Correlation between *Branhamella catarrhalis* Adherence to Oropharyngeal Cells and Seasonal Incidence of Lower Respiratory Tract Infections

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Since 1980, a sudden increase in the incidence of lower respiratory tract infections caused by *B. catarrhalis* has been noted in this department (Nagatake 1985). Other investigators (Ninane et al. 1978; Johnson et al. 1981; Aitken and Thornley 1983; McLeod et al. 1983; Slevin et al. 1984) have also reported that *B. catarrhalis* has become an important pathogen of the lower respiratory tract and should no longer be regarded as a commensal. Nevertheless, the reason for such a rapid increase in incidence is not clearly understood. There is however, cumulative evidence that the aptitude to produce β-lactamase might be
involved since the rise in incidence of lower respiratory tract infections with \textit{B. catarrhalis} is closely associated with an increase of $\beta$-lactamase positive strains as supported by the following data. From 1981 to 1984, $\beta$-lactamase producing \textit{B. catarrhalis} isolates collected in this department constituted 50\%, 60\%, 74\% (Nagatake 1985) and 90\%, respectively. Furthermore, compared to $\beta$-lactamase negative \textit{B. catarrhalis}, $\beta$-lactamase positive \textit{B. catarrhalis} strains were found to have a stronger affinity to adhere to oropharyngeal cells from patients with chronic pulmonary diseases (Rikitomi et al. 1986).

Adherence of pathogenic bacteria to the epithelial cells is now considered as the first step in the process of the invasion into the host organ(s). This study describes the relationship between adherence ability and the seasonal incidence of lower respiratory tract infections due to \textit{B. catarrhalis}. It also demonstrates that \textit{B. catarrhalis} adherence to oropharyngeal cells is closely associated with the occurrence of chronic pulmonary disease.

**MATERIALS AND METHODS**

Monthly records of hospitalized patients as well as outpatients with lower respiratory tract infection by \textit{B. catarrhalis} were studied from January 1984 to February 1986. Patients included in this study were selected on the basis of the existence of chronic pulmonary diseases. They were characterized as follows: 33 men and 15 women (age: mean, 61.8; range, 23 to 81). Twenty patients had chronic bronchitis and 10 had chronic pulmonary emphysema. Eight patients had bronchiectasis, 6 bronchial asthma and 4 inoperable lung cancer with multiple metastases. They were seen at 2 week intervals or more frequently according to the clinical course. Sputa cultures were routinely done when an infection was suspected. Criteria to determine the causative agent(s) have already been described (Nagatake 1985): First, more than $10^7$ c.f.u./ml of the causative agent(s) are cultured quantitatively from purulent sputum; second, Gram stain of the sputum show numerous polymorphonuclear leukocytes and the suspected bacteria intracellularly; third, disappearance or decrease in number of the infective bacteria coincides with clinical and laboratory improvement. An age-matched group of 5 females and 4 males was selected as control (age: mean, 65.5; range, 51-83). Two were healthy persons. Five patients had essential hypertension and 2 had mild diabetes mellitus controlled by diet alone. In common they had no history of cough or any other symptom related to ear, nose and throat. Smokers were excluded.

The basic method of in vitro adherence assay performed in this study is a modification of that of Stephens and McGee (1981) used in their recent work.

\textit{Oropharyngeal cells}. Cells were collected by vigorous scraping of the oropharynx with a cotton-tipped swab and then dislodged by twirling the swab in 4 ml of divalent cation-free PBS. Non-adherent bacteria were separated by centrifugation at 120 g for 15 min and the supernatant discarded. The procedure was repeated 2 times. The cells were counted with a hemacytometer and the suspension was adjusted to around $1 \times 10^5$ cells/ml in PBS containing Ca$^{++}$ and Mg$^{++}$ cations.

Recent \textit{B. catarrhalis} isolated from the sputum of a patient with exacerbation of chronic bronchitis was used in this study. Identification was made at follows: Gram negative diplooccci; growth on blood agar at 37°C in air; positive oxidase; failure to produce acid from glucose, maltose, lactose and fructose; negative citrate production and positive nitrate reduction. The strain was transferred from the blood agar sub-culture into different tubes of 1 ml of Mueller Hinton Broth (BBL) and stored at $-20^\circ$C. In each
RESULTS

Seasonal incidence of lower respiratory tract infections in our Department.

The number of patients who suffered from lower respiratory tract infections with *B. catarrhalis* varied according to the season. A high incidence was found in winter (January, February and December) whereas, the number of cases in summer (June, July and August) was particularly low. High average temperature was associated with low incidence, while the occurrence of lower respiratory tract infections with *B. catarrhalis* was markedly high when the average temperatures were low (Fig. 2).

