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Effect of Cyclic Adenosine 3',5'-Monophosphate Antagonists on Endotoxin-Induced Inhibition of Human Neutrophil Chemotaxis

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We reported previously that *Escherichia coli* endotoxin inhibited human neutrophil chemotaxis toward C5a. This effect of endotoxin was antagonized by anti-inflammatory steroids. We now report that dibutyryl cyclic adenosine 3',5'-monophosphate, prostaglandin E_1, isoproterenol, and cholera toxin also antagonize the suppression of chemotaxis by endotoxin. Each compound inhibited the effect of endotoxin in a dose-dependent fashion. To be effective, each compound except cholera toxin had to be present at the time of endotoxin challenge. Furthermore, propranolol blocked the protective effect of isoproterenol against endotoxin but not the protective effect of dibutyryl cyclic adenosine 3',5'-monophosphate or prostaglandin E_1. Dibutyryl cyclic guanosine 3',5'-monophosphate, adenosine 5'-monophosphate, phenylephrine, prostaglandin $F_{2\alpha}$, and carbachol did not modify the suppression of chemotaxis by endotoxin. Anti-inflammatory steroids and dibutyryl cyclic adenosine 3',5'-monophosphate are thought to stabilize phospholipids in certain cell membranes. This phospholipid-stabilizing action may contribute, at least in part, to the protective effect against endotoxin-mediated suppression of neutrophil chemotaxis.

The cell walls of gram-negative bacteria contain a lipopolysaccharide component called endotoxin. Endotoxin has a myriad of biological activities (4). Endotoxin can induce in humans and other species a shock syndrome which is often associated with disseminated intravascular coagulopathy. The pathogenesis of the shock syndrome and the accompanying coagulopathy may be related, at least in part, to the interaction of endotoxin with peripheral blood neutrophils (6, 26). For example, endotoxin has been shown in vitro to release endogenous pyrogens, lysosomal enzymes, and procoagulants from neutrophils (13). If these factors are released during the endotoxemia which accompanies gram-negative bacterial infection, they may contribute to the development of shock and disseminated intravascular coagulation.

Invasion of the host by gram-negative bacteria is in large part controlled by phagocytic cells such as neutrophils. To perform this function, neutrophils must migrate into the site of microbial invasion. We have shown recently that endotoxin in concentrations which do not affect cell viability causes a marked suppression of human neutrophil chemotaxis toward serum-derived chemotactic factors in vitro (14). Suppression of neutrophil movement by endotoxin may be important in vivo, since it could permit bacteria to grow and, therefore, produce more endotoxin in extravascular sites.

Methylprednisolone and other anti-inflammatory steroids prevent the suppression of neutrophil chemotaxis induced by endotoxin (16). Exactly how steroids protect the neutrophil against endotoxin is unclear. Studies employing other cell systems (8, 10, 12) suggest that the anti-inflammatory steroids inhibit the cellular synthesis of prostaglandins, which are known inflammatory mediators. The inhibition of prostaglandin synthesis appears to be due primarily to the suppression of arachidonic acid release from membrane phospholipids. More recently, Lapetina et al. (18) and Minkes et al. (21) reported that the thrombin-induced liberation of arachidonic acid from platelet phospholipids could be inhibited by dibutyryl cyclic adenosine 3',5'-monophosphate (dbcAMP). In view of these findings and to further understand the mechanisms regulating neutrophil responses to endotoxin, we examined what effect modulating neutrophil intracellular cAMP would have on endotoxin-mediated suppression of human neutrophil chemotaxis. We report here that agents which increase the level of cAMP in neutrophils, such as prostaglandin E_1 (PGE_1), isoproterenol,

cholera toxin, theophylline, and dbcAMP (2, 9, 24), antagonize the suppressive effect of endotoxin on neutrophil chemotaxis.

