Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 3496–3500, June 1984
Immunology

Antagonism by taxol of effects of microtubule-disrupting agents on lymphocyte cAMP metabolism and cell function

(lymphocyte-mediated cytolysis/3-deazaadenosine/colchicine/vincristine/nocodazole)

GERALD WOLBERG, CAROLYN R. STOPFORD, AND THOMAS P. ZIMMERMAN

Department of Experimental Therapy, The Wellcome Research Laboratories, Research Triangle Park, NC 27709

Communicated by George H. Hitchings, December 19, 1983

ABSTRACT **Several microtubule-disrupting agents (colchicine, demecolcine, vinblastine, vincristine, podophyllotoxin, and nocodazole) have been shown to inhibit lymphocyte-mediated cytolysis. These agents also enhanced the prostaglandin E_1-induced rise in cAMP levels in these cytolytic lymphocytes. Taxol, a natural product alkaloid that has been shown to enhance microtubule polymerization and to stabilize microtubules, antagonized both of these effects of the microtubule-disrupting agents in the cytolytic lymphocytes. Taxol also antagonized the enhancement of cAMP increases by colchicine in lymphocytes stimulated by 2-chloroadenosine, isoproterenol, and cholera toxin. The enhancement of the prostaglandin E_1-induced cAMP response caused by treatment of the lymphocytes with either cytochalasin B or 3-deazaadenosine in the presence or absence of L-homocysteine was not antagonized by taxol. Taxol, colchicine, or the combination of these two agents did not affect ATP levels in cytolytic lymphocytes. These results support a modulatory role for microtubules in both the cytolytic process and the production of cAMP in these lymphocytes.**

Taxol is a natural product diterpenoid that was originally isolated from the plant *Taxus brevifolia* and was demonstrated to have antitumor and antimitotic activity by Wani *et al.* (1). Studies *in vitro* have shown that taxol promotes and stabilizes microtubule assembly (2, 3). Further investigations established that taxol is inhibitory to a number of cellular activities, including fibroblast replication and migration (3), lymphocyte proliferation (4), secretion of plasma proteins by rat hepatocytes (5), human neutrophil spontaneous nondirectional migration as well as response to chemotactic factor (6), and glucose-stimulated insulin secretion by rat islets of Langerhans cells (7). The chemoattractant-induced tyrosinolation of tubulin in rabbit leukocytes (8) and the vincristine-induced shape change, microtubule assembly, and tubulin paracrystal formation in human platelets (9) were also inhibited. Taxol had no effect on Fc-mediated phagocytosis by murine cultured macrophages (10) and did not inhibit mouse lymphocyte capping by anti-Ig (11).

Colchicine, a microtubule-disrupting agent, has been reported to inhibit lymphocyte-mediated cytolysis (LMC) (12) and to enhance the hormonal stimulation of cAMP in leukocytes (13). The availability of taxol, a highly active and apparently specific agent, has allowed us to study in more detail the role of microtubules in the cytolytic activity and cAMP metabolism of specifically immune mouse lymphocytes. The results provide additional evidence for the involvement of microtubules in both of these processes and demonstrate that the microtubule-promoting and stabilizing effects of taxol can antagonize these two cellular effects of colchicine and other microtubule-disrupting agents.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Materials. Taxol was obtained from M. Suffness of the National Cancer Institute. Colchicine, podophyllotoxin, vinblastine, nocodazole, vincristine, demecolcine, β-lumicolchicine, γ-lumicolchicine, cytochalasin B, 2-chloroadenosine, *l*-isoproterenol, concanavalin A, prostaglandin E_1 (PGE_1), and L-homocysteine thiolactone were obtained from Sigma. Cholera toxin was a product of Schwarz/Mann. [2,8-^{3}H]Adenine (34 Ci/mmol; 1 Ci = 37 GBq) was a product of ICN. 3-Deazaadenosine was synthesized at the Wellcome Research Laboratories. Mice, C57BL mouse leukemia EL4 cells, $Na_2^{51}CrO_4$, and fetal calf serum were obtained from sources identified previously (14). RPMI 1640 medium supplemented with 10% heat-inactivated (56°C, 60 min) fetal calf serum plus 25 mM Hepes and adjusted to pH 7.2 was the medium used in all cell incubations (37°C, 5% CO_2/95% air).

