INFECTION AND IMMUNITY, May 1992, p. 2101–2105
0019-9567/92/052101-05$02.00/0
Copyright © 1992, American Society for Microbiology
Vol. 60, No. 5

Bordetella pertussis Induces Respiratory Burst Activity in Human Polymorphonuclear Leukocytes

LISA L. STEED,† EMMANUEL T. AKPORIAYE, AND RICHARD L. FRIEDMAN*
Department of Microbiology and Immunology, University of Arizona, Tucson, Arizona 85724

Received 6 January 1992/Accepted 11 February 1992

Virulent *Bordetella pertussis* strains survive intracellularly within human polymorphonuclear leukocytes (PMN), at least in part because of inhibition of phagosome-lysosome fusion (L. L. Steed, M. Setareh, and R. L. Friedman, J. Leukocyte Biol. 50:321–330, 1991). Further investigations were done to determine if *B. pertussis* also inhibited respiratory burst activity of PMN as an additional mechanism of intracellular survival. Chemiluminescence and flow cytometry assays showed that *B. pertussis* induced significant levels of hydrogen peroxide production. In contrast, ferricytochrome *c* reduction showed that *B. pertussis* suppressed extracellular release of superoxide. PMN intracellular reduction of nitroblue tetrazolium verified that superoxide was indeed produced intracellularly during *B. pertussis* phagocytosis. Therefore, *B. pertussis* does not inhibit production of superoxide but inhibits only its release. Thus, while phagosome-lysosome fusion is inhibited by *B. pertussis*, respiratory burst activity of PMN occurs at normal levels.

Bordetella pertussis is the etiologic agent of pertussis, a highly contagious, strictly human respiratory disease that has no known carriers, reservoirs, or vectors. Several studies have shown that *B. pertussis* can persist intracellularly within murine alveolar macrophages (12, 26), murine cerebral ependymal cells (29), HeLa cells (18, 19, 35), and human respiratory epithelial cells (19). *B. pertussis* has recently been reported to invade and survive inside human macrophages (10, 21, 43). We have recently demonstrated that *B. pertussis* also survives intracellularly within human polymorphonuclear leukocytes (PMN) (46). Internalization of these bacteria is immunoglobulin G (IgG) dependent, and survival depends, at least in part, on inhibition of phagosome-lysosome fusion (46). Two purified exoproducts of *B. pertussis*, pertussis toxin (PT) and adenylate cyclase toxin (ACT), have also been reported to inhibit phagocyte respiratory burst activity (7, 14, 22, 24, 36, 45, 49). Other bacteria have been demonstrated to inhibit phagosome-lysosome fusion, respiratory burst, or both as a mechanism for intracellular survival (1, 2, 6, 11, 23, 27, 28, 30, 33, 34, 38, 41).

During the respiratory burst process various toxic oxygen products, including superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals, are produced in the phagosome (for a review, see references 4 and 13). Thus, the ability of some microbes to block the respiratory burst and/or phagosome-lysosome fusion would protect them from oxygen-dependent and -independent killing mechanisms of PMN.

This study was performed to investigate the effect of intact virulent *B. pertussis* on PMN respiratory burst activity with a combination of in vitro intracellular and extracellular methods to assess the production of both hydrogen peroxide and superoxide. *B. pertussis*, in contrast to the suppression seen with purified PT and ACT, did not inhibit PMN respiratory burst activity.

Staphylococcus aureus, a clinical isolate, or *Escherichia coli* Bi 161-42 was used as a control in all assays. The virulent strains of *B. pertussis* used in this research were BP504, BP165, and a derivative of Tohama I, BP338 (46). Autologous normal sera (NS) were collected from clotted venous blood of PMN donors, held on ice, and used for opsonization of *S. aureus* and *E. coli* as described previously (46). Human anti-*B. pertussis* antibody (HAPA; Tosuman Berna; Swiss Serum and Vaccine Institute, Bern, Switzerland) is a purified IgG fraction of pooled human sera obtained from donors demonstrating high agglutinin titers against *B. pertussis*. An IgG fraction was made from pooled NS of individuals demonstrating high anti-whole-cell *B. pertussis* titers by enzyme-linked immunosorbent assay. HAPA and IgG fraction were used for opsonization of *B. pertussis* strains, as described previously (46). Anti-whole-cell *B. pertussis* titers of NS, HAPA, and IgG fraction against each of three virulent strains (range, 1:300 to 1:400) were not significantly different (46). Detailed characterization of HAPA, NS, and IgG fraction have been reported in our previous paper (46).

