INFECTION AND IMMUNITY, Feb. 1994, p. 639–643
0019-9567/94/$04.00+0

Vol. 62, No. 2

Effect of Tracheal Cytotoxin from *Bordetella pertussis* on Human Neutrophil Function In Vitro

D. R. CUNDELL,[1] K. KANTHAKUMAR,[1] G. W. TAYLOR,[2] W. E. GOLDMAN,[3] T. FLAK,[3] P. J. COLE,[1] AND R. WILSON[1]*

Host Defence Unit, Department of Thoracic Medicine, National Heart and Lung Institute, London SW3 6LR,[1] and Department of Clinical Pharmacology, Royal Postgraduate Medical School, London W12 ONN,[2] United Kingdom, and Department of Molecular Microbiology, School of Medicine, Washington University, St. Louis, Missouri[3]

Received 26 July 1993/Returned for modification 24 September 1993/Accepted 22 November 1993

The infiltration of neutrophils which phagocytose and kill microorganisms is an important defense mechanism against infections of the airways. *Bordetella pertussis* is a human respiratory pathogen which colonizes ciliated epithelium, causing whooping cough. We have investigated the effects of the peptidoglycan fragment tracheal cytotoxin (TCT) of *B. pertussis* on human neutrophil function in vitro. TCT (10^{-6} to 10^{-8} M) was toxic for human neutrophils, as measured by lactate dehydrogenase release and levels of intracellular ATP. TCT (10^{-9} to 10^{-15} M) did not stimulate neutrophil migration or chemiluminescence and did not affect neutrophil phagocytosis. Incubation of neutrophils for 20 min with TCT (10^{-9} to 10^{-11} M) significantly inhibited ($P < 0.05$) their subsequent migration toward the chemotactic factor *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP; 10^{-9} M). Incubation of neutrophils for 20 min with TCT (10^{-9} to 10^{-15} M) significantly inhibited ($P < 0.05$) chemiluminescence stimulated by FMLP (10^{-5} M). TCT (10^{-6} to 10^{-12} M) did not stimulate interleukin-1 alpha production by neutrophils or serum complement activation by the alternate pathway. We conclude that TCT at concentrations of $<10^{-8}$ M affects important neutrophil functions and at higher concentrations is toxic. TCT may therefore contribute to the survival of *B. pertussis* within the airways in vivo.

Bacterial infections of the lower respiratory tract are characterized by rapid infiltration of the airways by activated polymorphonuclear neutrophil granulocytes (8), which have been shown in animal models to be crucial for bacterial clearance (23). The most likely stimulus for this inflammatory response is the generation of endogenous and bacterially derived chemotactic factors (8, 21, 29, 30). However, it has also been suggested that chemoattractants alone are not sufficient to produce pulmonary injury in animals (29) and that other factors might act together in a proinflammatory capacity to enhance the neutrophil accumulation and its effects. A number of bacterial cell wall products which do not themselves act as chemoattractants but are able to induce pulmonary inflammation and neutrophil accumulation in animal models have been described (25, 26). These include gram-negative bacterial endotoxin (lipopolysaccharide [LPS]) and fragments of peptidoglycan from gram-positive bacteria. Peptidoglycan fragments are released during cell wall turnover and have been shown to possess a number of hematologic activities, including the ability to activate the complement cascade (14, 22) and to induce cytokine production by leukocytes (22, 27).

Although some bacterial products are proinflammatory, a fundamental requirement for bacterial persistence is to escape detection and elimination by phagocytic cells. Several bacterial species isolated from lung infections, including *Pseudomonas aeruginosa* (16), *Haemophilus influenzae* (7), *Staphylococcus aureus* (2), and *Bordetella pertussis* (1, 5), have been shown to generate factors affecting in vitro neutrophil functions. Some of these substances are cytotoxic (2), but some, such as pertussis toxin (1) and adenylate cyclase (5) from *B. pertussis*, have been found to interfere with cellular transduction mechanisms without affecting viability.