Analytical Procedures. cAMP levels in cytolytic lymphocytes were determined by radioimmunoassay after purification of acid-soluble extracts on sequential columns of aluminum oxide and Dowex 1-X8 and subsequent 2′-*O*-succinylation of these samples (15). Relative levels of lymphocyte [^{3}H]ATP were measured in [^{3}H]adenine-prelabeled cells (16) after a 60-min incubation with colchicine, taxol, or colchicine plus taxol. Trichloroacetic acid-soluble extracts of lymphocytes were analyzed for [^{3}H]ATP content by anion-exchange high-performance liquid chromatography (15).

Cytolytic Assay. Cytolytic lymphocytes were obtained from CD-1 mice 10, 11, or 12 days after intraperitoneal injection of leukemia EL4 cells (14). Erythrocytes were lysed by cold hypotonic (0.2% wt/vol) saline (20 sec) and adherent cells were removed by passage through a glass wool column (14). C57BL leukemia cells were maintained, harvested, and labeled with $Na_2^{51}CrO_4$ (14). The cytolytic assays were carried out in 12 × 75 mm plastic tubes (Falcon). Briefly, 2.5×10^5 cytolytic lymphocytes (in 0.90 or 0.95 ml of medium) were incubated for 15 min at 37°C and then 2.0 μl of taxol dissolved in 95% (vol/vol) ethanol (or ethanol alone as control) was added for an additional 15 min.* Two microliters of agents dissolved in ethanol (podophyllotoxin, vinblastine, nocodazole, or cytochalasin B) or 50 μl of saline-soluble agents was then added for an additional 60-min incubation. Then 2.5×10^5 ^{51}Cr-labeled EL4 cells (in 50 μl of medium) were added to give a total assay volume of 1.0 ml. The contents of the tubes were mixed, centrifuged at 185 × *g* for 10 min at room temperature, and incubated for 60 min at 37°C. After the addition of 1.0 ml of cold medium, the cells were resuspended and then centrifuged at 733 × *g* for 10 min, and the released ^{51}Cr was measured (15). The actual percentage

Abbreviations: LMC, lymphocyte-mediated cytolysis; PGE_1, prostaglandin E_1.

*Throughout this work taxol was added to the cells 15 min prior to each microtubule-disrupting agent even though taxol was equally antagonistic to colchicine when both agents were added simultaneously.

of specific lysis [(cpm released in the presence of cytolytic lymphocytes minus cpm released in the absence of lymphocytes) ÷ (cpm released by freeze–thaw of target cells)] varied among experiments between 15% and 39% (mean ± SEM, 26.2 ± 4.8%). Spontaneous release was always less than 10% of the cpm released by freeze–thaw treatment of the EL4 cells. Each point in Figs. 4 and 5 represents the mean of duplicate assays, which never varied from each other by more than 10%.

cAMP Incubations. Cytolytic lymphocytes (7.5–10 × 10^6 cells per 5.0 ml of medium) were incubated for 15 min at 37°C, 10 μl of taxol or ethanol was added for 15 min, and then 100 μl of the saline-soluble agents (or saline) or 10 μl of the ethanol-soluble agents (or ethanol) was added for 60 min. After this 60-min incubation, PGE_1, 2-chloroadenosine, or isoproterenol was added to the cell suspensions and the cells were incubated for either another 30 min (for PGE_1 and 2-chloroadenosine) or another 2.0 min (for isoproterenol) prior to trichloroacetic acid extraction of the cell suspensions for cAMP determinations (16). In the experiments involving cholera toxin, this agent was added to the cell suspensions at the start of the 60-min incubations and was in contact with the cells for a total of 90 min.

RESULTS

The effect of colchicine on the PGE_1-stimulated cAMP production in the cytolytic lymphocytes is illustrated in Fig. 1. PGE_1 alone induced a 3-fold rise in cAMP after a 1.0-min incubation with the lymphocytes. Prior incubation of the lymphocytes with 10 μM colchicine led to a marked enhancement of the PGE_1-induced rise in cAMP within 1.0 min after the addition of PGE_1, and no further increase of cAMP levels was observed over the next 30 min. Colchicine alone did not significantly affect cAMP levels.

Fig. 2 shows that this colchicine enhancement of the PGE_1-induced increase of cAMP was dose dependent over the range of 0.5–10 μM colchicine. Taxol at 2.0 μM antagonized this colchicine effect on cAMP metabolism at all concentrations of colchicine tested. The presence of 2.0 μM taxol resulted in an 89% antagonism of the effect of 10 μM colchicine on cAMP metabolism. This antagonistic effect of taxol was shown to be dose-dependent over the concentration range of 0.05–2.0 μM taxol (Fig. 3). Taxol itself had no effect on basal levels of cAMP and did not affect the increase in lymphocyte cAMP caused by PGE_1 in the absence of colchicine.

