Interaction of *Bordetella pertussis* Virulence Components with Neutrophils: Effect on Chemiluminescence Induced by a Chemotactic Peptide and by Intact Bacteria

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The effect of secreted virulence components of *Bordetella pertussis* on chemiluminescence (CL) of rabbit peritoneal neutrophils was determined with the chemotactic peptide *N*'-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) or intact *B. pertussis* as the stimulus. Pertussis toxin (PT) inhibited the response to fMLP in a dose-dependent manner, although only after the neutrophils had been exposed to the toxin for >15 min. Both filamentous haemagglutinin (FHA) and lipopolysaccharide (LPS) markedly enhanced the CL response to fMLP after ≥15 min incubation with the neutrophils. Similar effects to those of *B. pertussis* LPS were also seen with smooth and rough LPS from *Salmonella minnesota*. With the lowest dose of each component which elicited a maximal effect on CL, the inhibitory effect of PT overrode the enhancing effect of FHA and *B. pertussis* LPS. Pre-incubation of neutrophils with PT, FHA or *B. pertussis* LPS caused a slight reduction in the subsequent CL response to virulent *B. pertussis* Tohama. Virulent (phase I, or X-mode) organisms of *B. pertussis* 18334 and *B. pertussis* Tohama induced greater neutrophil CL than their avirulent (C-mode) derivatives. There appeared to be an inverse correlation between bacterial hydrophilicity and the ability to induce neutrophil CL: X-mode bacteria were significantly less hydrophilic than C-mode organisms. Three mutants, the adenylate cyclase (AC)- and haemolysin (HLY)-deficient *B. pertussis* BP348, the FHA-deficient *B. pertussis* BP353, and the PT-deficient *B. pertussis* BP357, generated similar levels of CL and had similar hydrophilicity values. The hydrophilicity value of the avirulent mutant *B. pertussis* BP347 (deficient in AC, HLY, FHA and PT) and the CL induced by this strain were similar to those of *B. pertussis* C-mode organisms. Thus, the interaction of *B. pertussis* with neutrophils appears to be complex, reflecting both the alteration of leucocyte function by secreted virulence components of the organism and, in the absence of opsonins, the surface properties of the bacterium.

INTRODUCTION

Bordetella pertussis is the cause of pertussis (whooping cough), a disease of the respiratory tract (Manclark & Cowell, 1984). Pertussis may be a severe disease in infants, and untreated cases are often complicated by secondary infections (Jamieson, 1973), suggesting that the normal defence mechanisms of the host are altered by *B. pertussis* infection. The lodgement and replication of *B. pertussis* in the respiratory tract would initiate an inflammatory response, with the subsequent recruitment of neutrophils to the infected area. Neutrophils play an important role in respiratory tract defence and patients with neutrophils which have chemotactic disorders are prone to recurrent respiratory illnesses (Smith *et al.*, 1972; Gallin *et al.*, 1980). The production of factors by *B. pertussis* which disrupt neutrophil function could therefore be important in pathogenesis of the disease and of related secondary infections.

Abbreviations: AC, adenylate cyclase; CL, chemiluminescence; FHA, filamentous haemagglutinin; fMLP, *N*'-formyl-L-methionyl-L-leucyl-L-phenylalanine; HBS, HEPES-buffered saline; HLY, haemolysin, PT, pertussis toxin.

Pertussis toxin (PT) is an important virulence component of *B. pertussis* (Weiss & Hewlett, 1986) and elicits a wide variety of biological responses (Wardlaw & Parton, 1983). It has profound effects on phagocyte function, including the inhibition of degranulation and chemotaxis of rabbit neutrophils *in vitro* induced by several chemotactic factors (Becker *et al.*, 1985). Chemotactic factors are important in mediating inflammation and in the recruitment of neutrophils to a site of infection (Wilkinson, 1982). Migration and chemotaxis of murine macrophages are also suppressed by PT (Meade *et al.*, 1984). Some of these effects on phagocytes are due to ADP-ribosylation of membrane-bound regulatory proteins by the toxin (Becker *et al.*, 1986).

