COLCHICINE STIMULATION OF PYROGEN PRODUCTION BY HUMAN BLOOD LEUKOCYTES*

By PHYLLIS BODEL

(From the Department of Medicine, Yale University School of Medicine, New Haven, Connecticut 06510)

Endogenous pyrogen (EP),[1] the protein which mediates fever, is normally synthesized and released by polymorphonuclear (PMN) and mononuclear (MN) phagocytes after stimulation by inflammatory agents such as endotoxin or by the phagocytosis of bacteria (1). Normal leukocytes contain no detectable pyrogen, and do not produce any during in vitro incubation. After stimulation, however, pyrogen production begins within a few hours and continues at a steady rate for 12 or more hours (2–4). New protein synthesis is required for the initiation of pyrogen production by both PMN and MN leukocytes, but only MN leukocytes require continuing protein synthesis in order for pyrogen production and release to proceed (4). The mechanisms by which inflammatory agents initiate production of EP, and the processes by which cells secrete this small protein, are entirely unknown.

Colchicine, an anti-inflammatory agent, inhibits many PMN leukocyte functions, including chemotaxis (5, 6), adhesiveness (7), locomotion (8), degranulation (9, 10), phagocytosis (11), and metabolic responses to particle ingestion (9, 11). It also inhibits pinocytosis and induction of lysosomal enzymes in cultured mouse macrophages (12). In other cell systems, colchicine inhibits secretion of various cellular products, including insulin (13), thyroid hormone (14), growth hormone and prolactin (15), plasma proteins (16), and catecholamines (17) and histamine (18). All these effects of colchicine have usually been ascribed to altered function of microtubular proteins, cell constituents to which colchicine binds selectively (19).

Since fever, due to EP production, commonly follows an inflammatory response, and since colchicine inhibits secretion of many other cellular products, the effect of this drug and related compounds on EP production and release by leukocytes was investigated. Contrary to expectation, the results of these studies indicate that colchicine not only does not suppress pyrogen production by stimulated human PMN leukocytes, but, in the absence of other inflammatory agents, micromolar concentrations of this compound stimulate EP production and release.

---

* This work was supported by grants from the U. S. Public Health Service (5RO1-A1-01564-18 and CA-14655-02).

[1] *Abbreviations used in this paper:* EP, endogenous pyrogen; MN, mononuclear; PMN, polymorphonuclear.

Materials and Methods

All materials, glassware, and reagents used in these studies were made sterile and pyrogen free, usually by heating at 160°C for 2 h or autoclaving for 90 min. Heat-labile compounds were filtered through a Millipore filter (Swinnex-25; Millipore Corporation, Bedford, Mass.).

Preparation and Incubation of Leukocytes. Human blood leukocytes were prepared from heparinized venous blood by dextran sedimentation as described previously (2). Incubations were carried out in modified Krebs-Ringer phosphate buffer, pH 7.4, containing 15% homologous serum, 10 U heparin, 1.5 mg glucose, 50 U penicillin, and 50 μg streptomycin/ml. Leukocyte concentrations were usually 10^7/ml, consisting of about 70% granulocytes and 3–5% monocytes. Identical methods were used to prepare rabbit blood leukocytes from heparinized blood obtained by cardiac puncture. Flasks were shaken for 2–3 h on a Dubnoff shaking incubator at 37°C, then incubated overnight in a stationary incubator. Human blood mononuclear cells were prepared from heparinized venous blood by flotation over Ficoll-Hypaque as described previously (4). These cells were suspended in Eagle's minimum essential medium (MEM: Auto-POW, Flow Laboratories, Inc., Rockville, Md.), and incubated overnight in a 5% CO_2-air incubator in Falcon tissue-culture flasks (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.). Differential counts ranged from 20–30% monocytes, 0–3% PMN leukocytes, and the remainder, small lymphocytes. Since lymphocytes do not produce pyrogen (3), these cells were usually not removed during the incubations.

In experiments where pyrogen production by whole blood leukocytes and monocytes was compared, some monocytes were exposed to dextran and then washed before incubation, to ensure that the different methods of preparation were not responsible for differences in cell function. PMN leukocytes which have been centrifuged through Ficoll-Hypaque and then washed are incapable of pyrogen release, however, so such cells could not be studied.