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**Fig. 1.** A representative oropharyngeal cell with adherent *B. catarrhalis* as viewed under light microscope (×850).

Experiment, one tube was incubated at 37°C for 18 to 24 hr. The bacterial suspension was washed 2 times at 1,500 × g for 15 min and resuspended to give a 1×10^7 c.f.u./ml suspension of *B. catarrhalis* in PBS containing Ca^{++} and Mg^{++} cations by reading O.D. at 620 nm.

**Adherence assay.** Cells and bacteria were mixed at a ratio of 1:100 in an experimental tube. Also, a control tube with washed cells alone was prepared. Both tubes were incubated at 37°C in a shaking bath for 30 min. Non adherent bacteria were separated by centrifugation at 120×g for 15 min. The procedure was repeated 4 times. The final suspension was centrifuged onto slide glasses at 80×g for 5 min, dried and Gram stained. The number of adherent *B. catarrhalis* was determined after 50 consecutive cell counts and given in terms of mean±s.d. A representative oropharyngeal cell with adherent *B. catarrhalis* as viewed under light microscope with oil immersion (1×1,000), is shown in Fig. 1.
Occurrence of bacterial infections in patients with chronic pulmonary diseases.

From January to December 1985, 27 (56%) of 48 patients with chronic pulmonary diseases had bacterial lower respiratory tract infections while, *B. catarrhalis* was isolated as the causative agent (single or associated with other bacteria) from sputa of 12 patients (25%) (Table 1).

**Table 1. Characteristics of patients with chronic pulmonary diseases**

<table>
<thead>
<tr>
<th></th>
<th>Chronic bronchitis</th>
<th>Chronic pulmonary emphysema</th>
<th>Bronchiectasis</th>
<th>Bronchial asthma</th>
<th>Lung cancer</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>20</td>
<td>10</td>
<td>8</td>
<td>6</td>
<td>4</td>
<td>48</td>
</tr>
<tr>
<td>Men/women</td>
<td>10-10</td>
<td>10-0</td>
<td>5-3</td>
<td>4-2</td>
<td>4-0</td>
<td>33-15</td>
</tr>
<tr>
<td>Age: mean</td>
<td>68.2</td>
<td>70.9</td>
<td>41.1</td>
<td>49.5</td>
<td>60.5</td>
<td>61.8</td>
</tr>
<tr>
<td>range</td>
<td>53-81</td>
<td>58-81</td>
<td>23-63</td>
<td>24-63</td>
<td>56-65</td>
<td>23-81</td>
</tr>
<tr>
<td>Bacterial infections in 1985</td>
<td>12</td>
<td>5</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>27</td>
</tr>
<tr>
<td><em>B. catarrh.</em> infections in 1985</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Number of studies</td>
<td>39</td>
<td>21</td>
<td>13</td>
<td>6</td>
<td>6</td>
<td>91</td>
</tr>
</tbody>
</table>
Seasonal change of adherence in patients with chronic pulmonary diseases.

The results of 91 investigations which were carried out in 1 year study are shown in Fig. 3. Closed circles (33 investigations) represent the mean of adherent *B. catarrhalis* to oropharyngeal cells from patients who had lower respiratory tract infection with this bacterium in 1985 and in 3 cases, this infection coincided with the time of investigation. Open circles represent the mean of adherent *B. catarrhalis* to oropharyngeal cells from patients who had not this infection in 1985.

![Seasonal Change of Bacterial Adherence](image)

*Fig. 3. Mean number of adherent *B. catarrhalis* per cell from patients with chronic pulmonary diseases.*

- •, Patients with episode (s) of lower respiratory tract infection by *B. catarrhalis* in 1985.
- ○, Patients without episode of lower respiratory tract infection in by *B. catarrhalis* in 1985.
- A, autumn; Sp, spring; Su, summer; W, winter.
- *p < 0.05; **p < 0.01.