B. pertussis generates a low-molecular-mass (921-Da) peptidoglycan fragment of its cell wall, called tracheal cytotoxin (TCT), which damages both human (28) and animal (6, 13, 15) airway epithelium in vitro. TCT is the major peptidoglycan fragment released by the bacterium, and its structure has been fully characterized and shown to consist of a disaccharide tetrapeptide containing glucosamine, muramic acid, alanine, glutamic acid, and diaminopimelic acid in the molar ratio 1:1:2:1:1 (6). Although the exact mechanism by which these toxic effects are mediated is not clear, a recent study by Heiss and colleagues (15) has suggested that interleukin-1 (IL-1) may be responsible for the toxicity of TCT to epithelial cells. The effects of this peptidoglycan fragment on the viability and functions of other cells, including neutrophils, have not previously been investigated.

We have therefore investigated the effects of TCT on neutrophil viability and a number of in vitro neutrophil functions—migration, phagocytosis, and chemiluminescence. We have also investigated whether TCT, like the peptidoglycan fragments of gram-positive bacteria, is able to activate complement (14) and have investigated its ability to stimulate neutrophils to produce the cytokine IL-1α

MATERIALS AND METHODS

TCT. TCT was purified from *B. pertussis* cultures by filtration, elution with trifluoroacetic acid, and reverse-phase high-pressure liquid chromatography as previously described (6). TCT was dissolved in colorless medium 199 (ICN Flow, High Wycombe, United Kingdom) at a concentration of 1 mM, aliquoted, and stored at −70°C until use. TCT purified in this fashion is free of contaminating proteins, as verified by amino

* Corresponding author. Mailing address: Host Defence Unit, Department of Thoracic Medicine, National Heart and Lung Institute, Emmanuel Kaye Building, Manresa Road, London SW3 6LR, United Kingdom. Phone: 071 351 8337. Fax: 071 351 8331.

acid analysis, and any endotoxin activity was below the sensitivity of the *Limulus* assay (0.05 U of endotoxin per ml) (6).

Neutrophil isolation. Assessment of the effects of TCT on neutrophil viability, migration, phagocytosis, and intracellular ATP levels was performed by using human neutrophils obtained on separate occasions from the peripheral venous blood of healthy volunteers by dextran (Sigma Chemical Co., Poole, United Kingdom) sedimentation (10). The neutrophils were washed (250 × *g*, 10 min) in Hanks balanced salt solution (HBSS) (Sigma) buffered to pH 7.4 with 30 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) and resuspended to a final concentration of 2×10^6 cells ml^{-1} in HBSS containing 0.2% ovalbumin (Sigma). Neutrophils accounted for >95% of cells and were >98% viable, as assessed by trypan blue exclusion.

Neutrophil viability. Cell viability was assessed following treatment of neutrophils (90 min, 37°C) with TCT (10^{-5} to 10^{-15} M) by lactate dehydrogenase (LDH) estimation, using a commercially available spectrophotometric kit (Sigma). Values of total cellular LDH were obtained after exposure of the cells to an equal volume of distilled water (10 min, 0°C) which was restored to isotonicity prior to assay. LDH release was expressed as a percentage of this value.

Intracellular ATP levels. Levels of ATP in neutrophils treated (20 min, 37°C) with TCT (10^{-6} to 10^{-15} M) in HBSS or HBSS alone were assessed using a spectrophotometric kit (Sigma). Cellular protein was assessed by the method of Lowry et al. (19), and levels of ATP were expressed as micromoles per microgram of cellular protein.

Neutrophil migration. (i) Chemotaxis. Neutrophil migration was assessed over 90 min at 37°C in response to TCT (10^{-9} to 10^{-15} M), HBSS alone, or *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP; 10^{-9} M) as a positive control, using the leading-front method of the modified Boyden technique (18). Chemoattractant activity of TCT was expressed as a percentage of the migratory response to FMLP.

(ii) Inhibition of migration. Neutrophils were incubated with TCT (10^{-9} to 10^{-15} M) or HBSS as a control for 20 min at 37°C. They were then washed twice in HBSS by centrifugation (250 × *g*, 10 min) and resuspension, and their migratory response to FMLP (10^{-9} M) was determined by the modified Boyden technique (18). Inhibition was expressed as the percentage reduction in migration toward the chemotaxin.

