

Species Specificity of *Bordetella* Adherence to Human and Animal Ciliated Respiratory Epithelial Cells

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Bacteria of the genus *Bordetella* adhere preferentially to ciliated respiratory epithelial cells. We investigated the specificity of this unique tropism by assessing the concentration-dependent adherence of the three *Bordetella* species to ciliated cells from different hosts. *Bordetella pertussis* and *Bordetella parapertussis* adhere better to human ciliated cells than to those from rabbits, mice, or hamsters. In contrast, *Bordetella bronchiseptica* demonstrates preferential adherence to nonhuman mammalian ciliated cells of rabbits, mice, and hamsters. There was no attachment of any *Bordetella* organisms to chicken ciliated cells. These observations suggest that specificities of attachment may explain the marked predominance of *B. pertussis* as the cause of whooping cough in humans and of *B. bronchiseptica* as a respiratory pathogen of many nonhuman mammals.

Bordetella species cause infections which are localized to the ciliated respiratory mucosa in humans and animals. *Bordetella pertussis* and *Bordetella parapertussis* produce natural disease only in humans (4), whereas *Bordetella bronchiseptica* causes disease predominantly in animals (1). The multiplication of bacteria attached to the ciliated respiratory epithelium is a unique attribute of the genus *Bordetella*. Noninvasive surface mucosal infection restricted to the ciliated epithelium and sparing the alveoli has been demonstrated at autopsy in humans who have died of whooping cough (8). A similar association of these organisms with cilia was seen during experimental infection of animals with *B. pertussis* and *B. bronchiseptica* (1, 10). Bacterial adherence to cilia is likely to be a critical step in the initiation of such a noninvasive mucosal infection. In this study we used an in vitro assay of the adherence of *Bordetella* species to ciliated respiratory epithelial cells to demonstrate the differences in the adherence of *Bordetella* species to ciliated cells from various hosts. Adherence specificities may explain the differences in host species susceptibility to infections by members of this genus.

MATERIALS AND METHODS

***Bordetella* organisms.** *B. pertussis* Br (UVA-1), *B. parapertussis* 501, and *B. bronchiseptica* 469 (Bureau of Biologics, U.S. Food and Drug Administration) were employed in these studies. Each was grown on Bordet-Gengou agar (Difco Laboratories, Detroit, Mich.) supplemented with 15% sheep blood for 48 h at

35.5°C in sealed petri dishes. Organisms were removed by sterile loop and transferred into medium 199S, consisting of medium 199 (Flow Laboratories, Rockville, Md.) supplemented with 0.3% bovine serum albumin (Miles Laboratories, Elkhart, Ind.), 10 mM L-glutamine, 0.15% NaHCO₃, and 25 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) (Calbiochem, Los Angeles, Calif.).

Adherence assay. The adherence assay was performed as previously described (12). *Bordetella* species were suspended in medium 199S, passed through a 21-gauge needle to disperse aggregates, and adjusted to the desired concentration, ranging from 5×10^7 to 5×10^{11} organisms per ml. Human ciliated cells obtained at bronchoscopy by brushing of normal-appearing trachea were eluted into medium 199S, providing 1×10^5 to 5×10^5 cells per ml. The following species of animals, *Bordetella*-free by culture of swabs of excised trachea, were examined: New Zealand white rabbits (Spring Valley Farm, Scottsville, Va.), Hubbard by Hubbard cross chickens (Heatwole Hatchery, Harrisonburg, Va.), golden Syrian hamsters, and CFW female mice from the Charles River Breeding Farm (Wilmington, Mass.). Tracheas were excised sterilely under pentobarbital anesthesia and were scraped with an ear curette. The resultant dispersed ciliated cell suspension was adjusted to a concentration of approximately 10^5 cells per ml in medium 199S.

