Interaction of Polymorphonuclear Neutrophils with *Escherichia Coli*

EFFECT OF ENTEROTOXIN ON PHAGOCYTOSIS, KILLING, CHEMOTAXIS, AND CYCLIC AMP

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ABSTRACT Enterotoxigenic *Escherichia coli* are associated with noninflammatory diarrhea and stimulate adenylate cyclase activity of mammalian cells, thereby increasing intracellular cyclic adenosine 3',5'-monophosphate (cyclic AMP). Increased concentrations of cyclic AMP in polymorphonuclear neutrophils (PMN) inhibit phagocytosis, candidacidal activity, granule discharge, and chemotactic responsiveness. We examined the effect of enterotoxin on the interaction of human PMN with *E. coli*. Enterotoxigenic and nonenterotoxigenic strains, including serotypes of *E. coli* identical except for the presence or absence of the plasmid coding for enterotoxin production, were utilized. Enterotoxigenic and nonenterotoxigenic *E. coli*, tumbled with PMN, were phagocytized and killed (>97%) equally well, and these strains stimulated PMN hexose monophosphate shunt activity equivalently.

However, a chemotaxis assay under agarose demonstrated that filtrates of 10 enterotoxigenic strains were less chemotactic for PMN by 15±2% total migration or 46±1% directed migration, when compared with 6 nonenterotoxigenic strains ($P < 0.001$). Inactivation of the enterotoxin by heat (65°C for 30 min) or antibodies formed to *E. coli* enterotoxin eliminated the inhibitory effect of the enterotoxic filtrates for PMN chemotaxis. Addition of purified *E. coli* enterotoxin directly to the PMN decreased chemotaxis to *E. coli* filtrates by 32 ±2% ($P < 0.001$). These data suggest that the effect was due to the heat-labile enterotoxin. The phosphodiesterase inhibitor, 1-methyl-3-isobutylxanthine (0.1 mM), which potentiates effects due to an increase in intracellular cyclic AMP, further decreased total PMN migration (random plus directed) toward enterotoxic filtrates to 46% of that to nonenterotoxic filtrates ($P < 0.001$). Addition of cholera toxin (1 μg/ml), which is similar to *E. coli* enterotoxin, to the PMN inhibited total migration toward nonenterotoxic filtrates by 16 ±2% ($P < 0.001$). Exogenous dibutyryl cyclic AMP (2 mM) inhibited total PMN migration toward *E. coli* filtrates by 32% ($P < 0.001$). PMN intracellular cyclic AMP levels increased by 220% after 2 h of incubation with purified *E. coli* enterotoxin. The decreased chemotactic attractiveness of enterotoxic *E. coli* filtrates appears to be related to the ability of enterotoxin to increase cyclic AMP in PMN. Enterotoxin production by *E. coli* may be advantageous to the microbe by decreasing its chemotactic appeal for PMN.

INTRODUCTION

Certain *Escherichia coli* produce a heat-labile enterotoxin which appears to cause noninflammatory watery diarrhea by stimulating intestinal mucosal adenylate cyclase (1–7). This enterotoxin, like cholera toxin, can also activate adenylate cyclase in various mammalian cells, thereby increasing intracellular concentrations of cyclic adenosine 3',5'-monophosphate (cyclic AMP)[1]

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[1] *Abbreviations used in this paper:* CHO, Chinese hamster ovary; cyclic AMP, cyclic adenosine 3',5'-monophosphate; MIX, 1-methyl-3-isobutylxanthine; PMN, polymorphonuclear neutrophils; Tox$^+$, enterotoxigenic; Tox$^-$, nonenterotoxigenic.

and inducing characteristic alterations of cell morphology and function (8–11).

Increased intracellular cyclic AMP levels in polymorphonuclear neutrophils (PMN) inhibit phagocytosis, candidacidal activity (12, 13), granule discharge (14, 15), and chemotactic responsiveness (16). Therefore, enterotoxin production may be of benefit to the microorganism by increasing PMN intracellular cyclic AMP and altering the ability of the phagocyte to approach, ingest, and kill the microbes. We examined the interaction of human PMN with enterotoxigenic *E. coli* to determine the effect of enterotoxigenicity on the PMN-bacterial interaction.

