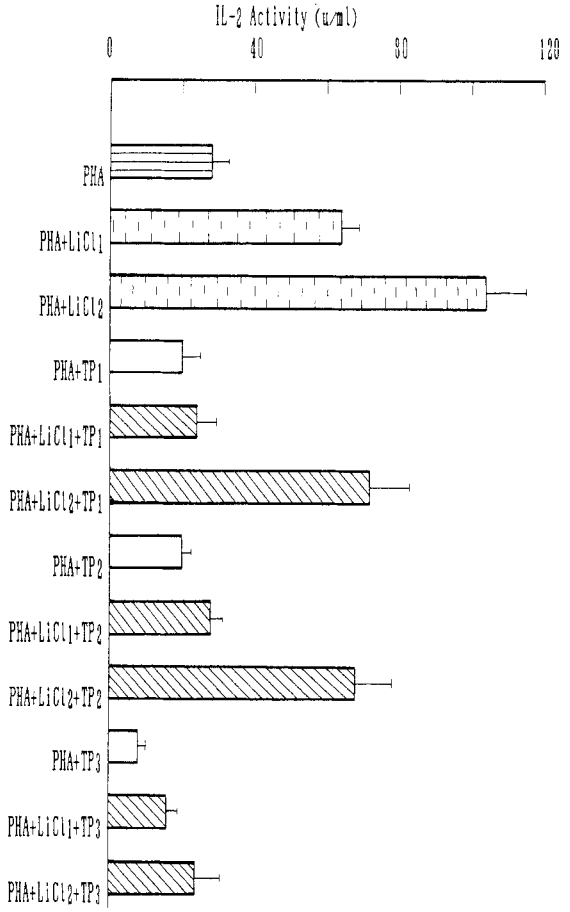

Figure 8. Effect of lithium on the inhibition of IL-2 production by TP. For details, see legend to Figure 7. Reagents: TP_1 (2.5×10^{-6} *M*), TP_2 (2.5×10^{-5} *M*), TP_3 (2.5×10^{-4} *M*), $LiCl_1$ (60 μg/ml), and $LiCl_2$ (120 μg/ml).

TPA is able to enhance IL-2 production. It was, therefore, of interest to ascertain whether the enhancing effect of lithium was similar to that of TPA. We used mAb anti-CD2 and anti-CD3 for further investigation. As shown in (Fig. 5), there was a marked inhibitory effect when anti-CD2 mAb was cultured in combination with PHA. However, this inhibitory effect could be overcome by TPA, but not by lithium. When a suboptimal concentration of anti-CD3 mAb was used in PBMC culture, TPA could synergistically enhance IL-2 production with anti-CD3 mAb, whereas lithium could not (Fig. 6). The above results indicate that the effect of lithium on IL-2 production shows some differences from that of TPA.

The enhancing effect of lithium on IL-2 production via a decrease in cAMP. The mechanisms of enhancing effect of lithium on IL-2 production have not been elucidated yet. In order to analyze the relationship between lithium and adenylate cyclase, we used two agents, TP and CTX, for investigating. CTX is an adenylate cyclase activator that can increase cAMP level. As shown in Figure 7, with the addition of CTX to PHA-stimulated PBMC culture, IL-2 activity was

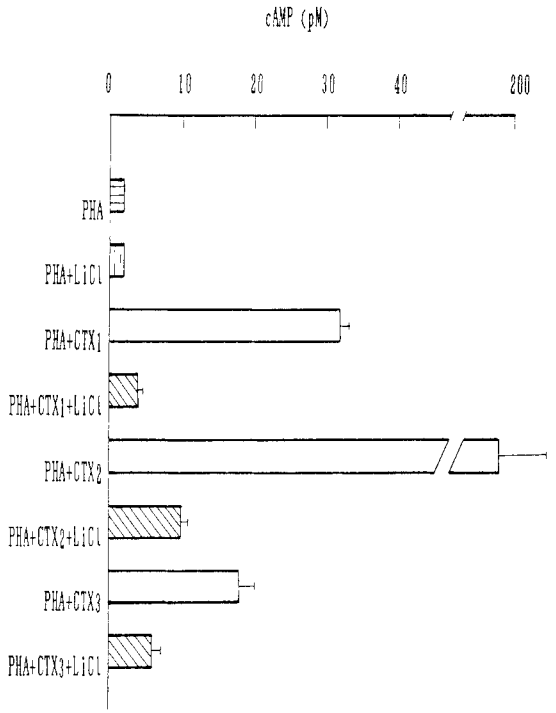

Figure 9. Lithium-mediated decrease of cAMP produced by CTX in PBMC. PBMC (2×10^6/ml) were incubated in the absence or presence of lithium (120 μg/ml) with either PHA or PHA + CTX at 37°C for 1 hr and the cAMP was assayed by the method described previously. The concentration of CTX was CTX_1 (1 ng/ml), CTX_2 (10 ng/ml), and CTX_3 (100 ng/ml).

