INFECTION AND IMMUNITY, Aug. 1993, p. 3123–3128
0019-9567/93/083123-06$02.00/0
Copyright © 1993, American Society for Microbiology
Vol. 61, No. 8

Interleukin-1 Is Linked to the Respiratory Epithelial Cytopathology of Pertussis

LINDA NIXON HEISS,[1] STEPHEN A. MOSER,[2]† EMIL R. UNANUE,[2] AND WILLIAM E. GOLDMAN[1]*

Department of Molecular Microbiology[1] and Department of Pathology,[2] Washington University School of Medicine, St. Louis, Missouri 63110

Received 3 March 1993/Accepted 6 May 1993

***Bordetella pertussis*, the causative agent of whooping cough, releases a muramyl peptide known as tracheal cytotoxin (TCT) that is responsible for destruction of ciliated epithelial cells lining the large airways. In vitro, TCT has been shown to cause this specific pathology in human or hamster respiratory epithelium and to inhibit the proliferation of cultured hamster trachea epithelial cells. The diverse biological actions of muramyl peptides, including adjuvanticity, somnogenicity, and pyrogenicity, have been correlated with the production and release of the inflammatory mediator interleukin-1 (IL-1). Consistent with its ability to reproduce other muramyl peptide actions, recombinant IL-1 caused TCT-like damage to the respiratory epithelium. In the nanogram-per-milliliter range, exogenous IL-1 inhibited DNA synthesis in hamster trachea epithelial cells and reproduced the pathology of TCT in hamster tracheal organ culture. Tumor necrosis factor alpha and IL-6, cytokines also associated with inflammation, were unable to reproduce TCT cytopathology. Furthermore, exposure of respiratory epithelial cells to TCT stimulated production of cell-associated IL-1α, which could be detected within 2 h of TCT treatment. In contrast, there was no evidence of TCT-triggered release of IL-1. Previous studies have suggested that intracellular IL-1α, as well as exogenous IL-1α and IL-1β, can inhibit cell proliferation. Our results therefore implicate IL-1α, produced by epithelial cells in response to TCT, as a potential intracellular mediator of the primary respiratory cytopathology of pertussis.**

Whooping cough (pertussis) is classically characterized in infants and children by violent, debilitating coughing episodes, a result of respiratory tract colonization by *Bordetella pertussis* (34). Although *B. pertussis* produces a variety of toxins, tracheal cytotoxin (TCT) is the only one able to reproduce the respiratory epithelial cytopathology that is the hallmark of pertussis (18). We have monitored the biological effects of TCT by using two in vitro models of the respiratory epithelium: hamster trachea epithelial (HTE) cells and hamster tracheal organ culture. HTE cells are a nontransformed, homogeneous epithelial cell population isolated from excised tracheal tissue (17). TCT inhibits DNA synthesis by HTE cells in a dose-dependent manner but has little effect on overall RNA or protein synthesis (18). Sectioned hamster tracheas serve as a parallel organ culture model to reproduce the dramatic ciliated cell pathology of human infection (6). When treated with purified TCT, the epithelium of tracheal rings exhibits pathological changes indistinguishable from those observed in human pertussis (7). Exposure of human nasal tissue explants to TCT has also been shown to result in the destruction of ciliated cells (40).

TCT is a 921-D disaccharide-tetrapeptide (8) that is a member of a large family of molecules known as muramyl peptides, which are fragments of bacterial cell wall peptidoglycan. Muramyl peptides have been shown to have a variety of biological activities in vivo, including somnogenicity, pyrogenicity, arthritogenicity, and adjuvanticity (1, 5, 27). There are many similarities between the biological effects of muramyl peptides and those of the inflammatory mediator interleukin-1 (IL-1), and several lines of evidence suggest that some muramyl peptide activities are the result of IL-1 induction. In vitro, muramyl peptides stimulate IL-1 production by monocytes and macrophages (13, 35), and in vivo production of IL-1 is seen when muramyl peptides are injected into experimental animals (36). The pyrogenic activities of muramyl peptides correspond to their ability to stimulate host production of endogenous pyrogens, including IL-1 (12). A correlation has also been demonstrated between the adjuvant activity of muramyl peptides and their ability to induce production of macrophage membrane-associated IL-1 (3). In addition, muramyl peptides may elicit their somnogenic effects via cytokines such as IL-1. The time courses of sleep effects stimulated by recombinant IL-1 or muramyl peptides in rabbits are consistent with the hypothesis that muramyl peptides modulate sleep by triggering the production of IL-1 (see reference 28). Given the correlations between IL-1 and muramyl peptide biological activities, we hypothesized that TCT might induce respiratory epithelial cells to produce IL-1, which in turn may be responsible for mediating TCT toxicity.