Neutrophil phagocytosis. Neutrophils were incubated (20 min, 37°C) with TCT (10^{-9} to 10^{-15} M), FMLP (10^{-3} M), or HBSS alone. For these experiments, FMLP was dissolved at a concentration of 10^{-2} M in 50 μl of 0.1 M NaOH plus 950 μl of HBSS. The final concentration of NaOH used in the assay had no effect on neutrophil function. Neutrophils were washed twice in HBSS by centrifugation (250 × *g*, 10 min) and resuspension and resuspended in HBSS-ovalbumin to a final concentration of 2×10^6 neutrophils per ml. Phagocytosis was assessed by light microscopy under oil immersion, using an opsonized latex bead model of ingestion (using gentian violet staining to test for the presence of beads) according to a method previously described by Cline and Lehrer for macrophages (4). Opsonized beads (1.3 μm; Sigma) were prepared by overnight incubation at 4°C with fetal calf serum (ICN Flow) and were used at a concentration of 1.0 mg/ml following washing (250 × *g*, 10 min) and resuspension in HBSS. The percentage of cells containing beads and the number of beads per cell were determined after 30 min.

Neutrophil chemiluminescence. Chemiluminescence was performed by the technique of Garbett et al. (12). Neutrophils were obtained from the peripheral blood of six human volunteers by sedimentation over dextran (Sigma) followed by density gradient centrifugation over Hypaque (Sigma). Erythrocytes were removed from the neutrophil layer by treatment with erythrocyte lysing agent (1:1 [vol/vol]; Sigma). The neutrophils were immediately pelleted by centrifugation (250 × *g*, 10 min) and resuspended in colorless medium 199 at a final concentration of 10^6 neutrophils per ml. Neutrophils accounted for >96% of the cells present and were >98% viable, as assessed by trypan blue exclusion. Following isolation, all cells were kept on ice until analysis, when they were equilibrated at 37°C for 15 min prior to use. Neutrophils were incubated with either TCT (10^{-6} to 10^{-16} M) or FMLP (10^{-5} M; Sigma) in medium 199 or medium 199 alone, and chemiluminescence was monitored for up to 1 h at 37°C in order to establish the optimum times at which the respiratory burst occurred in these treated cells. In separate experiments, the effect of TCT (10^{-6} to 10^{-16} M) on FMLP-induced chemiluminescence was assessed by first incubating the cells (20 min, 37°C) with TCT, then centrifuging the cells (250 × *g*, 10 min) and resuspending them in medium 199, and finally incubating the cells with FMLP (10^{-5} M); chemiluminescence was monitored for up to 40 min at 37°C.

Five hundred microliters of each cell suspension was mixed with 900 μl of luminol (Sigma), and chemiluminescence was assessed at 37°C by using an LKB Luminometer attached to an IBM computer (12). The maximal chemiluminescence observed following each treatment was recorded and expressed as a percentage of the FMLP control level following subtraction of background (medium 199 alone) values.

Complement activation. The ability of TCT to fix complement via the alternative pathway was assessed by using the blood hemolytic complement assay described by Platts-Mills et al. (20). Reagents were obtained from Sigma except where stated otherwise. All assays were performed with gelatin Veronal buffer containing final concentrations of 100 mM ethyleneglycol-bisaminotetraacetic acid (EGTA; pH 7.3) and 100 mM $MgCl_2$ (EGTA-Veronal buffer). EGTA was added to block complement fixation by the classical pathway (20). Rabbit erythrocytes (TCS Biologicals, Botolph Claydon, Buckinghamshire, United Kingdom) were suspended in EGTA-Veronal buffer at a concentration of 1.5×10^8/ml. Human serum which had been incubated (90 min, 37°C) with various dilutions of TCT (10^{-6} to 10^{-12} M) in HBSS, LPS (100 ng/ml) from *P. aeruginosa*, or HBSS alone was diluted in EGTA-Veronal buffer, and 0.4 ml was added to 0.2 ml of erythrocytes at 0°C. After incubation for 15 min at 37°C, 2.5 ml of cold EGTA-Veronal buffer was added, the tubes were centrifuged (600 × *g*, 10 min), and the optical density of the supernatant was measured at 412 nm. Serum hemolytic activity for treated and untreated serum was expressed as a reciprocal of the dilution producing 50% hemolysis. This value for the alternate pathway was defined as suggested by Platts-Mills and colleagues as the reciprocal of the quantity of serum required to achieve 50% hemolysis of 1.5×10^7 rabbit erythrocytes in 1 ml of reaction mixture after incubation for 15 min at 37°C.