MATERIALS AND METHODS

Human peripheral blood leukocytes were isolated and examined for chemotaxis under agarose as described by Nelson et al. (22), with minor modifications (14). Briefly, leukocytes were isolated by sedimentation, washed, and resuspended in medium 199 (M199) (Grand Island Biological Co., Grand Island, N.Y.). The chemotaxis assay consisted of establishing a concentration gradient of chemotactic factor or an appropriate control substance in agarose and exposing the leukocyte suspension to these gradients. Under these conditions, neutrophils migrated under the agarose on the surface of a tissue culture dish. After 2.5 h of incubation, the cells were fixed to the dish, the agarose was removed, and neutrophil migration was measured with a projecting microscope (×45 magnification). The linear distance that the cells moved toward the control substance was subtracted from the distance the cells moved toward the chemotactic factor. This difference was called the chemotactic differential (CD).

The chemotactic factor in these experiments was partially purified C5a. This was prepared by gel filtration of zymosan-activated human serum on Sephadex G-75 as described previously (14). The control substance was M199, which was the medium used to dilute the C5a.

Preparation of endotoxin. Highly purified *Escherichia coli* endotoxin was a kind gift of Kenneth Johnson, National Research Council, Ottawa, Ontario, Canada. The endotoxin was prepared as described previously (17). Briefly, the bacteria were disrupted by grinding with glass beads, treated with bovine pancreatic ribonuclease and deoxyribonuclease, extracted with phenol (29), and finally purified by differential centrifugation. The purified preparation satisfied the following criteria as set forth by Johnson and Perry (17): (i) elution as single symmetrical peaks at the void volumes of Sepharose 4B and 6B gel filtration systems; (ii) absence of detectable ribose or deoxyribose; (iii) lack of absorption maxima in the 210- to 300-nm region of the ultraviolet spectra; (iv) presence of discrete O-antigen and corepolysaccharides derived from mild acetic acid hydrolysis of intact endotoxin preparations, as shown by the elution of symmetrical peaks from columns of Sephadex G-50; and (v) presence of a single absorption maximum at 460 nm when preparations were reacted in the carbocyanine dye assay for endotoxin.

A stock solution of endotoxin (100 μg/ml) was prepared in 0.9% saline and stored in aliquots at −70°C for future use. A fresh aliquot was used for each experiment. After thawing, the aliquot of endotoxin was sonicated for 20 s and then diluted to the desired concentration in M199. Endotoxin was not chemotactic, and it did not activate complement at concentrations between 1 ng/ml and 10 μg/ml. The latter was determined by measuring the residual hemolytic complement activity of pooled human serum after incubation of this serum for 60 min at 37°C with varying concentrations of endotoxin.

Preparation of drugs. Isoproterenol bitartrate, theophylline, cyclic N^6, $O^{2'}$-dibutyryl adenosine 3',5'-monophosphoric acid, propranolol-HCl, phenylephrine-HCl, carbachol, 5'-AMP, and cyclic N^2,O^2-dibutyryl guanosine 3',5'-monophosphoric acid were purchased from Sigma Chemical Co., St. Louis, Mo. PGE_1 was a kind gift from C. R. Pace-Asciak, prostaglandin $F_{2\alpha}$ was from the late R. P. Orange, and cholera toxin (Schwartz-Mann, Orangeburg, N.Y.) was from P. Raybin. All of these agents were prepared just before use and diluted in M199.

Treatment of Leukocytes. Leukocytes were suspended in M199 and assayed at a cell concentration of 6×10^7 neutrophils per ml. When the leukocytes were treated with endotoxin, the endotoxin was added to the cell suspension just before the start of the chemotaxis assay. When the leukocytes were treated with one of the above agents, the agent was added to the cell suspension immediately before the addition of endotoxin unless otherwise indicated. Leukocyte viability, as judged by trypan blue exclusion, exceeded 95% and was not altered by any of the agents used. In addition, concentrations of endotoxin up to 100 times those required to suppress chemotaxis toward C5a (1 ng/ml) did not inhibit neutrophil chemotaxis toward two bacterial chemotactic factors (*E. coli* and *Staphylococcus epidermidis*) or toward the synthetic chemotactic peptide *N*-formyl-methionyl-phenylalanine (14; unpublished data). Furthermore, neutrophil hexose monophosphate pathway activity and the release of the cytoplasmic marker lactate dehydrogenase were not affected by concentrations of endotoxin between 1 and 100 ng/ml (15).

