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Detection of *Bordetella pertussis* Associated with the Alveolar Macrophages of Children with Human Immunodeficiency Virus Infection

KENNETH BROMBERG,[1,2]* GAYLENE TANNIS,[2] AND PHILLIP STEINER[2]

Departments of Medicine[1] and Pediatrics,[2] SUNY Health Science Center at Brooklyn, Box 49, 450 Clarkson Avenue, Brooklyn, New York 11203

Received 12 August 1991/Accepted 5 September 1991

In humans, infection with *Bordetella pertussis* is considered to be localized to an epithelial surface. However, an intracellular state in cultured cells and in the macrophages of infected animals has been shown. By using indirect immunofluorescence with a monoclonal antibody, it was found that 3 of 20 bronchoalveolar lavage specimens from children with human immunodeficiency virus infection had *B. pertussis* associated with pulmonary alveolar macrophages. None of the cultures from the patients grew *B. pertussis*. The *B. pertussis* appeared to be intracellular.

In humans, infection with *Bordetella pertussis* is considered to be a localized process at an epithelial surface (3, 7). However, in a mouse model of pertussis, Cheers and Gray visualized organisms within macrophages (4). Several investigators have described the presence of *B. pertussis* within HeLa 229 cells (5) as well as within rabbit macrophages (9), but the clinical relevance of these findings was unclear. To investigate the possibility that *B. pertussis* is associated with pulmonary alveolar macrophages in humans, we used indirect immunofluorescence with a monoclonal antibody to detect *B. pertussis* in bronchoalveolar lavage specimens from pediatric patients with human immunodeficiency virus infection.

B. pertussis infection in both adults (8) and children (1) with human immunodeficiency virus infection has now been described. Since bronchoalveolar lavage specimens contain numerous macrophages, and since *B. pertussis* has been associated with macrophages in animal studies (4, 9), an association of *B. pertussis* with these human macrophages was sought. The cytospin preparations of bronchoalveolar lavage fluid from 20 patients were examined for *B. pertussis*.

Bronchoalveolar lavage specimens were processed with a cytospin 2 (Shandon Instruments, Sewickley, Pa.) by using the provided cytospin fluid. Slides were then fixed in acetone at room temperature. All specimens were stained with an affinity-purified G-10 antibody directed against the lipooligosaccharide of *B. pertussis* (6) by an indirect immunofluorescence technique. The antibody was used at a dilution of 1/50, corresponding to a protein concentration of 2 μg/ml. A fluorescein-conjugated anti-mouse antibody was used at a dilution of 1/40 (Sigma) to detect the primary antibody. All specimens were examined with a Nikon epifluorescence microscope with a 100× glycerol lens and standard coverslips with an antifading agent in buffered glycerol (0.1 M *p*-phenylenediamine–1 M Tris-HCl, pH 8.0, in 9 parts glycerol). Photographs were made with the Nikon UFX-II system with a 2.5× projection lens for an initial magnification of ×250. All bronchoalveolar lavage specimens were cultured for *B. pertussis* with Regan-Lowe selective media (Remel) which had added cephalexin.

B. pertussis was associated with the macrophages of three patients (Fig. 1 through 3). *B. pertussis* did not grow in cultures of bronchoalveolar lavage fluid from any of the patients. Organisms were seen almost entirely associated with macrophages. The degree of macrophage-associated fluorescence of organisms was much less intense than that seen when the organisms were not associated with macrophages. Occasional groups of organisms that were not associated with macrophages were observed. Some organisms were isolated, and others seemed to be associated with cellular debris (Fig. 4). From 50 to 100 macrophages on each