IL-Iα estimation. Neutrophils (10^6 ml^{-1}) were incubated (90 min, 37°C) with TCT (10^{-6} to 10^{-12} M) in HBSS, LPS from *P. aeruginosa* (100 ng/ml; Sigma), or HBSS alone. The cells were then pelleted by centrifugation (120 × *g*, 10 min), and levels of IL-1α were determined in the supernatants by using a commercially available enzyme-linked immunosorbent assay (ELISA) obtained from Amersham International (Amersham, United Kingdom) with a lower limit of detection of 0.04 pg per well.

Statistical analysis. Control and test results were compared by nonparametric statistics, using the Wilcoxon signed rank

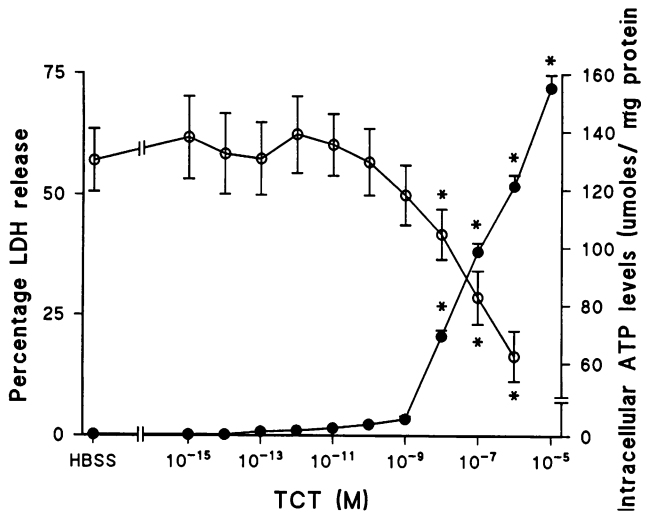

FIG. 1. Effect of TCT from *B. pertussis* on neutrophil viability. Neutrophil viability is assessed as the percentage of total cellular LDH released (●) and the levels of intracellular ATP (○) following a 90-min incubation. Spontaneous release of LDH from neutrophils was between 1 and 2% and has been subtracted from the values given. Data shown are means ± SEM of six experiments. *, $P < 0.05$ versus control values.

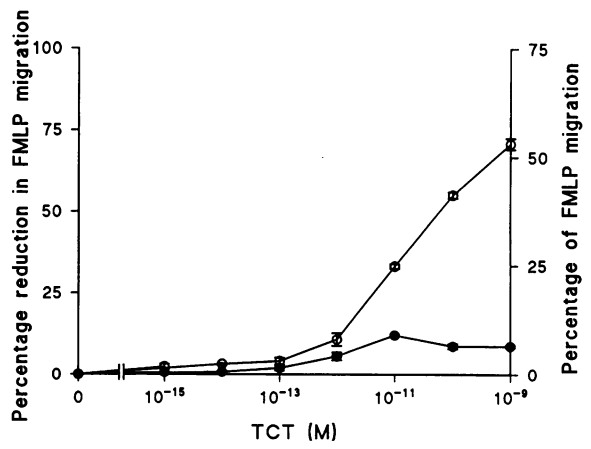

FIG. 2. Effect of TCT from *B. pertussis* on neutrophil migration. The chemoattractant activity of TCT (●) is expressed as a percentage of that caused by FMLP (10^{-9} M), and its ability to inhibit the migratory response toward FMLP is expressed as percentage inhibition of FMLP migration (○). Data shown are means ± SEM of six experiments.

test. Each series of experiments was repeated on six occasions with the blood from six healthy volunteers.

RESULTS

Effect of TCT on neutrophil viability. Neutrophils incubated with TCT at concentrations of $\geq 10^{-8}$ M for 90 min at 37°C demonstrated a significant loss in viability ($P < 0.05$) compared with medium 199-treated cells, as assessed by LDH release and falls in intracellular ATP (Fig. 1). Levels of intracellular ATP in neutrophils incubated with TCT (10^{-9} to 10^{-15} M) were found to be similar to those of control cells.

Effect of TCT on neutrophil migration and phagocytosis. TCT (10^{-9} to 10^{-15} M) was found to possess little neutrophil chemoattractant activity. Migration in response to TCT was at best (10^{-11} M) twice that of buffer alone and <8% of that of FMLP (Fig. 2). Incubation of neutrophils with TCT (10^{-9} to 10^{-12} M) caused a decrease in neutrophil migration which was not associated with a loss in viability, as assessed by LDH release (Fig. 1). Neutrophils exposed to TCT at concentrations of $>10^{-9}$ M were not sufficiently viable to be assessed for the presence of intracellular latex beads. TCT at 10^{-9} to 10^{-15} M did not affect the number of neutrophils phagocytosing latex beads or the number of latex beads per neutrophil compared with control values (Table 1).