Pyrogen Assay. Flask contents were centrifuged at 2,000 *g* for 30 min, cultured in thioglycollate broth to confirm sterility, and assayed for endogenous pyrogen by injection into rabbits as described previously (20). Usually supernate from 2×10^7 PMN leukocytes and 4×10^6 monocytes was employed as an assay dose per rabbit. In all experiments, both negative and positive control flasks of leukocytes were included, consisting of cells incubated in medium alone (negative control), and cells stimulated to release pyrogen, usually by addition of heat-killed staphylococci (positive control). Supernates from all flasks in each experiment were assayed for pyrogen in the same group of rabbits, with few exceptions. Rare experiments in which injection of supernates from either control flask produced inappropriate responses were discarded. Cell-associated pyrogen was assayed from supernates of leukocytes disrupted by freeze-thawing as described previously (2), except that the wash of the cell button was omitted.

Reagents. The following reagents were used: Colchicine, sterile ampule for intravenous injection, 0.5 mg/ml (Eli Lilly & Co., Indianapolis, Ind.); vinblastine sulfate (Velban) (Eli Lilly & Co.), sterile ampule for injection; puromycin dihydrochloride (ICN Nutritional Biochemicals Div., International Chemical & Nuclear Corp., Cleveland, Ohio), prepared as described previously (2); and lumicolchicine, 2.4 mg/ml solution in alcohol, kindly supplied by Dr. Leslie Wilson, Department of Pharmacology, Stanford Medical Center, Stanford, Calif.; *N*-desacetyl-*N*-methylcolchicine (Colcemid) (Ciba Corp., Summit, N.J.), dissolved in sterile water at 0.5 mg/ml, filtered, and diluted further as needed in normal saline. All materials were stored at 4°C or −20°C. Reagents were tested for pyrogenicity (endotoxin contamination) before use by dilution with Krebs-Ringer phosphate buffer and 10% normal rabbit serum, incubation at 37°C for 30 min, and injection into rabbits. Doses at least five times those used for incubations with cells were tested. Heat-killed *Staphylococcus albus* was prepared as described previously (2); 10–30 bacteria per leukocyte were used to stimulate pyrogen release. Endotoxin was supernatant typhoid vaccine, prepared as described previously (4).

Results

In initial experiments, human blood leukocytes were incubated with or without colchicine (10^{-5} M) for 1 h, heat-killed staphylococci were then added, and the amount of pyrogen produced was assayed after 18 h of incubation. Pretreatment with colchicine did not affect the amount of EP released by cells stimulated

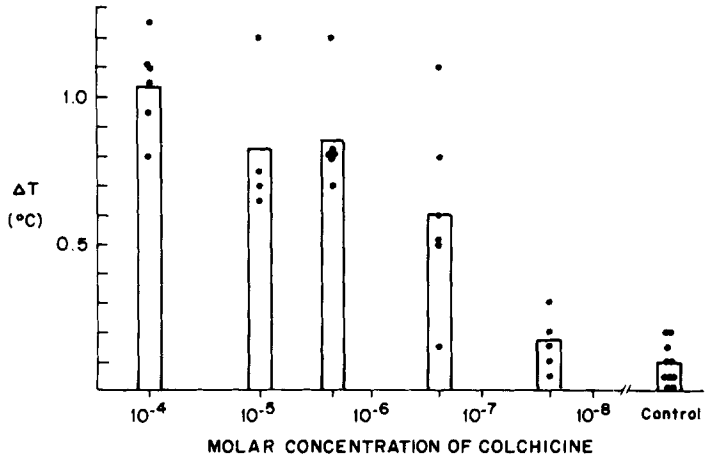

FIG. 1. Peak temperature elevations in rabbits after injection of supernates from human blood leukocytes incubated for 18 h with varying concentrations of colchicine. Supernate from 3×10^7 human leukocytes was given to each rabbit. Responses to supernate from cells incubated without colchicine (control) are shown on the far right. In this figure and in subsequent ones, each point represents an individual rabbit response; averages are indicated by the height of the bars. The results of five experiments are demonstrated.

by phagocytosis. In these experiments, as expected, control leukocytes incubated without colchicine or staphylococci released no pyrogen. However, cells incubated with colchicine alone produced as much pyrogen as cells stimulated by phagocytosis. This unexpected finding was confirmed in a series of further experiments (see Fig. 1). Colchicine consistently induced pyrogen release from human blood cells when added at concentrations greater than 10^{-7} M, and a dose-response effect was observed. Proof that the pyrogen-inducing activity of colchicine was not due to contaminating endotoxin was obtained by experiments with rabbit blood leukocytes (described below).

Since the results of these studies indicated that concentrations of colchicine below 10^{-7} M did not stimulate significant EP production, further attempts were made to inhibit pyrogen release after phagocytosis, using very low concentrations of colchicine. Human blood leukocytes were incubated for 1 h with colchicine at 5 or 2.5×10^{-8} M, staphylococci were added, and pyrogen release was assayed. There was again no difference between the amount of EP released by colchicine-treated, compared to normal, cells stimulated by phagocytosis.