Other *B. pertussis* components implicated in virulence include adenylate cyclase (AC), filamentous haemagglutinin (FHA), and lipopolysaccharide (LPS). *B. pertussis* AC has a wide range of antiphagocytic properties (Confer & Eaton, 1982) but unlike the other virulence factors may not be released into the growth medium (Hewlett & Gordon, 1988). FHA is an adhesin of *B. pertussis* and its absence from the organism has been associated with loss of adherence to human ciliated respiratory-epithelial cells *in vitro* (Tuomanen & Weiss, 1985). *B. pertussis* LPS has properties similar to those of other Gram-negative bacteria, such as pyrogenicity and the ability to promote non-specific immunity in mice (Nakase *et al.*, 1970; Ayme *et al.*, 1980).

The relationship between expression of virulence components by *B. pertussis* and its effect on neutrophil bactericidal responses is poorly understood. Light-production (chemiluminescence, CL) may be used as an indirect assay for neutrophil bactericidal responses and can be enhanced by 5-amino-2,3-dihydro-1,4-phthalazinedione (luminol), which is oxidized by products of the neutrophil myeloperoxidase system (DeChatelet *et al.*, 1982; Dahlgren & Stendahl, 1983). The aim of the present investigation was to determine the effects of secreted virulence components of *B. pertussis* on luminol-enhanced CL of neutrophils induced by a soluble (the chemotactic factor *N*'-formyl-L-methionyl-L-leucyl-L-phenylalanine; fMLP) and by a particulate (intact bacteria) stimulus. Strains of *B. pertussis* that are altered in a range of virulence components have been derived by transposon-insertion mutagenesis (Weiss *et al.*, 1983) or can be produced by modifying the growth medium to yield either virulent (phase I, or X-mode) or avirulent (C-mode) organisms (Lacey, 1960; Robinson *et al.*, 1986). The luminol-enhanced CL response of neutrophils to these different strains was also determined. Rabbit neutrophils were used for our study since the rabbit is susceptible to *B. pertussis* aerosol infection and therefore constitutes a relevant model for the disease in humans (Preston *et al.*, 1980; Ashworth *et al.*, 1982).

METHODS

Bacteria and growth conditions. The *B. pertussis* strains used and their relevant properties are listed in Table 1. Strains Tohama and 18334 were from departmental stock cultures. The other four strains were kindly supplied by Dr A. A. Weiss (Department of Microbiology and Immunology, Box 678, Medical College of Virginia, Richmond, Virginia 23298-0001, USA) and were derived from *B. pertussis* strain Tohama as described by Weiss *et al.* (1983).

A loopful of growth from a 72 h culture grown at 37 °C on Bordet-Gengou medium, containing 20% (v/v) sterile, defibrinated horse blood, was used to inoculate each litre of Stainer-Scholte medium (Stainer & Scholte, 1971) modified such that the Tris concentration was 1.25 g l⁻¹. Bacteria were grown either in X-mode (NaCl-containing) medium or in C-mode medium (containing MgSO₄ instead of NaCl) for 48 h at 37 °C in an orbital incubator at 80 r.p.m. (Brownlie *et al.*, 1985). Cultures were centrifuged (6000 g for 30 min at 4 °C) and the sedimented bacteria stored at -70 °C in 9.6 mM-phosphate-buffered saline (PBS; 0.285 M-NaCl, 0.006 M-KCl, 0.016 M-Na₂HPO₄, 0.003 M-KH₂PO₄, pH 7.3) containing 10% (v/v) glycerol until used.

