Adherence of *Streptococcus pneumoniae* to Human Bronchial Epithelial Cells (BEAS-2B)

JOHN E. ADAMOU, THERESA M. WIZEMANN, PHILIP BARREN, AND SOLOMON LANGERMANN*

Department of Immunology and Molecular Genetics, MedImmune, Inc., Gaithersburg, Maryland 20878

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Pneumococcal adherence to alveolar epithelial cells and nasopharyngeal epithelial cells has been well characterized. However, the interaction of *Streptococcus pneumoniae* with bronchial epithelial cells has not been studied. We have now shown that pneumococci bind specifically to a human bronchial epithelial cell line (BEAS-2B cells). Pneumococci adhered to BEAS-2B cells in a time- and dose-dependent manner. These results suggest that the bronchial epithelium may serve as an additional site of attachment for pneumococci and demonstrate the utility of the BEAS-2B cell line for studying mechanisms of pneumococcal infection.

The gram-positive pathogen *Streptococcus pneumoniae* is a major cause of pneumonia, otitis media, septicemia, and meningitis (17, 18). The adherence of *S. pneumoniae* to eukaryotic cells is the initial step in colonization and infection of the host. In some cases this results in subsequent mucosal invasion and systemic disease. The primary site of colonization for pneumococci is the nasopharynx. Otitis media results from pneumococcal migration from the nasopharynx into the middle ear via the eustachian tube. Bacteria colonizing the nasopharynx may also be aspirated into the alveoli of the lower respiratory tract and cause pneumonia. In some cases, pneumococci may penetrate the mucosal epithelium of the nasopharynx or lung and enter the vascular compartment, causing sepsis and meningitis (4).

The ability of *S. pneumoniae* to adhere to specific sites on host tissues has been investigated by using various cell culture models, such as human nasopharyngeal epithelial cells (1) and alveolar type II epithelial cells (5), as well as human vascular endothelial cells (6). However, the ability of *S. pneumoniae* to adhere to bronchial epithelial cells, which pneumococci may encounter as they pass from the nasopharynx to the lower respiratory tract, has not been demonstrated. In this study we investigated the ability of pneumococci to attach to bronchial cells by using a well-characterized, human bronchial epithelial cell line (BEAS-2B) (8). Earlier studies have shown that these cells express bronchial-cell-specific characteristics (7, 8, 13).

*S. pneumoniae* strains used in this study were D39 (serotype 2) (2) and its unencapsulated derivative Rx1 (16), which were provided by H. R. Masure (The Rockefeller University, New York, N.Y.), as well as two clinical isolates of *S. pneumoniae* (serotypes 4 and 14), which were provided by I. Aabege (National Institute of Public Health, Oslo, Norway). For adherence tests, pneumococci were grown to mid-logarithmic phase (optical density at 620 nm, 0.4 to 0.6) in Todd-Hewitt broth with 0.5% yeast extract (Difco, Detroit, Mich.) at 37°C, centrifuged, and resuspended in bronchial tracheal epithelial cell growth medium (BEGM; Clonetics Corp., San Diego, Calif.) at a density ranging from 1.5 × 10^7 to 3.6 × 10^7 CFU/ml. BEAS-2B (12, 13), a transformed human bronchial epithelial cell line (ATCC CRL-9609), was passaged in coated (10) tissue culture flasks in serum-free BEGM without antibiotics at 37°C in 5% CO₂. The attachment of *S. pneumoniae* to BEAS-2B cells was assessed by a 24-well tissue culture plate (Costar, Cambridge, Mass.) adherence assay (15). BEAS-2B cells were seeded at a density of 2 × 10^4 cells/well and grown to confluence (approximately 5 × 10^4 cells/well). One-milliliter aliquots of bacterial suspensions (adjusted to a final concentration of 2 × 10^7 CFU/ml) were inoculated onto washed epithelial cell monolayers and incubated for 2 h at 37°C in 5% CO₂. The monolayers were then washed five times with sterile HEPES-buffered saline (pH 7.4) (HBS; Clonetics Corp.), detached by treatment with 0.25% trypsin–0.01% EDTA–0.5% polyvinylpyrrolidone (Sigma Chemical Company, St. Louis, Mo.) for 10 min at 37°C, and lysed with 0.025% Triton X-100. The total number of adherent bacteria was determined by serial dilution in HBS and quantitative culture on BBL tryptic soy agar plates containing 5% sheep blood (Becton Dickinson, Cockeysville, Md.). The viability of BEAS-2B cells was determined by trypan blue dye exclusion and was found to be ≥95% for each experiment. Differences between groups were tested by Tukey’s studentized range test.

All four *S. pneumoniae* strains tested were able to bind to bronchial epithelial cells in vitro (Table 1). The unencapsulated Rx1 strain demonstrated the highest level of adherence to human BEAS-2B cells. Encapsulated isogenic strain D39 (serotype 2) and the serotype 14 strain exhibited moderate levels of adherence, whereas the serotype 4 strain demonstrated relatively low levels of adherence to BEAS-2B cells. The adherence of Rx1 and serotype 14 pneumococci to BEAS-2B cells was demonstrated to be time-dependent (with an inoculum of approximately 2 × 10^7 CFU/well), reaching a plateau within 30 min (data not shown).