Statistical analysis. The Student *t* test for small samples was used to determine *P* values.

RESULTS

Chemotaxis of human neutrophils toward C5a in the presence of varying concentrations of *E. coli* endotoxin is shown in Fig. 1. For these experiments, endotoxin was added to the leukocyte suspension at the beginning of the chemotaxis assay. Endotoxin caused a dose-dependent suppression of chemotaxis toward C5a. Neutrophil chemotaxis was inhibited by 50% when endotoxin was present at a concentration of 0.3 ng/ml. This inhibition increased to 85% in the presence of 1.0 ng of endotoxin per ml. Random migration was slightly, but significantly, inhibited in the presence of all concentrations of endotoxin (control, 4.3 ± 1.0 cm [mean ± standard deviation] versus 3.6 ± 0.4 cm; $P < 0.05$). Similar inhibition of neutrophil chemotaxis was observed when two commercially available endotoxins were tested. *E. coli* 0111-B4 endotoxin (Difco Laboratories, Detroit, Mich.) inhibited chemotaxis by 50% at a concentration of 0.02 μg/ml (14), and *Salmonella enteritidis* endotoxin (Difco) caused 50% inhibition at 0.08 μg/ml (unpublished data).

The effect of dbcAMP on the endotoxin-in-

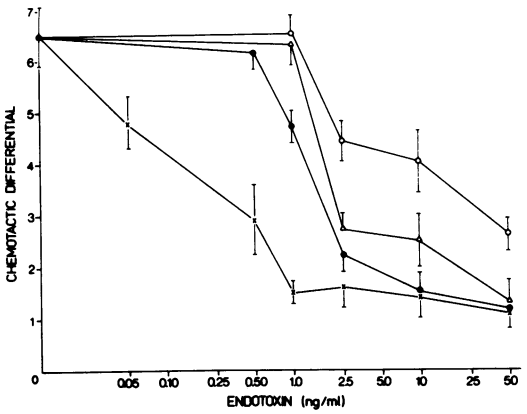

FIG. 1. *Effect of endotoxin on neutrophil chemotaxis toward partially purified C5a in the presence or absence of dbcAMP, PG E_1, or isoproterenol. Leukocytes (6 × 10^7 polymorphonuclear leukocytes per ml) were suspended in M 199. The CD was determined in the presence of endotoxin alone (x), of 10 μM isoproterenol and endotoxin (●), of 5 μM PGE_1 and endotoxin (△), and of 1.25 mM dbcAMP and endotoxin (○). The mean ± standard error of the mean for at least three experiments is shown.*

duced suppression of neutrophil chemotaxis is summarized in Fig. 1. For these experiments, dbcAMP was added to the leukocyte suspension at 37°C in a final concentration of 1.25 mM at 3 to 4 min before the addition of endotoxin. dbcAMP by itself had no effect on chemotaxis, but it caused a significant shift to the right in the endotoxin dose-response curve. Under these conditions, the 50% inhibitory concentration for endotoxin increased from 0.3 ng/ml in the absence of dbcAMP to 22 ng/ml.

The effect of PGE_1 on the endotoxin-induced suppression of neutrophil chemotaxis is summarized in Fig. 1. In these experiments, PGE_1 was added to the leukocytes in a final concentration of 5 μM at 3 to 4 min before the addition of endotoxin. PGE_1 by itself had no effect on chemotaxis, but it caused a significant shift to the right in the endotoxin dose-response curve. Under these conditions, the 50% inhibitory concentration for endotoxin increased from 0.3 to 2.2 ng/ml.