Reagents. Bordet-Gengou medium and defibrinated horse blood were both obtained from Gibco. PT (kindly supplied by Dr V. Y. Perera, Department of Microbiology, Glasgow University), was purified by the method of Perera *et al.* (1985). Briefly, the toxin was isolated by fetuin-Sepharose affinity chromatography of cytoplasmic fractions of *B. pertussis* 18334, phase I, grown for 48 h at 37 °C in cyclodextrin liquid medium (Imaizumi *et al.*, 1983). No detectable AC activity was found in a sample (10 µg protein per assay tube) of the purified toxin. The AC assay was performed as described by Brownlie *et al.* (1985) except that calmodulin (bovine brain) was added to the reaction mixture to a final concentration of 0.3 µM to increase sensitivity. The assay could detect a minimum of 0.2 pM-cAMP per assay tube. FHA was kindly provided by Dr L. A. E. Ashworth (Public Health Laboratory Service Centre for Applied Microbiology and Research, Porton Down, Wilts SP4 0JG, UK). LPS from *B. pertussis* 165 (phase I) was obtained from LIST Biological Laboratories. *Salmonella minnesota* (smooth) LPS, *S. minnesota*

Table 1. *B. pertussis* strains used

Organism (X-mode)	Virulence component(s) absent
<i>B. pertussis</i> 18334	None
<i>B. pertussis</i> Tohama	None
<i>B. pertussis</i> BP347	AC, FHA, PT, haemolysin (HLY), heat-labile toxin
<i>B. pertussis</i> BP348	AC, HLY
<i>B. pertussis</i> BP353	FHA
<i>B. pertussis</i> BP357	PT*

* Some cell-associated PT present (Weiss *et al.*, 1983).

Re595 (rough) LPS, calmodulin, EDTA, oyster glycogen, HEPES, luminol and fMLP were all obtained from Sigma. All LPS preparations were reconstituted according to the supplier's instructions except that HEPES-buffered saline (HBS) was used in place of distilled water. Normal HBS contained (g l⁻¹): NaCl, 8; KCl, 0.4; MgCl₂·6H₂O, 0.2; CaCl₂, 0.14; glucose, 1; and HEPES, 2.388. Divalent-cation-free HBS-EDTA was prepared by the omission of calcium and magnesium salts and addition of 0.292 g EDTA l⁻¹. Both solutions were adjusted to pH 7.4 using 1 M-NaOH.

Hydrophilicity assay. Hydrophilicity of intact bacteria was measured by a modification of the method of Robinson *et al.* (1983). Bacterial suspensions were washed twice in PBS by centrifugation (9000 *g* for 1 min) and resuspended in PBS to an OD₅₅₀ of 1.00. *N*-Octane (300 µl) was added to 4 ml of the bacterial suspension and the suspension vortex-mixed for 1 min. After standing for 20 min at room temperature, the upper, hydrophobic layer was removed and the OD₅₅₀ of the remaining aqueous layer determined. This value, after correction for a PBS control, was multiplied by 100 and expressed as the percentage hydrophilicity of the organism.

Neutrophil preparation. Peritoneal neutrophils were obtained from healthy female New Zealand White rabbits with no history of *Bordetella bronchiseptica* infection. Briefly, 500 ml 0.85% (w/v) NaCl containing 0.1% (w/v) oyster glycogen was injected intraperitoneally and the peritoneal exudate collected 4 h later (Lackie, 1977). The exudate was stored at 4 °C and used within 2 d of isolation. Before use the cells were washed once in divalent-cation-free HBS-EDTA by centrifugation (700 *g* for 60 s at 4 °C). Contaminating erythrocytes were then removed from the cell pellet by resuspension in 2 ml distilled water for 5 s, restoration of isotonicity with HBS, and re-centrifugation of the cell suspension. Washed cells were used within 2 h of preparation. To obtain a monodisperse population of cells for the assay the neutrophils were passed through a 10 µm pore size Nitex filter (Plastok Associates). Cell suspensions contained >95% neutrophils as assessed by microscopy and cell viability was >95% as determined by Trypan Blue exclusion. Cell numbers were standardized by counting with a haemocytometer.