PMN used in these studies were isolated daily from heparinized venous blood through Ficoll-Hypaque as described previously (46). PMN were resuspended in Hanks' balanced salt solution containing 0.1% gelatin at a concentration of 2×10^6 PMN per ml. PMN viability was assayed by trypan blue exclusion before and after each assay and was consistently >95%. For all respiratory burst assays described, the bacterium-to-PMN ratio of 60:1 was used, unless otherwise stated.

In all the assays used to monitor respiratory burst activity of PMN, the optimum opsonin for each microbe was used, unless otherwise stated, to induce equal levels of phagocytosis. In a previous study we reported that the optimum opsonin for *B. pertussis* PMN phagocytosis is anti-*B. pertussis* antibody (IgG), which is present in both HAPA and IgG fraction (isolated from NS) (46). No complement is contained in either preparation (46). The optimum opsonin for *S. aureus* is complement (48), while *E. coli* requires IgM and complement (31), which are present in NS.

Chemiluminescence (CL) assays were performed by the method of Friedman et al. (22). Results were expressed as the average counts per minute (experimental counts per minute minus background counts per minute) and were

* Corresponding author.
† Present address: Department of Pathology, College of Medicine, University of Utah, Salt Lake City, UT 84132.

presented as the percentage of maximum CL relative to the percentage of maximum CL induced by *S. aureus* opsonized in NS (control). Superoxide anion production assays were performed by the method of Akporiaye et al. (1). Phorbol myristate acetate (PMA; Sigma Chemical Co.; 100 ng/ml) was used as a positive control for induction of superoxide release. In some experiments, 100 μg of superoxide dismutase (from bovine erythrocytes; Sigma) per ml was added to determine the amount of superoxide dismutase-inhibitable superoxide produced. The amount of superoxide formed was calculated by using the extinction coefficient of 2.1×10^4 $M^{-1}cm^{-1}$ at 550 nm. Results are given as nanomoles of superoxide produced per 5×10^5 PMN per 60 min. Nitroblue tetrazolium (NBT) reduction assays were done to monitor intracellular production of superoxide by phagocytosing PMN. Assays, done in duplicate from four donors, contained 0.1 ml of NBT solution (0.1% in phosphate-buffered saline containing gelatin), 0.1 ml of PMN (2×10^6/ml), and 0.1 ml of opsonized *B. pertussis*, *E. coli*, *S. aureus* (as prepared above), or PMA (100 ng/ml), and mixtures were incubated for 30 min at 37°C. Cells were fixed by the addition of 10% formalin, centrifuged, and resuspended in normal serum, and cytocentrifuge preparations were made. Slides were air dried, fixed in 100% methanol for 60 s, and then counterstained in 0.05% safranin O for 5 min. The percentage of cells that were formazan positive was determined microscopically by counting 100 cells under oil immersion. Flow cytometric analysis of reactive oxygen molecules was conducted by the method of Bass et al. (5) with modification. PMN were loaded with a final concentration of 5 μM dichlorofluorescin diacetate (DCFH-DA; Eastman Kodak, Rochester, N.Y.) for 15 min in a shaking 37°C water bath. After the loading period, 20 μl (each) of 0.25 mM EDTA and 0.25 mM sodium azide per ml of PMN was added. Background fluorescence (fluorescence of unstimulated PMN) was determined on a Becton Dickinson FACScan analyzer. DCFH-DA-loaded PMN were added to NS-opsonized *E. coli*, HAPA- or IgG fraction-opsonized *B. pertussis*, or 100 ng of PMA per ml. FACScan analysis was conducted with the same parameters as previously described for the flow cytometric analysis of phagocytosis (46). Results were presented as the average maximum fluorescence intensity (FI) associated with the stimulated PMN (experimental maximum FI minus background maximum FI).