### Table 2. Seasonal mean of adherent *Branhamella catarrhalis* to oropharyngeal cells from patients with chronic pulmonary diseases

<table>
<thead>
<tr>
<th>Month</th>
<th>Mean ± SD</th>
<th>Month</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>3.20 ± 1.49</td>
<td>March</td>
<td>1.12 ± 0.46</td>
</tr>
<tr>
<td>February</td>
<td>2.70 ± 1.00</td>
<td>April</td>
<td>0.96 ± 0.45</td>
</tr>
<tr>
<td>December</td>
<td>3.17 ± 1.35</td>
<td>May</td>
<td>1.35</td>
</tr>
<tr>
<td>Winter</td>
<td>3.11 ± 1.30</td>
<td>Spring</td>
<td>1.70 ± 0.45</td>
</tr>
<tr>
<td>June</td>
<td>0.26 ± 0.16</td>
<td>September</td>
<td>1.09 ± 0.91</td>
</tr>
<tr>
<td>July</td>
<td>0.32 ± 0.22</td>
<td>October</td>
<td>2.05 ± 1.48</td>
</tr>
<tr>
<td>August</td>
<td>0.59 ± 0.22</td>
<td>November</td>
<td>2.35 ± 1.12</td>
</tr>
<tr>
<td>Summer</td>
<td>0.40 ± 0.25</td>
<td>Autumn</td>
<td>1.84 ± 1.30</td>
</tr>
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</table>
Eleven studies (12%) were performed in patients who were staying in the hospital while 80 (88%) were conducted in outpatients. Oropharyngeal cells from patients with chronic pulmonary diseases had a high mean level of adherent *B. catarrhalis* in winter (3.11 ± 1.30 bact./cell). However, as the average temperature rose with the coming of spring, the receptivity of oropharyngeal cells towards *B. catarrhalis* gradually declined to reach the lowest level in summer (0.40 ± 0.25 bact./cell). From late summer to early autumn, the adherence activity gradually rose again to reach the highest value in winter. The mean score of adherent *B. catarrhalis* was moderate in spring (1.07 ± 0.45 bact./cell) and autumn (1.84 ± 1.30 bact./cell) (Table 2). The adherence activity of *B. catarrhalis* in winter, spring, summer and autumn was correlated to the incidence of lower respiratory tract infections with this bacterium in respective seasons. Patients who had lower respiratory tract infections due to *B. catarrhalis* in 1985, showed a higher adherence activity. Retrospectively, it was found that 10 patients were investigated two times or more in different seasons. Similarly, the number of adherent *B. catarrhalis* to oropharyngeal cells within the same patient was high in winter and low in summer. (Fig. 4). Significant difference (*p* < 0.01) in adherence value was observed between winter and spring, between spring and summer, between summer and autumn and between autumn and winter. The adherence rate was higher in autumn than in spring (*p* < 0.05).

**Adherence in the control group.**

Nine investigations were undertaken in 9 control subjects. None were staying in the hospital. Studies were performed in winter (December) because the strain adhered more readily to cells from patients with chronic pulmonary diseases in winter. Cells from the control group however, had a mean level of adherent *B.
Seasonal Change of Bacterial Adherence

Seasonal change in the incidence of lower respiratory tract infections by *B. catarrhalis* seems to be an outstanding characteristic. The important finding in the present study is that the incidence lower respiratory tract infection due to *B. catarrhalis* in each season is closely correlated to the mean number of *B. catarrhalis* adherent to oropharyngeal cells. Oropharyngeal cells from these patients seem to be the easy targets for bacteria to adhere to, particularly in periods with high incidence of lower respiratory tract infections with *B. catarrhalis*. In addition, the same phenomenon was observed in patients who were successively studied in the same seasons, suggesting that an increase in adherent capacity of *B. catarrhalis* in vitro precedes the occurrence of lower respiratory tract infections. Likewise, assessment of buccal epithelial cell adherence of Gram-negative bacilli in vitro served to indentify patients who are at risk of bacillary colonization of the respiratory tract (Johanson et al. 1980).

Specificity in adherence of *B. catarrhalis* to oropharyngeal cells might be involved since this bacterium adhered poorly to similar cells from subjects

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**DISCUSSION**

Seasonal change in the incidence of lower respiratory tract infections by *B. catarrhalis* seems to be an outstanding characteristic. The important finding in the present study is that the incidence lower respiratory tract infection due to *B. catarrhalis* in each season is closely correlated to the mean number of *B. catarrhalis* adherent to oropharyngeal cells. Oropharyngeal cells from these patients seem to be the easy targets for bacteria to adhere to, particularly in periods with high incidence of lower respiratory tract infections with *B. catarrhalis*. In addition, the same phenomenon was observed in patients who were successively studied in the same seasons, suggesting that an increase in adherent capacity of *B. catarrhalis* in vitro precedes the occurrence of lower respiratory tract infections. Likewise, assessment of buccal epithelial cell adherence of Gram-negative bacilli in vitro served to identify patients who are at risk of bacillary colonization of the respiratory tract (Johanson et al. 1980).