METHODS

Bacteria and bacterial filtrates

Three enterotoxigenic [334 (015:H11), 711K12(P155)$_2$, and 408-3(078:H12)] and three nonenterotoxigenic [10405, 711K12, and 408-4(078:H12)] *E. coli* strains were used to evaluate phagocytosis and killing by PMN. These strains were also used in measuring PMN postphagocytic hexose monophosphate shunt activity. Culture filtrates for chemotaxis assays were obtained from ten enterotoxigenic strains of *E. coli* [334, 711K12(P155)$_2$, 408-3(078:H12), 19662, 9116, 17974, 19094, B2C(06:H16), B7A(0148:H28), and 19437] and five nonenterotoxigenic strains [10405, 711K12, 408-4(078:H12), 1624(0144:K?:H-), and 1272(0124:K72:H-)]. The paired strains 408-3 (078:H12) and 408-4(078:H12), as well as 711K12(P155)$_2$ and 711K12, are identical serotypes except that strains 408-3(078:H12) and 711K12(P155)$_2$ contain a plasmid coding for the production of heat-labile enterotoxin (3, 5, 17, 18). Also studied was *E. coli* strain B44, which produces only a heat-stable toxin (11, 19), and strains 1624 and 1272, which are invasive but nonenterotoxigenic.

To prepare bacterial culture filtrates, organisms were incubated for 24 h at 37°C in Media 199 (Microbiological Associates, Bethesda, Md.). The cultures were centrifuged at 2,000 *g* for 15 min, and the resulting supernatants were filtered through a 0.45-μm micropore filter (Millipore Corporation, Bedford, Mass.).

Purified E. coli enterotoxin

Heat-labile *E. coli* enterotoxin was purified essentially as previously described (20–22). Crude toxin was extracted using polymyxin B and further purified by chromatography on inactivated Affi-Gel 202 (Bio-Rad Laboratories, Richmond, Calif.) affinity chromatography, gel filtration on Ultro-gel AcA-44 (LKB), and passage through a P-150 (Bio-Rad) column. The resulting ultraviolet (UV)-absorbing peak that contained toxic activity, as demonstrated in the Chinese hamster ovary (CHO) cell assay (11), was dialyzed overnight against 0.1 M NH_4HCO_3 and lyophilized. This material gives a single band on polyacrylamide electrophoresis. Antiserum against *E. coli* toxin was prepared in rabbits against enterotoxin from enterotoxigenic *E. coli* strain 23505. This enterotoxin was partially purified by polymyxin release, $(NH_4)_2SO_4$ precipitation, and agarose-gel chromatography. The antiserum, designated antiserum 441, has been previously described (21), and it neutralizes the biologic effects of purified cholera toxin and *E. coli* enterotoxin in CHO cells at dilutions greater than 1:8 and 1:64, respectively (23). Enterotoxin was used in a concentration of 0.33 mg/ml for all enterotoxin experiments. Purified cholera toxin, prepared by Dr. R. A. Finkelstein (24), was provided by Dr. Carl E. Miller (National Institute of Allergy and Infectious Diseases, Bethesda, Md.).

Determination of enterotoxigenicity by CHO cell assay

Enterotoxin production by strains of *E. coli* was determined by quantitating morphological alterations of CHO cells as previously described (11). The characteristic change in CHO morphology, from epithelial to elongated forms, appears to be mediated by enterotoxin activation of adenylate cyclase and a corresponding increase in intracellular cyclic AMP. This is supported by the observations that elevated cyclic AMP concentrations coincide with observed changes in CHO morphology, by the fact that phosphodiesterase inhibition potentiates this observed morphological effect, and by the occurrence of the morphological elongation after the addition of exogenous dibutyryl cyclic AMP (25).

Microscopic observations of phagocytosis

Leukocyte monolayers were prepared as previously described (26). The interaction of PMN with toxigenic and nontoxigenic *E. coli* was observed on a 37°C air curtain stage with phase contrast microscopy. Giemsa-stained smears of PMN-*E. coli* interaction were also prepared and examined.

Phagocytosis of ^{14}C-labeled bacteria

Modification of the method of Downey and Diedrich (27) was employed. Toxigenic or nontoxigenic *E. coli* were cultured for 18 h in trypticase soy broth with 10 μCi/ml of ^{14}C-labeled amino acid mixture (New England Nuclear, Boston, Mass.) and then washed in saline solution. 5×10^8 bacteria/ml were added to 5×10^6 PMN/ml, which were tumbled at 37°C, and 0.2-ml samples were removed at 0, 5, 10, and 20 min. The samples were placed in 4 ml of iced Hanks' balanced salt solution (Microbiological Associates), with 10% fetal bovine serum and centrifuged at 100 *g* for 5 min at 4°C. The cell buttons were washed, digested with Protosol (New England Nuclear), and counted in a Beckman LS250 scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.).