In order to determine whether other compounds which alter microtubular function would also stimulate pyrogen production in vitro, human blood leukocytes were incubated with Colcemid and vinblastine. The results of these experiments are shown in Fig. 2. Like colchicine, both Colcemid and vinblastine induced human blood cells to release pyrogen. On the other hand, lumicolchicine, a light-altered derivative of colchicine which has very little affinity for microtubules (21), did not stimulate pyrogen release even when added at 100 times the concentration at which colchicine is effective.

Since stimulated blood monocytes produce large quantities of EP (4, 22, 23), it was of interest to determine whether mononuclear leukocytes from blood would

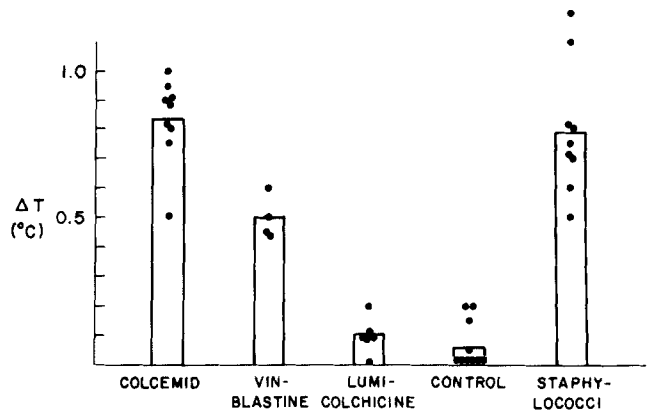

FIG. 2. Peak temperature elevations in rabbits after injection of supernates from human blood leukocytes incubated for 18 h without stimulus (control), or with Colcemid (2.5×10^{-5} M), vinblastine (4.5 or 9×10^{-5} M), lumicolchicine (2×10^{-5} M), or heat-killed staphylococci.

also release pyrogen during incubation with colchicine. Preparations of human blood monocytes were therefore incubated with several different concentrations of colchicine, and pyrogen release was measured. The results of these experiments, shown in Fig. 3, indicate that monocytes release pyrogen when incubated with colchicine at concentrations above 2.5×10^{-8} M. A dose-response relationship was observed, similar to that shown in Fig. 1 for human blood (predominantly PMN) leukocytes. The higher average temperature responses recorded with supernates from monocytes (Fig. 3) are due to incomplete adjustment of the assay for the greater yield of pyrogen from monocytes compared to that of PMN leukocytes (4, 23).

In order to exclude the possibility that the pyrogenic activity of colchicine was due to contamination of the preparations by small amounts of endotoxin, rabbit leukocytes suspended in a rabbit serum-buffer medium were incubated with varying amounts of the drug, and EP release was assayed. Although as little as 0.001 μg of endotoxin will induce EP production under such conditions (24), colchicine did not induce pyrogen production by rabbit leukocytes, even at concentrations as high as 10^{-4} M (see Fig. 4). The viability and pyrogen-producing capacity of the rabbit cells were confirmed by addition of staphylococci to other flasks, either in the presence or absence of colchicine (right-hand bar, Fig. 4). Similar experiments using both large and minimally stimulating doses of endotoxin, with and without colchicine, also failed to demonstrate either stimulatory or inhibitory activity of this agent on rabbit leukocytes.

Experiments were next performed to examine some characteristics of the process by which colchicine or Colcemid induces production and release of EP by human leukocytes. In initial experiments, varying concentrations of Colcemid were added to human blood monocytes, and the pyrogen released by these cells was compared to that released by whole-blood leukocytes. As shown in Fig. 5, at low concentrations of Colcemid (2.5 or 5.0×10^{-6} M),[2] blood leukocytes, predominantly granulocytes, produced significant amounts of pyrogen, whereas mono-

[2] With a subsequent preparation of Colcemid, 2.5×10^{-7} M was the critical concentration.

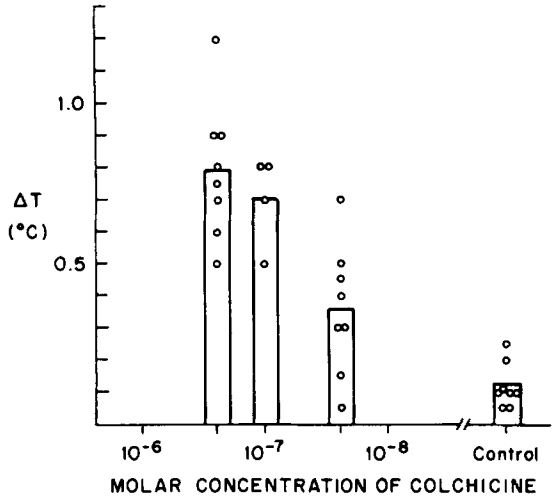

FIG. 3. Peak temperature elevations in rabbits after injection of supernates from human blood monocytes incubated for 18 h with varying concentrations of colchicine. Responses to supernate from cells incubated without colchicine (control) are shown on the far right. Supernate from about 4×10^6 monocytes was given to each rabbit.