Figure 1 shows the results of CL responses of PMN to *B. pertussis* and *S. aureus*. CL response of PMN to all NS-opsonized *B. pertussis* strains tested was 13% or less of *S. aureus* control values and in some cases was essentially nonexistent ($P < 0.01$) (Fig. 1). NS has anti-*B. pertussis* titers similar to those of HAPA, yet when used as an opsonin for *B. pertussis*, NS does not induce uptake by PMN. This suggests the presence of a blocking agent in NS that prevents opsonic anti-*B. pertussis* antibody from binding to *B. pertussis* (46). When *B. pertussis* was opsonized with HAPA, the CL response was dramatically different with BP338, most closely attaining *S. aureus* CL levels (96.2%). The CL response induced by HAPA-opsonized BP338 was not significantly different from *S. aureus* levels, while HAPA-opsonized BP504 and BP165 induced significantly lower ($P < 0.05$) CL response levels than *S. aureus*. The CL response induced by HAPA-opsonized BP338 was significantly higher than levels induced by either HAPA-opsonized BP504 or BP165 ($P < 0.05$).

PMN superoxide production was monitored by reduction of ferricytochrome *c*. The amount of superoxide production induced by opsonized *E. coli*, *S. aureus*, and *B. pertussis*

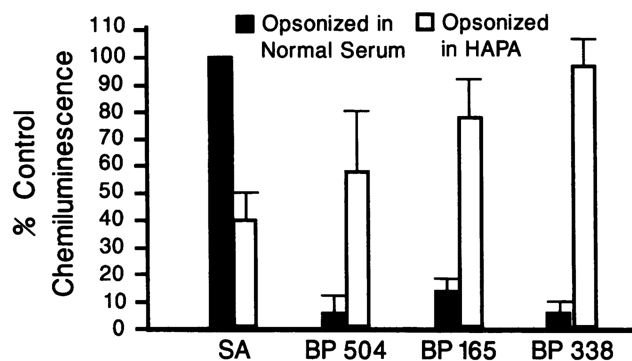

FIG. 1. Induction of PMN CL by *B. pertussis* strains and *S. aureus* opsonized in NS or HAPA. CL response was assayed as described in Materials and Methods. CL response is given as the maximum CL intensity observed compared with that of the control (*S. aureus* opsonized in NS). Values represent the mean ± standard deviation of at least three experiments done in duplicate.

(BP338) was compared with the superoxide production of unstimulated PMN from four individuals done in duplicate. Unstimulated PMN produced 1.5 ± 1.5 nmol of superoxide. Both *E. coli* and *S. aureus* stimulated superoxide production exceeding background levels, with means of 26.8 ± 19.0 and 17.8 ± 14.4 nmol of superoxide produced, respectively. BP338 was unable to stimulate superoxide production, with −2 ± 8.3 nmol of superoxide produced. Normal PMN superoxide production was indicated by the production of 548.9 ± 71.8 nmol of superoxide in response to PMA. Detection of superoxide production stimulated by all three microbes and PMA was inhibited more than 95% by the addition of exogenous superoxide dismutase.

It is possible that the superoxide produced by PMN in response to *B. pertussis* is being rapidly degraded to hydrogen peroxide before it can leave the cell and reduce ferricytochrome *c*, since *B. pertussis* produces high levels of superoxide dismutase (20b). However, this does not seem to be a problem in assessing superoxide production induced by other superoxide dismutase-producing microbes like *Mycobacterium tuberculosis* (38, 50) and *Coxiella burnetii* (1). Another possibility is that *B. pertussis*-associated PT and ACT induce increased intracellular cyclic AMP levels within PMN which could interfere with extracellular release of superoxide and thereby impede ferricytochrome *c* reduction.

When *B. pertussis* was added simultaneously with *E. coli*, *S. aureus*, or PMA or if PMN were pretreated with *B. pertussis* for 30 min prior to the addition of other respiratory burst stimuli, no inhibition of superoxide release was observed by using the ferricytochrome *c* reduction assay (data not shown). Thus, while *B. pertussis* phagocytosis fails to trigger PMN superoxide release, it does not prevent superoxide release induced by other particulate or soluble stimuli.

