Moraxella catarrhalis in Chronic Obstructive Pulmonary Disease

Burden of Disease and Immune Response

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Rationale: ***Moraxella catarrhalis*** **is frequently present in the sputum of adults with chronic obstructive pulmonary disease (COPD). Little is known about the role of *M. catarrhalis* in this common disease.** ***Objective:*** **To elucidate the burden of disease, the dynamics of carriage, and immune responses to *M. catarrhalis* in COPD.** ***Methods:*** **Prospective cohort study of 104 adults with COPD in an outpatient clinic at the Buffalo Veterans Affairs Medical Center.** ***Measurements:*** **Clinical information, sputum cultures, molecular typing of isolates, and immunoassays to measure antibodies to *M. catarrhalis*.** ***Main Results:*** **Over 81 months, 104 patients made 3,009 clinic visits, 560 during exacerbations. Molecular typing identified 120 episodes of acquisition and clearance of *M. catarrhalis* in 50 patients; 57 (47.5%) of the acquisitions were associated with clinical exacerbations. No instances of simultaneous acquisition of a new strain of another pathogen were observed. The duration of carriage of *M. catarrhalis* was shorter with exacerbations compared with asymptomatic colonization (median, 31.0 vs. 40.4 days; $p = 0.01$). Reacquisition of the same strain was rare. The intensity of the serum IgG response was greater after exacerbations than asymptomatic colonization ($p = 0.009$). Asymptomatic colonization was associated with a greater frequency of a sputum IgA response than exacerbation ($p = 0.009$).** ***Conclusions:*** ***M. catarrhalis*** **likely causes approximately 10% of exacerbations of COPD, accounting for approximately 2 to 4 million episodes annually. The organism is cleared efficiently after a short duration of carriage. Patients develop strain-specific protection after clearance of *M. catarrhalis* from the respiratory tract.**

Keywords: chronic bronchitis; mucosal immunity; respiratory tract infection

Bacterial infection of the respiratory tract has long been recognized as playing a role in the course and pathogenesis of chronic obstructive pulmonary disease (COPD) (1–3). However, the precise role of bacteria is poorly understood and controversial. Bacteria cause many of the exacerbations that characterize the disease and, through chronic colonization, contribute to airway inflammation that is a hallmark of the disease (4, 5). COPD is the fourth most common cause of death in the world and is associated with enormous morbidity and health care costs (6, 7). Elucidating the dynamics of bacterial infection and characterizing the role of bacterial pathogens in causing exacerbations have important implications in managing these patients and in designing better therapy.

Studies performed decades ago emphasized the role of *Haemophilus influenzae* and *Streptococcus pneumoniae* in COPD (8–12). Interestingly, in classic studies by May (8) and Howell (9), *Moraxella catarrhalis* (then known as *Neisseria catarrhalis*) was isolated more often than *H. influenzae* and *S. pneumoniae* from sputum of adults with COPD. However, the bacterium was described as an "organism whose pathogenic propensities are known to be slight or non-existent" (8) and was thus ignored for decades. Point prevalence studies indicate that *M. catarrhalis* colonizes the respiratory tract of 5 to 32% of adults with COPD at any one time (8, 9, 13–16). Little is known about the duration of carriage, the relative frequency of *M. catarrhalis* as a cause of exacerbations or the human immune response, and its relationship to the clinical expression of carriage and disease. Studies of the human immune response to *M. catarrhalis* have been limited by the use of heterologous laboratory strains in immunoassays. The use of immunoassays that detect antibodies that bind to epitopes on the bacterial surface of the homologous infecting strain is important to detect potentially protective immune responses.

We are conducting a prospective study in which we obtain clinical information, sputum, and serum samples monthly and during exacerbations in a cohort of patients with COPD. In previous work, on the basis of 57 months of follow-up, we have demonstrated that acquisition of a new strain of *H. influenzae, M. catarrhalis*, or *S. pneumoniae* is associated with the occurrence of an exacerbation (17). Furthermore, in previous work, we studied serum and sputum supernatants from 21 patients with exacerbations associated with *M. catarrhalis* to develop immunoassays to measure serum IgG and sputum IgA to antigens on the bacterial surface (18). The present study has elucidated the dynamics of carriage of *M. catarrhalis* in COPD on the basis of 81 months of follow-up, measured the systemic and mucosal antibody responses in 106 sets of serum and sputum samples to homologous infecting strains of *M. catarrhalis*, and related these immune responses to clinical aspects of carriage. Results of clinical data, colonization patterns, and immunoassays were analyzed to elucidate the dynamics of carriage and the role of *M. catarrhalis* in the clinical course of COPD.