The effect of isoproterenol is also summarized in Fig. 1. Isoproterenol was added to the leukocytes in a final concentration of 10 μM at 3 to 4 min before the addition of endotoxin. Isoproterenol by itself had no effect on chemotaxis, but it caused a significant shift to the right in the endotoxin dose-response curve (Fig. 1). In the presence of isoproterenol, 50% inhibitory concentration for endotoxin increased from 0.3 to 1.7 ng/ml.

Increasing concentrations of dbcAMP were used to determine whether dbcAMP modified the endotoxin effect in a dose-dependent fashion. Table 1 shows that 1.25 mM dbcAMP, in the presence of 1 ng of endotoxin per ml (CD, 6.5 ± 0.4) completely prevented the effect of endotoxin (CD, 1.5 ± 0.2). dbcAMP at 0.5 mM was about half as effective (CD, 3.9 ± 0.4), whereas 2.5 mM dbcAMP (CD, 5.2 ± 0.4) was higher than the optimal concentration required to antagonize the effect of endotoxin. dbcAMP alone at concentrations of 2.5 mM or higher inhibited neutrophil chemotaxis (data not shown).

For dbcAMP to be maximally effective it had to be added to the leukocyte suspension just before the addition of endotoxin. When dbcAMP was added at 5 min or more after the endotoxin, the protective effect of dbcAMP was diminished.

The specificity of dbcAMP in preventing endotoxin-induced suppression of chemotaxis was examined. Two agents were studied: 5'-AMP and dibutyryl cyclic guanosine monophosphate. Neither of these nucleotides at concentrations of 0.25 to 2.5 mM inhibited neutrophil chemotaxis (CD, 6.6 and 6.3, respectively). Furthermore, in the presence of 1 ng of endotoxin per ml, neither 5'-AMP at concentrations of 0.25 to 2.5 mM (CD, 1.9 ± 0.5) nor dibutyryl cyclic guanosine monophosphate at concentrations of 0.05 to 2.5 mM (CD, 1.5 ± 0.3) influenced the inhibitory effect of endotoxin on neutrophil chemotaxis.

Increasing concentrations of PGE_1 were examined to determine whether PGE_1 modified the endotoxin effect in a dose-dependent fashion.

TABLE 1. *Effect of PGE_1 and dbcAMP on endotoxin-induced suppression of neutrophil chemotaxis*[a]

Presence of endotoxin (1 ng/ml)	Drug	Concn of drug	CD
−	None		6.5 ± 0.3[b]
+	None		1.5 ± 0.2
−	dbcAMP	0.25–1.25 mM	6.2 ± 0.3
+		0.25 mM	2.5 ± 0.5
+		0.5 mM	3.9 ± 0.4
+		1.25 mM	6.5 ± 0.4
+		2.5 mM	5.2 ± 0.4
−	PGE_1	0.05–5 μM	6.9 ± 0.3
+		0.05 μM	1.2 ± 0.4
+		0.5 μM	4.3 ± 0.3
+		5 μM	6.1 ± 0.4

[a] Leukocytes (6 × 10^7 polymorphonuclear leukocytes per ml) were suspended in M199. Immediately before assay, dbcAMP or PGE_1 and/or endotoxin was added to the leukocyte suspension in the final concentrations indicated. The chemotactic stimulus was partially purified C5a.

[b] Mean of at least three experiments ± standard error of the mean.

Table 1 shows that 5 μM PGE_1, in the presence of 1 ng of endotoxin per ml completely prevented the effect of endotoxin. PGE_1 at 0.5 μM was less effective, and at 0.05 μM it had no effect. For maximal effect, PGE_1 had to be added to the leukocyte suspension before the addition of endotoxin.