NBT reduction, which detects intracellular production of superoxide and H_2O_2, was monitored in PMN that had phagocytosed *B. pertussis* to verify that superoxide was generated intracellularly, but not released, from the phagocyte. *B. pertussis* induced NBT reduction in PMN at levels equal to those induced by *E. coli* and *S. aureus* (77, 82, and 81%, respectively). PMA stimulated NBT reduction in 98% of PMN, while unstimulated PMN controls showed only 3% NBT reduction. These results confirm that phagocytosis of *B. pertussis* does indeed induce a respiratory burst. Two other microbes have been reported to stimulate respiratory

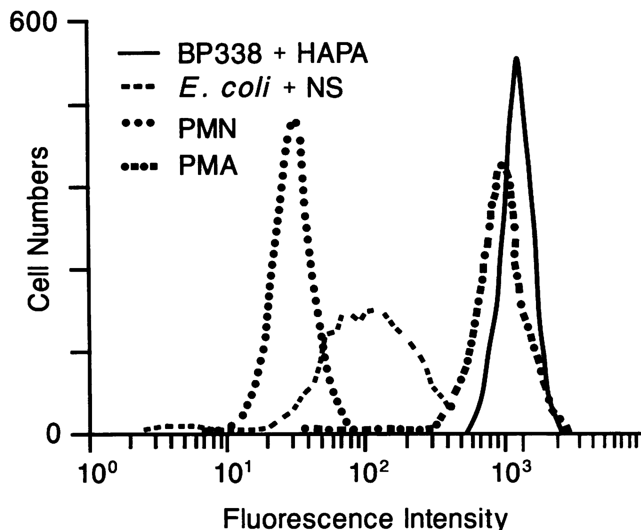

FIG. 2. Flow cytometric analysis of PMN respiratory burst activity. The stimuli used were *E. coli* opsonized in NS, BP338 opsonized in HAPA, and PMA. Opsonized bacteria or PMA (100 ng/ml) were incubated with PMN loaded with DCFH-DA at a 60:1 ratio. Ten thousand events per sample were collected. The histogram of the maximum fluorescence intensity of each stimulus was graphed in comparison with the background fluorescence of unstimulated PMN. This is a group of representative experiments performed at least four different times with almost identical results.

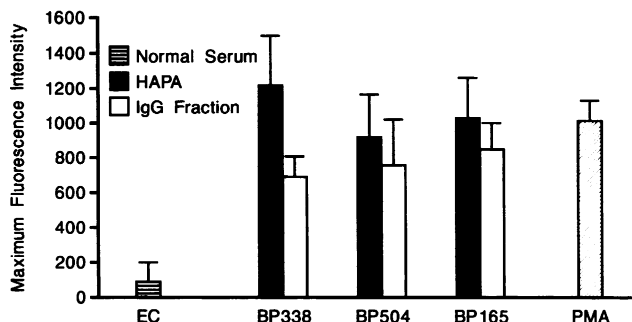

FIG. 3. Flow cytometric analysis of PMN respiratory burst activity induced by *E. coli*, *B. pertussis* strains, and PMA. *E. coli* cells were opsonized with NS while *B. pertussis* BP338, BP504, and BP165 were opsonized with either HAPA or IgG fraction. Opsonized bacteria were incubated with PMN loaded with DCFH-DA in a 60:1 ratio. PMA (100 ng/ml) was added as a positive control stimulator of respiratory burst activity. Ten thousand events per sample were collected, and the mean maximum fluorescence intensities of the stimuli were calculated. The values represent the mean ± standard deviation of at least three experiments.

burst activity of phagocytes but to block extracellular release of superoxide. *Neisseria gonorrhoeae* stimulates a respiratory burst without superoxide or H_2O_2 release upon interaction with human PMN (40), and the fungus *Histoplasma capsulatum* fails to induce superoxide release from macrophages and human PMN (17, 44).

Bass et al. (5) developed a technique which can directly monitor respiratory burst activity occurring within phagocytes by using the compound DCFH-DA, which becomes oxidized and fluoresces in the presence of H_2O_2. Flow cytometry is used to measure the resulting fluorescence of individual phagocytes. This technique has been used to study PMN respiratory burst response to *S. aureus*, *E. coli*, and *Candida albicans* (8, 25, 32, 42, 47).