A suspension of *Bordetella* species (0.5 ml) was incubated with 0.5 ml of ciliated cell suspension for 3 h at 37°C. Ciliated cells were washed free of nonadherent organisms over a 3- μ m polycarbonate filter, eluted off the filter into suspension by agitation, centrifuged, and spread on a slide. Fluorescein-conjugated antibody to whole *B. pertussis* (Difco) was used to stain the adherent organisms. Although the antibody used in preparation of this conjugate is raised against *B.*

pertussis, the less bright staining of *B. parapertussis* and *B. bronchiseptica* was adequate for identification and enumeration of the organisms. Slides were coded and mixed for reading by one observer. Twenty-five ciliated cells were located with phase microscopy, and fluorescent antibody-stained organisms adherent to the ciliated ends of the cells were enumerated with fluorescence microscopy. The reproducibility of the standard assay, in which 0.5×10^5 ciliated cells are incubated with 2.5×10^9 bacteria for 3 h, is illustrated by the data from 10 experiments: the mean number of adherent organisms per ciliated cell \pm the standard error of the mean was 5.3 ± 0.4 , with a range of 5.0 to 5.8. This variation was not statistically significant by the goodness of fit test ($P > 0.1$).

Statistical methods. The adherence of each *Bordetella* species to each host species of ciliated cell was classified as high or low. By simultaneous inference procedures for binomial proportions (9), the three bacterial species were then compared for adherence to each host species, and the four host species were compared for adherence of each bacterial species. One experiment, in which *B. pertussis* organisms were completely absent from an entire series of human cell samples, was omitted from the data analysis.

RESULTS

This assay has two major attributes. First, the source of ciliated cells can be varied, thereby providing the opportunity to compare, in parallel, the adherence of each *Bordetella* species to ciliated cells from different host species. The capability of assessing adherence to human cells *in vitro* is a particular advantage. Second, the assessment of adherence in this assay system is uncomplicated by bacterial growth before the detection of adherent organisms, since the incubation time required to reach maximal adherence (3 h) is less than the generation time of the organisms under these conditions (12). Adherence is consistently limited to the ciliated end of epithelial cells. Organisms are not adequately identifiable on phase microscopy but are clearly seen with fluorescence. Specificity of the assay is evidenced by the lack of *Bordetella* species adherence to squamous epithelial cells and by the absence of adherence of pneumococci to ciliated cells (12). Specific adherence to ciliated cells appears to be a characteristic of pathogenic *Bordetella* species in that avirulent strains derived from serial laboratory passage do not adhere (Tuomanen and Hendley, *in press*).

The three *Bordetella* species were assessed for their adherence to human, rabbit, hamster, mouse, and chicken ciliated cells. In the following discussion, all reported differences are significant at $P < 0.05$. Marked differences were observed in the degree of adherence of the three *Bordetella* species to various host cells (Fig. 1). When human ciliated respiratory cells were assayed, *B. pertussis* exhibited the highest, *B.*

bronchiseptica the lowest, and *B. parapertussis* an intermediate degree of adherence. A mean of 4.9 ± 0.3 (standard error of the mean) adherent organisms per human cell was achieved with an inoculum of 10^9 *B. pertussis*. A similar number of adherent organisms per cell required a 100-fold increase in the inoculum of *B. bronchiseptica*. Although *B. parapertussis* was similar to *B. pertussis* in that it adhered most readily to human ciliated cells, at a given bacterial concentration the number of adherent organisms was lower than for *B. pertussis*.

B. bronchiseptica demonstrated preferential adherence to the nonhuman ciliated cells assayed. The number of adherent organisms was similar to that of *B. pertussis* to human cells. *B. parapertussis* adhered poorly to nonhuman mammalian cells, with the detection of even low level adherence requiring a 100-fold higher bacterial concentration than that for human cells. Mouse, hamster, and rabbit cells showed a similar order in the degree of adherence: *B. bronchiseptica* was more adherent than *B. pertussis*, which was more adherent than *B. parapertussis*. In contrast, no *Bordetella* species adhered to chicken ciliated cells.

DISCUSSION

Bordetella species clearly exhibit host preference in adherence to ciliated cells. Although for each species at least 10^8 bacteria were required to demonstrate adherence, it is unlikely that such high concentrations are necessary in natural infections. This high inoculum requirement appears to reflect limitations of the *in vitro* assay, particularly the relatively limited contact of organisms with ciliary tufts and the substitution of a liquid medium for the air-mucous-cilia interface found *in vivo*.