Phagocytosis and leukocyte bactericidal activity

10 ml of heparinized venous blood from normal donors was sedimented for 60 min with an equal volume of 3% dextran. The supernatant fluid containing leukocytes was collected, erythrocytes remaining in the supernatant were lysed with iced distilled water, and tonicity was restored with hypertonic saline. Leukocytes were then centrifuged at 200 *g* for 12 min, and the resulting cell button was resuspended in Hanks' balanced salt solution with 10% autologous serum. 4 ml of suspension with 5×10^6 bacteria and 5×10^6 PMN/ml was tumbled at 37°C, and samples were removed at specified times for determination of total, supernatant, and sediment viable bacterial counts (28). PMN were omitted in some experiments to determine the effect of serum on bacterial viability. Experiments were done with (*a*) *E. coli* washed three times with saline, (*b*) whole culture suspensions, (*c*) washed nonenterotoxigenic *E. coli* with addition of 2 μg/ml purified cholera toxin to the PMN 1 h before the addition of the bacteria, (*d*) washed enterotoxigenic *E. coli* resuspended in filtrate with

or without enterotoxin, and (*e*) washed nonenterotoxigenic *E. coli* resuspended in filtrate with or without enterotoxin.

Determination of hexose monophosphate shunt activity of PMN

Oxidation of glucose by PMN, *E. coli*, and PMN plus *E. coli* via the hexose monophosphate shunt was determined using [1-^{14}C]glucose, as previously described (29).

Chemotaxis studies

A chemotaxis assay under agarose, as described by Nelson et al. (30), was used with agarose (Litex, Denmark; available from Accurate Chemical & Scientific Corp., Hicksville, N. Y.) replacing agarose Indubiose. Leukocyte suspensions containing PMN and mononuclear cells were utilized, and chemotaxis was measured after 2 h of incubation. Nelson et al. have shown that PMN make up the vast majority of migrating cells after this period of incubation. Each agarose chemotaxis plate consists of six triplets of wells arranged in radial fashion, with one experiment performed in each triplet. The distance from the outer edge of the well containing PMN to the leading border of migrating cells was measured in coded plates without knowledge of well contents, utilizing a ×40-μm eyepiece on a dissecting microscope.

PMN to filtrates and media. The following chemotaxis assays were run to determine migration toward enterotoxic (Tox$^+$) and nonenterotoxic (Tox$^-$) filtrates vs. Media 199 (Fig. 1): (*a*) PMN (center well) migrating to Tox$^-$ filtrates and to Media 199 (outer wells); (*b*) PMN (center well) migrating to Tox$^+$ filtrates and to Media 199 (outer wells). For these assays, chemotactic (directed) migration was determined by subtracting the distance moved toward the media control from the distance moved towards the bacterial filtrates.

PMN to filtrates. To compare migration to filtration from the same well of PMN, we set up the following assays (Fig. 2): (*a*) PMN (center well) migrating to Tox$^+$ and Tox$^-$ filtrates (outer wells); (*b*) PMN (center well) to heated (65°C for 30 min) Tox$^+$ and heated or nonheated Tox$^-$ filtrates (outer wells); (*c*) PMN (center well) to Tox$^+$ filtrates with antiserum to enterotoxin and Tox$^-$ filtrates with or without antiserum to enterotoxin (outer wells); and (*d*) PMN plus 0.1 mM 1-methyl-3-isobutylxanthine (MIX) (Aldrich Chemical Co., Inc., Milwaukee, Wis.) (center well) to Tox$^+$ and Tox$^-$ filtrates (outer wells). Results of these assays are expressed by considering total migration distance to Tox$^-$ filtrates as 100%.

PMN plus additions. To test additions to PMN migrating to the same chemotactic stimulus, we performed the following assays (Fig. 3): (*a*) PMN (outer well) and PMN plus cholera toxin (other outer well) to Tox$^-$ filtrate (center well); (*b*) PMN (outer well) and PMN plus dibutyryl cyclic AMP (other outer well) to Tox$^-$ filtrate or Media 199 (center well); (*c*) PMN (outer well) and PMN plus MIX (other outer well) to Tox$^-$