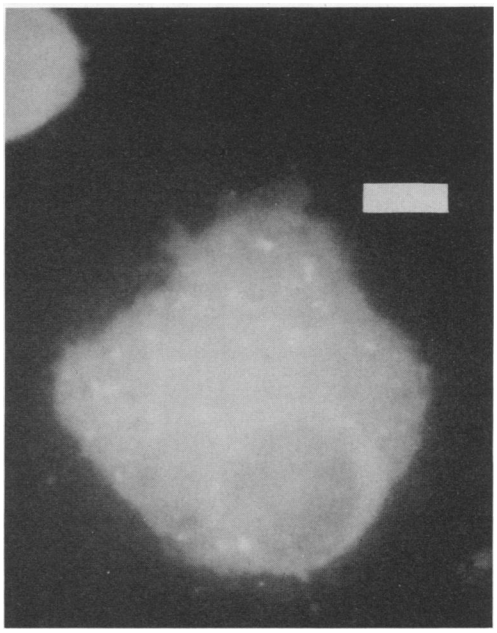

FIG. 1. Cytospin preparation of bronchoalveolar lavage specimen from patient 1. White bar = 10 μm. Note multiple intracellular *B. pertussis* organisms.

* Corresponding author.

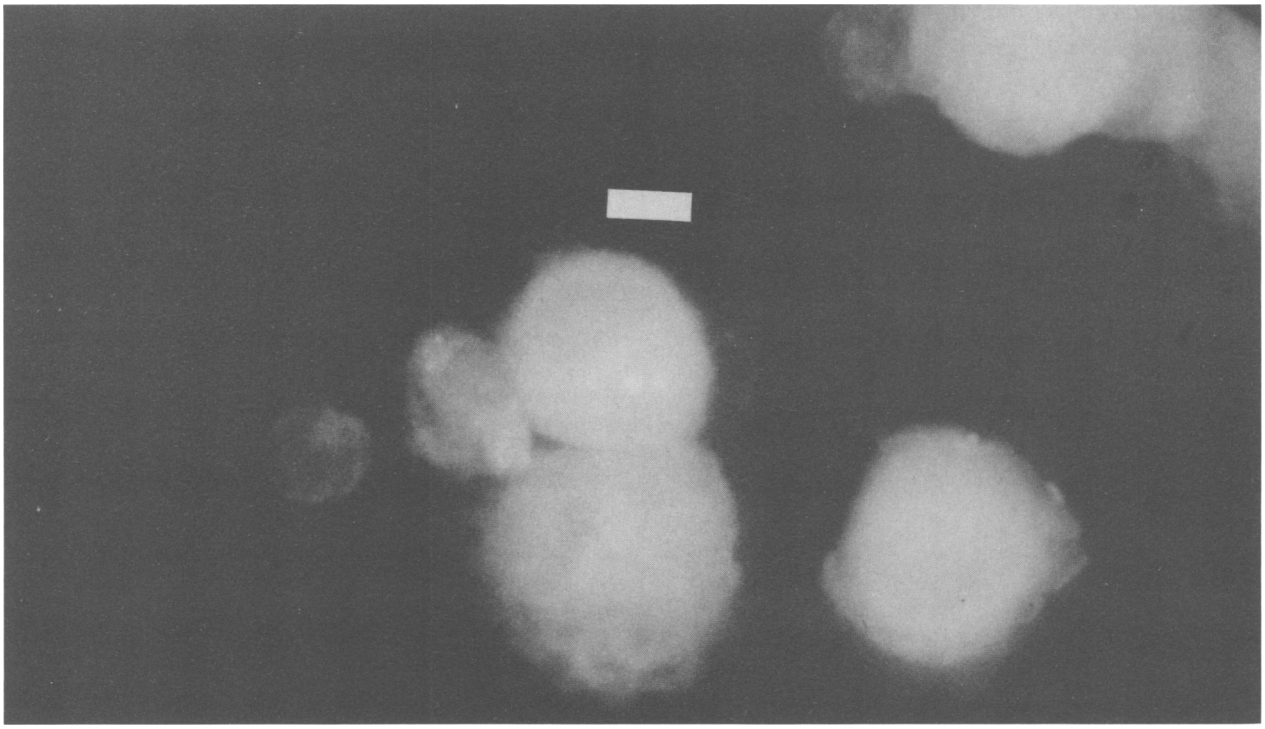

FIG. 2. Cytospin preparation of bronchoalveolar lavage specimen from patient 2. White bar = 10 μm. Note two prominent organisms in the upper macrophage.