Five other microtubule-disrupting agents—demecolcine, vinblastine, vincristine, nocodazole, and podophyllotoxin—also enhanced the PGE_1-induced rise in lymphocyte cAMP levels, and these effects were also antagonized by taxol (Table 1). Again, taxol alone had little or no effect on basal or PGE_1-induced levels of cAMP but greatly reduced the enhancement of PGE_1-induced cAMP increase caused by the microtubule-disrupting agents and also antagonized the increase in lymphocyte cAMP caused by these microtubule-disrupting agents alone. β- and γ-lumicolchicine at concentrations as high as 25 μM did not enhance the PGE_1 induction of cAMP (data not given).

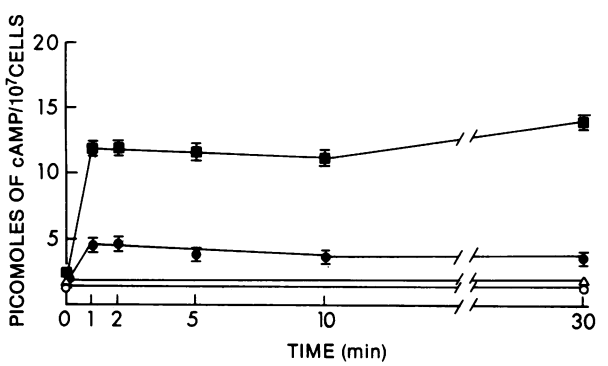

FIG. 1. Effect of colchicine on the PGE_1 stimulation of cAMP in cytolytic lymphocytes. Cells (1.0 × 10^7 cells per 5.0 ml of medium) were incubated for 60 min in the absence (●, ○) or presence (△, ■) of 10 μM colchicine prior to the addition of ethanol (○, △) or 2.0 μM PGE_1 (●, ■). The cell suspensions were acid extracted for cAMP determinations at the indicated times after addition of PGE_1. All incubations were performed in duplicate and each column-purified extract was radioimmunoassayed in duplicate for cAMP. Each point represents the mean ± SEM for four determinations.

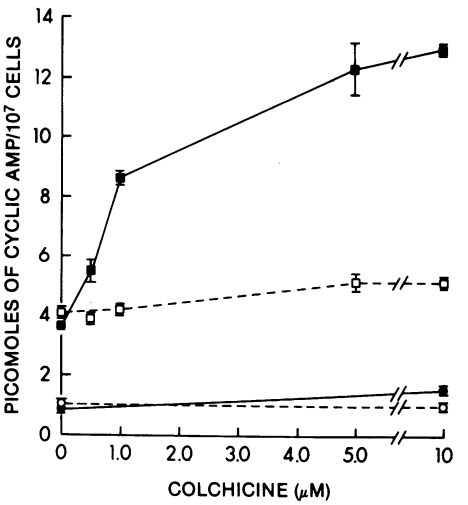

FIG. 2. Dose-dependent enhancement of the lymphocyte cAMP response to PGE_1 caused by colchicine and its antagonism by taxol. Lymphocytes (7.0 × 10^6 cells per 5.0 ml of medium) were incubated in the absence (○, ■) or presence (●, □) of 2.0 μM taxol for 15 min before the addition of the indicated concentrations of colchicine for 60 min. Ethanol (●, ○) or PGE_1 (2.0 μM) (■, □) was then added and the cell suspensions were acid extracted for cAMP determinations after an additional 30-min incubation. All incubations were performed in duplicate and each column-purified extract was radioimmunoassayed in duplicate for cAMP. Each point represents the mean ± SEM for four determinations.