Specific pneumococcal binding to BEAS-2B cells was fur-

### TABLE 1. Adherence of *S. pneumoniae* strains to BEAS-2B cells

<table>
<thead>
<tr>
<th>Strain</th>
<th>Serotype</th>
<th>Adherence* (CFU/well)</th>
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<tbody>
<tr>
<td>Clinical isolate</td>
<td>4</td>
<td>415 ± 92</td>
</tr>
<tr>
<td>Clinical isolate</td>
<td>14</td>
<td>8,340 ± 1,258</td>
</tr>
<tr>
<td>D39</td>
<td>2</td>
<td>6,030 ± 650</td>
</tr>
<tr>
<td>Rx1</td>
<td>Unencapsulated</td>
<td>39,500 ± 6,364</td>
</tr>
</tbody>
</table>

* Adherence was determined after BEAS-2B monolayers (5 × 10^4 cells/well) were incubated at a bacterial cell/epithelial cell ratio of approximately 40:1 for each of the indicated strains. The total number of pneumococci in each well was determined by quantitative culture. Data are the means ± standard deviations for triplicate wells from a representative experiment of three similar experiments.
Pneumococcal adherence to A549, a human alveolar carcinoma (type II pneumocyte) cell line (ATCC CRL-185), was used as a positive control for these experiments given that pneumococci have been shown to bind to these cells in other assay systems (5). A549 cells were cultured in nutrient mixture Ham’s F-12K medium (Gibco Laboratories) supplemented with 10% heat-inactivated fetal calf serum (BioWhittaker Inc., Walkersville, Md.) without antibiotics at 37°C in 5% CO₂. Following their removal from the flask by incubation with 0.1% trypsin, epithelial cells were incubated at 37°C in spinner flasks (Bellco Glass Inc., Vineland, N.Y.) overnight. Pneumococci were harvested from overnight cultures on tryptic soy agar plates containing 5% sheep blood and were labeled with fluorescein isothiocyanate (FITC; 1.0 mg/ml; Sigma Chemical Company) as previously described (6). For adherence assays, labeled pneumococci were incubated with $5 \times 10^5$ BEAS-2B or A549 cells at bacterial cell/epithelial cell ratios of 1,000:1 to 60:1. After gentle mixing for 30 min at 37°C, samples were assayed by flow cytometry with a Becton Dickinson FACStar Plus. Mean channel fluorescence was used as an indicator of FITC-labeled bacteria bound to viable BEAS-2B or A549 cells.

The attachment of FITC-labeled bacteria to BEAS-2B (Fig. 1A) or A549 (Fig. 1B) cells occurred in a dose-dependent and saturable manner. Fluorescence microscopy confirmed the attachment of pneumococci to the cell surfaces of BEAS-2B (Fig. 1C) or A549 cells (Fig. 1D). The viability of the epithelial cells was inversely proportional to the ratio of bacteria to cells, as determined by propidium iodide staining (2.5 μg; Boehringer Mannheim) (Fig. 1, insets). The dose-dependent attachment of serotype 14 pneumococci to BEAS-2B cells was confirmed by the 24-well tissue culture plate adherence assay described above. As the inoculum size increased, the number of organisms recovered increased, until saturation at approximately $2 \times 10^8$ CFU/well (data not shown). Similar saturation results were obtained with the unencapsulated Rx1 strain (data not shown).

As shown in Table 1, the unencapsulated *S. pneumoniae* strain Rx1 adhered to a much greater extent to bronchial epithelial cells than the encapsulated parental strain D39 (*P* < 0.05). This may be due to the fact that adhesins present on the *S. pneumoniae* surface normally shielded by the capsule are exposed in the unencapsulated strain and are available for binding to the cell surface. Furthermore, preliminary results from recent experiments have shown that the higher levels of adherence by *S. pneumoniae* may correlate with enhanced invasive capacity of bacteria. What relevance this may have to the pathogenesis of *S. pneumoniae* in the respiratory tract remains unclear.

Unlike respiratory pathogens, such as *Haemophilus influenzae*, that initiate infection in the nasopharynx and often descend into the bronchi causing bronchitis, pneumococci do not typically cause bronchial infections although they have been associated with a minority of cases of chronic bronchitis (3, 14). Nonetheless, pneumococci which migrate from the nasopharynx to the lower respiratory tract must traverse bronchial epithelial cells. Hence, bronchial cells may serve as transient, secondary sites for attachment by virulent pneumococci.

Our finding that pneumococci bind to human bronchial epithelial cells is significant in that understanding more about interactions between pneumococci and human epithelial cells of the respiratory tract may elucidate host-pathogen interactions involved in establishing pneumococcal infection in addition to those already defined. These studies may also aid in the identification of novel therapeutic intervention strategies to block pneumococcal infection.

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**REFERENCES**