METHODS

COPD Study Clinic

A prospective study of 104 adults with COPD was conducted between March 1994 and December 2000. Patients were seen at the Buffalo Veterans Affairs Medical Center monthly and whenever they had symptoms suggestive of an exacerbation. At each clinic visit, clinical information and sputum and serum samples were obtained. Details are described in the online supplement.

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Sputum Samples

Study personnel who processed sputum samples were unaware of the clinical status of patients. Samples were subjected to culture, and supernatants were prepared. Details are provided in the online supplement. All isolates were subjected to molecular typing by pulsed-field gel electrophoresis (17).

Immunoassays

Serum IgG that bound to intact bacteria was determined with whole cell ELISA (18). Sputum supernatant samples were subjected to flow cytometry to identify IgA antibodies that bound to the surface of bacteria (18). All assays were performed with homologous infecting strains. Results were expressed as a percentage of change between the samples from before and after the episode of carriage of *M. catarrhalis*. In this way, the antibody responses being detected were specific to the strain isolated from the current episode of carriage, thus controlling for preexisting or background antibody. The percentage of change that represents a significant increase between the paired samples was determined by assays with a series of negative control samples as previously described (18).

Statistical Methods

Patients who experienced at least one episode of *M. catarrhalis* carriage were compared with patients who experienced no such episodes by unpaired *t* test for continuous data and by χ^2 analysis for nominal data.

Antibody levels in blood and sputum samples were transformed logarithmically to approximate a normal distribution. In addition, antibody levels were expressed in a binary form and coded as 1 for a significant increase after carriage and 0 when there was no significant change. Generalized estimating regressions (19) were used to determine the relation between antibody levels and with exacerbation or colonization (coded as 1 and 0, respectively). A Gaussian and binomial family were used for continuous and binary dependent variables, respectively, with an exchangeable correlation structure.

The Cox proportional hazards model with the Andersen-Gill modification for multiple events occurring in the same patient was used to determine the role of potential predictor variables on the time between acquisitions of *M. catarrhalis* and on the duration of carriage (20). These predictor variables included the immune response, the presence or absence of an exacerbation, concomitant corticosteroid therapy, and antibiotic therapy. Positive coefficients in these models indicate that a higher level of predictor variable is associated with a longer duration between events. The proportional hazards assumption was verified from the Schoenfeld residuals (21).

RESULTS

Burden of Disease Associated with *M. catarrhalis*

A total of 104 adults with COPD were enrolled in the study clinic over 81 months. These patients completed 3,009 clinic visits, 560 during exacerbations and 2,449 during clinically stable periods (Table E1 in the online supplement). Sputum samples from 2,727 clinic visits were available for analysis.

Cultures of sputum grew *M. catarrhalis* in 210 of 2,727 cultures (7.7%). Analysis of results of pulsed-field gel electrophoresis revealed 120 episodes of carriage of *M. catarrhalis* in 50 patients during which a patient acquired a strain and subsequently cleared the strain. No difference in age, race, smoking, severity of disease, or overall rate of exacerbations was noted in these patients compared with those who never acquired *M. catarrhalis* (Table 1). Patients with *M. catarrhalis* were followed longer than those from whom *M. catarrhalis* was never isolated (40.3 ± 4.3 vs. 18.4 ± 2.3 visits, $p < 0.001$). Fifty-seven (47.5%) of the 120 acquisitions were associated with symptoms of exacerbation and 63 were asymptomatic colonization. Therefore, 57 of 560 (10.2%) total exacerbations recorded in the study clinic occurred simultaneous with the acquisition of a new strain of *M. catarrhalis*.

Dynamics of Carriage

The median duration of carriage of an individual strain was 34 days (interquartile range [IQR], 31.2; range, 7–315 days). Furthermore, 88 of the 120 episodes of carriage were a single clinic visit in duration. The duration of carriage was longer for colonization than for exacerbation (median, 40.4 days; IQR, 67.5 vs. 31.0 days; IQR, 18.2; $p = 0.01$; Figure 1). Exacerbations were more likely to have received antibiotic compared with colonizations (55 of 57 vs. 4 of 63, $p < 0.001$). The duration of carriage was significantly shorter in the 59 episodes at which antibiotics were administered on acquisition compared with the 61 episodes at which antibiotics were not administered (median, 31.0 days; IQR, 16.5 vs. 42 days; IQR, 68.0; $p = 0.001$). The duration of carriage was no different in the 20 episodes at which systemic corticosteroids were administered compared with the 100 episodes at which corticosteroids were not administered (median, 31.5 days; IQR, 10.5 vs. 35 days; IQR, 32.7; $p = 0.22$). No difference was noted in the time to a subsequent acquisition of *M. catarrhalis* after exacerbation compared with colonization (median, 234 days; IQR, 404.5 vs. 267.5 days; IQR, 376.5; p = nonsignificant).