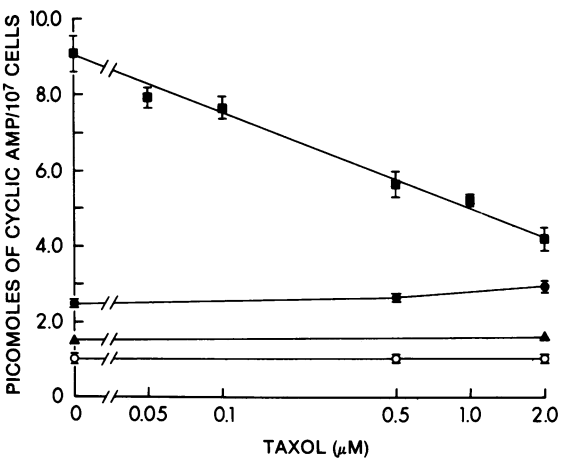

FIG. 3. Concentration-dependent antagonism by taxol of colchicine enhancement of the lymphocyte cAMP response to PGE_1. Cells (8.4 × 10^6 per 5.0 ml of medium) were incubated in the absence or presence of the indicated concentration of taxol (15 min) followed by the addition of saline or 10 μM colchicine (60 min). Ethanol or PGE_1 (2.0 μM) was then added for the final 30 min of incubation, after which the suspensions were acid extracted for cAMP determinations. All incubations were performed in duplicate and each column-purified extract was assayed in duplicate for cAMP. Each point represents the mean ± SEM for four determinations. ○, Taxol alone; ▲, taxol plus colchicine; ●, taxol plus PGE_1; ■, taxol plus PGE_1 and colchicine.

Table 1. Effect of taxol on the enhancement of the lymphocyte cAMP response to PGE_1 caused by various agents

Exp.	Taxol (2.0 μM)	Pretreatment	Picomoles of cAMP/10^7 cells: No PGE_1	Picomoles of cAMP/10^7 cells: With PGE_1
I	−	None	1.67 ± 0.10	3.58 ± 0.18
	+	None	1.38 ± 0.14	4.02 ± 0.12
	−	Vinblastine (1.0 μM)	2.08 ± 0.07	13.74 ± 0.54
	+	Vinblastine (1.0 μM)	1.48 ± 0.05	4.94 ± 0.35
	−	Podophyllotoxin (1.0 μM)	2.27 ± 0.06	15.97 ± 0.36
	+	Podophyllotoxin (1.0 μM)	1.47 ± 0.03	6.22 ± 0.45
II	−	None	1.88 ± 0.06	5.03 ± 0.29
	+	None	2.11 ± 0.10	5.50 ± 0.08
	−	Nocodazole (10 μM)	2.53 ± 0.10	19.57 ± 0.64
	+	Nocodazole (10 μM)	1.76 ± 0.07	4.77 ± 0.28
	−	Cytochalasin B (10 μM)	1.87 ± 0.11	6.23 ± 0.41
	+	Cytochalasin B (10 μM)	1.88 ± 0.09	5.81 ± 0.37
	−	Cytochalasin B (40 μM)	2.24 ± 0.05	8.22 ± 0.33
	+	Cytochalasin B (40 μM)	2.14 ± 0.13	12.94 ± 0.79
III	−	None	2.94 ± 0.20	7.29 ± 0.21
	+	None	3.15 ± 0.23	9.28 ± 0.24
	−	Vinblastine (5.0 μM)	8.68 ± 0.16	37.74 ± 1.09
	+	Vinblastine (5.0 μM)	3.58 ± 0.14	14.55 ± 0.21
	−	Concanavalin A (25 μg/ml)	4.34 ± 0.12	16.54 ± 0.46
	+	Concanavalin A (25 μg/ml)	4.16 ± 0.23	16.78 ± 0.52
IV	−	None	0.42 ± 0.06	2.35 ± 0.14
	+	None	0.36 ± 0.06	2.33 ± 0.05
	−	Vincristine (1.0 μM)	0.79 ± 0.09	5.80 ± 0.24
	+	Vincristine (1.0 μM)	0.58 ± 0.07	2.60 ± 0.10
	−	Demecolcine (1.0 μM)	1.38 ± 0.12	11.08 ± 0.46
	+	Demecolcine (1.0 μM)	0.88 ± 0.07	4.04 ± 0.21

Cytolytic lymphocytes (5.8–9.0 × 10^6 cells per 5.0 ml of medium) were incubated in the presence or absence of 2.0 μM taxol for 15 min followed by a 60-min incubation in the presence of the specified pretreatment agents. Solvent (0.07% ethanol) or 2.0 μM PGE_1 was then added to the cell suspensions and these incubations were terminated 30 min later by acid extraction for subsequent cAMP radioimmunoassay. All incubations were performed in duplicate and each column-purified extract was assayed in duplicate for cAMP. Each value represents the mean ± SEM for four determinations.

Cytochalasin B, an agent that affects microfilaments (17) and enhances hormone-stimulated cAMP accumulation in S49 lymphoma cells (18), was shown to enhance the PGE_1-induced increase in cAMP in the cytolytic lymphocytes at 40 μM drug (Table 1, Exp. II). Taxol did not antagonize this effect of cytochalasin B; in fact, in two separate experiments taxol (2.0 μM) was observed to enhance the cAMP accumulation induced by the combination of PGE_1 and cytochalasin B. Concanavalin A, another agent that has been reported to enhance the cAMP response of macrophages to PGE_1 (19), was found to produce this effect in cytolytic lymphocytes as well (Table 1, Exp. III); however, taxol (2.0 μM) had no effect upon this activity of concanavalin A.