MATERIALS AND METHODS

Reagents. TCT was purified from *B. pertussis* Tohama I or Tohama III culture supernatant as described previously (7). Recombinant murine IL-1β and the anti-IL-1α monoclonal antibody (MAb) 161.1-ALF were gifts from K. Hogquist and D. Chaplin (Washington University School of Medicine). Recombinant murine IL-6 and recombinant murine tumor necrosis factor alpha (TNF-α) were purchased from Genzyme (Cambridge, Mass.). *Escherichia coli* O26:B6 lipopolysaccharide (LPS) was purchased from Sigma Chemical Co. (St. Louis, Mo.).

Cell culture. HTE cells were isolated from a Golden Syrian hamster as described previously (17) and cultured in F-12

* Corresponding author. Electronic mail address: goldman@borcim.wustl.edu.

† Present address: Department of Pathology, University of Alabama-Birmingham, Birmingham, AL 35294.

tissue culture medium (GIBCO, Grand Island, N.Y.) containing 10% fetal bovine serum (Hyclone Sterile Systems, Logan, Utah), 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 2 mM L-glutamine. In certain experiments (e.g., those whose results are shown in Fig. 1 and Table 2), 3 ng of epidermal growth factor (EGF; Sigma) per ml was included during culture. EGF is not required for TCT toxicity, but growth rate and longevity of HTE cells may be improved by culture with EGF. Cells were maintained in 95% air–5% CO_2 at 37°C.

HTE cell toxicity assay. Inhibition of DNA synthesis by HTE cells was assessed in a modification of the microtiter assay described previously (18). Briefly, HTE cells resuspended in minimal essential medium (GIBCO) containing 2.5% fetal bovine serum were plated in 96-well culture plates at a density of 12,000 cells per well. In experiments where cells were cultured with EGF, 0.1 ng of EGF per ml was included. Incubation for 24 h synchronized these cells in the nonproliferative phase of the cell cycle. The nonproliferative cultures were incubated for 4 h with the test compound in serum-free minimal essential medium containing 5 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; pH 7.3; U.S. Biochemicals, Cleveland, Ohio). Cells were then stimulated with 15% fetal bovine serum, pulsed with [^{3}H]thymidine, and allowed to incubate for 26 h. The cells were harvested on cotton-tipped applicators, and the amount of DNA synthesis was measured as trichloroacetic acid-precipitable ^{3}H counts. The relative inhibition of DNA synthesis was calculated by comparing incorporated counts per minute of treated cells to untreated controls. The data shown are the means and the standard deviations of quadruplicate samples.

E. coli LPS was included at a concentration of 10 ng/ml as a cofactor for TCT toxicity. Toxicity of purified TCT for HTE cells was previously shown to require 1 μM bovine serum albumin (BSA), which was thought to act as a protectant or carrier protein (4). Recent experiments determined that it is actually low-level endotoxin contamination of BSA that is responsible for this enhanced TCT toxicity (manuscript in preparation). Approximately 10 ng of *E. coli* LPS or *B. pertussis* lipooligosaccharide per ml can substitute for BSA in toxicity assays with HTE cells; this corresponds to lipooligosaccharide levels that we have measured in *B. pertussis* culture supernatant (7). However, even high levels (10 μg/ml) of LPS alone do not produce significant toxicity. In the tracheal ring assays described below, there is no corresponding requirement for BSA, LPS, or lipooligosaccharide.

Hamster tracheal ring toxicity assay. Damage to hamster tracheal epithelium was assessed as described previously (18). Tracheas from male Golden Syrian hamsters (Charles Rivers Laboratories, Wilmington, Mass.) at least 90 days old were dissected and sectioned into rings. Tracheal rings were cultured in F-12 medium containing the compound to be tested under an atmosphere of 95% air–5% CO_2 at 37°C. Tracheal rings were screened for ciliated cell damage daily by light microscopy over the course of 102 h. Toxicity was defined as ciliostasis and extrusion of ciliated cells from the tracheal epithelium after exposure to the test compound for 4 to 5 days.

Transmission electron microscopy. Tracheal rings were fixed overnight at 4°C in Tyrode's buffer containing 2% glutaraldehyde and then postfixed for 1 to 2 h in 1% osmium tetroxide at 4°C. Fixed samples were embedded with Poly/Bed 812 (Polysciences, Inc., Warrington, Pa.) after passage through 70, 95, and 100% ethanol and propylene oxide. Thin sections were cut with a glass knife, stained with 2% uranyl acetate and 0.4% lead citrate, and then examined on a Philips model 400 transmission electron microscope.