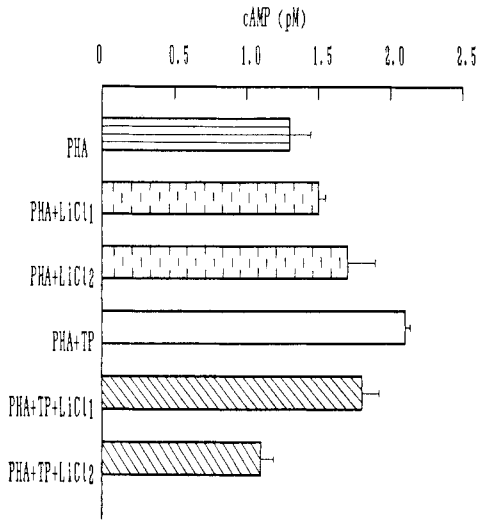

Figure 10. Lithium-mediated decrease of cAMP produced by TP in PBMC. For details, see legend to Figure 9. Reagents: $LiCl_1$ (60 μg/ml), $LiCl_2$ (120 μg/ml), and TP (10^{-5} *M*).

significantly suppressed by CTX in the 0.5–500 ng/ml range. The IL-2 activity was down to 18.11 ± 1.80 units/ml, 9.04 ± 0.3 units/ml, 5.82 ± 0.73 units/ml, and 6.64 ± 1.04 units/ml, respectively. The control of lithium alone or lithium and CTX did not produce any detectable IL-2 activity. However, as lithium was added in the presence of PHA and CTX, IL-2 activity was

partially or completely restored. These results were similar to our findings with TP, a phosphodiesterase inhibitor (Fig. 8), suggesting that lithium might effect on a cAMP level. In order to directly approach the mechanisms underlying the increase of the cyclic nucleotide, the cAMP level was determined. CTX induced a relatively high increase in the intracellular levels of cAMP in PHA-stimulated PBMC. The maximal level of cAMP was observed at a dose of 10 ng/ml of CTX. Lithium alone did not affect any cAMP level. However, as lithium was added in the presence of PHA and CTX, cAMP levels were markedly suppressed, particularly at the low dose of CTX (Fig. 9). When TP was added instead of CTX, the results were similar, although TP was less effective in increasing cAMP level than CTX. The level of cAMP was significantly suppressed by lithium in the concentration of 120 μg/ml ($P < 0.01$) (Fig. 10). Thus, there is a very good negative correlation between the IL-2 activity and cAMP inducible in lithium-treated PBMC, indicating that the enhancing effect of lithium on IL-2 production is via a decrease in cAMP level.

Discussion

Lithium is known to interfere with many sodium- and potassium-dependent enzymes, to inhibit the action of adenylate cyclase, and to reduce the intracellular level of cAMP (9). Recently, it was reported that an enhancement of IL-2 production by lithium had been detected (2). Here, we comparatively studied the effect of lithium with other elements (selenium, zinc, and TPA) on IL-2 production. Using three different systems (PBMC, MLA144, and Jurkat cells), the enhancing effect of three elements on IL-2 production was different. Both selenium and lithium markedly enhanced IL-2 production in the MLA144 cell system. Using PBMC, a vigorous response could only be seen in lithium. In contrast, there was no response to lithium in Jurkat cells. Our results are in accordance with Brown *et al.*'s (10) findings. However, there was a discrepancy between our findings and those initially reported by Lin and Robb (2). They found that lithium could enhance IL-2 production in Jurkat cells. There are two possible explanations: (i) They stimulated Jurkat cells with PHA and TPA, whereas we used only PHA; and (ii) The different results may be due to the different clones used. Taken together, our results indicate that these agents are useful probes for investigating the different mechanisms of IL-2 production in these three systems.

It has already been shown that TPA is an effective agent to enhance IL-2 production (11). We have found that the effects of lithium on lymphocytes showed some differences from that of TPA in two respects: (i) TPA could reverse the inhibitory effect of anti-CD2 mAb on IL-2 production, whereas lithium could not; and (ii) Lithium was unable to synergistically induce IL-2 production with anti-CD3 mAb, as TPA did. Thus, lithium may act in a different way from TPA to augment IL-2 production.

So far, little is known about the regulatory mechanisms of IL-2 production by lithium. It is possible that the enhancing effect of lithium may be mediated by the decrease in cAMP. In order to prove the decrease of cyclic nucleotide by lithium, the intracellular cAMP was assayed. Our data suggest that lithium acts by lowering levels of cAMP, and an early step of the T cell response may be affected. In supporting such a hypothesis, we have found that pretreated PBMC with lithium is also able to augment IL-2 production. Taken together, these observations point out that lithium affects IL-2 production by directly regulating cyclic AMP levels in the adenylate cyclase system.

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