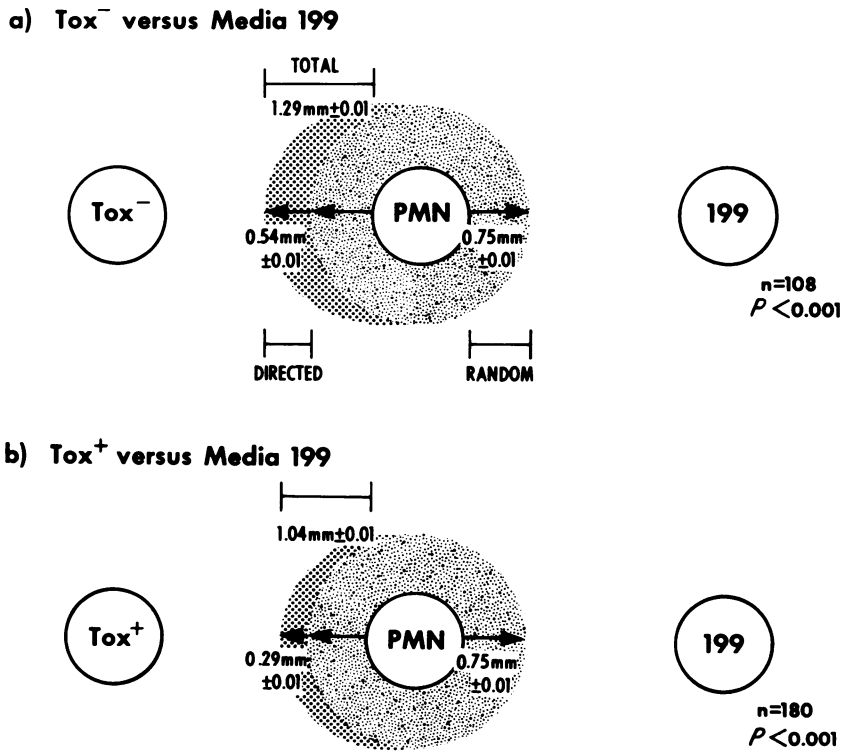

FIGURE 1 PMN migration toward *E. coli* filtrates and Media 199. Migration of PMN toward equidistant *E. coli* filtrates and Media 199 was quantitated after 2-h incubation. Random migration is the distance the cell front moves toward Media 199; directed (chemotactic) migration is the distance the cell front moves toward the filtrate minus the random migration. Migration distances are expressed as mean±SEM; n = number of observations; P values obtained with Student's t test using paired data.

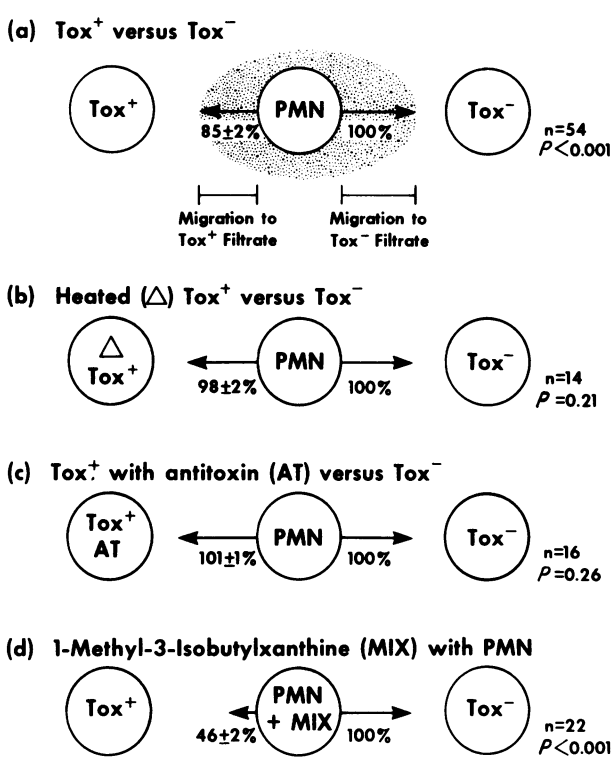

FIGURE 2 PMN migration toward *E. coli* filtrates. Migration of PMN toward two equidistant chemotactic stimuli was quantitated after a 2-h incubation. The migration distance toward Tox⁻ filtrate is considered to be 100%. Migration toward Tox⁺ filtrates is expressed as the mean±SEM percent of migration toward Tox⁻ filtrate.

filtrate or Media 199 (center well). Results of these assays are expressed by considering total migration distance of PMN without additions as 100%.

PMN plus purified E. coli enterotoxin. To study the effect of the purified enterotoxin, we performed the following experiments (Fig. 4): (*a*) PMN (center well) to Tox⁻ filtrate with or without purified *E. coli* enterotoxin (outer wells); (*b*) PMN with or without purified *E. coli* enterotoxin (outer wells) to Tox⁻ filtrate incubated for 2 h; (*c*) as *b*, but incubated for 4 h.