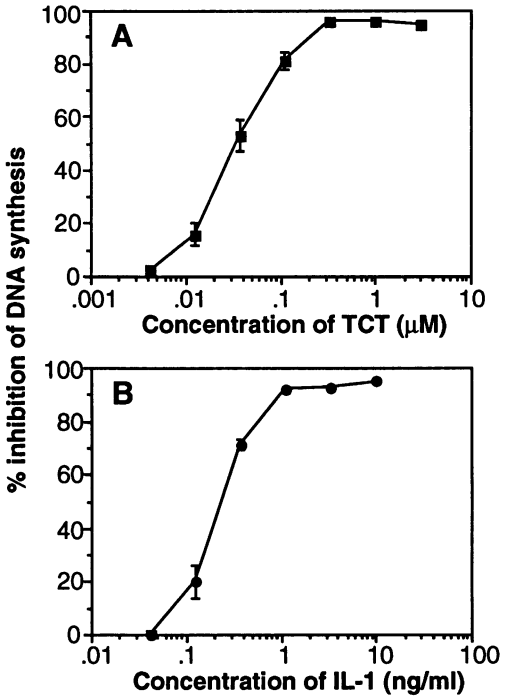

FIG. 1. Inhibitory effects of TCT and IL-1 on the proliferation of HTE cells. HTE cells were exposed to the indicated concentrations of TCT (A) or recombinant IL-1β (B) for 30 h. The relative inhibition of DNA synthesis was calculated by comparing incorporated counts per minute of treated cells with those of untreated controls. [^{3}H]thymidine incorporation for untreated controls was 70,471 ± 1365 cpm. The data shown are the means and standard deviations for quadruplicate samples.

Induction of IL-1 and IL-1 bioassay. HTE cells were seeded for synchronization and treated with 3 μM TCT in the presence of 10 ng of LPS per ml. To assess IL-1 production, culture supernatants were collected, and the cell monolayers were washed with pH 3.0 medium for 2 min (acidic eluate) or subjected to three cycles of freeze-thawing in minimal essential medium-HEPES. Cell culture supernatants, acidic eluates, and cell lysates were tested for IL-1 activity as described previously (29) by using the D10.G4.1 T-cell clone as an indicator (25). Samples were added to 2×10^4 D10.G4.1 cells plus 2.5 μg of concanavalin A per ml in microtiter wells. The indicator cells were pulsed with 0.2 μCi of [^{3}H]thymidine per well during the last 24 h of a 72-h incubation at 37°C.

RESULTS

Toxic effects of TCT and IL-1 for respiratory epithelial cells. HTE cells were treated with TCT or IL-1 for 30 h to determine whether exogenous IL-1 could reproduce the ability of TCT to inhibit proliferation of HTE cells. At concentrations as low as 50 pg/ml, IL-1 inhibited DNA synthesis in HTE cells in a concentration-dependent manner (Fig. 1). The toxicity curve for IL-1 was similar to that obtained for TCT.

IL-1 was also tested for the ability to generate pertussis-like destruction of ciliated respiratory epithelium. Hamster