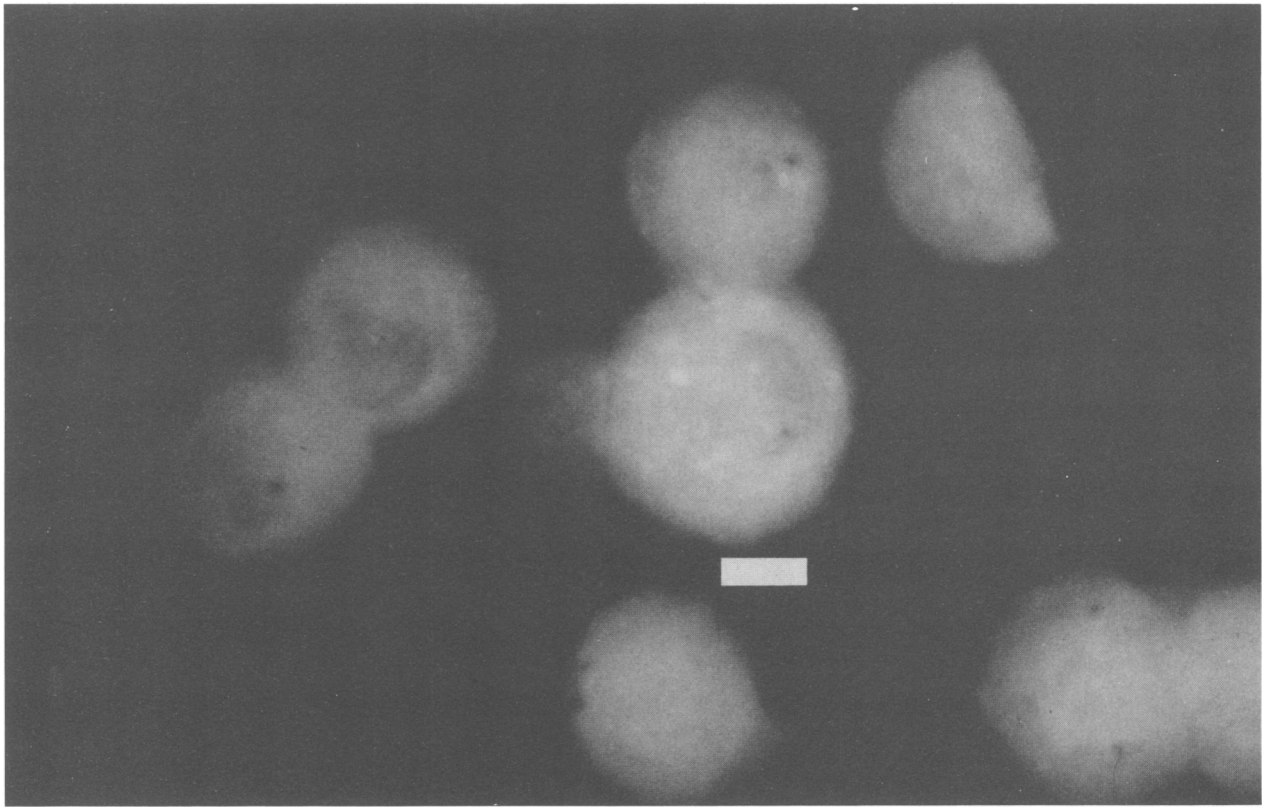

FIG. 3. Cytospin preparation of bronchoalveolar lavage specimen from patient 3. White bar = 10 μm. Note several organisms in the central macrophage.