Effect of TCT on neutrophil chemiluminescence. Experiments in which neutrophils were treated with TCT (10^{-6} to 10^{-16} M) and chemiluminescence was monitored for up to 1 h demonstrated maximal chemiluminescence responses at incubation times of between 10 and 30 min. An incubation time of 20 min was therefore chosen for use in further experiments of the effect of TCT on these cells. Exposure of neutrophils to TCT (10^{-6} to 10^{-14} M) produced only small changes in chemiluminescence which were not significantly greater than those with buffer alone (<5% of the effect produced by treatment with 10^{-5} M FMLP) (Fig. 3). Neutrophils that had been treated with TCT (10^{-6} to 10^{-15} M) for 20 min showed a significant depression in the subsequent FMLP (10^{-5} M)-induced chemiluminescence peak during 40 min compared with neutrophils which had been treated with medium 199 alone for a similar time ($P < 0.01$). This effect was abolished by diluting the TCT to concentrations of $<10^{-15}$ M (Fig. 3).

Effect of TCT on human serum complement activation. TCT (10^{-6} to 10^{-12} M) had no effect on serum hemolytic activity compared with HBSS buffer alone, with the reciprocal means ± standard errors of the means (SEM) of the serum dilutions producing 50% hemolysis being 41.3 ± 2.7, 38.9 ± 1.4, 36.9 ± 2.3, and 39.9 ± 2.7 for 10^{-6}, 10^{-8}, 10^{-10}, and 10^{-12} M TCT, respectively, versus control values of 38.8 ± 3.6. Activation of the alternate pathway was observed ($P < 0.05$) in serum treated with LPS from *P. aeruginosa* (100 ng/ml). The reciprocal mean ± SEM of the serum dilutions producing 50% hemolysis was 73.2 ± 5.4 versus the same control values of 38.8 ± 3.6.

Effect of TCT on neutrophil IL-1α production. Levels of IL-1α produced by neutrophils incubated (90 min, 37°C) with either TCT (10^{-6} to 10^{-12} M) in HBSS or HBSS alone were found to be below the lower limit of detection of the ELISA ($n = 6$). Treatment with LPS from *P. aeruginosa* (100 ng/ml) for 90 min resulted in the generation of 4.3 ± 1.0 pg of IL-1α per ml ($n = 6$).

TABLE 1. Effect of TCT on neutrophil phagocytosis

Treatment	Phagocytosis (mean ± SEM; $n = 6$)	
	% of cells containing beads at 30 min	No. of beads/cell at 30 min
Control (HBSS)	50.9 ± 2.1	5.0 ± 1.7
FMLP (10^{-3} M)	94.5 ± 3.7	15.5 ± 5.3
TCT		
10^{-9} M	49.7 ± 2.3	4.6 ± 1.9
10^{-10} M	51.9 ± 1.7	4.5 ± 1.1
10^{-11} M	48.4 ± 2.5	4.9 ± 1.7
10^{-12} M	51.2 ± 2.8	5.3 ± 2.1
10^{-13} M	50.7 ± 1.0	5.1 ± 1.8
10^{-14} M	52.6 ± 2.6	5.1 ± 1.9
10^{-15} M	51.3 ± 1.9	5.0 ± 1.7

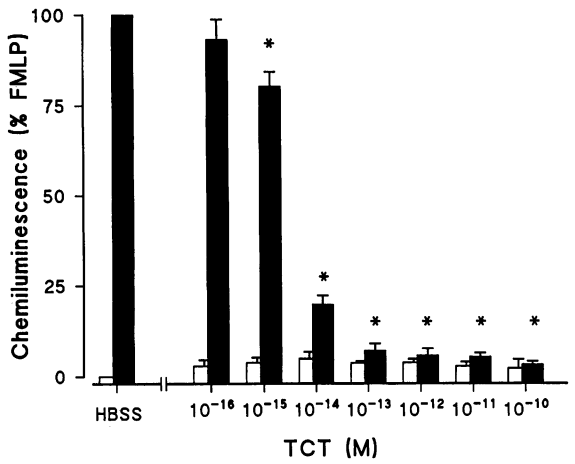

FIG. 3. Effect of TCT from *B. pertussis* on neutrophil chemiluminescence (□) and on that stimulated by FMLP (10^{-5} M; ■). Data are maximal chemiluminescence responses observed over a 40-min incubation period and are expressed as means ± SEM of six experiments, with chemiluminescence being expressed as a percentage of that produced in response to FMLP (10^{-5} M) alone. *, $P < 0.05$ fall in FMLP-induced chemiluminescence of TCT-treated cells versus cells treated with FMLP alone.