To assess the development of protective responses after clearance of *M. catarrhalis*, the rate at which patients experienced reacquisition after clearing a strain was assessed. Once a strain of *M. catarrhalis* was cleared, reacquisition of the same strain was rare. Reacquisition of the same strain occurred in 1 of 50 patients (2%; confidence interval, 0–10.9%). Of the 120 episodes of carriage of *M. catarrhalis* over 3,183 patient-months of follow-up, reacquisition of the same strain was observed three times (2.5%; confidence interval, 0.05–7.2%), and the three episodes occurred in a single patient.

TABLE 1. CHARACTERISTICS OF PATIENTS WHO EXPERIENCED AT LEAST ONE EPISODE OF CARRIAGE OF *M. CATARRHALIS* COMPARED WITH THOSE WITH NO CARRIAGE OVER 81 MONTHS

Characteristic (*mean ± SEM unless otherwise noted*)	Patients with at Least One Episode of *M. catarrhalis* Carriage (*n = 50*)	Patients with No Episodes of *M. catarrhalis* Carriage (*n = 54*)	p Value
Age, yr	66.3 ± 1.4	67.9 ± 1.3	0.42
White, no.	42	45	0.99
Yr since diagnosis	11.9 ± 1.7	10.5 ± 1.5	0.54
FEV_1	1.65 ± 0.10	1.48 ± 0.09	0.23
FEV_1, % predicted	48.4 ± 2.0	43.9 ± 2.5	0.23
Smoker at enrollment, no.	14	20	0.40
Pack-yr smoking	80.1 ± 5.5	85.0 ± 5.3	0.52
Exacerbations/yr	2.3 ± 0.2	3.4 ± 0.7	0.18
Duration of follow-up, clinic visits	40.3 ± 3.4	18.4 ± 2.3	< 0.001

To assess seasonal variation of carriage of *M. catarrhalis*, results of sputum cultures were analyzed by month of the year during 81 months (Figure 2). The rate of positive cultures was higher during winter months ($p < 0.001$, χ^2) as was the rate of new acquisitions of *M. catarrhalis* ($p < 0.001$). When the results were normalized for number of clinic visits per month, the same result was obtained.

Concomitant Pathogens

Of the 210 clinic visits with positive sputum cultures for *M. catarrhalis*, *H. influenzae* was isolated from 56 (26.7%), *S. pneumoniae* was isolated from 12 (5.7%), and *Pseudomonas aeruginosa* was isolated from 10 sputum samples (4.8%). In the exacerbation visits associated with acquisition of a new strain of *M. catarrhalis*, no instances of simultaneous acquisition of a new strain of another pathogen were observed. The presence of a concomitant pathogen had no effect on the occurrence of exacerbation versus colonization on acquisition of a new strain of *M. catarrhalis* (54.5% exacerbation with no concomitant pathogen vs. 41.9% exacerbation with a concomitant pathogen, $p = 0.29$).

Immune Responses

Paired serum and sputum samples were available for study for 106 episodes of carriage. Seventy-six of 106 (71.7%) episodes of acquisitions of *M. catarrhalis* were followed by a systemic or mucosal antibody response to the homologous strain. Development of new serum IgG and sputum IgA were not correlated ($p =$ nonsignificant), indicating that systemic and mucosal immune responses to *M. catarrhalis* occurred independently.

Colonization was associated with a significantly greater likelihood of developing a sputum IgA response compared with exacerbation (53.2 vs. 26.0%, $p = 0.009$; Table 2). Although the proportion of patients who developed serum IgG was not different between exacerbation and colonization, exacerbation resulted in a significantly greater intensity of serum IgG response compared with colonization (median, 41.7% increase; IQR, 118.2 vs. 13.5%; IQR, 47.4; $p = 0.009$; Table 2). The development of a new antibody response had no effect on the time to subsequent acquisition of another strain of *M. catarrhalis*.