Effect of purified E. coli enterotoxin and MIX on PMN cyclic AMP concentrations

PMN were obtained by Ficoll-Hypaque separation (31) followed by dextran sedimentation and hypotonic lysis of the residual erythrocytes. 5×10^6 PMN (0.4–0.8 mg of protein) were tumbled at 37°C in 0.5 ml of Hanks' balanced salt solution without bicarbonate with purified *E. coli* enterotoxin (0.33 mg/ml), MIX (0.05 mM), both, or neither. Samples were run in duplicate in experiment 1 and in triplicate in experiment 2. In experiment 2, cell suspensions were incubated at 37°C for 15 min before any additions were made. After 2 h,incubations were terminated with the addition of 50 μl of 60% trichloroacetic acid. Samples were extracted, acetylated, and assayed for cyclic AMP with a radioimmunassay procedure (32), as described previously (33). Protein was determined by the method of Lowry et al. (34).

RESULTS

Microscopic observations of phagocytosis

Examination of stained smears and of living preparations with phase contrast microscopy suggested that most cell-associated *E. coli* were internalized.

Phagocytosis of ^{14}C-labeled bacteria

Both toxigenic and nontoxigenic *E. coli* became cell associated to a similar rate and degree (Fig. 5), suggesting equivalent phagocytosis by PMN.

Phagocytosis and killing

The *E. coli* strains utilized were not killed by serum alone. Enterotoxigenic and nonenterotoxigenic *E. coli* were removed from the supernate at a similar rate and to a similar degree after incubation with PMN. After 60 min, 98.0±1.7% (n = 5) of the Tox⁻ *E. coli* and 98.9

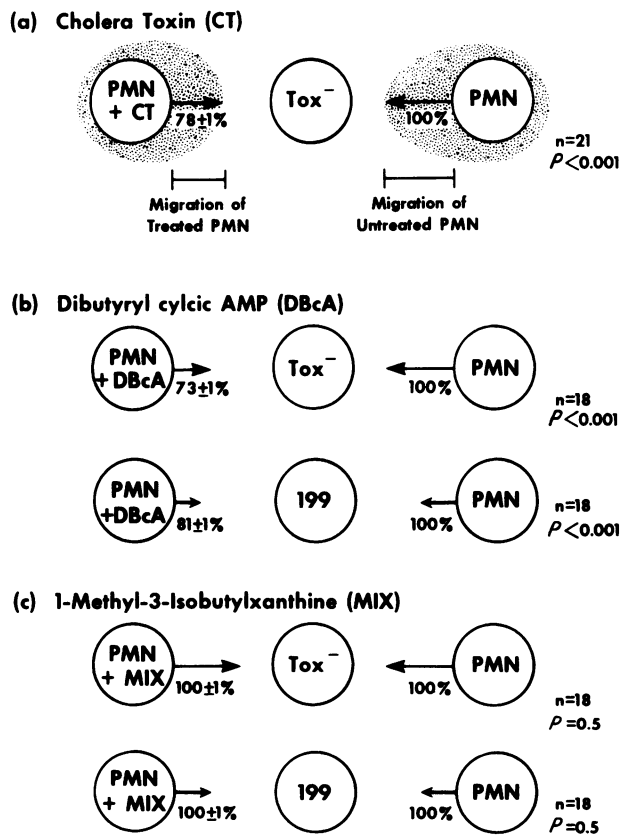

FIGURE 3 Effects of additives on PMN migration toward *E. coli* filtrates and Media 199. Migration of PMN toward Tox⁻ filtrates was compared with migration of PMN plus either cholera toxin (1 μg/ml), dibutyryl cyclic AMP (2 mM), or MIX (0.1 mM) toward the same stimulus. PMN were pretreated with cholera toxin for 90 min. The migration distance of the untreated PMN is considered to be 100%.

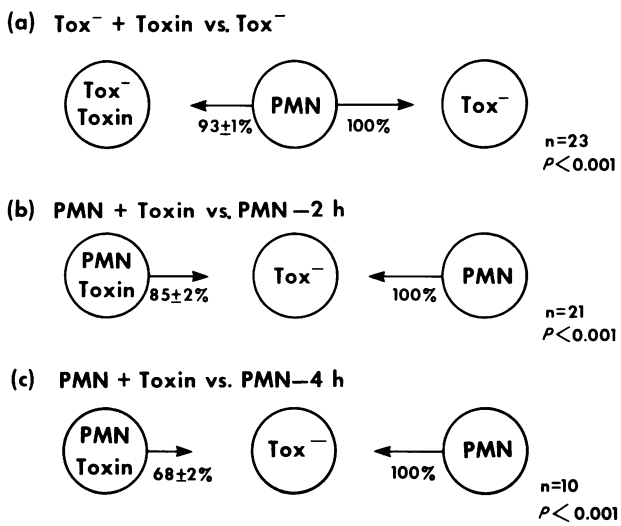

FIGURE 4 Effect of purified *E. coli* enterotoxin on PMN migration. Migration of PMN toward Tox⁻ filtrates (migration distance considered as 100%) was compared with migration toward Tox⁻ filtrate plus purified enterotoxin. (*b*) and (*c*) depict experiments done with the addition of purified enterotoxin directly to the PMN. Migration distance of the untreated PMN is considered to be 100%.