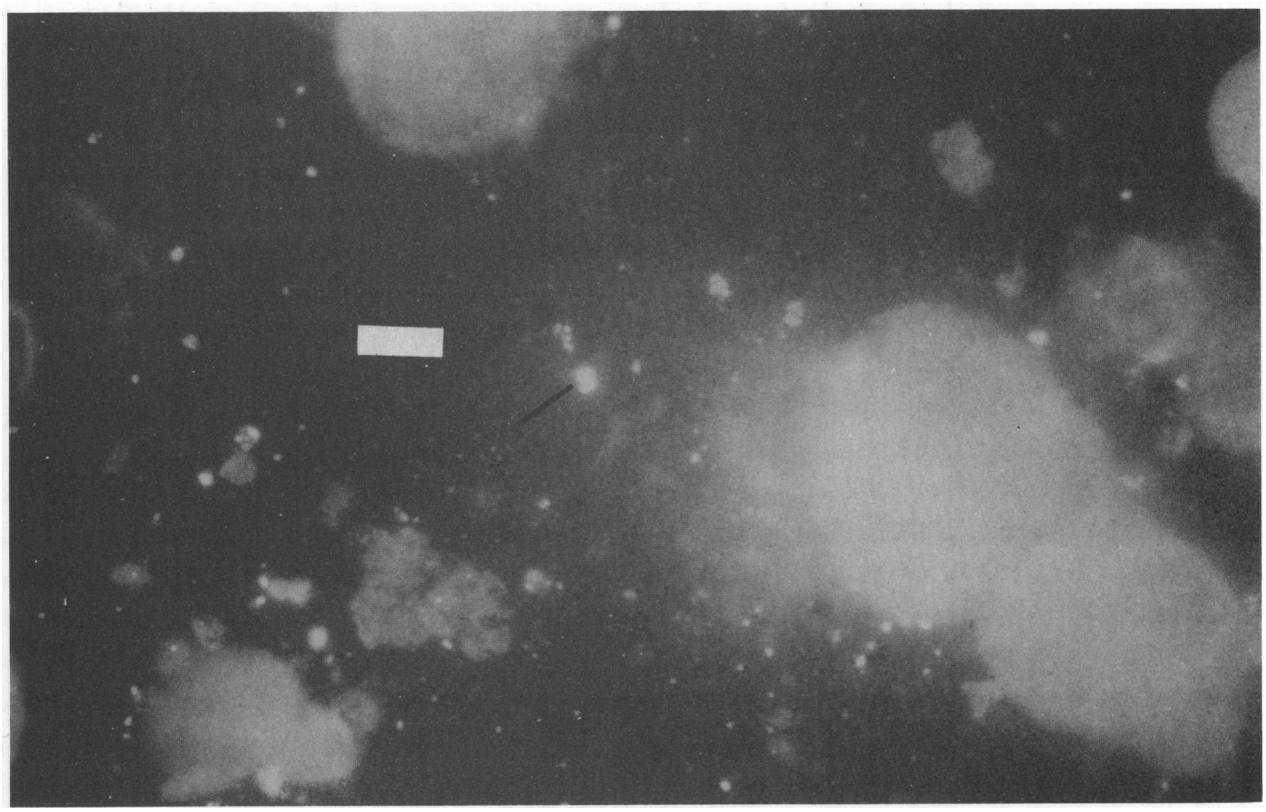

FIG. 4. Cytospin preparation of bronchoalveolar lavage specimen from patient 1. Note the extracellular *B. pertussis* organism (black line) and amorphous material resembling a fragmented cell. White bar = 10 μm.

slide were examined. Each macrophage contained 2 to 30 organisms. At least 10% of the macrophages had organisms present.

The clinical details for the three patients are as follows.

Patient 1 was 18 months of age. He was hospitalized because of an 8-day history of rhinorrhea and 3 days of cough associated with vomiting. He had one documented diphtheria-tetanus-pertussis vaccine (DTP) given at 2 months of age. His chest X ray showed diffuse infiltrates on the right side with no focal consolidation. When the bronchoalveolar lavage was performed because of presumed pneumonia, he had received intravenous trimethoprim-sulfamethoxazole for 48 h. All results of studies for mycobacteria, fungi, pathogenic bacteria, and *Pneumocystis carinii* were negative.

Patient 2 was 4 years of age. She was hospitalized because of exposure to tuberculosis. She had a cough for several weeks and a reticular-nodular pattern on her chest X ray. Her 5-U tuberculin skin test was negative. She was receiving either intravenous gamma globulin or placebos as part of a study (information not available). She had received four doses of DTP. She had received several courses of oral trimethoprim-sulfamethoxazole over a 3-month period. A bronchoalveolar lavage was done for diagnostic purposes. All results of studies for mycobacteria, fungi, pathogenic bacteria, and *P. carinii* were negative.