CL assay. This assay was performed using a Wallac LKB 1251 luminometer connected to an Acorn BBC B microcomputer. CL emission was measured in mV at 37 °C. The number of neutrophils per assay tube was 10⁶ in a final volume of 700 µl. The luminometer was run in continuous mixing mode throughout all assays, each sample being assayed in duplicate or triplicate. When fMLP was the stimulus, samples were counted for 0.5 s every 90 s over a period of approximately 30 min, which gave 20 counts per sample. When bacteria were the stimulus, samples were counted for 12 s every 225 s over a period of approximately 60 min, which gave 17 counts per sample. Thus, although the CL curves are shown as continuous traces, they are derived from a finite number of observations (17 or 20 depending on the stimulus), each observation being the mean of replicate samples. In some cases the peak values and total CL counts for each sample were tabulated for ease of comparison. The peak value was the highest CL value obtained over the period of the assay while total CL was the sum of the individual CL counts over the period of the assay. The final concentration of luminol in all assays was 10 µM. When fMLP was the stimulus, both fMLP and luminol were added as a mixture at the start of each assay by automated dispenser. The final concentration of fMLP in the assays was 0.1 µM. Bacterial suspensions used as stimuli were first washed twice in HBS then standardized to an OD₆₀₀ of 0.5 (equivalent to a bacterial dry weight of 255 µg ml⁻¹). A sample (50 µl) of this suspension constituted the bacterial stimulus and was added to pre-heated (20 min at 37 °C) neutrophils prior to automated dispensing of luminol. The ratio of bacteria to neutrophils in the assay was approximately 15:1.

For pre-treatment with a bacterial component(s), neutrophils were pre-incubated in the presence of the component(s) in the luminometer at 37 °C for 60 min, unless otherwise stated. The assay was then started by addition of the stimulus and luminol and counting of the samples.

The Student *t*-test or the Fisher-Behrens test was used to evaluate the significance of differences between the two population means in various experiments (Campbell, 1981).

RESULTS

Effect of individual virulence components on neutrophil CL response to fMLP

Human peripheral neutrophils gave a bimodal response in the presence of fMLP and luminol (Bender & Van Epps, 1983). A similar bimodal response was observed with rabbit peritoneal neutrophils (Fig. 1). In these cells, pre-incubation with PT for 60 min caused a dose-dependent inhibition of the CL induced by fMLP: 6 ng caused a slight suppression and 160 ng an almost complete suppression of CL with 10^6 cells. However, similar pre-incubation with the toxin had no effect on luminol-enhanced CL of neutrophils in the absence of fMLP (Table 2).

Pre-treatment with FHA for 60 min enhanced the CL of neutrophils induced by fMLP at doses of 10–50 ng per 10^6 cells (Fig. 2a). A dose of 250 ng had approximately the same enhancing effect as a dose of 50 ng over the first 10 min of the assay, although a slight inhibitory effect was observed after 20 min (data not shown). Similar pre-treatment with a dose of 50 ng of FHA slightly stimulated luminol-enhanced CL even in the absence of fMLP (Table 2).

Pre-incubation with *B. pertussis* LPS for 60 min enhanced neutrophil CL induced by fMLP at doses $\geq 1 \mu\text{g}$ per 10^6 cells (Fig. 2b). Maximal enhancement occurred with a dose of 10 μg . Although increased neutrophil CL was obtained with doses of LPS exceeding 10 μg , cell viability was diminished by such treatment. As with FHA, pre-treatment with *B. pertussis* LPS for 60 min slightly enhanced neutrophil CL in the absence of fMLP (Table 2).