DISCUSSION

Peptidoglycans constitute an integral part of the cell wall of both gram-negative and gram-positive organisms. They are structurally complex molecules which consist of long chains of *N*-acetylmuramic acid and *N*-acetylglucosamine residues cross-linked by amino acids (26). Peptidoglycan fragments of various sizes are released into bacterial growth medium during cell wall turnover, and although the majority are reincorporated (9), those obtained from gram-positive organisms have been shown to possess a number of hematologic, pyrogenic, and somnogenic activities (17). These include the activation of complement via the alternate pathway (14), neutrophil recruitment and generation of pulmonary inflammation in animal models (25), stimulation of the release of the inflammatory cytokine IL-1α by blood monocytes (21), and inhibition of macrophage chemotaxis (25).

Large fragments of peptidoglycan (35 to 40 kDa) may also stimulate human mononuclear cells to produce noncytotoxic factors which inhibit neutrophil chemotaxis toward formyl peptides (9). Peptidoglycan fragments may also directly affect neutrophil function. We have recently demonstrated that at least eight low-molecular-weight glycopeptide fragments which can inhibit the in vitro neutrophil migration and chemiluminescence stimulated by chemotactic factors are released into the growth medium of nontypable *H. influenzae* (7).

In these studies, we have investigated the effects of a purified fragment of peptidoglycan, TCT, on human neutrophil function. TCT at dilutions of between 10^{-6} and 10^{-8} M was found to be toxic for human neutrophils over a 90-min incubation period, producing a significant release of LDH from the cells and a fall in intracellular ATP. TCT was only weakly chemoattractant and did not produce a significant increase in chemiluminescence compared with medium 199 alone but did significantly inhibit the migration and chemiluminescence responses produced by the chemotactic factor FMLP. TCT had no effect on neutrophil phagocytosis and was unable to activate complement in human serum or stimulate the release of IL-1α from human neutrophils after 90 min of incubation at any dilution (10^{-6} to 10^{-12} M) tested.

TCT cytotoxicity has been previously documented in both hamster tracheal (6) and human nasal (28) organ cultures incubated with 10^{-6} M TCT. We have shown that TCT at 10^{-11} M is able to significantly inhibit important human neutrophil functions in vitro, and TCT is thus more potent in this biological assay system than in those previously described (6, 28). The concentrations of TCT present in the airways of patients infected with *B. pertussis* are not known, although in vitro studies have shown that under optimal conditions, 10^{-6} M TCT may be elaborated in broth culture (6). The adherence of *B. pertussis* to the cilia of epithelial cells may increase levels of TCT in the microenvironment around adherent bacteria, which may be important in generating sufficient concentration of toxin to kill these cells.

Some of the biological activities of TCT are linked to those of the cytokine IL-1, and a recent study by Heiss and colleagues (15) has suggested that IL-1 may be responsible for the toxicity of TCT. Heiss and colleagues (15) demonstrated that exposure of cultures of respiratory epithelial cells to 10^{-6} M TCT for 2 h resulted in the generation of intracellular IL-1 activity and that IL-1 added directly to the cells could mimic the toxic effects of TCT. In vitro, muramyl peptides have been shown to stimulate IL-1 production by monocytes and macrophages (22). Our studies were unable to demonstrate the production of any measurable IL-1α following 90 min of incubation with 10^{-6} to 10^{-12} M TCT. This may be because neutrophils possess a receptor signalling pathway different from that used by TCT in the production of IL-1 from respiratory epithelial cells or that the IL-1 is not released extracellularly.