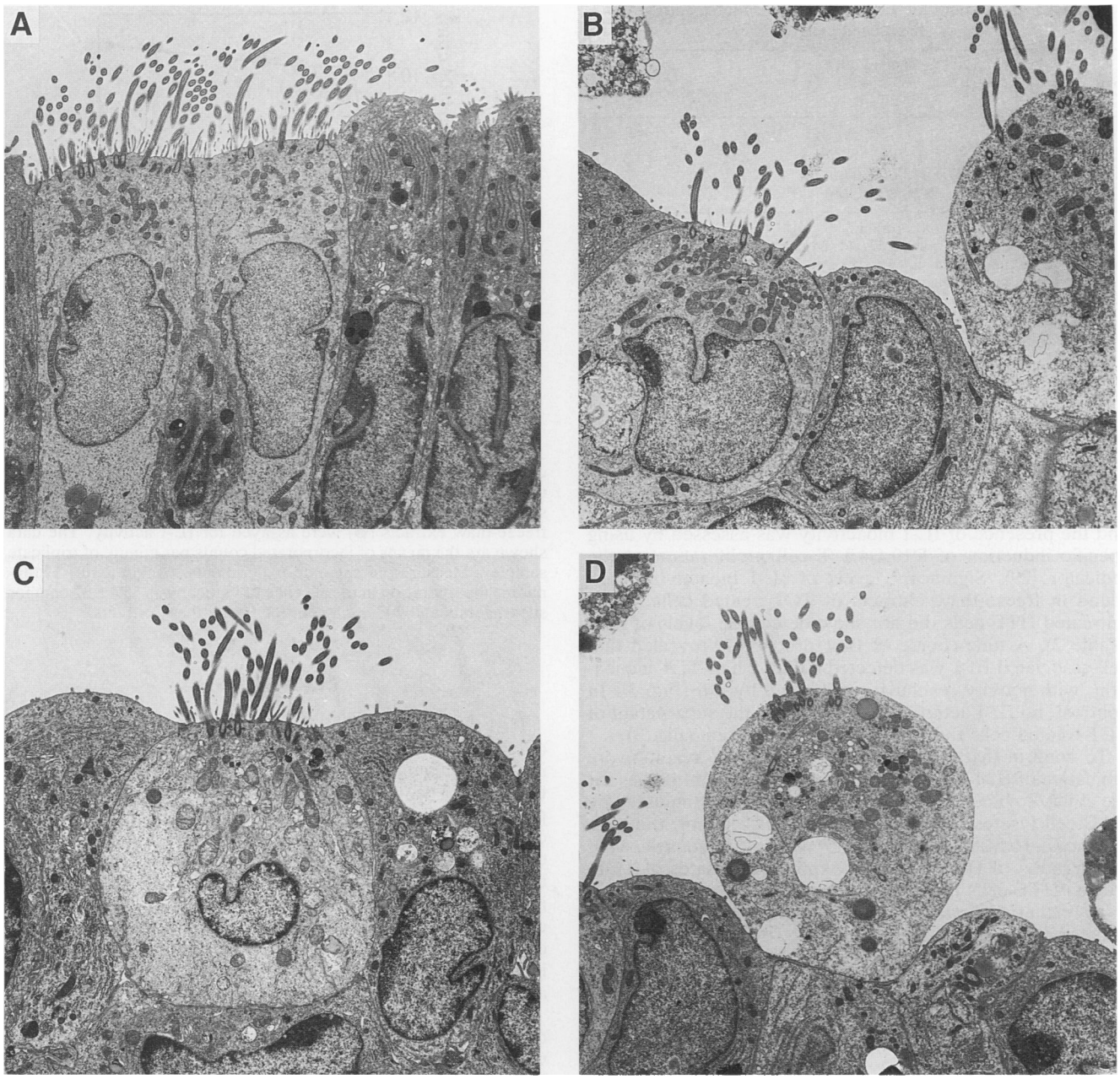

FIG. 2. Electron photomicrographs representative of the destruction of ciliated cells in hamster tracheal rings treated with TCT or IL-1. Sectioned hamster tracheas from male Golden Syrian hamsters were treated with TCT or IL-1 in F-12 medium for 102 h. (A) Control ring; (B) ring treated with 3 μM TCT; (C and D) ring treated with 40 ng of recombinant IL-1β per ml. Magnification, ×5,250.

tracheal rings treated with IL-1 or TCT were monitored by light microscopy over a period of 102 h to screen for TCT-like damage. At a concentration of 40 ng of recombinant IL-1 per ml, marked ciliostasis and extrusion of ciliated cells from the epithelium were visible at 72 to 96 h. Electron microscopy of tracheal rings treated for 102 h showed that the pathology generated by IL-1 is indistinguishable from that triggered by TCT (Fig. 2). At this time point, most of the ciliated cells had been extruded from the epithelium, while other cell types showed no ultrastructural abnormalities. Those ciliated cells still remaining were rounded, had fewer cilia, and contained abnormally swollen mitochondria.

Cytokine specificity of respiratory epithelial cell toxicity. In further experiments, we wanted to compare the respiratory epithelial cell toxicity of IL-1 with that of other cytokines that are known to inhibit cell proliferation. TNF-α and IL-6 were tested for toxicity because they have been previously shown to be produced by epithelial cells (39). High concentrations of TNF-α or IL-6 had no effect on HTE cell proliferation (Table 1). TNF-α and IL-6 were also tested for toxicity to hamster tracheal rings and neither induced ciliostasis at the concentrations tested (Table 1).