Figure 2 shows representative fluorescence histograms for *E. coli*-, BP338-, and PMA-stimulated PMN compared with those of unstimulated PMN. Similar histograms were obtained for BP504 and BP165 (data not shown). Respiratory burst activity induced by *B. pertussis* was equivalent to that induced by PMA and was much greater than that induced by *E. coli*. Figure 3 shows the mean maximum FI induced by normal serum-opsonized *E. coli*, by all three *B. pertussis* strains opsonized in both HAPA and IgG fraction, and by PMA. The levels of respiratory burst induced by all *B. pertussis* strains, opsonized with either opsonin, and by PMA were all significantly higher than levels induced by *E. coli* ($P < 0.01$). There was no statistical difference observed in the levels of respiratory burst induced by the three *B. pertussis* strains compared with that induced by PMA. These results indicate that the respiratory burst is quite active in PMN that have phagocytosed *B. pertussis*.

Highly active *B. pertussis* superoxide dismutase conversion of phagosome superoxide to hydrogen peroxide may explain the ability of *B. pertussis* to induce very high levels of respiratory burst activity detected by flow cytometry. Another possibility is that *E. coli* did not induce as high a response as *B. pertussis* in this assay because of the difference in opsonins used for each microbe. IgG (opsonin for *B. pertussis*) may stimulate different levels of intracellular H_2O_2 than do complement and IgM (opsonins for *E. coli*).

In the present studies we assessed PMN respiratory burst activity induced by *B. pertussis*. In contrast to the ferricytochrome *c* reduction assay, CL assays and flow cytometry demonstrated that whole-cell *B. pertussis* induced significant levels of PMN respiratory burst activity. The flow cytometry technique measures the amount of hydrogen peroxide present within individual PMN (5). CL assays monitor hydrogen peroxide released into the extracellular medium as well as that within PMN, because luminol can enter phagocytic cells (9, 16). CL assays chiefly monitor products from the interaction of hydrogen peroxide and myeloperoxidase (9, 16). The fact that these two techniques which measure hydrogen peroxide show similar results suggests that *B. pertussis* does indeed induce PMN respiratory burst activity. Craig et al. previously reported that *B. pertussis* induces a CL response in rabbit PMN, yet CL levels induced were not compared with those of other control microorganisms (15).

It is surprising that intact *B. pertussis* does not inhibit respiratory burst activity, since purified PT and ACT both have been reported to inhibit this response (14, 15, 22, 24, 36, 45). It is conceivable that the concentrations of PT and ACT used in these studies were in excess of what PMN would be subjected to in vivo upon direct interaction with *B. pertussis*. In support of the purified ACT studies, Mouallem et al. (39) demonstrated that both purified ACT and intact virulent *B. pertussis* increased cyclic AMP in Chinese hamster ovary (CHO) cells. Unfortunately, CHO cells do not have a respiratory burst. The observed inhibition of respiratory burst activity of phagocytes by using purified PT and ACT may be an in vitro artifact. Yet, the levels of PT and ACT that are actually produced and released in a patient with pertussis is unknown.

While *B. pertussis* induces normal levels of respiratory burst activity, the microbe inhibits phagosome-lysosome fusion (46). *M. tuberculosis* also prevents phagosome-lysosome fusion but has no effect on respiratory burst activity (38). In contrast, *Brucella abortus* inhibits respiratory burst

activity but not phagosome-lysosome fusion (41). *Legionella pneumophila* inhibits both respiratory burst activity and phagosome-lysosome fusion (23, 30). The fact that phagosome-lysosome fusion does not occur in response to internalized *B. pertussis* at least partially explains how this microbe can survive intracellularly.

While phagosome-lysosome fusion is inhibited, phagosomes containing *B. pertussis* will still contain high levels of superoxide and hydrogen peroxide, produced via the respiratory burst, which could reach toxic levels. *B. pertussis* produces both superoxide dismutase and catalase (20a), which may break down these toxic oxygen products to oxygen and water, allowing for increased survival in the phagosome. Franzon et al. (20) demonstrated that superoxide dismutase, and not catalase, is critical for intracellular survival of *Shigella flexneri*, while catalase expression is critical for *S. aureus* intracellular survival (37). Studies are in progress to determine the role of *B. pertussis* superoxide dismutase and catalase in intracellular survival.

The residency of *B. pertussis* within PMN may simply be a mechanism for avoidance of host immune responses. It does not appear likely that *B. pertussis* multiplies within PMN, because of the long generation time of the bacteria and the short life span of PMN. *B. pertussis* may multiply intracellularly within longer-life alveolar macrophages or respiratory epithelial cells rather than PMN. These *B. pertussis*-phagocyte interactions may help explain the longevity of pertussis as well as the incidence of complicating secondary infections.