Previous studies on peptidoglycan fragments from gram-positive bacteria are in agreement with our findings that peptidoglycan fragments do not stimulate neutrophil migration per se (24). Many of the biological effects of peptidoglycan are thought to be mediated by its ability to activate complement by the alternate pathway (14). The inability of TCT to activate complement may be due to its small molecular size of 921 Da. Studies performed by Tuomanen and colleagues (26) using fragments of peptidoglycan obtained from gram-positive bacteria have suggested that the ability of these fragments to produce and maintain inflammation via the activation of complement is size related. Small or degraded peptidoglycan fragments were not proinflammatory in vivo in an animal model (25).

The production of TCT in vivo in the microenvironment of the airway mucosa may contribute to the survival of *B. pertussis* within the airways by impairing neutrophil chemotaxis and oxidative metabolism. We previously demonstrated that low-molecular-mass (<8-kDa) glycopeptide fragments from nontypable *H. influenzae* which were without neutrophil chemoattractant or phagocytic activity significantly impaired the human neutrophil migratory response to both FMLP and leukotriene B_4 (7). All gram-negative bacteria studied to date have been shown to possess one chemical type of peptidoglycan, and variations have been limited to degree of muropeptide cross-linkage, degree of amidation of the carboxyl groups of the peptide subunit, and O acetylation of the disaccharide (23). These findings may therefore be relevant to peptidoglycan fragments obtained from other gram-negative bacteria.

ACKNOWLEDGMENTS

This work was supported by Lilly Industries and Public Health Service grant A122243 from the National Institutes of Health.