Expression of IL-1 biological activity in HTE cells treated with TCT. The respiratory tract includes many possible

TABLE 1. Toxicity of TCT and cytokines for respiratory epithelial cells

Sample	Toxicity for HTE cells (IC_{50})[a]	Toxicity for tracheal rings[b]
TCT	30 nM	+
IL-1	250 pg/ml	+
IL-6[c]	>3,000 U/ml	−
TNF-α[c]	>10,000 U/ml	−

[a] IC_{50}, 50% inhibitory concentration.
[b] Toxicity is defined as ciliostasis and the extrusion of ciliated cells after exposure to test compound for 4 to 5 days. Concentrations of toxic compounds required for maximal tracheal ring cytopathology were 100 × IC_{50} for HTE cells.
[c] IL-6 and TNF-α did not exhibit toxicity for HTE cells or tracheal rings at the concentrations shown, which were the highest tested.

cellular sources of inflammatory cytokines such as IL-1. Because our simplest model for evaluating TCT toxicity is a homogeneous respiratory epithelial cell population (HTE cells), we investigated the possibility that HTE cells produce IL-1 upon TCT exposure. HTE cells were treated with TCT, and the presence of IL-1 bioactivity was assessed by using specific induction of D10.G4.1 T cell proliferation as the indicator (29). Significant levels of IL-1 bioactivity were found in freeze-thaw extracts of TCT-treated cells, while untreated HTE cells did not show detectable levels of IL-1 (Table 2). A time-course of IL-1 production revealed that cell-associated IL-1 was detected within 2 h of TCT incubation, with activity reaching a maximum by 4 h (Fig. 3). In contrast, no IL-1 activity was detected in the supernatant of TCT-treated cells throughout a 30-h incubation (Fig. 3).

To confirm that the D10.G4.1 cell bioassay results were indicative of IL-1 activity, we tested anti-IL-1 antibodies for the ability to block D10.G4.1 cell proliferation stimulated by HTE cell lysates. At a concentration of 2 μg/ml, the anti-murine IL-1α MAb ALF-161.1 (15) was able to neutralize the proliferation of D10.G4.1 cells exposed to lysates of TCT-treated HTE cells (Table 2).

To further evaluate our failure to detect released IL-1, we assayed acidic eluates from the surface of TCT-treated HTE cells. Similar elution conditions have been used previously to demonstrate IL-1 occupancy of high-affinity cell surface receptors on endothelial cells, although culture supernatants had no IL-1 activity (9). We were unable to detect any receptor-associated extracellular IL-1 by this technique (Table 2).

TABLE 2. Production of intracellular IL-1α by HTE cells treated with TCT

Sample	[^{3}H]thymidine incorporation by D10.G4.1 T cells (cpm)[a]	
	−anti-IL-1α MAb	+anti-IL-1α MAb
Recombinant IL-1α (1 ng/ml)	111,336 ± 22,398	7,686 ± 400
Lysate of untreated HTE cells	8,691 ± 995	ND
Lysate of TCT-treated HTE cells	100,896 ± 7223	8,139 ± 1,429
pH 3.0 eluate from TCT-treated HTE cells	12,154 ± 706	ND

[a] The data shown are the means and the standard deviations of the incorporated counts per minute for triplicate samples. Background [^{3}H]thymidine incorporation was 12,213 ± 914 cpm. ND, not determined.

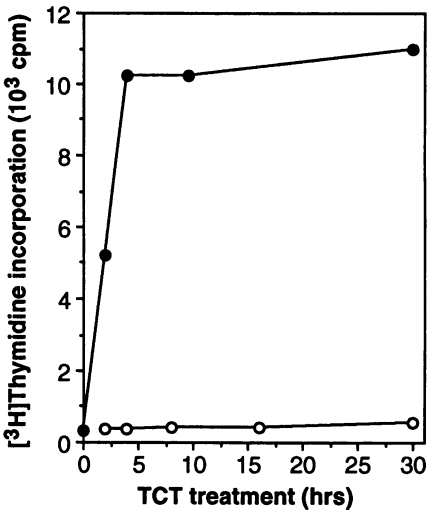

FIG. 3. Time-course of IL-1 production by TCT-treated HTE cells. HTE cells were treated with 3 μM TCT, and, at the indicated time, the culture supernatants were removed and the cells were subjected to three cycles of freeze-thawing. Supernatants (○) and freeze-thaw extracts (●) were assayed for IL-1 activity. The data shown are the means of incorporated counts per minute of triplicate samples. Measured in separate experiments, background [^{3}H]thymidine incorporation in the absence of inducer was 557 ± 32 cpm for supernatants and 207 ± 58 cpm for freeze-thaw extracts.