Neither antibiotic nor corticosteroid administration had an effect on the frequency or intensity of new serum IgG or sputum IgA responses. Serum IgG antibody response was observed in 47% of patients who did not receive antibiotics compared with 58% when antibiotics were given. Sputum IgA response was observed in 46% of patients who did not receive antibiotics compared with 31% when antibiotics were given. Serum IgG response was observed in 55% of patients who did not receive systemic corticosteroids compared with 42% of patients who were treated with systemic corticosteroids. Sputum IgA response was observed in 40% of patients who did not receive systemic corticosteroids compared with 29% in those who did. None of these differences were statistically significant.

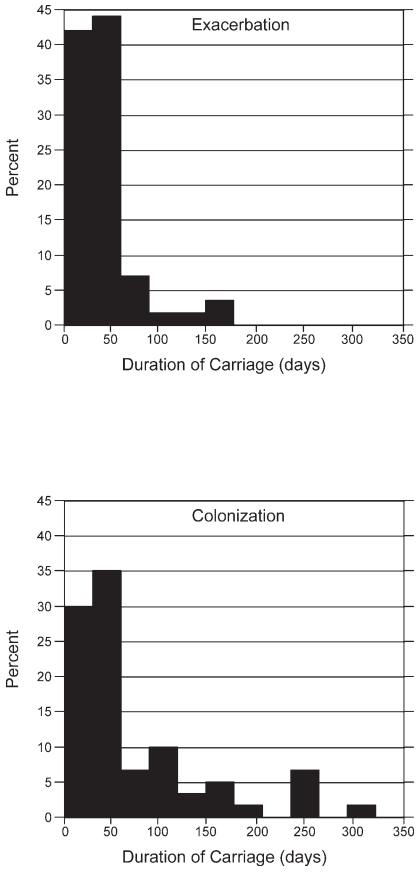

Figure 1. Duration of carriage of episodes of exacerbation (*top*) and colonization (*bottom*) with *M. catarrhalis*. The *x* axis is duration of carriage in days. The *y* axis is episodes expressed as percentage of total.

DISCUSSION

We evaluated the role of *M. catarrhalis* in the course of COPD in a prospective study involving 3,183 patient-months of observation. Several important new observations were made in this study. Using the criteria of the new onset of clinical symptoms of exacerbation occurring simultaneously with the acquisition of a new strain of *M. catarrhalis* (17), 10.2% of 560 exacerbations were likely caused by *M. catarrhalis.* Approximately 20 million adults in the United States have COPD and exacerbations occur in individuals at a rate of 1 to 2 annually (22). On the basis of these estimates, *M. catarrhalis* causes approximately 2 to 4 million exacerbations of COPD annually in the United States. The observation that the only difference between patients who acquired *M. catarrhalis* and those who did not was duration of follow-up suggests that all patients with COPD are susceptible to infection by *M. catarrhalis*.

An important consideration in the present study is the difficulty in establishing the etiology of an individual exacerbation with absolute certainty. The presence of a bacterium in sputum alone does not indicate causation. This study used three criteria to assess causation: (*1*) clinical evidence of an exacerbation with simultaneous acquisition of a new strain of *M. catarrhalis*, (*2*) absence of acquisition of a new strain of another bacterial pathogen,

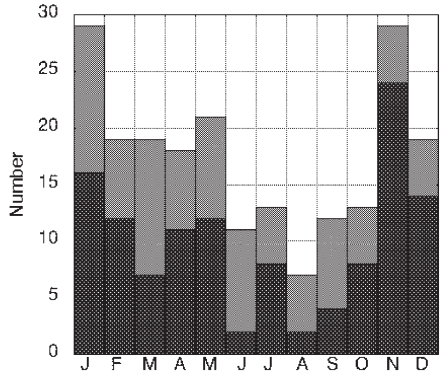

Figure 2. Frequency of positive sputum cultures (*gray bars*) and new acquisitions (*black bars*) of *M. catarrhalis* by month of the year. The *x* axis shows months from January (J) through December (D).