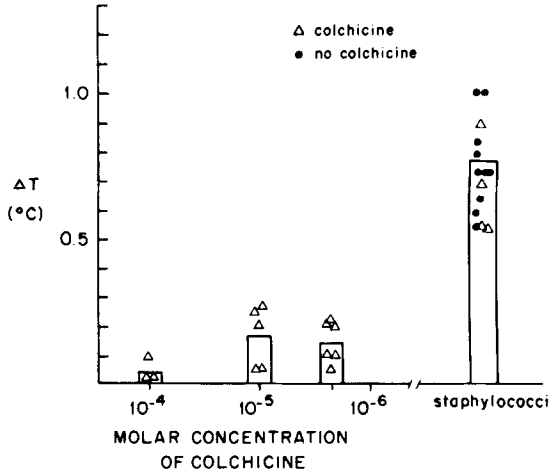

FIG. 4. Peak temperature elevations in rabbits after injection of supernates from $3\text{-}6 \times 10^7$ rabbit blood leukocytes incubated for 18 h with varying concentrations of colchicine. Responses to supernates from cells incubated with staphylococci and colchicine, or with staphylococci alone, are shown on the far right.

cytes produced much less. At higher concentrations of Colcemid (not shown), both cell preparations were stimulated equally. Since no methods are presently available to obtain pure preparations of blood PMN leukocytes suitable for studies of pyrogen production, this differential sensitivity of the two cell types to low concentrations of Colcemid provided an opportunity to study the mechanism of PMN leukocyte activation by this agent.

First, human blood leukocytes were incubated with Colcemid for ½ or 2 h, the cells were washed once in buffer, then resuspended in fresh media without

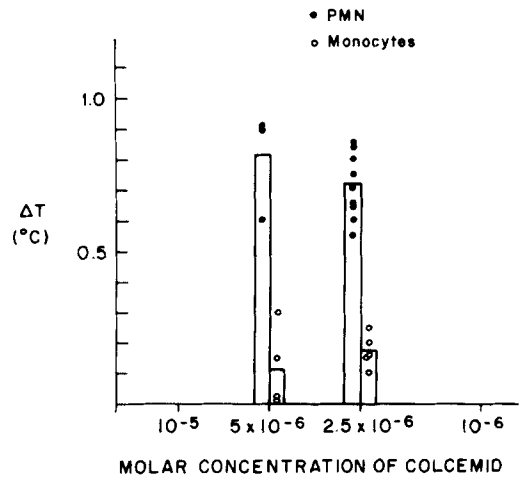

FIG. 5. Peak temperature elevations in rabbits after injection of supernates from human blood (predominantly PMN) leukocytes, or from preparations of human blood mononuclear cells, incubated for 18 h with Colcemid. Supernate from about 2×10^7 PMN leukocytes or 4×10^6 monocytes was given to each rabbit.

the drug. Other cells were incubated with Colcemid for 18 h as usual. As shown in Table I, a short contact with medium containing Colcemid was sufficient to induce nearly normal pyrogen release during the subsequent 16–18 h of incubation. These results are similar to those previously observed when staphylococci or endotoxin were used as stimuli (3).

Next, to examine the time-course of EP production in this system, the pyrogen content of the medium was assayed at different times after the beginning of leukocyte incubation with Colcemid (see Table II). Whereas pyrogen from leukocytes stimulated by phagocytosis is normally detectable in the medium 3 or 4 h after addition of bacteria (2, 3), pyrogen did not appear in the medium in these experiments until 7 or 8 h after the beginning of incubation with Colcemid. Assays for cell-associated pyrogen were also negative during these 7–8 h (not shown), but became positive when pyrogen appeared in the medium.