We thank John Galgiani and Richard Rest for their input and critical review of these studies. We also thank John Roy for the statistical analysis done on these studies.

This work was supported in part by a grant from the Arizona Disease Control Research Commission and Public Health Service grant R01-A1 22822 to R.L.F. L.L.S. was supported by Public Health Service grant CA 39827.

REFERENCES

1. **Akporiaye, E. T., D. Stefanovich, V. Tsosie, and G. Baca.** 1990. *Coxiella burnetii* fails to stimulate human neutrophil superoxide anion production. Acta Virol. **34:**64–70.
2. **Anderson, S. E., Jr., and J. S. Remington.** 1974. Effect of normal and activated human macrophages on *Toxoplasma gondii*. J. Exp. Med. **139:**1154–1174.
3. **Arthur, M. J. P., P. Kowalski-Sanders, S. Gurney, R. Tolcher, F. G. Bull, and R. Wright.** 1987. Reduction of ferricytochrome c may underestimate superoxide production by monocytes. J. Immunol. Methods **98:**63–69.
4. **Babior, B. M.** 1984. The respiratory burst of phagocytes. J. Clin. Invest. **73:**599–601.
5. **Bass, D. A., J. W. Parce, L. R. DeChatelet, P. Szejda, M. C. Seeds, and M. Thomas.** 1983. Flow cytometric studies of oxidative product formation by neutrophils: a graded response to membrane stimulation. J. Immunol. **130:**1910–1917.
6. **Beaman, B. L., C. M. Black, F. Doughty, and L. Beaman.** 1985. Role of superoxide dismutase and catalase as determinants of pathogenicity of *Nocardia asteroides*: importance in resistance to microbicidal activities of human polymorphonuclear neutrophils. Infect. Immun. **47:**135–141.
7. **Becker, E. L., J. C. Kermode, P. H. Naccache, R. Yassin, J. J. Munoz, M. L. Marsh, C. K. Huang, and R. I. Sha'afi.** 1986. Pertussis toxin as a probe of neutrophil activation. Fed. Proc. **45:**2151–2155.
8. **Bjerknes, R., H. Vindenes, J. Pitkanen, J. Ninnemann, O. D. Laerum, and F. Abyholm.** 1989. Altered polymorphonuclear neutrophilic granulocyte functions in patients with large burns. J. Trauma **29:**847–855.
9. **Briheim, G. O. S., and C. Dahlgren.** 1984. Intra- and extracellular events in luminol-dependent chemiluminescence of polymorphonuclear leukocytes. Infect. Immun. **45:**1–5.
10. **Bromberg, K., G. Tannis, and P. Steiner.** 1991. Detection of *Bordetella pertussis* associated with the alveolar macrophages of children with human immunodeficiency virus infection. Infect. Immun. **59:**4715–4719.
11. **Charnetzy, W. T., and W. W. Shuford.** 1985. Survival and growth of *Yersinia pestis* within macrophages and the effect of the loss of the 47-megadalton plasmid on growth in macrophages. Infect. Immun. **47:**234–241.
12. **Cheers, C., and D. F. Gray.** 1969. Macrophage behavior during the complaisant phase of murine pertussis. Immunology **17:**875–887.
13. **Clark, R. A.** 1990. The human neutrophil respiratory burst oxidase. J. Infect. Dis. **161:**1140–1147.
14. **Confer, D. L., and J. W. Eaton.** 1982. Phagocyte impotence caused by an invasive bacterial adenylate cyclase. Science **217:**948–950.
15. **Craig, F. F., J. M. Lackie, R. Parton, and J. H. Freer.** 1988. Interaction of *Bordetella pertussis* virulence components with neutrophils: effect on chemiluminescence induced by a chemotactic peptide and intact bacteria. J. Gen. Microbiol. **134:**2201–2211.
16. **Dahlgren, C., H. Anianssoin, and K. E. Magnusson.** 1985. Pattern of formylmethionyl-leucyl-phenylalanine-induced luminol- and lucigenin-dependent chemiluminescence in human neutrophils. Infect. Immun. **47:**326–328.
17. **Eissenberg, L. G., and W. E. Goldman.** 1987. *Histoplasma capsulatum* fails to trigger release of superoxide from macrophages. Infect. Immun. **55:**29–34.
18. **Ewanowich, C. A., A. R. Melton, A. A. Weiss, R. K. Sherburne, and M. S. Peppler.** 1989. Invasion of HeLa 229 cells by virulent *Bordetella pertussis*. Infect. Immun. **57:**2698–2704.
19. **Ewanowich, C. A., R. K. Sherburne, S. F. P. Man, and M. S. Peppler.** 1989. *Bordetella parapertussis* invasion of HeLa 229 cells and human respiratory epithelial cells in primary culture. Infect. Immun. **57:**1240–1247.
20. **Franzon, V. L., J. Arondel, and P. J. Sansonetti.** 1990. Contribution of superoxide dismutase and catalase to *Shigella flexneri* pathogenesis. Infect. Immun. **58:**529–535.