The antagonistic effect of taxol towards the effects of microtubule-disrupting agents on cAMP metabolism was also apparent in the LMC counterpart to these cAMP experi-

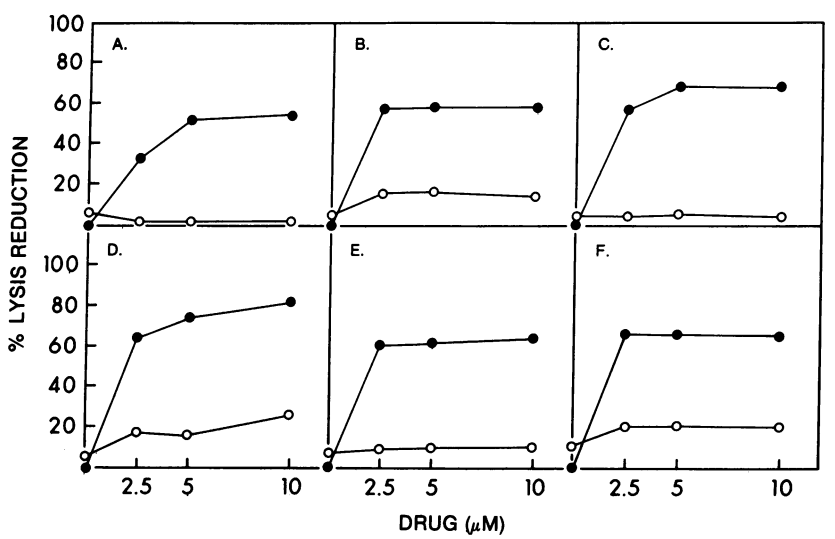

FIG. 4. Effects of colchicine (*A*), demecolcine (*B*), vinblastine (*C*), vincristine (*D*), nocodazole (*E*), and podophyllotoxin (*F*) on LMC. All agents were tested after a 15-min incubation in the absence (●) or presence (○) of 2.0 μM taxol.

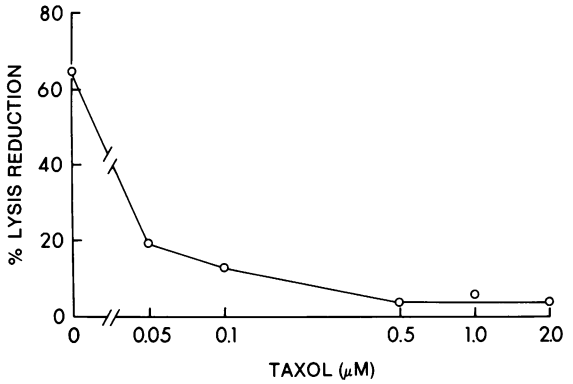

FIG. 5. Concentration-dependent antagonism by taxol of colchicine inhibition of LMC. Cytolytic lymphocytes were incubated for 15 min with the indicated concentrations of taxol before the addition of 10 μM colchicine and the start of the cytolysis assays.

ments. Colchicine (Fig. 4*A*), demecolcine (Fig. 4*B*), vinblastine (Fig. 4*C*), vincristine (Fig. 4*D*), nocodazole (Fig. 4*E*), and podophyllotoxin (Fig. 4*F*) all inhibited LMC by 50% at concentrations below 5.0 μM; taxol (2.0 μM) reduced this effect of each of these agents, tested at 10 μM concentration, to below 25% inhibition of LMC. This effect of taxol on the colchicine inhibition of LMC was dose dependent and was 70% of its maximum at only 0.05 μM taxol (Fig. 5).

β- and γ-lumicolchicine (25 μM) were not inhibitory to LMC. Cytochalasin B inhibited LMC by 56% at 10 μM, and this effect was not altered by 2.0 μM taxol. Similarly, concanavalin A inhibited LMC by approximately 50% at 25 μg/ml, and taxol (2.0 μM) had no effect upon this LMC inhibitory activity of concanavalin A (data not presented).