Finally, the effect of puromycin, an inhibitor of protein synthesis, on the process of EP release was examined. Previous studies (2, 25) have shown that puromycin prevents production and release of pyrogen from PMN leukocytes only if added within 1–2 h after a phagocytic stimulus. After this interval, although pyrogen production and release are just beginning, inhibitors of new RNA or protein synthesis do not alter the process. To determine whether a similar sequence of events occurs during induction of pyrogen production by Colcemid, puromycin (10^{-5} M) was added to preparations of leukocytes 2, 4, 6, or 8 h after Colcemid treatment. Incubations were continued for a total of 18 h, and pyrogen release, as well as cell-associated pyrogen content, was then assayed. The results, shown in Table III, indicate that when puromycin was added 2 or 4 h after Colcemid, subsequent release of EP into the medium was completely prevented. Cell-associated pyrogen also did not develop (not shown), indicating that pyrogen production, as well as its release, is inhibited. When puromycin

TABLE I
Effect of Time of Exposure to Colcemid on Leukocyte Pyrogen Production

Incubation with Colcemid (2.5×10^{-6} M)	ΔT (°C) ± SEM*			
	0 min	30 min	2 h	18 h
Pyrogen in 18-h supernate	0.07 ± 0.03 (8)‡	0.58 ± 0.04 (6)	0.52 ± 0.11 (9)	0.74 ± 0.05 (10)

* Average maximum temperature elevations in rabbits after injection of supernates ± standard errors of the mean.
‡ Number of responses in parentheses.

TABLE II
Effect of Time of Incubation with Colcemid on Pyrogen Production

Time of incubation with colcemid (2.5×10^{-6} M)	ΔT (°C) ± SEM*		
	4 h	6-8 h	18 h
Pyrogen in supernate	0.10 ± 0.03 (6)‡	0.27 ± 0.07 (13)	0.70 ± 0.09 (9)

* Average maximum temperature elevations in rabbits after injection of supernates ± standard errors of the mean.
‡ Number of responses in parentheses.

was added 6-8 h after Colcemid, however, nearly normal pyrogen production occurred (see Table III). These results, then, indicate that a brief incubation of PMN leukocytes with Colcemid is sufficient to induce EP production, that leukocytes stimulated by Colcemid do not produce or release pyrogen for many hours, and that inhibition of protein synthesis during these hours will prevent subsequent EP production.

Discussion

When this investigation was begun, it was anticipated that colchicine might inhibit pyrogen release from human leukocytes stimulated by phagocytosis, but no such effect was observed, even when concentrations of colchicine below 0.1 μM were used. In contrast, the results of the studies presented here indicate that colchicine, and the related compound Colcemid, activate human blood leukocytes to produce and release endogenous pyrogen. This effect is dose related, occurring with concentrations of colchicine above 0.1 μM. Both PMN leukocytes and monocytes are stimulated to release pyrogen when incubated with these agents. Although contamination of colchicine and Colcemid solutions with endotoxin would result in similar activation of leukocytes, this possibility is effectively ruled out by the failure of these compounds to activate rabbit blood leukocytes. Rabbit leukocytes produce pyrogen when exposed to very small amounts of endotoxin (24), yet they failed to become activated by 100 μM colchicine, a dose 400 times that required to stimulate human leukocytes.

Previous studies have shown that colchicine either inhibits, or has no effect on, various functions of leukocytes (5-12). Similarly, most investigators have reported inhibitory effects of colchicine on secretion of cellular products, such as hormones and amines (13-18). In a few instances, however, stimulatory actions

TABLE III
*Effect of Puromycin on Pyrogen Production by Leukocytes Incubated with Colcemid**

Time puromycin (10^{-5} M) added after incubation begun	ΔT (°C) ± SEM‡			
	2 h	4 h	6–8 h	None
Pyrogen in 18-h supernate	0.10 ± 0.03 (11)§	0.07 ± 0.07 (6)	0.63 ± 0.07 (6)	0.81 ± 0.06 (13)

*2.5×10^{-6} M
‡ Average maximum temperature elevations in rabbits after injection of supernates ± standard errors of the mean.
§ Number of responses in parentheses.

of colchicine on the secretory processes of cells have been observed. Explants of synovial tissue exposed to colchicine exhibit increased synthesis and release of collagenase (26) and neutral protease (27), as well as prostaglandins (28). Colchicine also markedly stimulates steroid secretion by adrenal tumor cells in culture (29). In a recent abstract, colchicine was reported to stimulate secretion of elastase and collagenase by cultured mouse macrophages (30). Concentrations of the drug which were effective in these experiments ranged from 0.01 to 1 μM, levels comparable to those used to stimulate pyrogen release from leukocytes in the studies reported here.

Both inhibitory and stimulatory effects of colchicine have usually been ascribed to an action on microtubules, protein constituents of many cells, including leukocytes, to which colchicine binds selectively (19). Colcemid and vinblastine, like colchicine, interfere with the assembly of microtubules, whereas lumicolchicine, a light-altered derivative of colchicine, has almost no affinity for these structures (21). Since Colcemid and vinblastine, but not lumicolchicine, stimulated EP production by normal leukocytes, altered function of microtubules may in some way lead to EP production and release.