REFERENCES

1. **Becker, E. L., J. C. Kermode, P. H. Naccache, R. Yassin, M. L. Marsh, J. J. Munoz, and R. I. Sha'afi.** 1985. The inhibition of neutrophil granule enzyme secretion and chemotaxis by pertussis toxin. J. Cell Biol. **100:**1641–1646.
2. **Bhakdi, S., and J. T. Jensen.** 1991. Alpha-toxin of *Staphylococcus aureus*. Microbiol. Rev. **55:**733–751.
3. **Boxer, L. A., M. Yoder, S. Bonsib, M. Schmidt, P. Ho, et al.** 1979. Effects of a chemotactic factor N-formylmethionyl peptide on adherence, superoxide generation, phagocytosis and microtubule assembly of human polymorphonuclear leukocytes. J. Lab. Clin. Med. **93:**506–514.
4. **Cline, M. J., and R. I. Lehrer.** 1968. Phagocytosis by human monocytes. Blood **32:**423–435.
5. **Confer, D. L., and J. W. Eaton.** 1983. Phagocyte-host jujitsu; phagocyte impotence caused by internalised bacterial adenylate cyclase. Trans. Assoc. Am. Physicians **45:**1–7.
6. **Cookson, B. T., H. L. Cho, L. A. Herwaldt, and W. E. Goldman.** 1989. Biological activities and chemical composition of purified tracheal cytotoxin of *Bordetella pertussis*. Infect. Immun. **57:**2223–2229.
7. **Cundell, D. R., G. W. Taylor, K. Kanthakumar, M. Wilks, S. Tabaqchali, E. Dorey, J. L. Devalia, D. E. Roberts, R. J. Davies, R. Wilson, and P. J. Cole.** 1993. Inhibition of neutrophil migration in vitro by low molecular-mass products of nontypeable *Haemophilus influenzae*. Infect. Immun. **61:**2419–2424.
8. **Currie, D. C., A. M. Peters, P. George, B. Strickland, J. P. Lavender, and P. J. Cole.** 1990. Indium-111 labelled granulocyte scanning to detect inflammation in the lungs of patients with chronic sputum expectoration. Thorax **45:**541–544.
9. **Donabedian, H.** 1985. Human mononuclear cells exposed to staphylococci rapidly produce an inhibitor of neutrophil chemotaxis. J. Infect. Dis. **152:**24–32.
10. **Doyle, R. J., J. Chaloupka, and V. Winter.** 1988. Turnover of cell walls in microorganisms. Microbiol. Rev. **52:**554–567.
11. **Fontan, P. A., C. R. Amira, V. E. Garcia, M. C. Cerquetti, and D. O. Sordelli.** 1992. Preliminary characterization of *Pseudomonas aeruginosa* peptide chemotactins for polymorphonuclear leukocytes. Infect. Immun. **60:**2465–2469.
12. **Garbett, N. D., G. S. Matharu, and P. J. Cole.** 1989. Defective opsonization of *Haemophilus influenzae* by sera of elderly patients. Clin. Exp. Immunol. **76:**73–75.
13. **Goldman, W. E., D. G. Klapper, and J. B. Baseman.** 1982. Detection, isolation, and analysis of a released *Bordetella pertussis* product toxic to cultured tracheal cells. Infect. Immun. **36:**782–794.
14. **Greenblatt, J., R. J. Boackle, and J. H. Schwab.** 1978. Activation of the alternate complement pathway by peptidoglycan from streptococcal cell wall. Infect. Immun. **19:**296–303.
15. **Heiss, L. N., S. A. Moser, E. R. Unanue, and W. E. Goldman.** 1993. Interleukin-1 is linked to epithelial cytopathology of pertussis. Infect. Immun. **61:**3123–3128.
16. **Jaeger, K. E., A. Kharazmi, and N. Hoiby.** 1990. Extracellular lipase of *Pseudomonas aeruginosa*: biochemical characterization and effect on human neutrophil and monocyte function *in vitro*. Microb. Pathog. **10:**173–182.
17. **Johannsen, L., L. A. Toth, R. S. Rosenthal, M. R. Opp, F. Obel, A. B. Cady, and J. M. Krueger.** 1989. Somnogenic, pyrogenic and hematologic effects of bacterial peptidoglycan. Am. J. Physiol. **258:**R182–R186.
18. **Lee, T. H. L., L. Nagy, T. Nagakura, M. Walport, and A. B. Kay.** 1982. Identification and characterization of an exercise-induced neutrophil chemotactic factor in bronchial asthma. J. Clin. Invest. **69:**889–898.
19. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
20. **Platts-Mills, T. A. E., and K. Ishizaka.** 1974. Activation of the alternate pathway of human complement by rabbit cells. J. Immunol. **113:**348–358.
21. **Ras, G., R. Wilson, H. Todd, G. Taylor, and P. Cole.** 1990. Effect of bacterial products on neutrophil migration *in vitro*. Thorax **45:**276–280.
22. **Riesenfeld-Orn, I., S. Wolpe, J. F. Garcia-Bustos, M. K. Hoffmann, and E. Tuomanen.** 1989. Production of interleukin-1 but not tumor necrosis factor by human monocytes stimulated with pneumococcal cell surface components. Infect. Immun. **57:**1890–1893.
23. **Schleifer, K. H., and O. Kandler.** 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriol. Rev. **36:**407–477.
24. **Toews, G. B., W. C. Vial, and E. J. Hansen.** 1985. Role of C5 and recruited neutrophils in early clearance of nontypable *Haemophilus influenzae* from murine lungs. Infect. Immun. **50:**207–212.
25. **Tuomanen, E., H. Liu, B. Hengstler, O. Zak, and A. Tomasz.** 1985. The induction of meningeal inflammation by components of the pneumococcal cell wall. J. Infect. Dis. **151:**859–868.
26. **Tuomanen, E., R. Rich, and O. Zak.** 1987. Induction of pulmonary inflammation by components of the pneumococcal cell surface. Am. Rev. Respir. Dis. **135:**869–874.
27. **Vaara, K. P., D. Banda, and I. M. Goldstein.** 1987. Bacterial lipopolysaccharide-induced release of lactoferrin from human polymorphonuclear leukocytes: role of monocyte-derived tumor necrosis factor. Infect. Immun. **55:**2956–2961.
28. **Wilson, R., R. Read, M. Thomas, A. Rutman, K. Harrison, V. Lund, B. Cookson, W. Goldman, H. Lambert, and P. Cole.** 1991. Effects of *Bordetella pertussis* infection on human respiratory epithelium in vivo and in vitro. Infect. Immun. **59:**337–345.
29. **Worthen, G. S., C. Haslett, A. J. Rees, S. Gumbay, J. E. Henson, and P. M. Henson.** 1987. Neutrophil-mediated pulmonary vascular injury. Synergistic effect of trace amounts of lipopolysaccharide and neutrophil stimuli on vascular permeability and neutrophil sequestration in the lung. Am. Rev. Respir. Dis. **136:**19–28.
30. **Young, S. K., G. C. Worthen, C. Haslett, M. G. Tonnensen, and P. M. Henson.** 1990. Interaction between chemoattractants and bacterial lipopolysaccharide in the induction and enhancement of neutrophil adhesion. Am. J. Respir. Cell Mol. Biol. **2:**523–532.