B. pertussis LPS is a rough LPS (Peppler, 1984) and was compared to the smooth LPS from *Salmonella minnesota* and the rough LPS from *S. minnesota* Re595 to determine if LPS enhancement of neutrophil CL was specific to *B. pertussis* LPS, or typical of LPSs in general. After pre-treatment of neutrophils with LPS for 60 min, *S. minnesota* rough LPS was the most active, *B. pertussis* the second most active, while *S. minnesota* smooth LPS was the least active enhancer of neutrophil CL induced by fMLP (Table 3). The higher dose (10 μg) of *S. minnesota* smooth LPS was not as active as the lower dose (1 μg), suggesting a slight degree of toxicity at the higher dose. Pre-incubation with any of the three LPS types stimulated neutrophil CL in the absence of fMLP (Table 2).

Influence of pre-incubation time on activity of the virulence components

The time of pre-incubation of neutrophils with individual virulence components of *B. pertussis* profoundly affected the subsequent CL response to fMLP (Fig. 3). Neutrophils had to be exposed to PT for > 15 min before any inhibitory effects were observed. An enhanced CL response following exposure to FHA or LPS was first detectable 15 min after addition of these agents to the neutrophils.

Effect of mixtures of virulence components on neutrophil CL response to fMLP

PT inhibited fMLP-induced CL of rabbit peritoneal neutrophils, whereas FHA enhanced it. Since both factors are released during bacterial growth, it was of interest to determine which effect would predominate. After pre-incubation for 60 min with equal doses (50 ng) of both factors, the suppressive effect of PT overrode the enhancing effect of FHA (Table 4). Pre-incubation of neutrophils with 50 ng of FHA and a higher dose of PT (160 ng) caused a further reduction in CL. Similarly, with 10 μg of *B. pertussis* LPS and an inhibitory dose of PT (160 ng), the inhibitory activity of PT almost completely suppressed the enhancing effect of LPS (Table 4).

Effect of purified virulence components on neutrophil CL response to virulent B. pertussis

To further determine the influence of secreted virulence components of *B. pertussis* on neutrophil responses, CL studies were performed using *B. pertussis* Tohama X-mode as the stimulus (Fig. 4). The CL peak values occurred within the first 60 min of the assay, although the responses took up to 2 h to decline to background levels. Most experiments were run for 60 min, which was sufficient to detect any differences in the CL patterns generated. Pre-treatment of neutrophils with 160 ng PT for 60 min caused about 30% inhibition ($P < 0.05$) of the peak value and total CL induced by the bacterial stimulus. Pre-treatment for 60 min with either 50 ng FHA or 10 μg LPS induced an initial increase in the rate of CL generation followed by a decrease, the overall effect being a slight reduction in the peak value and total CL production ($P < 0.05$).

Fig. 1

Fig. 1. Inhibition of fMLP-induced CL of neutrophils by PT. The figure shown is a mean of triplicate observations from one assay and is representative of seven separate experiments. The highest SEM value recorded was ± 0.6 for the first peak and ± 0.5 for the second peak.

Fig. 2

Fig. 2. Enhancement of fMLP-induced CL of neutrophils by (a) FHA and (b) *B. pertussis* LPS. The figures shown are a mean of triplicate observations from one assay and are representative of four separate experiments. The highest SEM value noted was ± 4 for the first peak and ± 2 for the second peak.

Table 2. Stimulation of neutrophil CL by purified virulence components in the absence of fMLP

Test substance	Dose per assay mixture	CL (mV) $\pm$ SEM†	
		Experimental	Control
PT	160 ng	18 $\pm$ 1	18 $\pm$ 1
FHA	50 ng	21 $\pm$ 1**	18 $\pm$ 1
<i>B. pertussis</i> LPS	1 µg	26 $\pm$ 1**	19 $\pm$ 1
<i>S. minnesota</i> smooth LPS	1 µg	24 $\pm$ 1**	19 $\pm$ 1
<i>S. minnesota</i> rough LPS	1 µg	62 $\pm$ 1***	19 $\pm$ 1

† After pre-incubation, individual CL counts were summed over 30 min and the mean value obtained for each sample ($n > 4$ observations). For experimental versus control values: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, otherwise not significantly different from control value ($P > 0.05$).