In contrast to PGE_1, prostaglandin $F_{2\alpha}$, which does not increase the level of cAMP in neutrophils (9, 24), did not modify the inhibiting effect of endotoxin on neutrophil chemotaxis when used at concentrations of 0.01 to 5 μM (CD, 1.6 ± 0.3).

The effect of various concentrations of isoproterenol were also studied. Table 2 shows that 1 μM isoproterenol in the presence of 1 ng of endotoxin per ml was nearly as effective as 50 μM isoproterenol in modifying the effect of endotoxin. At 0.1 μM, isoproterenol was without effect. The protective effect of 10 μM isoproterenol was prevented when the beta-receptor blocking agent propranolol (0.1 mM) was added to the leukocyte suspension before the addition of isoproterenol (for 10 μM isoproterenol plus 1 ng of endotoxin per ml, CD = 4.7 ± 0.3; for 0.1 mM propranolol plus 10 μM isoproterenol plus 1 ng of endotoxin per ml, CD = 2.0 ± 0.5). Propranolol (0.1 mM) alone had no effect on chemotaxis (CD, 7.5 ± 0.5). In contrast, propranolol did not modify the protective effect of either PGE_1 (CD, 7.5 ± 0.5) or dbcAMP (CD, 6.4 ± 0.3).

Non-beta-receptor agonists, such as the alpha-adrenergic agent phenylephrine and the acetylcholine analog carbachol, were also tested. Phenylephrine alone (0.1 to 50 μM) increased neutrophil chemotaxis (CD, 9 ± 0.5). Carbachol alone (0.1 to 50 μM) had no effect on chemotaxis (CD, 7.2 ± 0.6). In the presence of either phenylephrine or carbachol, suppression of neutrophil chemotaxis mediated by endotoxin (1 ng/ml) was unaffected (CD, 1.1 and 1.7, respectively).

The effect of various concentrations of cholera toxin (1 to 500 ng/ml) was examined. This agent at 10 ng/ml did not affect chemotaxis in the absence of endotoxin. However, in the presence of endotoxin it increased chemotaxis and increased the 50% inhibitory concentration for endotoxin from 0.3 to 2.8 ng/ml (data not shown). Table 3 shows that for optimal protection against endotoxin the leukocytes had to be incubated for 60 to 90 min at 37°C with cholera toxin before the addition of endotoxin. When leukocytes were preincubated with 10 ng of cholera toxin per ml for 30 min or less, very little protection against the inhibitory effect of 1 ng of endotoxin per ml was observed. In contrast, preincubation of the leukocytes with cholera toxin for 90 min completely prevented the effect of endotoxin on chemotaxis (CD, 6.2 ± 0.3; for the control without endotoxin, CD = 6.5 ± 0.3).

The effect of theophylline on the endotoxin-induced suppression of neutrophil chemotaxis was also examined. This drug alone, at concentrations of 0.5 to 2.5 mM, had no effect on chemotaxis (CD, 6.4 ± 0.4). However, 2.5 mM theophylline largely prevented the inhibitory effect of 1 ng of endotoxin per ml (CD 5.2 ± 0.4; for endotoxin alone, CD = 1.5 ± 0.2).

In all of the above experiments, the random migration of the neutrophils was slightly suppressed by endotoxin, and this effect was not altered by the drugs tested.

TABLE 2. *Effect of isoproterenol on endotoxin-induced suppression of neutrophil chemotaxis*[a]

Presence of endotoxin (1 ng/ml)	Drugs	Concn of drug (μM)	CD
−	None		6.5 ± 0.3[b]
+	None		1.5 ± 0.2
−	Isoproterenol	0.1–10	6.3 ± 0.4
+		0.1	2.0 ± 0.6
+		1	4.2 ± 0.5
+		10	4.7 ± 0.3
+		50	5.3 ± 0.6

[a] Leukocytes (6 × 10^7 polymorphonuclear leukocytes per ml) were suspended in M199. Immediately before assay, isoproterenol and/or endotoxin was added to the leukocyte suspension in the final concentrations indicated. The chemotactic stimulus was partially purified C5a.