DISCUSSION

IL-1 is a key biological mediator produced by many different cell types in response to a number of stimuli including inflammatory agents, injury, and microbial infection (11). The two forms of IL-1, IL-1α and IL-1β, are produced as cytosolic peptides that are proteolytically processed upon secretion to the forms of the proteins found in culture supernatants (16, 23). Both IL-1α and IL-1β bind to the same receptor on the cell surface (26) and share biological activities. IL-1β is the major form of IL-1 released into culture supernatants, while IL-1α is found to be primarily cytoplasmic as well as membrane associated (14, 30). The intracellular pro-IL-1α, but not pro-IL-1β, is biologically active (14, 22).

Because IL-1 is linked to some of the biological activities of muramyl peptides (see Introduction), we investigated whether IL-1 might also be responsible for the actions of TCT, the muramyl peptide toxin of *B. pertussis*. IL-1 was able to reproduce TCT-specific damage in two in vitro models of the respiratory epithelium. Furthermore, TCT was shown to trigger the production of cell-associated IL-1α by tracheal epithelial cells. Taken together, these findings implicate intracellular IL-1α as a putative mediator of TCT toxicity and hence the primary cytopathology of pertussis.

As a regulator of cell proliferation, IL-1 may have either growth inhibitory or stimulatory effects, and it has also been shown to be directly cytotoxic for some cells (11). Thus, it is not surprising that our in vitro models suggest that IL-1 may have a dual role in pertussis infection. IL-1 may be responsible not only for the destruction of the ciliated cells in the large airways but also for delaying the restoration of the ciliated epithelium after *B. pertussis* infection. This is suggested most directly by the results with HTE cells, which are growth inhibited by TCT. HTE cells may represent the basal cell population that divides and differentiates into ciliated

cells, and it is likely that inhibition of DNA synthesis in basal cells would slow this regeneration process.

Epithelial cell lines originating from the human urinary tract have been recently shown to produce IL-1α after infection with *E. coli*, suggesting that epithelial-derived cytokines may participate in mucosal immunity (2). In contrast to this beneficial role for epithelial IL-1, our results suggest that IL-1 may act in respiratory epithelial cells to generate the ciliated cell damage induced by TCT. Although the pathology of other bacterial toxins, including LPS (33) and toxic-shock-syndrome-associated staphylococcal and streptococcal exotoxins (4, 20, 24, 37), have been attributed to cytokines including IL-1, this is the first indication that a bacterial toxin generates target cell damage directly by stimulating production of epithelial-derived IL-1.

Although IL-1 frequently acts as an intercellular biological mediator, there are many indications that IL-1α may act intracellularly. Evidence suggests that the activity of intracellular IL-1α may regulate the senescence of human endothelial cells (31). Pro-IL-1α contains a putative nuclear translocation sequence that may target endogenous pro-IL-1α to the nucleus of IL-1-producing cells, and nuclear binding sites for IL-1 have been demonstrated (19). Additionally, it has been shown that exogenous IL-1 is localized to the nucleus (32, 38), still bound to its receptor (10), which may itself contain a nuclear translocation sequence (19). Targeting of intracellular and exogenous IL-1 to the nucleus may explain shared biological activities. The finding that epithelial cells produce an intracellular form of the IL-1 receptor antagonist that may act as an antagonist of endogenous or internalized IL-1 further implicates an intracellular role for IL-1 (21). The intracellular nature of the IL-1 produced by HTE cells exposed to TCT explains our inability to block the toxic effects of TCT for HTE cells by using anti-IL-1α MAb or exogenous IL-1 receptor antagonist (unpublished observation).

Our results indicate that IL-1 may be the central factor linking the pathology of pertussis to the other biological activities of muramyl peptides. Although other muramyl peptide actions have been correlated to the production of IL-1 as an intercellular mediator, our results imply that muramyl peptides may also act directly on their target cells through the induction of intracellular IL-1 activity. This mechanism would represent a unique enlistment by *B. pertussis* of a natural host cytokine to trigger cell-specific pathophysiology.

ACKNOWLEDGMENTS

We thank T. Flak for purifying TCT, K. Hogquist and D. Chaplin for their gifts of recombinant murine IL-1β and anti-IL-1α MAb, K. Lairmore for technical assistance, and J. Woods for helpful advice.

This work was supported by Public Health Service grant AI22243 from the National Institutes of Health, the Monsanto/Washington University Biomedical Research Contract, Public Health Service training grant AI07172 from the National Institutes of Health to Washington University School of Medicine, and a fellowship from Calgon-Vestal/Merck (to L.N.H.).