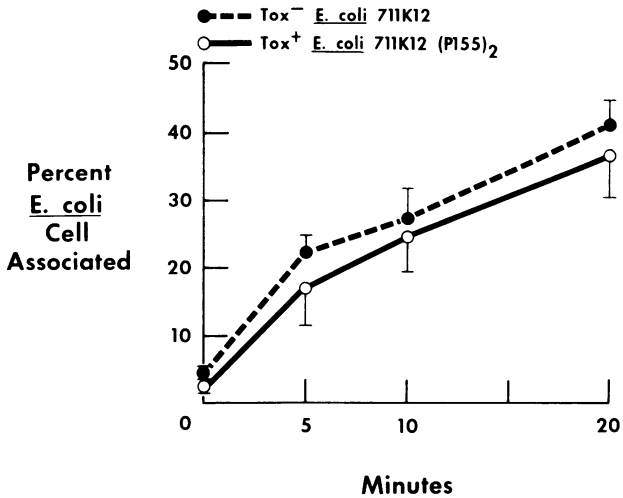

FIGURE 5 Phagocytosis of ^{14}C-labeled bacteria. The percent of ^{14}C-labeled toxigenic and nontoxigenic *E. coli* associated with PMN is depicted at various incubation times. Mean ±SEM for nine determinations at each time period are shown. The two curves can be considered identical by comparison of the regression lines $P = 0.42$ (35).

±1.0% ($n = 5$) of the Tox⁺ *E. coli* had been removed from the supernate and 97.6±1.5% ($n = 5$) and 98.7 ±0.7% ($n = 5$), respectively, had been killed. By 120 min, 99.3±0.5% ($n = 5$) of the Tox⁻ *E. coli* and 99.5 ±0.4% ($n = 5$) of the Tox⁺ *E. coli* had been removed from the supernate and 98.1±1.5% ($n = 5$) and 99.3 ±0.5% ($n = 5$), respectively, had been killed. These differences are not significant and thus PMN ingest and kill toxigenic and nontoxigenic *E. coli* equally well in this tumbling system. Suspension of the bacteria in culture media or incubation of PMN for 1 h with cholera toxin (2 μg/ml) or purified *E. coli* enterotoxin (0.33 μg/ml) did not alter the results.

Bacterial stimulation of PMN hexose monophosphate shunt activity.

There were equivalent increases in hexose monophosphate shunt activity after incubation with both the enterotoxigenic and nonenterotoxigenic strains of *E. coli*, suggesting equivalent ingestion and PMN stimulation (Table I).

Chemotaxis

Both enterotoxigenic and nonenterotoxigenic strains of *E. coli* showed equivalent growth rates over an 18-h growth period, each reaching 1.8–2.0 × 10^9 colony forming units/ml. Thus filtrates were prepared from cultures with equivalent growth.

PMN to filtrates and media. PMN total migration toward Tox⁻ filtrates was 1.29±0.01 mm and toward Tox⁺ filtrates was 1.04±0.01 mm. Since migration toward Media 199 (random migration) was 0.75±0.01 mm, chemotactic migragtion toward Tox⁻ filtrates was 0.54±0.01 mm and toward Tox⁺ filtrates was 0.29±0.01 mm ($P < 0.001$). Chemotactic migration toward Tox⁺ filtrates was thus 46% less than that toward Tox⁻ filtrates (Fig. 1).

PMN to filtrates. PMN migration toward filtrates from paired enterotoxigenic and nonenterotoxigenic *E. coli* strains also showed a significant decrease in total (random plus chemotactic) migration toward the en-

TABLE I
Hexose Monophosphate Shunt Activity

	Experiment 1	Experiment 2	Experiment 3
		$^{14}CO_2$cpm	
E. coli (toxigenic), no PMN	4,055	8,697	5,637
E. coli (nontoxigenic), no PMN	4,281	6,397	4,761
PMN	2,224	1,732	1,271
PMN plus *E. coli* (toxigenic)	7,903	14,097	13,337
PMN plus *E. coli* (nontoxigenic)	9,676	12,225	12,541

[1-^{14}C]glucose oxidation by 5 × 10^8 *E. coli*, 10^7 PMN, and 5 × 10^8 *E. coli* plus 10^7 PMN. Both enterotoxigenic *E. coli* [408-3(078:H12)] and nonenterotoxigenic *E. coli* [408-4 (078:H12)] caused an equivalent increase in [1-^{14}C]glucose oxidation by PMN.