The failure of colchicine or Colcemid to induce pyrogen production by rabbit leukocytes is unexplained. However, different responses of rabbit and human leukocytes to both pyrogenic and antipyrogenic stimuli have been reported before, in studies with etiocholanolone, hydrocortisone, and estrogen (31). Colchicine in a concentration of 10^{-4} M does not inhibit chemotaxis of rabbit leukocytes (32), whereas much lower concentrations inhibit chemotaxis of human leukocytes (5, 6). Rabbit and human leukocytes also differ in morphology and contain different proteins and enzymes (33, 34).

The process of EP production by human blood PMN leukocytes, stimulated by phagocytosis or endotoxin, has been studied previously (2, 25). After a stimulus is added to normal cells, EP production begins within 2–3 h and continues for about 12 h. During an interval of 1–2 h between addition of the activator and the first release of pyrogen, addition of inhibitors of new RNA and protein synthesis prevents subsequent production and release of pyrogen. If added later, however, these inhibitors, as well as 10^{-2} M sodium fluoride, have no effect. Investigation of the mechanism of action of Colcemid in the present studies indicated that although the time required for contact between activator and cell is brief, EP

production and release do not begin for about 6 h. If puromycin is added during this time interval, but not afterward, subsequent EP production is inhibited. This finding suggests that Colcemid induces EP production and release by a process similar to that of other activators studied, although the prolonged induction phase distinguishes it from all agents investigated previously except etiocholanolone (35). This pyrogenic steroid, however, may activate only monocytes, not granulocytes, whereas Colcemid, in the concentration used, probably activated mainly granulocytes. This conclusion is supported by the observation that puromycin did not suppress late release of pyrogen by Colcemid (see Table II), whereas late synthesis and release of EP by blood monocytes are inhibited by puromycin (4).

The results of the experiments reported here raise the possibility that disassembly of microtubules and the resulting disorganization of cellular structures which occurs when colchicine or vinblastine is incubated with leukocytes in vitro (36–38) somehow initiate or stimulate pyrogen production and release. Although microtubules could also be involved in the initiation of EP production by inflammatory stimuli such as endotoxin or phagocytosis, evidence for such a mechanism is lacking. Alternatively, colchicine may act by a mechanism not involving microtubules – perhaps by altering the structure or function of membranes, as has been suggested previously (16, 39). The fact that colchicine stimulates secretion of certain cellular products while it inhibits release of others, may provide information about different pathways for stimulation and secretion of various cellular products. Further studies of the action of colchicine on mouse macrophages (12, 30), cells which synthesize and secrete pyrogen (40) as well as other protein enzymes (41–43), may be especially helpful in examining these relationships.

Finally, since colchicine has not been reported to be pyrogenic in patients, it is unlikely that the in vitro effects seen here are related to its therapeutic actions. Concentrations of 0.1 μM, the minimum level for stimulation of pyrogen production in vitro, are about 10 times higher than those achieved in serum after a full therapeutic dose of the drug (44, 45). When concentrations of 0.05 or 0.025 μM were tested for inhibitory activity on pyrogen release from leukocytes stimulated by phagocytosis, no effect was observed. It is improbable, therefore, that the antipyretic effects of colchicine seen clinically, as, for example, in familial Mediterranean fever (46), are directly due to inhibition of pyrogen production by leukocytes. More likely, its anti-inflammatory properties, such as effects on the adhesiveness and chemotaxis of neutrophils, prevent the initial stimulation of the cells, which in turn prevents subsequent pyrogen release.

Summary

The effect of colchicine, an anti-inflammatory agent, on endogenous pyrogen (EP) production by human blood leukocytes in vitro was examined. Colchicine not only failed to suppress EP production by human leukocytes stimulated by phagocytosis, but, in the absence of other stimuli, micromolar concentrations of the drug induced pyrogen production and release by both polymorphonuclear (PMN) and mononuclear leukocytes. The response was dose related, occurring at concentrations above 0.1 μM. Colcemid and vinblastine, other agents which

bind to microtubular protein, also induced pyrogen release from human leukocytes, whereas lumicolchicine, a light-altered derivative of colchicine without affinity for microtubules, was ineffective. Colchicine did not induce EP production by rabbit leukocytes, even at 100 μM concentration. Studies of the mechanism of PMN leukocyte activation by Colcemid indicated that although the time required for contact between drug and leukocyte was brief, pyrogen production and release did not begin for 6 or more hours. If added during this time, puromycin prevented subsequent production and release of pyrogen. These results indicate that agents which interfere with the assembly of microtubules induce EP production and secretion by human leukocytes in vitro.