20a. **Friedman, R. L.** Personal communication.

20b. **Friedman, R. L., and D. Deshazer.** Personal communication.

21. **Friedman, R. L., and P. Z. Detsky.** 1989. Multiplication of *Bordetella pertussis* in human monocytes, abstr. D-128, p. 103. Abstr. 89th Annu. Meet. Am. Soc. Microbiol. 1989. American Society for Microbiology, Washington, D.C.
22. **Friedman, R. L., R. L. Fiederlein, L. Glasser, and J. N. Galgiani.** 1987. *Bordetella pertussis* adenylate cyclase: effects of affinity-purified adenylate cyclase on human polymorphonuclear leukocyte functions. Infect. Immun. **55:**135–140.
23. **Friedman, R. L., J. E. Lochner, R. H. Bigley, and B. H. Iglewski.** 1982. The effects of *Legionella pneumophila* toxin on oxidative processes and bacterial killing of human polymorphonuclear leukocytes. J. Infect. Dis. **146:**328–334.
24. **Galgiani, J. N., E. L. Hewlett, and R. L. Friedman.** 1988. Effects of adenylate cyclase toxin from *Bordetella pertussis* on human neutrophil interactions with *Coccidioides immitis* and *Staphylococcus aureus*. Infect. Immun. **56:**751–755.
25. **Gordon, D. L., J. L. Rice, and P. J. McDonald.** 1989. Regulation of human neutrophil type 3 complement receptor (iC3b receptor) expression during phagocytosis of *Staphylococcus aureus* and *Escherichia coli*. Immunology **67:**460–465.
26. **Gray, D. F., and C. Cheers.** 1967. The steady state in cellular immunity. II. Immunological complaisance in murine pertussis. Aust. J. Exp. Biol. Med. Sci. **45:**417–426.
27. **Hammerschlag, M. R., K. Sunthralingam, and S. Fikrig.** 1985. The effect of *Chlamydia trachomatis* on luminol-dependent chemiluminescence of human polymorphonuclear leukocytes: requirements for opsonization. J. Infect. Dis. **151:**1045–1051.
28. **Holzer, T. J., K. E. Nelson, V. Schauf, R. G. Crispen, and B. R. Andersen.** 1986. *Mycobacterium leprae* fails to stimulate phagocytic cell superoxide anion generation. Infect. Immun. **51:**514–520.
29. **Hopewell, J. W., L. B. Holt, and T. R. Desombre.** 1972. An

electron microscope study of intracerebral infection of mice with low-virulence *Bordetella pertussis*. J. Med. Microbiol. 5:154–157.