Pre-incubation of neutrophils with a mixture of PT (50 ng) and FHA (50 ng) for 60 min reduced both the subsequent CL peak value and total CL response to *B. pertussis* Tohama, and the extent of reduction appeared to be a cumulative effect of PT and FHA alone (Table 4).

Neutrophil CL induced by different strains of *B. pertussis*

The effect on neutrophil CL of the loss of a range of cell-associated virulence components by the process of antigenic modulation of two *B. pertussis* strains, 18334 and Tohama, was

Fig. 3. Influence of pre-incubation time on the effect of *B. pertussis* virulence components on neutrophil CL induced by fMLP. The individual CL counts were summed over 30 min and expressed as percentage CL relative to the control (= 100%). Each point shown is the mean of six observations obtained from duplicate samples in three separate experiments; bars represent SEM.

Table 3. Influence of *B. pertussis* and *S. minnesota* LPSs on neutrophil CL induced by fMLP

Test substance	Dose per assay mixture (µg)	CL (mV) ± SEM†	
		Peak value	Total
Control	—	26 ± 3	347 ± 11
<i>B. pertussis</i> LPS	1	90 ± 13***	667 ± 22***
	10	151 ± 4***	963 ± 39**
<i>S. minnesota</i> smooth LPS	1	75 ± 10**	579 ± 22***
	10	51 ± 3***	362 ± 26
<i>S. minnesota</i> rough LPS	1	193 ± 2***	847 ± 22***
	10	176 ± 6***	912 ± 29**

† Derived as in Table 2; *n* = 6 observations.

Table 4. Influence of mixtures of *B. pertussis* virulence components on neutrophil CL induced by fMLP or by *B. pertussis* Tohama X-mode organisms

Stimulus	Test substance (dose per assay mixture)	CL (mV) ± SEM†	
		Peak value	Total
fMLP	Control	20 ± 1	292 ± 16
	PT (50 ng)	7 ± 2**	83 ± 23***
	PT (160 ng)	3 ± 1**	40 ± 9**
	FHA (50 ng)	117 ± 16**	868 ± 73**
	PT (50 ng) + FHA (50 ng)	14 ± 4	142 ± 42*
	PT (160 ng) + FHA (50 ng)	7 ± 1***	65 ± 12***
	LPS (10 µg)	108 ± 3**	898 ± 14***
	PT (160 ng) + LPS (10 µg)	5 ± 1***	51 ± 2***
<i>B. pertussis</i>	Control	333 ± 14	4338 ± 154
	PT (50 ng)	276 ± 14*	3654 ± 166**
	FHA (50 ng)	292 ± 7*	3851 ± 93*
	PT (50 ng) + FHA (50 ng)	241 ± 9***	3085 ± 104***

† Derived as in Table 2; *n* = 9 observations.

Fig. 4

Fig. 5

Fig. 4. Effect of purified virulence components of *B. pertussis* on generation of neutrophil CL to *B. pertussis* Tohama X-mode organisms. Each figure shown is the mean of triplicate observations from one assay and is representative of at least four separate experiments. The highest SEM value recorded was ± 25 .

Fig. 5. Stimulation of neutrophil CL by various strains of *B. pertussis*. The mode of the organism is given in parentheses. The figure shown is the mean of eight observations obtained from duplicate samples in four separate experiments. The highest SEM value observed was ± 14 . The overall CL responses stimulated by *B. pertussis* Tohama (C), *B. pertussis* BP347 (X) and *B. pertussis* 18334 (C) were not significantly different ($P > 0.05$).

investigated. *B. pertussis* Tohama and 18334 C-mode organisms induced lower CL peaks and lower total levels of CL than their respective virulent X-mode counterparts (Fig. 5). The *B. pertussis* Tohama X-mode strain induced a significantly higher CL peak value and higher total CL than the *B. pertussis* 18334 X-mode strain ($P < 0.001$), perhaps reflecting inter-strain differences in expression of biologically active products. A transposon-induced avirulent

Fig. 6. A comparison of hydrophilicity (open bars) of strains of *B. pertussis* with their generation of neutrophil CL (hatched bars). The CL results are the total counts over 60 min. Percentage CL was expressed relative to the level of CL induced by the *B. pertussis* Tohama (X) strain (taken as 100%). Each point shown is the mean of between 8 and 12 observations obtained from duplicate samples in four to six separate experiments; bars represent SEM.

mutant, *B. pertussis* BP347, grown in X-mode culture medium, induced a similar CL pattern to the C-mode organisms.