REFERENCES

1. **Adam, A., and E. Lederer.** 1984. Muramyl peptides: immunomodulators, sleep factors, and vitamins. Med. Res. Rev. **4:**111–152.
2. **Agace, W., S. Hedges, U. Andersson, J. Andersson, M. Ceska, and C. Svanborg.** 1993. Selective cytokine production by epithelial cells following exposure to *Escherichia coli*. Infect. Immun. **61:**602–609.
3. **Bahr, G. M., L. A. Chedid, and K. Behbehani.** 1987. Induction, in vivo and in vitro, of macrophage membrane interleukin-1 by adjuvant-active synthetic muramyl peptides. Cell. Immunol. **107:**443–454.
4. **Bohach, G. A., D. J. Fast, R. D. Nelson, and P. M. Schlievert.** 1990. Staphylococcal and streptococcal pyrogenic toxins involved in toxic shock syndrome and related illnesses. Crit. Rev. Microbiol. **17:**251–272.
5. **Chedid, L.** 1983. Muramyl peptides as possible endogenous immunopharmacological mediators. Microbiol. Immunol. **27:**723–732.
6. **Collier, A. M., L. P. Peterson, and J. B. Baseman.** 1977. Pathogenesis of infection with *Bordetella pertussis* in hamster tracheal organ culture. J. Infect. Dis. **136**(Suppl.):S196–S203.
7. **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.
8. **Cookson, B. T., A. N. Tyler, and W. E. Goldman.** 1989. Primary structure of the peptidoglycan-derived tracheal cytotoxin of *Bordetella pertussis*. Biochemistry **28:**1744–1749.
9. **Cozzolino, F., M. Torcia, D. Aldinucci, M. Ziche, F. Almerigogna, D. Bani, and D. M. Stern.** 1990. Interleukin 1 is an autocrine regulator of human endothelial cell growth. Proc. Natl. Acad. Sci. USA **87:**6487–6491.
10. **Curtis, B. M., M. B. Widmer, P. deRoos, and E. E. Qwarnstrom.** 1990. IL-1 and its receptor are translocated to the nucleus. J. Immunol. **144:**1295–1303.
11. **Dinarello, C. A.** 1989. Interleukin-1 and its biologically related cytokines. Adv. Immunol. **44:**153–205.
12. **Dinarello, C. A., R. J. Elin, L. Chedid, and S. M. Wolff.** 1978. The pyrogenicity of the synthetic adjuvant muramyl dipeptide and two structural analogues. J. Infect. Dis. **138:**760–767.
13. **Dinarello, C. A., and J. M. Krueger.** 1986. Induction of interleukin 1 by synthetic and naturally occurring muramyl peptides. Fed. Proc. **45:**2545–2548.
14. **Fuhlbrigge, R. C., S. M. Fine, E. R. Unanue, and D. D. Chaplin.** 1988. Expression of membrane interleukin 1 by fibroblasts transfected with murine pro-interleukin 1α cDNA. Proc. Natl. Acad. Sci. USA **85:**5649–5653.
15. **Fuhlbrigge, R. C., K. C. Sheehan, R. D. Schreiber, D. D. Chaplin, and E. R. Unanue.** 1988. Monoclonal antibodies to murine IL-1α. Production, characterization, and inhibition of membrane-associated IL-1 activity. J. Immunol. **141:**2643–2650.
16. **Giri, J. G., P. T. Lomedico, and S. B. Mizel.** 1985. Studies on the synthesis and secretion of interleukin 1. I. A 33,000 molecular weight precursor for interleukin 1. J. Immunol. **134:**343–349.
17. **Goldman, W. E., and J. B. Baseman.** 1980. Selective isolation and culture of a proliferating epithelial cell population from the hamster trachea. In Vitro. **16:**313–319.
18. **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.
19. **Grenfell, S., N. Smithers, K. Miller, and R. Solari.** 1989. Receptor-mediated endocytosis and nuclear transport of human interleukin 1α. Biochem. J. **264:**813–822.
20. **Hackett, S. P., and D. L. Stevens.** 1992. Streptococcal toxic shock syndrome: synthesis of tumor necrosis factor and interleukin-1 by monocytes stimulated with pyrogenic exotoxin A and streptolysin O. J. Infect. Dis. **165:**879–885.
21. **Haskill, S., G. Martin, L. Van Le, J. Morris, A. Peace, C. F. Bigler, G. J. Jaffe, C. Hammerberg, S. A. Sporn, S. Fong, W. P. Arend, and P. Ralph.** 1991. cDNA cloning of an intracellular form of the human interleukin 1 receptor antagonist associated with epithelium. Proc. Natl. Acad. Sci. USA **88:**3681–3685.
22. **Hazuda, D., R. L. Webb, P. Simon, and P. Young.** 1989. Purification and characterization of human recombinant precursor interleukin 1β. J. Biol. Chem. **264:**1689–1693.
23. **Hazuda, D. J., J. C. Lee, and P. R. Young.** 1988. The kinetics of interleukin 1 secretion from activated monocytes. Differences between interleukin 1α and interleukin 1β. J. Biol. Chem. **263:**8473–8479.
24. **Ikejima, T., C. A. Dinarello, D. M. Gill, and S. M. Wolff.** 1984.