Specificity in adherence of *B. catarrhalis* to oropharyngeal cells might be involved since this bacterium adhered poorly to similar cells from subjects.
without any underlying pulmonary disease even in winter. This observation indicates that chronic pulmonary diseases are an important determinant risk factor of lower respiratory tract infection due to *B. catarrhalis*. McLeod (1983) and others (Ninane et al. 1978; Johnson et al. 1981; Slevin et al. 1984) have also isolated this bacterium mainly in sputa of patients with chronic pulmonary diseases. However, *B. catarrhalis* adherence to oropharyngeal cells did not depend on the type of the underlying pulmonary disease. No age difference was observed either.

The degree of adherence of micro-organism may differ under different conditions even in the same mucosal cell. Previous in vitro studies demonstrated that oropharyngeal cells from patients with rheumatic fever bind much more *alpha*-streptococcus associated with this disease than the control group (Reed et al. 1980). On the other hand, clinically isolated *Escherichia coli* adhered more strongly to uroepithelial cells from patients with recurrent urinary tract infections than to those from the control group (Eden et al. 1976; Fowler and Stamey 1977). Similarly, adherence and colonization of pathogenic bacteria occur when the defence mechanism of the epithelium surface become defective. A markedly enhanced susceptibility to colonization of the oropharynx by Gram negative bacilli occurs in serious illness (Johanson et al. 1969; Niederman et al. 1984) because of the rise in protease activity of the saliva which leads to the loss of the cell surface fibronectin (Woods et al. 1981a) as this macromolecule prevents the oropharynx from being colonized by these bacilli (Woods et al. 1981b; Simpson et al. 1982). In addition, Johanson et al. (1980) observed that cells from the epithelium which was colonized by Gram negative bacilli bind much more *Pseudomonas aeruginosa* in vitro. In the present study, cells from patients who had lower respiratory tract infection due to *B. catarrhalis* in 1985 showed a higher adherence activity. However, similar adhesive properties were found among patients without *B. catarrhalis* infection in the same period. Hence it is conceivable that they were in danger of lower respiratory tract infection with *B. catarrhalis* at any moment. In fact, some had infection due to *B. catarrhalis* beyond the investigation period.

Previous reports showed that prior viral infection enhances adherence of pathogenic bacteria to epithelial cells (Davison and Sanford 1982; Jones and Menna 1982). In the present study it was found that the viral infection did not affect significantly the seasonal rate of adherence, because in 60% of investigations conducted in winter (data not shown) were done on patients with clinical status which remain unchanged at least 3 weeks before and after the time they were studied. However, they kept a higher adherence rate in winter. Furthermore, 4 of 10 patients in whom experiment was done two times or more in successive seasons, had a clinical status which was stable throughout the year. The present study did not determine the critical adherence value at which patients are exposed to lower respiratory tract infections with *B. catarrhalis*. Neverthe-
less, factors other than adherence activity are expected to be involved in initiating lower respiratory tract infections due to this bacterium. Further studies are required to determine such factors. On the other hand, neither the duration nor the severity of the underlying disease was significantly associated with an increased rate of adherence.

The investigations focused on bacterial determinant of adherence indicated that serial subcultures lead to damage of the bacterial pili (De Voe and Gilchrist 1975) which are thought to be involved in attachment (Buchnan and Pearce 1976; Beachey 1981; Stephens and McGee 1981). In the present study, this possibility could be excluded, because the fresh isolates were stored in separate tubes of which one each was used for one experiment alone. Thus the adhesive properties of the strain remained unchanged throughout this study so that it is obvious to conclude that the oropharyngeal cell factors involved in bacterial adherence might be responsible for making \textit{B. catarrhalis} to have a higher adherence activity in winter.

Selective adherence of the normal flora to specific surfaces is already proposed as a critical ecological determinant affecting their colonization in the mouth (Gibbons and Van Houte 1971). Furthermore, it is known that the normal flora enhances the human capacity to resist infection (Sprunt and Redman 1968; Sanders 1969; Crowe et al. 1973). Gibbons and Van Houte (1975) reviewed factors which had been postulated to account for the selection of the resident bacteria flora of the mouth. Many including narrow range of temperature, pH, redox potential, inhibitory substance as lysozyme, bacterial products including hydrogen peroxide, bacteriocins are expected to influence bacterial selection in the mouth (Malamud 1985; Cole 1985) thus, modifying the resistance aptitudes on surface of the epithelium. The present data demonstrate that the adherence properties of the oropharyngeal epithelium towards \textit{B. catarrhalis} in patients with chronic pulmonary diseases are subject to cyclic changes and the high incidence in winter of lower respiratory tract infections with this bacterium might be closely related to the above factors. The nature of cyclic changes in cold months in the microenvironment of the oropharynx remains to be clarified.

Acknowledgments

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References