[b] Mean of at least three experiments ± standard error of the mean.

TABLE 3. *Effect of preincubation time with cholera toxin on neutrophil chemotaxis in the presence of endotoxin*[a]

Preincubation time (min)	CD
0	1.9 ± 0.3[b]
30	2.7 ± 0.4
60	5.0 ± 0.3
90	6.2 ± 0.3

[a] Leukocytes (6 × 10^7 polymorphonuclear leukocytes per ml) were suspended in M199 containing 10 ng of cholera toxin per ml and incubated at 37°C for various times, as indicated. Then endotoxin (1 ng/ml) was added to the leukocyte suspension, and the leukocytes were assayed for chemotaxis. The chemotactic stimulus was partially purified C5a.

[b] Mean of at least three experiments ± standard error of the mean.

DISCUSSION

Neutrophil chemotaxis is important for host defense against bacterial infection. Endotoxin is a factor derived from gram-negative bacteria which can suppress neutrophil chemotaxis in vivo (27) and in vitro (14). Our findings in vitro

(14) suggested that the inhibitory effect of endotoxin did not require another cell type. Furthermore, inhibition was not dependent on the extracellular release of a diffusible substance, such as neutrophil immobilizing factor (7).

We report here that dbcAMP, PGE_1, isoproterenol, theophylline, and cholera toxin can antagonize the inhibition of chemotaxis by endotoxin. This is indicated by the shift to the right in the endotoxin dose-response curves in the presence of the above compounds (Fig. 1). Furthermore, these compounds acted in a dose-dependent fashion.

PGE_1, dbcAMP, isoproterenol, cholera toxin, and theophylline, at the concentrations which we employed in this study, have been shown to increase the intracellular level of cAMP in neutrophils (2, 9, 24). It is likely that the ability of these five structurally different compounds to antagonize the suppression of chemotaxis by endotoxin is associated with elevated levels of neutrophil cAMP. This conclusion is supported by the finding that 5'-AMP, dibutyryl cyclic guanosine monophosphate, and prostaglandin $F_{2\alpha}$, which does not increase the level of neutrophil cAMP (9, 24), were not effective against endotoxin (see above).

In neutrophils, the stimulation of beta receptors by isoproterenol is associated with increased levels of cAMP (9, 24). Propranolol, a beta-receptor antagonist, blocked the protective effect of isoproterenol but not that of dbcAMP or PGE_1 (see above). Furthermore, the alpha-adrenergic agent phenylephrine did not modify the effect of endotoxin. These findings suggest that the effect of isoproterenol was related to its interaction with beta receptors.

Theophylline is thought to increase neutrophil cAMP, by inhibiting cAMP phosphodiesterase (24). When theophylline was combined with isoproterenol, a synergistic increase of cAMP in rabbit neutrophils was observed (24). In our experiments, a high concentration of theophylline (2.5 mM) modified the effect of endotoxin on chemotaxis. However, we did not consistently observe a synergistic effect against endotoxin when suboptimal concentrations of theophylline were combined with either isoproterenol or PGE_1 (data not shown). Our failure to demonstrate a synergistic effect may have been because theophylline has only a marginal effect on human leukocyte cAMP levels (2), in contrast to its more potent effect on rabbit neutrophil cAMP (24).