To assess the role of individual, cell-associated, virulence components of *B. pertussis* on the neutrophil CL response, several transposon-induced mutants deficient in either one or two virulence components were assayed and compared with the CL response to virulent *B. pertussis*. The total CL generated by all strains correlated well with the CL peak values, and hence only total CL values are shown for these strains (Fig. 6). The CL responses generated by the AC- and HLY-deficient mutant *B. pertussis* BP348, the FHA-deficient mutant *B. pertussis* BP353, and the PT-deficient mutant *B. pertussis* BP357 were not significantly different ($P > 0.05$). Although reduced compared to Tohama X-mode organisms ($P < 0.01$), the CL stimulated by these three mutants was still higher than that generated in response to any of the C-mode bacteria and was similar to that to 18334 X-mode organisms.

Hydrophilicity of the B. pertussis strains

The hydrophilicity of a bacterium is important in determining its interaction with other cells, and so the hydrophilicity values of the *B. pertussis* strains used in the CL studies were measured. *B. pertussis* BP347 and the *B. pertussis* C-mode organisms were significantly more hydrophilic ($P < 0.01$) than *B. pertussis* X-mode organisms (Fig. 6). *B. pertussis* BP347 was the only strain to differ significantly ($P < 0.01$) in hydrophilicity when compared to other X-mode strains.

DISCUSSION

A significant observation in this study was that FHA and LPS enhanced, whereas PT inhibited, neutrophil CL induced by fMLP. In addition, PT overrode the enhancing effect of FHA or *B. pertussis* LPS. PT inhibits both rabbit and human neutrophil chemotaxis and degranulation induced by fMLP (Becker *et al.*, 1985; Lad *et al.*, 1985). Our results suggest that neutrophil myeloperoxidase-associated responses to fMLP would also be suppressed during a

pertussis infection, even in the presence of stimulatory components such as FHA or *B. pertussis* LPS. The suppression of neutrophil responses to chemotactic factors by PT could inhibit stimulation of bactericidal activity and leucocyte recruitment to an area of infection. This inhibition may prolong survival of *B. pertussis* or secondary invaders *in vivo*.

The stimulation of neutrophil CL by *B. pertussis* LPS, in the absence or presence of fMLP, was non-specific since both *S. minnesota* smooth and rough LPS had similar effects. There was an association between roughness of LPS and stimulation of neutrophil CL. Similar results have been reported with smooth and rough *Escherichia coli* LPS and human neutrophils in the absence of fMLP (Henricks *et al.*, 1983).

The susceptibility of a bacterium to interaction with phagocytes decreases as bacterial hydrophilicity increases (Stendahl, 1983). In agreement with earlier studies (Robinson *et al.*, 1983), we found that *B. pertussis* C-mode strains were more hydrophilic than their X-mode counterparts. There appeared to be an inverse correlation between hydrophilicity and neutrophil CL induced by *B. pertussis* X- and C-mode organisms. An association between bacterial hydrophilicity and induction of poor CL by human neutrophils with unopsonized *Escherichia coli* was reported by Topley *et al.* (1984). Since CL is an indicator of neutrophil bactericidal activity (Horan *et al.*, 1982), *B. pertussis* C-mode bacteria seem to stimulate rabbit neutrophils less than their respective X-mode strains, but it is not known if this is the case with *B. pertussis* strains and human neutrophils.