Prior treatment of the cytolytic lymphocytes with 3-deazaadenosine also increased the cAMP levels induced by subsequent treatment of the cells with PGE_1 (Fig. 6). As was seen with cytochalasin B-treated cells, the presence of taxol

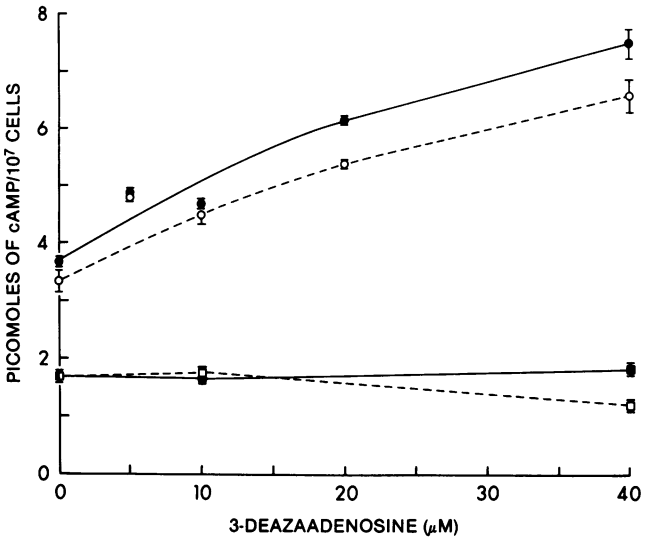

FIG. 6. Effect of taxol on the 3-deazaadenosine enhancement of the lymphocyte cAMP response to PGE_1. Cells (8.8×10^6 per 5.0 ml of medium) were incubated in the absence (○, □) or presence (●, ■) of 2.0 μM taxol (15 min) followed by addition of the indicated concentrations of 3-deazaadenosine for 60 min. Ethanol (■, □) or PGE_1 (2.0 μM) (●, ○) was then added and the cell suspensions were acid extracted for cAMP determinations after an additional 30-min incubation. All incubations were performed in duplicate and each column-purified extract was radioimmunoassayed in duplicate for cAMP. Each point represents the mean ± SEM for four determinations.

Table 2. Effect of taxol on the colchicine enhancement of the lymphocyte cAMP response to various activators of adenylate cyclase

Pretreatment	Stimulant	Picomoles of cAMP/10^7 cells
None	None	2.09 ± 0.11
Taxol		1.97 ± 0.03
Colchicine		3.13 ± 0.48
Colchicine + taxol		1.86 ± 0.08
None	2-Chloroadenosine	3.78 ± 0.19
Taxol		4.17 ± 0.36
Colchicine		19.25 ± 0.36
Colchicine + taxol		6.49 ± 0.50
None	Cholera toxin	7.49 ± 1.00
Taxol		10.82 ± 0.29
Colchicine		147.60 ± 10.20
Colchicine + taxol		13.45 ± 1.48
None*	None	1.55 ± 0.06
Taxol*		1.27 ± 0.05
Colchicine*		2.65 ± 0.04
Colchicine + taxol*		2.19 ± 0.23
None	Isoproterenol	1.82 ± 0.03
Taxol		1.77 ± 0.09
Colchicine		4.76 ± 0.12
Colchicine + taxol		2.01 ± 0.11

Cytolytic lymphocytes (6.9×10^6 cells per 5.0 ml of medium) were incubated for 15 min at 37°C. Ethanol or taxol was added to the cell suspensions (final concentration, 2.0 μM taxol and 0.2% ethanol) and the cells were incubated for a further 15 min, at which time saline, cholera toxin (10 μg/ml), and/or colchicine (10 μM) was added. After a further 60 min of incubation, saline, 2-chloroadenosine (5.0 μM), or isoproterenol (20 μM) was added to the cell suspensions. The cell suspensions were then acid extracted after a total incubation of 90 min with cholera toxin, 30 min with 2-chloroadenosine, or 5.0 min with isoproterenol. All incubations were performed in duplicate and each column-purified extract was radioimmunoassayed in duplicate for cAMP. Each value represents the mean ± SEM for four determinations.
*Controls for isoproterenol incubations.

did not antagonize this cAMP effect of 3-deazaadenosine but actually appeared to increase it slightly. Taxol was also unable to antagonize the inhibition of LMC by 3-deazaadenosine. In addition, taxol did not antagonize the inhibition of LMC or the enhancement of the PGE_1-induced rise in cAMP (20) due to the combination of 3-deazaadenosine and L-homocysteine thiolactone (data not shown).