The human pathogen, *B. pertussis*, adheres most readily to human ciliated cells. In contrast, *B. bronchiseptica* adheres best to nonhuman mammalian cells. *B. parapertussis*, present in a minority of human infections, adheres preferentially to human cells, but to a lesser degree than *B. pertussis*. Since the number of cilia per cell is comparable among the different species of target cells, differences in the number of adherent organisms must reflect species differences in the bacterial-ciliary interaction. These data suggest that the host and bacterial species specificity of adherence may account for the predominance of *B. pertussis* in human whooping cough and of *B. bronchiseptica* in disease in animals.

Bordetella serve as an example of cell-specific adherence in the respiratory tract. No other bacterial respiratory pathogens are known to resist clearance along the mucociliary escalator by their adherence to cilia. Studies in the gastro-

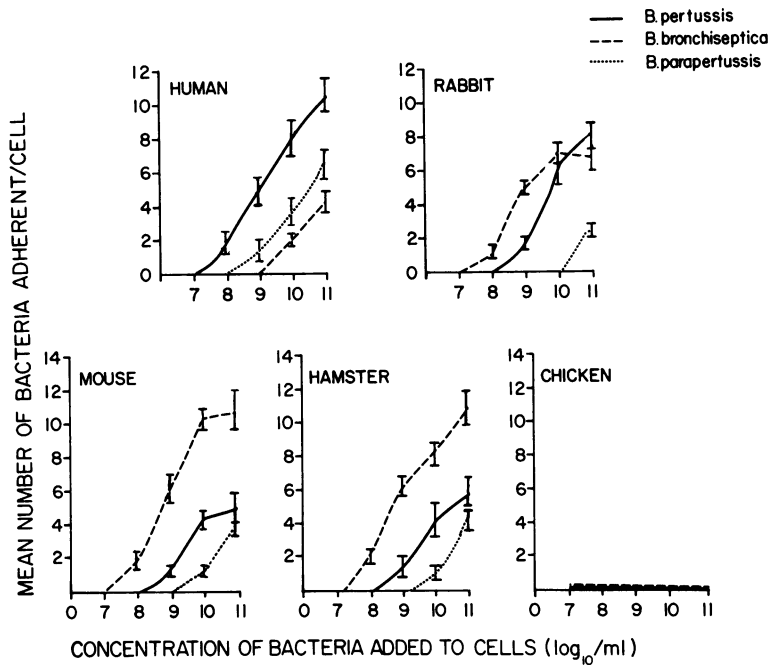


FIG. 1. Concentration-dependent adherence of *Bordetella* species to ciliated cells. The adherence assay was performed as described in the text. Each data point represents the mean (\pm standard error of the mean) number of bacteria per ciliated cell based on an examination of 25 cells. Conditions were assayed in duplicate for each *Bordetella* species and ciliated cell. Data represent experiments repeated at least four times. Statistically significant differences between the curves for each target cell ($P < 0.05$) are indicated by $>$. For human cells at all bacterial concentrations, *B. pertussis* $>$ *B. parapertussis* $>$ *B. bronchiseptica*. For rabbit, mouse, and hamster cells at 10^9 and 10^{10} bacteria, *B. bronchiseptica* $>$ *B. pertussis* $>$ *B. parapertussis*. For mouse and hamster cells at 10^{11} bacteria, *B. bronchiseptica* $>$ *B. pertussis* and *B. parapertussis*. For rabbit cells at 10^{11} bacteria, *B. pertussis* $>$ *B. bronchiseptica* $>$ *B. parapertussis*.

intestinal tract, however, provide ample precedent for the concept that specific adherence can overcome the clearance mechanisms of mucosal surfaces (6). For example, adherence is a well-known virulence factor for *Vibrio cholerae* (7), *Escherichia coli* (2, 5, 11), and oral streptococci (3).

The unique features of *Bordetella* species adherence are the striking dependence on both bacterial and host species and the specificity for a histological type of target cell. These factors, important in the pathogenesis of mucosal infections, may be critical determinants of host susceptibility to disease. Furthermore, this study suggests that a broader investigation of the tropism of other bacterial pathogens for respiratory tract cells may be fruitful.

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