Induction of human interleukin-1 by a product of *Staphylococcus aureus* associated with toxic shock syndrome. J. Clin. Invest. **73**:1312–1320.

25. **Kaye, J., S. Gillis, S. B. Mizel, E. M. Shevach, T. R. Malek, C. A. Dinarello, L. B. Lachman, and C. A. Janeway, Jr.** 1984. Growth of a cloned helper T cell line induced by a monoclonal antibody specific for the antigen receptor: interleukin 1 is required for the expression of receptors for interleukin 2. J. Immunol. **133**:1339–1345.
26. **Kilian, P. L., K. L. Kaffka, A. S. Stern, D. Woehle, W. R. Benjamin, T. M. Dechiara, U. Gubler, J. J. Farrar, S. B. Mizel, and P. T. Lomedico.** 1986. Interleukin 1α and interleukin 1β bind to the same receptor on T cells. J. Immunol. **136**:4509–4514.
27. **Kotani, S., M. Tsumimoto, T. Koga, S. Nagao, A. Tanaka, and S. Kawata.** 1986. Chemical structure and biological activity relationship of bacterial cell walls and muramyl peptides. Fed. Proc. **45**:2534–2540.
28. **Krueger, J. M.** 1990. Somnogenic activity of immune response modifiers. Trends Pharmacol. Sci. **11**:122–126.
29. **Kurt-Jones, E. A., D. I. Beller, S. B. Mizel, and E. R. Unanue.** 1985. Identification of membrane-associated interleukin 1 in macrophages. Proc. Natl. Acad. Sci. USA **82**:1204–1208.
30. **Lonnemann, G., S. Endres, J. W. Van der Meer, J. G. Cannon, K. M. Koch, and C. A. Dinarello.** 1989. Differences in the synthesis and kinetics of release of interleukin 1α, interleukin 1β, and tumor necrosis factor from human mononuclear cells. Eur. J. Immunol. **19**:1531–1536.
31. **Maier, J. A. M., P. Voulalas, D. Roeder, and T. Maciag.** 1990. Extension of the life-span of human endothelial cells by an interleukin-1α antisense oligomer. Science **249**:1570–1574.
32. **Mizel, S. B., P. L. Kilian, J. C. Lewis, K. A. Paganelli, and R. A. Chizzonite.** 1987. The interleukin 1 receptor. Dynamics of interleukin 1 binding and internalization in T cells and fibroblasts. J. Immunol. **138**:2906–2912.
33. **Ohlsson, K., P. Bjork, M. Bergenfeldt, R. Hageman, and R. C. Thompson.** 1990. Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. Nature (London) **349**:550–552.
34. **Olsen, L. C.** 1975. Pertussis. Medicine (Baltimore) **54**:427–469.
35. **Oppenheim, J. J., A. Togawa, L. Chedid, and S. Mizel.** 1980. Components of mycobacteria and muramyl dipeptide with adjuvant activity induce lymphocyte activating factor. Cell. Immunol. **50**:71–81.
36. **Parant, M., G. Riveau, F. Parant, C. A. Dinarello, S. M. Wolff, and L. Chedid.** 1980. Effect of indomethacin on increased resistance to bacterial infection and on febrile responses induced by muramyl dipeptide. J. Infect. Dis. **142**:708–715.
37. **Parsonnet, J., R. K. Hickman, D. D. Eardley, and G. B. Pier.** 1985. Induction of human interleukin-1 by toxic-shock-syndrome toxin-1. J. Infect. Dis. **151**:514–522.
38. **Qwarnstrom, E. E., R. C. Page, S. Gillis, and S. K. Dower.** 1988. Binding, internalization, and intracellular localization of interleukin-1β in human diploid fibroblasts. J. Biol. Chem. **263**:8261–8267.
39. **Swope, V. B., Z. Abdel-Malek, L. M. Kassem, and J. J. Nordlund.** 1991. Interleukins 1α and 6 and tumor necrosis factor-α are paracrine inhibitors of human melanocyte proliferation and melanogenesis. J. Invest. Dermatol. **96**:180–185.
40. **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.