Patient 3 was 11 months old. He was hospitalized because of a 1-week history of cough associated with vomiting and a diffuse interstitial infiltrate on chest X ray. He had received two doses of DTP. A bronchoalveolar lavage was done because of suspected *P. carinii* infection. All results of studies for mycobacteria, fungi, pathogenic bacteria, and *Pneumocystis carinii* were negative.

B. pertussis will attach to respiratory epithelial cilia both in vitro (11) and in vivo (7) (Fig. 5). Autopsy findings have included *B. pertussis* enmeshed in the ciliated epithelium of the respiratory tract. However, previous work with *B. pertussis* has described an association with macrophages in animals (4) or certain cell lines (5) only but not with macrophages obtained from infected humans.

It is unlikely that the association of *B. pertussis* with macrophages in specimens from humans would have been seen in nonimmunocompromised patients, since nonimmunocompromised patients with symptoms compatible with pertussis do not usually have bronchoalveolar lavage performed. In our experience, nasopharyngeal aspirates or nasopharyngeal wire specimens that are obtained from nonimmunocompromised patients rarely contain macrophages. In addition, deaths associated with *B. pertussis* infection are rare, and since most deaths are associated with bacterial superinfection, light microscopy would not be able to distinguish between a superinfecting pathogen and *B. pertussis* in macrophages seen at autopsy.

The monoclonal antibody used to examine these specimens does cross-react with *Bordetella bronchiseptica* (6), and it is possible that these patients were not infected with *B. pertussis*. However, *B. bronchiseptica* is easy to grow and is not usually associated with infection in humans.

The *B. pertussis* that was observed in association with these macrophages appeared to be intracellular. The cluster-