The transposon-induced avirulent mutant *B. pertussis* BP347 (Weiss *et al.*, 1983) is similar to C-mode organisms in that both are deficient in a number of virulence components (Robinson *et al.*, 1986). *B. pertussis* BP347 was similar to C-mode organisms in being more hydrophilic than X-mode organisms and in stimulating a low level of neutrophil CL. Three other transposon-induced mutants, *B. pertussis* BP348, *B. pertussis* BP353 and *B. pertussis* BP357, are deficient in only one or two virulence components. These three mutants had similar hydrophilicity values and induced levels of CL comparable to those of X-mode organisms. Thus, the transition of *B. pertussis* from the hydrophobic to hydrophilic state is not solely due to loss in AC and HLY, FHA or PT.

Although CL was similar with virulent *B. pertussis* 18334 and FHA- or PT-deficient mutants as stimulus, neutrophils pre-treated with exogenous FHA or PT gave slightly suppressed CL responses to virulent *B. pertussis*. With the ratio of bacteria to neutrophils used there may not have been enough of each virulence factor on the surface of the washed bacterial cells to cause inhibition of CL. Also this may explain the discrepancy between reports of purified AC inhibiting neutrophil CL induced by bacteria (Friedman *et al.*, 1987) and the present study showing that an AC- and HLY-deficient mutant of *B. pertussis* induced less CL than a strain which produced both components.

The data of Friedman *et al.* (1987) showed that PT has only a slight inhibitory effect on the CL of human neutrophils induced by *Staphylococcus aureus*. We found a similar pattern with rabbit neutrophils and *B. pertussis*. In addition, PT has only a slight inhibitory effect on neutrophil release of myeloperoxidase to another particulate stimulus, opsonized zymosan (Spangrude *et al.*, 1985). Thus, the interaction of particulate stimuli with neutrophils is more resistant to disruption by PT than are responses stimulated by chemotactic factors. This suggests that the PT-sensitive regulatory protein involved in stimulation by chemotactic factors is not so important in mediating the neutrophil response to particulate stimuli.

FHA and *B. pertussis* LPS had different effects on the overall response of neutrophils to fMLP and to *B. pertussis*. However, both components had an initial stimulatory effect, over about the first 15 min of each assay, with either stimulus. The influence of these components on neutrophil responses may depend on the nature of the stimulus and on the time-course over which the stimulus has its major effect. To our knowledge this is the first report of FHA showing biological activity with phagocytes. The effects of FHA on neutrophil CL responses suggest that this factor may also influence other neutrophil functions. FHA is a major adhesin of *B. pertussis* (Manclark & Cowell, 1984; Tuomanen & Weiss, 1985), and like other bacterial adhesins (Scheffer *et al.*, 1985), it could play a role in pathogenesis by induction of the release of inflammatory mediators from neutrophils.

In conclusion, exogenous FHA and LPS enhanced fMLP-induced CL of neutrophils but had an overall slight inhibitory effect on the neutrophil response to virulent *B. pertussis*. However, the inhibitory effect of PT overrode the enhancing effect of both FHA and *B. pertussis* LPS on the neutrophil response to fMLP, suggesting that inhibition of CL could provide a sensitive, *in vitro* assay for PT (Craig *et al.*, 1988). The neutrophil CL pattern induced by an individual strain of unopsonized *B. pertussis* appears to be influenced by strain hydrophilicity. Although unopsonized *B. pertussis* C-mode organisms are more resistant to interaction with neutrophils than are X-mode organisms, the production of virulence factors, such as PT and AC (Weiss & Hewlett, 1986), by X-mode bacteria would probably suppress neutrophil bactericidal responses *in vivo* and therefore prolong the survival of the bacterium or secondary pathogens. Thus, the interaction of *B. pertussis* with neutrophils appears to be complicated, depending on disruption of leucocyte function by secreted virulence components and, in the absence of opsonins, on surface components of the organism.

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