The constructive advice of Dr. Stephen Malawista, the expert technical assistance of Ms. Judy Smillie and Ms. Jean Buiter, and the continuing support of Dr. Elisha Atkins are gratefully acknowledged.

Received for publication 26 January 1976.

References

1. Atkins, E., and P. T. Bodel. 1974. Fever. *In* The Inflammatory Process, 2nd edition. B. W. Zweifach, L. Grant, and R. T. McCluskey, editors. Academic Press, Inc., New York. 467–513.
2. Bodel, P. 1970. Studies on the mechanism of endogenous pyrogen production. I. Investigation of new protein synthesis in stimulated human blood leucocytes. *Yale J. Biol. Med.* 43:145.
3. Root, R. K., J. J. Nordlund, and S. M. Wolff. 1970. Factors affecting the quantitative production and assay of human leukocytic pyrogen. *J. Lab. Clin. Med.* 75:679.
4. Bodel, P. 1974. Studies on the mechanism of endogenous pyrogen production. III. Human blood monocytes. *J. Exp. Med.* 140:954.
5. Caner, J. E. Z. 1965. Colchicine inhibition of chemotaxis. *Arthritis Rheum.* 8:757.
6. Phelps, P., and A. J. McCarty. 1969. Crystal-induced arthritis. *Postgrad. Med.* 45:87.
7. Penny, R., D. A. G. Galton, J. T. Scott, and V. Eisen. 1966. Studies on neutrophil function. I. Physiological and pharmacological aspects. *Br. J. Haematol.* 12:623.
8. Ramsey, W. B., and A. Harris. 1973. Leucocyte locomotion and its inhibition by antimitotic drugs. *Exp. Cell Res.* 82:262.
9. Bodel, P., and S. Malawista. 1967. The dissociation by colchicine of phagocytosis from increased oxygen consumption in human leukocytes. *J. Clin. Invest.* 46:786.
10. Zurier, R. B., S. Hoffstein, and G. Weissman. 1973. Mechanisms of lysosomal enzyme release from human leukocytes. I. Effect of cyclic nucleotides and colchicine. *J. Cell Biol.* 58:27.
11. Lehrer, R. I. 1973. Effects of colchicine and chloramphenicol on the oxidative metabolism and phagocytic activity of human neutrophils. *J. Infect. Dis.* 127:40.
12. Pesanti, E. L., and S. G. Axline. 1975. Colchicine effects on lysosomal enzyme induction and intracellular degradation in the cultivated macrophage. *J. Exp. Med.* 141:1030.
13. Lacy, P. E., S. L. Howell, D. A. Young, and C. J. Fink. 1968. New hypothesis of insulin secretion. *Nature (Lond.).* 219:1177.
14. Williams, J. A., and J. Wolff. 1970. Possible role of microtubules in thyroid secretion. *Proc. Natl. Acad. Sci. U. S. A.* 67:1901.
15. Gautvik, K. M., R. F. Hoyt, Jr., and A. H. Tashjian, Jr. 1973. Effects of colchicine and 2-Br-a-Ergocryptine-methane-sulfonate (CB 154) on the release of prolactin and

growth hormone by functional pituitary tumor cells in culture. *J. Cell. Physiol.* 82:401.