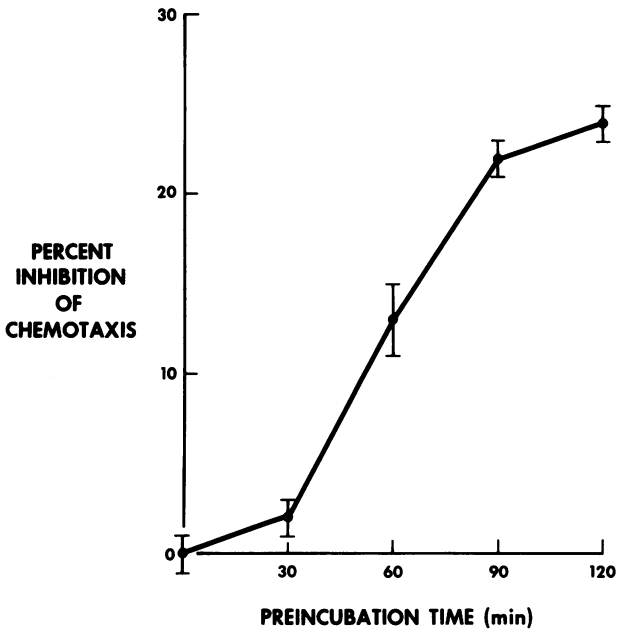

FIGURE 6 Time course of cholera toxin effect on PMN chemotaxis. PMN were preincubated with cholera toxin (1 μg/ml), and then migration toward nonenterotoxigenic *E. coli* filtrates was measured. Results were compared with PMN incubated without cholera toxin. The percent inhibition of chemotaxis is plotted against incubation time. Mean±SEM for 13–21 determinations at each time period are shown. Percent inhibition of chemotaxis = 1 − (Migration distance of PMN + CT/Migration distance of PMN).

terotoxic filtrate from each pair (Fig. 2 *a*). Migration toward the nonenterotoxic filtrates was considered to be 100% for each pair. Invasive *E. coli* and *E. coli* that produce only the heat-stable toxin were as chemotactic for PMN as were nonenterotoxigenic *E. coli*. The inhibition of PMN chemotaxis by enterotoxic filtrates was eliminated by heating at 65°C for 30 min to destroy the heat-labile enterotoxin (Fig. 2 *b*) and by the addition of antisera prepared against *E. coli* enterotoxin (Fig. 2 *c*). Neither heat nor antiserum prepared against *E. coli* enterotoxin influenced migration to Tox⁻ filtrates.

Addition of the phosphodiesterase inhibitor, MIX, to the PMN (Fig. 2 *d*), in order to potentiate any effect due to an increase in intracellular concentrations of cyclic AMP, resulted in a threefold increase in inhibition of PMN chemotaxis toward enterotoxic filtrate, compared with chemotaxis toward nonenterotoxic filtrate. MIX alone did not affect migration to Tox⁻ filtrates or to Media 199 (Fig. 3 *c*).

PMN plus additions. Addition of cholera toxin (1 μg/ml) directly to the PMN well decreased migration of PMN to *E. coli* filtrates after a lag period (Fig. 3 *a*). Although addition of cholera toxin (1 μg/ml) to the nonenterotoxic filtrate did not diminish migration of PMN, incubation of cholera toxin in the wells for 4 h before addition of PMN and the Tox⁻ filtrates decreased migration by 13±2% ($n = 18, P < 0.001$). The relationship of inhibition of chemotaxis and time of incubation with cholera toxin is shown in Fig. 6. The effect could not be eliminated by washing off the cholera toxin after preincubation with PMN for only 5 min.

Addition of exogenous dibutyryl cyclic AMP to PMN resulted in marked inhibition of PMN movement toward a chemotactic stimulus and also inhibited random movement (Fig. 3 *b*).

PMN plus purified E. coli enterotoxin. Addition of the purified *E. coli* enterotoxin directly to the Tox⁻ filtrates resulted in a 7% diminution of chemotaxis. When added directly to the PMN with a 2-h or 4-h incubation, there was 15% and 32%, respectively, diminished total migration towards Tox⁻ filtrates (Fig. 4).

Effect of purified E. coli enterotoxin on PMN cyclic AMP

Incubation with enterotoxin significantly increased PMN cyclic AMP levels, and this was amplified with the addition of MIX (Table II).