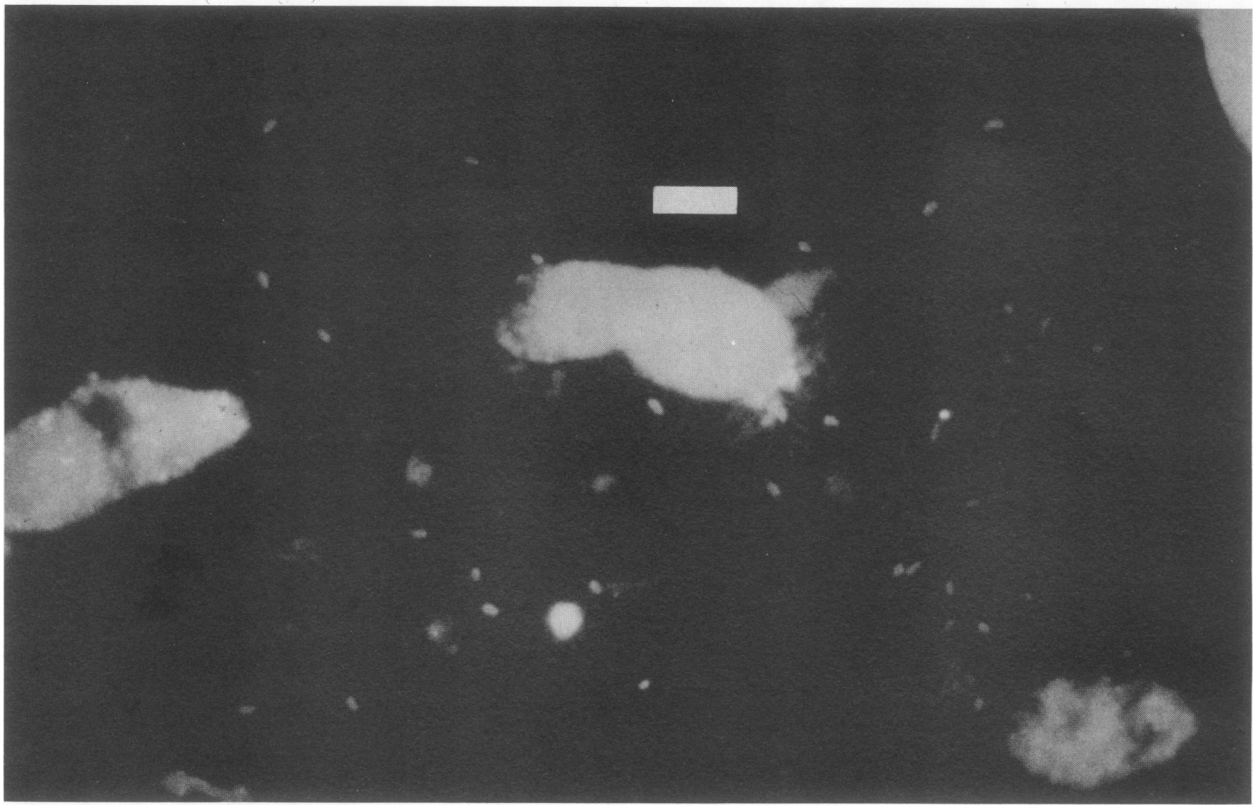

FIG. 5. Cytospin preparation of nasopharyngeal aspirate from patient with pertussis. Note the presence of *B. pertussis* enmeshed in the cilia of a nasal epithelial cell. G-10 antibody was used for staining, as for the previous bronchoalveolar lavage specimens. White bar = 10 μm.

ing on the slide of the brightly staining organisms surrounded by debris is suggestive of prior concentration within a cell which subsequently underwent lysis (Fig. 4). Although the macrophage-associated organisms might be membrane bound, the diminution of intensity of staining is consistent with internalization.

Even if these organisms were intracellular, they were not viable as determined by culture. However, two of the three patients had been receiving antibiotics which may have had an effect on the viability of the organisms.

The locations of these organisms could have been determined by using differential fixation (10). However, this was a retrospective study, and all of the specimens were fixed in acetone.

If these organisms were intracellular, then this observation has implications for the understanding of *B. pertussis* pathogenesis. An intracellular state of *B. pertussis* would be consistent with the inability to show a carrier state by using standard culture. An intracellular state would also explain why eradication of *B. pertussis* from the nasopharynx requires prolonged antibiotic therapy (2). Whether *B. pertussis* exists in an intracellular state cannot be determined from these studies. However, *B. pertussis* is surely associated with pulmonary macrophages in humans and may be intracellular. A prospective study of bronchoalveolar lavage specimens prepared with differential fixation from patients likely to have *B. pertussis* infection would clarify these observations.

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REFERENCES

1. **Adamson, P. C., T. C. Wu, B. D. Meade, M. R. Rubin, C. R. Manclark, and P. A. Pizzo.** 1989. Pertussis in a previously immunized child with human immunodeficiency virus infection. J. Pediatr. **115**:589–591.
2. **Bass, J. W.** 1983. Use of erythromycin in pertussis outbreaks. Pediatrics **72**:748–749. (Letter.)
3. **Bromberg, K.** 1989. Whooping cough (pertussis), p. 346–350. *In* P. D. Hoeprich and M. C. Jordan (ed.), Infectious diseases, 4th ed. J. B. Lippincott, Philadelphia.
4. **Cheers, C., and D. F. Gray.** 1969. Macrophage behavior during the complaisant phase of murine pertussis. Immunology **17**:875–887.
5. **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.
6. **Li, Z. M., J. L. Cowell, M. J. Brennan, D. L. Burns, and C. R. Manclark.** 1988. Agglutinating monoclonal antibodies that specifically recognize lipooligosaccharide A of *Bordetella pertussis*. Infect. Immun. **56**:699–702.
7. **Mallory, F. B., and A. A. Horner.** 1912. Pertussis: the histological lesions in the respiratory tract. J. Med. Res. **27**:115–122.
8. **Ng, V. L., M. York, and W. K. Hadley.** 1989. Unexpected isolation of *Bordetella pertussis* from patients with acquired immunodeficiency syndrome. J. Clin. Microbiol. **27**:337–338.

9. **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.
10. **Storrie, B., and P. J. Edelson.** 1977. Distribution of concanavalin A in fibroblasts: direct endocytosis versus surface capping. Cell **11**:707–717.
11. **Tuomanen, E., H. Towbin, G. R. Rosenfelder, D. Braun, G. Larson, G. C. Hanson, and R. Hill.** 1988. Receptor analogs and monoclonal antibodies that inhibit adherence of Bordetella pertussis to human ciliated respiratory epithelial cells. J. Exp. Med. **168**:267–277.