The colchicine enhancement of cAMP accumulation caused by three other stimulators of adenylate cyclase—2-chloroadenosine, cholera toxin, and isoproterenol—was also greatly antagonized by taxol (Table 2). This effect was especially marked with cholera toxin. Colchicine increased the cholera toxin-induced levels of cAMP 13.6-fold, and this effect of colchicine was antagonized greater than 95% by 2.0 μM taxol.

Taxol (2.0 μM), colchicine (10 μM), or the combination of these two agents did not affect the cellular level of [^{3}H]ATP in lymphocytes whose adenine nucleotide pools had previously been labeled metabolically with [^{3}H]adenine (data not shown).

DISCUSSION

The availability of taxol has made possible studies on the role of microtubules in a number of cell functions. Previous studies concerning the role of microtubules in various cellu-

lar reactions have involved the use of agents such as colchicine which, while known to disrupt microtubules, also appear to have actions unrelated to their tubulin-binding property (21–24). Taxol, in contrast to colchicine, promotes microtubule polymerization and thus may specifically prevent the microtubule-disrupting activity of colchicine. This property of taxol has allowed us to study the role of microtubules in the lysis of tumor cells by specifically sensitized cytolytic lymphocytes as well as their role in the hormonal stimulation of cAMP synthesis.

In confirmation of the results with human leukocytes (13), the cAMP response of the mouse cytolytic lymphocytes to PGE_1 is enhanced by colchicine and other microtubule-disrupting agents. In contrast to the large (2.5- to 5.0-fold) enhancement by these agents, cytochalasin B, a microfilament-disrupting agent, increased the PGE_1-induced cAMP levels by only 24 and 63% at 10 and 40 μM, respectively (Table 1). Also, while the increase in the PGE_1 induction of cAMP by the microtubule-disrupting agents is prevented by taxol, this increase in the PGE_1 induction of cAMP by cytochalasin B is not. In fact, the effect of 40 μM cytochalasin B is enhanced significantly ($P < 0.01$) by taxol. These results indicate a greater relative importance of microtubules, compared with microfilaments, in modulating the hormonal stimulation of cAMP production in these cells.

It is clear from this work and that of others (25, 26) that the integrity of microtubules and microfilaments is very important for lymphocyte function. Microtubule-disrupting agents of very diverse chemical structure, as well as a microfilament-disrupting agent, were very inhibitory to LMC in the low micromolar range. The prevention of both the LMC-inhibitory activity and the cAMP-increasing activity of the microtubule-disrupting agents by taxol was striking although, as can be seen in Figs. 3 and 4, the two dose–response curves for taxol differ somewhat: 0.05 μM taxol was much more effective in preventing colchicine inhibition of LMC than it was in preventing the colchicine enhancement of PGE_1 stimulation of cAMP. This difference in the two dose–response curves for taxol in antagonizing the effects of colchicine on lymphocyte cAMP responsiveness and on LMC indicates that these two cellular processes may be differentially sensitive to the integrity of cellular microtubules.

3-Deazaadenosine in the presence (20) or absence (Fig. 6) of L-homocysteine thiolactone also increases PGE_1-stimulated cAMP levels and inhibits LMC (27). However, these activities of 3-deazaadenosine are not antagonized by taxol. This result indicates that 3-deazaadenosine must be acting in these cells by a mechanism different from that of colchicine and the other microtubule-disrupting agents.

It is possible that taxol antagonizes the activity of the microtubule-disrupting agents by preventing their uptake. However, this possibility seems unlikely since taxol selectively antagonized the activities of six different microtubule-disrupting agents, some of these differing considerably in chemical structure, but did not reduce the cellular activities of other agents, such as cytochalasin B and 3-deazaadenosine. The fact that β- and γ-lumicolchicine, which do not disrupt microtubules (28), also do not inhibit LMC or increase the PGE_1-stimulated cAMP levels in these lymphocytes provides additional evidence for the role of microtubules in these two cellular processes.

In conclusion, the present report has evaluated the influence of taxol on microtubule-disrupting agent-induced inhibition of LMC and enhancement of PGE_1-stimulated cAMP production. Both of these effects of the various microtubule-disrupting agents were antagonized by taxol, thus suggesting that both types of effects involve microtubules. Antagonism by taxol of the inhibition of a cellular function caused by a microtubule-disrupting agent has not been described previously, to our knowledge.

Note Added in Proof. After this manuscript was submitted for review, DoKhac *et al.* (29) reported that colchicine, vinblastine, and nocodazole enhance the prostaglandin E_1- and I_2-stimulated elevation of cAMP in rat myometrium and that taxol antagonizes this effect of the microtubule-disrupting agents. However, in this latter tissue, the microtubule-disrupting agents failed to enhance the cAMP response to either isoproterenol or cholera toxin.