TABLE 2. SYSTEMIC AND MUCOSAL ANTIBODY RESPONSE TO HOMOLOGOUS *M. CATARRHALIS* AFTER EXACERBATION AND COLONIZATION

	Frequency of Immune Response, No. Positive/No. Tested (%)			Intensity of Immune Response, Median % Increase (*IQR*)		
	Exacerbation	Colonization	p Value	Exacerbation	Colonization	p Value
Serum IgG*	31/54 (57.4)	23/52 (44.2)	0.11	41.7 (118.2)	13.5 (47.4)	0.009
Sputum IgA†	13/50 (26.0)	25/47 (53.2)	0.009	28.5 (114.3)	99.9 (174.9)	0.07

Definition of abbreviation: IQR = interquartile range.
* Serum IgG determined by whole cell ELISA.
† Sputum IgA determined by flow cytometry.

and (*3*) absence of clinical evidence of alternative causes of exacerbations.

This study demonstrates that adults with COPD clear *M. catarrhalis* from the respiratory tract efficiently. Most individuals carry the organism for only a single monthly clinic visit. The relatively short duration of carriage by *M. catarrhalis* is strikingly different from that observed for *H. influenzae*, for example, which colonizes subsets of patients for substantially longer periods of time (23–25). Differences in pathogen adaptation to the human respiratory tract likely account for these findings, which need further exploration.

Interesting differences in duration of carriage of *M. catarrhalis* based on clinical status and antibiotic administration were observed. Exacerbations were associated with a shorter duration of carriage than asymptomatic colonization. However, because most patients with exacerbations received antibiotic (55 of 57) and most patients with colonization did not receive antibiotic (59 of 63), it is not possible to determine whether host factors, antibiotic administration, or a combination of these accounted for the difference. Because cultures were performed monthly and 88 of the 120 episodes of carriage were only a single culture, the actual duration of carriage may be shorter than our estimate based on monthly cultures. The observation that patients clear *M. catarrhalis* quickly, even in the absence of antibiotic therapy (median, 42 days), demonstrates that host mechanisms effectively clear *M. catarrhalis* from the respiratory tract. These data have important implications in interpreting results of cultures in antibiotic trials; for example, the apparent eradication of β-lactamase–producing strains of *M. catarrhalis* by amoxicillin is not surprising in view of this new information (26, 27).

The observation that acquisition and clearance of a strain of *M. catarrhalis* results in long-lasting, strain-specific protection from reacquisition has important implications in vaccine development. The observation supports the concept that humans make protective responses that are capable of clearing *M. catarrhalis* from the respiratory tract and preventing reacquisition. An effective vaccine would replicate this response but generate such protective responses for all strains of *M. catarrhalis*. Identification of the antigens to which the new antibodies are directed will be rewarding in elucidating the targets of protective immune responses. Subsequent work will focus on inducing similar protective responses to antigens that are conserved among strains of *M. catarrhalis*.

Studies on immune responses to bacterial pathogens in COPD have yielded confusing and conflicting results (1, 28, 29). The present study, using homologous infecting strains, evaluating both systemic and mucosal antibody responses, and using assays that detect antibodies specifically to surface-exposed epitopes, demonstrated that the majority of adults who acquire *M. catarrhalis* develop an immune response, regardless of whether symptoms of an exacerbation occur. Mucosal and systemic immune responses occurred independently of one another. Interestingly, asymptomatic colonization was more often associated with the development of a mucosal immune response compared with exacerbation. One might speculate that a vigorous mucosal immune response decreases local invasion and inflammation and is thus capable of protecting against clinical symptoms of disease.

Exacerbations were associated with systemic immune responses of greater intensity, suggesting greater systemic exposure to bacterial antigens during exacerbations compared with asymptomatic colonization. One might speculate that clinical symptoms of exacerbations were actually caused in part by this more robust immune response.

In addition to the limitations of expectorated sputum cultures noted previously, another limitation of the study is that we did not identify infections caused by viruses and atypical bacteria. Respiratory virus infection is present in approximately one-third of exacerbations and there is serologic evidence of infection with *Chlamydia pneumoniae* in 5 to 10% of exacerbations. An additional limitation of the present study is that because the work was performed in patients with chronic bronchitis, the observations may not pertain to patients with classic emphysema without chronic sputum production.

In summary, *M. catarrhalis* likely causes approximately 10% of exacerbations of COPD, accounting for 2 to 4 million exacerbations annually in the United States. The majority of adults with COPD make new systemic and/or mucosal antibody responses to their homologous strain after acquisition. Adults with COPD efficiently clear *M. catarrhalis* from the respiratory tract after a relatively short duration of carriage and develop strain-specific protection.

Conflict of Interest Statement: T.F.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; A.L.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; B.J.B.G. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; S.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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