30. **Horwitz, M. A.** 1983. The legionnaires' disease bacterium (*Legionella pneumophila*) inhibits phagosome-lysosome fusion in human monocytes. J. Exp. Med. **158**:2108–2126.
31. **Horwitz, M. A., and S. C. Silverstein.** 1980. Influence of the *Escherichia coli* capsule on complement fixation and on phagocytosis and killing by human phagocytes. J. Clin. Invest. **65**:82–94.
32. **Jacobs, A. A., R. A. Ward, S. R. Wellhausen, and K. R. McLeish.** 1989. Polymorphonuclear leukocyte function during hemodialysis: relationship to complement activation. Nephron **52**:119–124.
33. **Kossack, R. E., R. L. Guerrant, P. Densen, J. Schadelin, and G. L. Mandell.** 1981. Diminished neutrophil oxidative metabolism after phagocytosis of virulent *Salmonella typhi*. Infect. Immun. **31**:674–678.
34. **Kreutzer, D. L., L. A. Dreyfus, and D. C. Robertson.** 1979. Interaction of polymorphonuclear leukocytes with smooth and rough strains of *Brucella abortus*. Infect. Immun. **23**:737–742.
35. **Lee, C. K., A. L. Roberts, T. M. Finn, S. Knapp, and J. J. Mekalanos.** 1990. A new assay for invasion of HeLa 229 cells by *Bordetella pertussis*: effect of inhibitors, phenotypic modulation, and genetic alterations. Infect. Immun. **58**:2516–2522.
36. **Leusch, M. S., S. Paulaitis, and R. L. Friedman.** 1990. Adenylate cyclase toxin of *Bordetella pertussis*: production, purification and partial characterization. Infect. Immun. **58**:3621–3626.
37. **Mandell, G. L.** 1975. Catalase, superoxide dismutase, and virulence of *Staphylococcus aureus*. *In vitro* and *in vivo* studies with emphasis on staphylococcal-leukocyte interaction. J. Clin. Invest. **55**:561–566.
38. **May, M. E., and P. J. Spagnuolo.** 1987. Evidence for activation of a respiratory burst in the interaction of human neutrophils with *Mycobacterium tuberculosis*. Infect. Immun. **55**:2304–2307.
39. **Mouallem, M., Z. Farfel, and E. Hanski.** 1990. *Bordetella pertussis* adenylate cyclase toxin: intoxication of host cells by bacterial invasion. Infect. Immun. **58**:3759–3764.
40. **Naids, F. L., and R. F. Rest.** 1991. Stimulation of human neutrophil oxidative metabolism by non-opsonized *Neisseria gonorrhoeae*. Infect. Immun. **59**:4383–4390.
41. **Riley, L. K., and D. C. Robertson.** 1984. Ingestion and intracellular survival of *Brucella abortus* in human and bovine polymorphonuclear leukocytes. Infect. Immun. **46**:224–230.
42. **Rothe, G., and G. Valet.** 1990. Flow cytometric analysis of respiratory burst activity in phagocytes with hydroethidine and 2′,7′-dichlorofluorescin. J. Leukocyte Biol. **47**:440–448.
43. **Saukkonen, K., C. Cabellos, M. Burroughs, S. Prasad, and E. Tuomanen.** 1991. Integrin-mediated localization of *Bordetella pertussis* within macrophages: role in pulmonary colonization. J. Exp. Med. **173**:1143–1149.
44. **Schnur, R. A., and S. L. Newman.** 1990. The respiratory burst response to *Histoplasma capsulatum* by human neutrophils: evidence for intracellular trapping of superoxide anion. J. Immunol. **144**:4765–4772.
45. **Spangrude, G. J., F. Sacchi, H. R. Hill, D. E. Van Epps, and R. A. Daynes.** 1985. Inhibition of lymphocyte and neutrophil chemotaxis by pertussis toxin. J. Immunol. **135**:4135–4143.
46. **Steed, L. L., M. Setareh, and R. L. Friedman.** 1991. Host-parasite interactions between *Bordetella pertussis* and human polymorphonuclear leukocytes. J. Leukocyte Biol. **50**:321–330.
47. **Szeda, P., J. W. Parce, M. S. Seeds, and D. A. Bass.** 1984. Flow cytometric quantitation of oxidative product formation by polymorphonuclear leukocytes during phagocytosis. J. Immunol. **133**:3303–3307.
48. **Verhoef, J., P. K. Peterson, Y. Kim, L. D. Sabath, and P. G. Quie.** 1977. Opsonic requirements for staphylococcal phagocytosis. Immunology **33**:191–197.
49. **Weiss, A. A., and E. L. Hewlett.** 1986. Virulence factors of *Bordetella pertussis*. Annu. Rev. Microbiol. **40**:661–686.
50. **Zhang, Y., R. Lathigra, T. Garbe, D. Catty, and D. Young.** 1991. Genetic analysis of superoxide dismutase, the 23 kilodalton antigen of *Mycobacterium tuberculosis*. Mol. Microbiol. **5**:381–391.