DISCUSSION

E. coli enterotoxin stimulates the adenylate cyclase activity of mammalian cells and subsequently increases intracellular cyclic AMP. Intracellular cyclic nucleo-

TABLE II
Effect of E. coli Enterotoxin and MIX on Cyclic AMP Levels in Incubations of Polymorphonuclear Leukocytes

Addition	Cyclic AMP
	pmol/mg protein
Experiment 1	
None	5.97/3.91
Enterotoxin	14.65/13.00
MIX	6.69/9.79
Enterotoxin + MIX	32.76/31.28
Experiment 2	
None	2.63±0.31
Enterotoxin	4.28±0.11*
MIX	5.04±0.79*
Enterotoxin + MIX	10.07±0.51*‡

5×10^6 PMN were incubated with or without 0.05 mM MIX and 0.33 mg/ml purified *E. coli* enterotoxin. In experiment 2, cells were preincubated for 15 min before additions. After 120-min incubation, cyclic AMP was determined by radioimmune assay. Values in experiment 1 are from duplicate incubations and values in experiment 2 are means±SEM of triplicate incubations.

* Significantly different from "none" ($P < 0.03$).

‡ Significantly different from enterotoxin alone and MIX alone ($P < 0.002$).

tides may function as an important modulator of PMN function. Increased intracellular concentrations of cyclic guanosine 3′,5′-monophosphate (cyclic GMP) enhance chemotaxis (36, 37), while increased intracellular concentrations of cyclic AMP inhibit rabbit PMN chemotaxis (16). Studies also suggest that the ability of PMN to phagocytize, degranulate, and kill may be inhibited by cyclic AMP (12–15). We noted that PMN were able to ingest and kill enterotoxigenic and nonenterotoxigenic *E. coli* equally well. The phagocytosis system used entails tumbling bacteria and PMN together so that active movement by PMN plays a relatively minor role.

In contrast to the lack of effect of enterotoxin upon phagocytosis and killing in suspensions of PMN, there was a consistent impairment of chemotaxis of PMN toward enterotoxigenic *E. coli* filtrates. One possible explanation for this effect is that chemotactic factors are less active in the Tox$^+$ filtrates. A second possibility is that a substance in the Tox$^-$ filtrates destroys a chemotaxis inhibitor. Against both of these possibilities are the observations that chemotaxis to Tox$^+$ filtrates is restored to that of Tox$^-$ filtrates by inactivating the heat-labile enterotoxin with specific antiserum or heat. Furthermore, chemotaxis inhibition by enterotoxin is enhanced by MIX, an effect that is best explained by the action of enterotoxin upon PMN adenylate cyclase.

Our data strongly suggest that inhibition of PMN migration toward Tox$^+$ filtrates is due to the enterotoxin itself. This is substantiated by the following observations: (*a*) Filtrates from enterotoxigenic *E. coli* are consistently less chemotactic for human PMN than are nonenterotoxigenic *E. coli* filtrates prepared from serotypes of *E. coli* identical except for the plasmid coding for enterotoxin. (*b*) Inactivation of the heat-labile enterotoxin at 65°C for 30 min, or inactivation by antisera to enterotoxin, eliminates the inhibition of chemotaxis noted with Tox$^+$ filtrates. (*c*) Addition of purified *E. coli* enterotoxin to nonenterotoxic filtrates or directly to PMN significantly decreases PMN chemotactic migration.

The inhibition of PMN chemotaxis by enterotoxin appears to be due to an increase in intracellular cyclic AMP induced by the enterotoxin because of the following: (*a*) The reports of others (16) and our observations with the direct addition of dibutyryl cyclic AMP to PMN show that increased cyclic AMP concentrations are associated with decreased chemotactic responsiveness. (*b*) The time course of cholera toxin effect on PMN chemotaxis is consistent with the temporal effects of cholera toxin on adenylate cyclase activation in intestinal tissue (38) and on cyclic AMP increases in other tissues (6, 9, 10, 11); (the addition of cholera toxin to wells containing Tox$^-$ filtrate inhibits PMN chemotaxis only after sufficient time is allowed for the larger cholera toxin molecule [84,000 daltons vs. 23,500 daltons] to diffuse through the agar [39, 40]). (*c*) The addition of a phosphodiesterase inhibitor decreased migration to Tox$^+$ filtrates but not to Tox$^-$ filtrates, probably by potentiating increased intracellular cyclic AMP induced by the enterotoxin. (*d*) Finally, we noted significant increases in PMN cyclic AMP concentrations after incubation with purified *E. coli* enterotoxin. Furthermore, cyclic AMP effects in PMN incubated with enterotoxin are enhanced by the phosphodiesterase inhibitor MIX. The use of purified cell preparations in these studies indicates that the cylic AMP increases occur specifically in PMN.

In conclusion, the heat-labile enterotoxin produced by *E. coli* may be advantageous to the microbe by decreasing its chemotactic appeal for PMN.

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