All of the agents which we used to elevate neutrophil cAMP levels are also reported to inhibit human neutrophil chemotaxis in the Boyden chamber assay (5, 11). With the agarose chemotaxis assay we have observed similar results. For example, isoproterenol (100 to 500 μM), dbcAMP (5 to 20 mM), and PGE_1 (25 to 250 μM) inhibited chemotaxis by approximately 25 to 60%. Previous reports regarding the effect of PGE_1 or PGE_2 differ. Hill et al. (11) reported that both agents inhibited neutrophil chemotaxis at a concentration of 1 μM. However, Van Epps et al. (28) recently reported that low concentrations (10^{-9} to 10^{-6} M) of PGE_2 enhanced neutrophil migration, whereas higher concentrations (10 to 100 μM) inhibited migration. Hill et al. (11) also reported that cholera toxin at 1 and 10 ng/ml inhibited chemotaxis. In our studies (unpublished data) and those of Bergman et al. (1), who also used the agarose chemotaxis assay, 500 to 1,000 ng of cholera toxin per ml was required to inhibit chemotaxis by approximately 30%. Some of these differences may be due to the use of different assays to measure chemotaxis. Our observations demonstrate that adenyl cyclase agonists at concentrations which were too low to inhibit neutrophil chemotaxis could prevent endotoxin-induced suppression of chemotaxis.

To obtain consistent protection against the effect of endotoxin, PGE_1, dbcAMP, isoproterenol, and theophylline had to be added to the leukocytes at the same time as, or a few minutes before, the addition of endotoxin. This suggests that the level of cAMP in the neutrophils had to be elevated before the interaction of endotoxin with the cells and that cAMP may, in some way, make the neutrophils relatively refractory to the suppression of chemotaxis by endotoxin. This hypothesis is best supported by the cholera toxin experiments. This agent did not protect against endotoxin after a 30-min preincubation. However, it was very effective against endotoxin when the preincubation was 60 to 90 min. This may be explained by the finding that cholera toxin has little effect on human neutrophil cAMP during the first 30 min of incubation (2). After this time, cAMP increases and peaks at 90 min.

Anti-inflammatory steroids also antagonize the suppression of human neutrophil chemotaxis by endotoxin (16). Logsdon et al. (19) reported that hydrocortisone stimulates adenyl cyclase activity in human leukocytes, and Parker et al. (23) showed that this steroid could increase cAMP in human lymphocytes. These findings could explain the similar effect of anti-inflammatory steroids and cAMP agonists against the suppression of chemotaxis by endotoxin.

Anti-inflammatory steroids are reported to inhibit the liberation of arachidonic acid from the phospholipids of various cells (8, 10, 12). More

recently, dbcAMP and PGE_1 have been shown to inhibit the liberation of arachidonic acid from the phospholipids of platelets (18, 21). Thus, anti-inflammatory steroids and agents which increase intracellular cAMP appear to stabilize the phospholipids in certain cell membranes, perhaps by regulating the activity of phospholipase A_2. It is possible that these agents have a similar stabilizing action on neutrophil membrane phospholipids. Thus, endotoxin may inhibit chemotaxis by a perturbation of neutrophil membrane phospholipids. Anti-inflammatory steroids and agents which increase intracellular cAMP may therefore inhibit the effect of endotoxin by preventing the breakdown of membrane phospholipids.

The inhibition of human neutrophil chemotaxis by endotoxin is of potential importance to host defense because such an effect could indirectly permit the growth of gram-negative bacteria in extravascular sites, resulting in the continued production of endotoxin. The in vivo experiments of Conti et al. (3) support this hypothesis. They demonstrated with a rabbit model of staphylococcal cellulitis that the administration of endotoxin systemically or intradermally together with *Staphylococcus aureus* suppressed the infiltration of neutrophils into infected lesion. More recently, Smith et al. (25) and MacGuire and Wallis (20) observed that endotoxin markedly suppressed the migration of leukocytes into inflammatory sites in rats. Finally, Territo and Golde (27) reported that neutrophil chemotaxis decreased after the injection of a small dose of *Pseudomonas* endotoxin into humans.

The observations reported here indicate that it is possible to antagonize the suppression of chemotaxis by endotoxin in vitro. Studies such as this may further our understanding of the interaction of endotoxin and neutrophils and perhaps suggest ways of improving the treatment of endotoxemia.

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