16. Redman, C. M., D. Banerjee, K. Howell, and G. L. Palade. 1975. Colchicine inhibition of plasma protein release from rat hepatocytes. *J. Cell Biol.* 66:42.
17. Poesner, A. M., and J. Bernstein. 1971. A possible role of microtubules in catecholamine release from the adrenal medulla: effects of colchicine vinca alkaloids and deuterium oxide. *J. Pharmacol. Exp. Ther.* 177:102.
18. Gillespie, E., R. J. Levine, and S. E. Malawista. 1968. Histamine release from rat peritoneal mast cells: inhibition by colchicine and potentiation by deuterium oxide. *J. Pharmacol. Exp. Ther.* 164:158.
19. Weisenberg, R. C., G. G. Borisy, and E. W. Taylor. 1968. The colchicine-binding protein of mammalian brain and its relation to microtubules. *Biochemistry.* 1:4466.
20. Bodel, P. 1974. Studies on the mechanism of endogenous pyrogen production. II. Role of cell products in the regulation of pyrogen release from blood leucocytes. *Infect. Immun.* 10:451.
21. Wilson, L., and M. Friedkin. 1967. The biochemical events of mitosis. II. The *in vivo* and *in vitro* binding of colchicine in grasshopper embryos and its possible relation to inhibition of mitosis. *Biochemistry.* 6:3126.
22. Bodel, P., and E. Atkins. 1967. Release of endogenous pyrogen by human monocytes. *N. Engl. J. Med.* 276:1002.
23. Dinarello, C. A., N. P. Goldin, and S. M. Wolff. 1974. Demonstration and characterization of two distinct human leukocytic pyrogens. *J. Exp. Med.* 139:1369.
24. Moore, D. M., S. F. Cheuk, J. D. Morton, R. D. Berlin, and W. B. Wood, Jr. 1970. Studies on the pathogenesis of fever. XVIII. Activation of leukocytes for pyrogen production. *J. Exp. Med.* 131:179.
25. Nordlund, J. J., R. K. Root, and S. M. Wolff. 1970. Studies on the origin of human leukocytic pyrogen. *J. Exp. Med.* 131:727.
26. Harris, E. D., Jr., and S. M. Krane. 1971. Effects of colchicine on collagenase in cultures of rheumatoid synovium. *Arthritis Rheum.* 14:669.
27. Harris, E. D., Jr., and S. M. Krane. 1972. An endopeptidase from rheumatoid synovial tissue culture. *Biochim. Biophys. Acta.* 258:566.
28. Robinson, D. R., H. Smith, M. B. McGuire, and L. Levine. 1975. Prostaglandin synthesis by rheumatoid synovium and its stimulation by colchicine. *Prostaglandins.* 10:67.
29. Temple, R., and J. Wolff. 1973. Stimulation of steroid secretion by anti-microtubular agents. *J. Biol. Chem.* 248:2691.
30. Werb, Z., and S. Gordon. 1975. Selective stimulation of secretion of elastase and collagenase from macrophages by colchicine and cytochalasin B. *J. Cell Biol.* 67:452a. (Abstr.)
31. Dillard, G. M., and P. Bodel. 1970. Studies on steroid fever. II. Pyrogenic and antipyrogenic activity in vitro of some endogenous steroids of man. *J. Clin. Invest.* 49:2418.
32. Chang, Y. H. 1975. Mechanism of action of colchicine. II. Effects of colchicine and its analogs on phagocytosis and chemotaxis *in vitro*. *J. Pharmacol. Exp. Ther.* 194:159.
33. Baggiolini, M. 1972. The enzymes of the granules of polymorphonuclear leukocytes and their functions. *Enzyme (Basel).* 13:132.
34. Lehrer, R. I., K. M. Ladra, and R. B. Hake. 1975. Nonoxidative fungicidal mechanisms of mammalian granulocytes: demonstration of components with candidacidal activity in human, rabbit, and guinea pig leukocytes. *Infect. Immun.* 11:1226.
35. Bodel, P., and M. Dillard. 1968. Studies on steroid fever. I. Production of leukocyte pyrogen in vitro by etiocholanolone. *J. Clin. Invest.* 47:107.

36. Malawista, S. M., and K. Bensch. 1969. Microtubular crystals in mammalian cells. *J. Cell Biol.* 40:95.
37. Bhisey, A. N., and J. J. Freed. 1971. Ameboid movement induced in cultured macrophages by colchicine or vinblastine. *Exp. Cell Res.* 64:419.
38. Bhisey, A. N., and J. J. Freed. 1971. Altered movement of endosomes in colchicine-treated cultured macrophages. *Exp. Cell Res.* 64:430.
39. Oliver, J. M., T. E. Ukena, and R. D. Berlin. 1974. Effects of phagocytosis and colchicine on the distribution of lectin-binding sites on cell surfaces. *Proc. Natl. Acad. Sci. U. S. A.* 71:394.
40. Bodel, P., and H. Miller. 1976. Pyrogen from mouse macrophages causes fever in mice. *Proc. Soc. Exp. Biol. Med.* 151:93.
41. Gordon, S., J. Todd, and Z. Cohn. 1974. In vitro synthesis and secretion of lysozyme by mononuclear phagocytes. *J. Exp. Med.* 139:1228.
42. Unkeless, J. C., S. Gordon, and E. Reich. 1974. Secretion of plasminogen activator by stimulated macrophages. *J. Exp. Med.* 139:834.
43. Werb, Z., and S. Gordon. 1975. Secretion of a specific collagenase by stimulated macrophages. *J. Exp. Med.* 142:346.
44. Wallace, S. L., B. Omokoku, and N. H. Ertel. 1970. Colchicine plasma levels: implications as to pharmacology and mechanism of action. *Am. J. Med.* 48:443.
45. Wallace, S. L., and N. H. Ertel. 1973. Plasma levels of colchicine after oral administration of a single dose. *Metabolism.* 22:749.
46. Dinarello, C. A., S. M. Wolff, S. E. Goldfinger, D. C. Dale, and D. W. Alling. 1974. Colchicine therapy for familial Mediterranean fever: a double-blind trial. *N. Engl. J. Med.* 291:934.