We gratefully acknowledge the skilled technical assistance of Robert L. Veasey and Mrs. Marvin S. Winston. We express our gratitude to Mrs. Beverly Nobles and Ms. Nancy Jackson for their preparation of the graphics and to Mrs. Sherron Paris for typing this manuscript.

1. Wani, M. C., Taylor, H. L., Wall, M. E., Coggon, P. & McPhail, A. T. (1971) *J. Am. Chem. Soc.* **93,** 2325–2327.
2. Schiff, P. B., Fant, J. & Horwitz, S. B. (1979) *Nature (London)* **277,** 665–667.
3. Schiff, P. B. & Horwitz, S. B. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 1561–1565.
4. Cuthbert, J. A. & Shay, J. W. (1983) *J. Cell. Physiol.* **116,** 127–134.
5. Oda, K. & Ikehara, Y. (1982) *Biochem. Biophys. Res. Commun.* **107,** 561–567.
6. Roberts, R. L., Nath, J., Friedman, M. M. & Gallin, J. I. (1982) *J. Immunol.* **129,** 2134–2141.
7. Howell, S. L., Hii, C. S., Shaikh, S. & Tyhurst, M. (1982) *Biosci. Rep.* **2,** 795–801.
8. Nath, J., Flavin, M. & Schiffmann, E. (1981) *J. Cell Biol.* **91,** 232–239.
9. White, J. G. & Rao, G. H. R. (1982) *Blood* **60,** 474–483.
10. Horwitz, S. B., Chia, G. H., Harracksingh, C., Orlow, S., Pifko-Hirst, S., Schneck, J., Sorbara, L., Speaker, M., Wilk, E. W. & Rosen, O. M. (1981) *J. Cell Biol.* **91,** 798–802.
11. Paatero, G. I. L. & Brown, D. L. (1982) *Cell Biol. Int. Rep.* **6,** 1033–1040.
12. Henney, C. S. & Bubbers, J. E. (1973) *J. Immunol.* **111,** 85–90.
13. Rudolph, S. A., Greengard, P. & Malawista, S. E. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3404–3408.
14. Wolberg, G., Hiemstra, K., Burge, J. J. & Singler, R. C. (1973) *J. Immunol.* **111,** 1435–1443.
15. Zimmerman, T. P., Rideout, J. L., Wolberg, G., Duncan, G. S. & Elion, G. B. (1976) *J. Biol. Chem.* **251,** 6757–6766.
16. Duncan, G. S., Wolberg, G., Schmitges, C. J., Deeprose, R. D. & Zimmerman, T. P. (1982) *J. Immunopharmacol.* **4,** 79–100.
17. Wessells, N. K., Spooner, B. S., Ash, J. F., Bradley, M. O., Luduena, M. A., Taylor, E. L., Wrenn, J. T. & Yamada, K. M. (1971) *Science* **171,** 135–143.
18. Insel, P. A. & Koachman, A. M. (1982) *J. Biol. Chem.* **257,** 9717–9723.
19. Gemsa, D., Steggemann, L., Till, G. & Resch, K. (1977) *J. Immunol.* **119,** 524–529.
20. Zimmerman, T. P., Schmitges, C. J., Wolberg, G., Deeprose, R. D., Duncan, G. S., Cuatrecasas, P. & Elion, G. B. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5639–5643.
21. Fitzgerald, T. J., Williams, B. & Uyeki, E. M. (1971) *Proc. Soc. Exp. Biol. Med.* **136,** 115–120.
22. Mizel, S. B. & Wilson, L. (1972) *Biochemistry* **11,** 2573–2578.
23. Furcht, L. T. & Scott, R. E. (1975) *Exp. Cell Res.* **96,** 271–282.
24. Landowne, D., Larsen, J. B. & Taylor, K. T. (1983) *Science* **220,** 953–954.
25. Greene, W. C., Parker, C. M. & Parker, C. W. (1976) *J. Immunol.* **117,** 1015–1022.
26. Katz, P., Zaytoun, A. M. & Lee, J. H., Jr. (1982) *J. Immunol.* **129,** 2816–2825.
27. Zimmerman, T. P., Wolberg, G. & Duncan, G. S. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 6220–6224.
28. Dustin, P. (1978) *Microtubules* (Springer, New York).
29. DoKhac, L., Tanfin, Z. & Harbon, S. (1983) *Biochem. Pharmacol.* **32,** 2535–2541.