CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Nov. 1996, p. 717–721 Vol. 3, No. 6
1071-412X/96/$04.00+0

Serum Antibody Response to Outer Membrane Proteins of *Moraxella* (*Branhamella*) *catarrhalis* in Patients with Bronchopulmonary Infection

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Received 23 February 1996/Returned for modification 3 June 1996/Accepted 29 July 1996

A Western blot (immunoblot) method for detecting antibodies against outer membrane protein (OMP) epitopes of *Moraxella* (*Branhamella*) *catarrhalis* was evaluated. Paired serum samples from patients suspected of *M. catarrhalis* ($n = 38$) and non-*M. catarrhalis* ($n = 25$) bronchopulmonary infection were examined for the presence of antibodies of the immunoglobulin M (IgM), IgG, and IgA classes to OMPs from *M. catarrhalis* by a gel electrophoresis-immunoperoxidase technique (Western blotting); sera from 40 healthy adult blood donors were also included. A significantly ($P = 0.004$) more frequent occurrence of IgM-class antibodies and/or an increase in the number of IgG-class antibodies against different *M. catarrhalis* OMPs from acute- to convalescent-phase serum samples was found for patients with *M. catarrhalis* (79%) than for patients without *M. catarrhalis* (40%). IgM-class antibodies against OMPs of *M. catarrhalis* were found in acute- and/or convalescent-phase serum samples from 58% of patients with *M. catarrhalis* and 32% of patients without *M. catarrhalis*. Fifty percent of patients with *M. catarrhalis* and 16% of patients without *M. catarrhalis* had, from acute- to convalescent-phase serum samples, an increased number of IgG-class antibodies directed against different OMPs. A total of 34% of patients with *M. catarrhalis* and 4% of patients without *M. catarrhalis* had, from acute- to convalescent-phase serum samples, an increased number of IgA-class antibodies directed against different OMPs. The present study indicates that *M. catarrhalis* is one of the bacteria involved in acute exacerbations of chronic bronchitis.

During the past 15 years *Moraxella* (*Branhamella*) *catarrhalis* has been reported with increasing frequency as a cause of acute exacerbations in patients with chronic obstructive pulmonary disease and of acute otitis media in children (3). The isolation of *M. catarrhalis* from clinical samples is not sufficient evidence of its role as a pathogen, because nasopharyngeal carriage is common in healthy persons, especially children and adults more than 60 years old (22).

Among possible targets for the immune system are outer membrane proteins (OMPs). OMP preparations of *M. catarrhalis* have been used in an enzyme-linked immunosorbent assay (ELISA) to examine immune responses in children with acute otitis media (11), but they have not previously been used to investigate immunoglobulin responses in patients with bronchopulmonary infection.

In the study described here, a Western blot (immunoblot) method for detecting antibodies against OMP epitopes of *M. catarrhalis* was evaluated for its ability to detect cross-reactivity to related species and other respiratory tract pathogens in an immunoadsorption experiment. Paired serum samples from patients suspected of *M. catarrhalis* and non-*M. catarrhalis* bronchopulmonary infection were then examined for the presence of immunoglobulin M (IgM)-, IgG- and IgA-class antibodies to the OMPs of *M. catarrhalis* by using a gel electrophoresis-immunoperoxidase technique.

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MATERIALS AND METHODS

Strains. The *M. catarrhalis* type strain (strain CCUG 353) and the following six clinical *M. catarrhalis* isolates were examined for OMPs by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE): three strains from respiratory tract specimens (one strain each from Sweden, Denmark, and the United States [strain F 48, received from T. F. Murphy] [19]), two blood culture strains (one received from R. E. Weaver, Centers for Disease Control and Prevention, Atlanta, Ga., and the other isolated in Denmark), and one strain isolated from an eye specimen (received from R. E. Weaver).

In the Western blot analysis experiments for the detection of antibodies, a mixture of all strains except strain F 48 was used.

For immunoadsorption experiments the following strains were used: *M. catarrhalis* CCUG 353^T, *Moraxella* (*Branhamella*) *caviae* CCUG 355^T, *Moraxella* (*Branhamella*) *cuniculi* CCUG 2154^T, *Moraxella* (*Branhamella*) *ovis* CCUG 354^T, *Moraxella bovis* ATCC 10900, *Moraxella lacunata* ATCC 17967, *Moraxella nonliquefaciens* ATCC 19975, *Moraxella osloensis* ATCC 19976^T, *Moraxella phenylpyruvica* CCUG 351^T, *Neisseria cinerea* CCUG 2156^T, *Neisseria gonorrhoeae* CCUG 26876^T, *Neisseria lactamica* CCUG 5853^T, *Neisseria meningitidis* group A CCUG 3269, *Neisseria flavescens* ATCC 13120^T, *Neisseria mucosa* CCUG 26877^T, *Haemophilus influenzae* ATCC 9795, and *Streptococcus pneumoniae* (clinical strain, type 23 F).

Isolation of outer membranes. Outer membranes were isolated from broth culture supernatants as described by Bartos and Murphy (1). The protein concentration was adjusted to 10 g/liter in sample buffer (containing 4.0 ml of distilled water, 1.0 ml of 0.5 M Tris-HCl [pH 6.8], 0.8 ml of glycerol, 1.6 ml of 10% [wt/vol] SDS, 0.4 ml of 2-β-mercaptoethanol, and 0.2 ml of 0.05% [wt/vol] bromphenol blue). The OMPs of all strains were characterized by using strain F 48 from Murphy as a reference strain.

SDS-PAGE. The outer membranes of the *M. catarrhalis* strains prepared as described above were heated at 100°C for 5 min (15). PAGE was performed with 12.5% running gels at 180 V and 4% stacking gels at 120 V. The peptide bands were visualized with Coomassie blue.

Western blot analysis. Transfer of *M. catarrhalis* OMPs from the SDS-polyacrylamide gel onto a nitrocellulose membrane was performed in a Trans Blot Chamber (Bio-Rad); this was followed by washing with Tris-buffered saline (TBS) and blocking with ovalbumin and Tween 20 for 1 h as described previously (8). After the addition of patient serum, diluted 1:100 in blocking solution, and further incubation at 22°C overnight, the paper was washed in TBS containing 0.01% Tween 20. Horseradish peroxidase-labelled rabbit antibodies to human IgG, IgM, or IgA (Dako, Glostrup, Denmark; unwanted antibodies were re-

TABLE 1. Data for patients suspected of bronchopulmonary infection from whom *M. catarrhalis* was or was not isolated from the lower respiratory tract

Characteristic	Patients with *M. catarrhalis* (n = 38)	Patients without *M. catarrhalis* (n = 25)
Median age (yr [range])	70 (4–86)	73 (61–83)
Sex (no. of females/no. of males)	18/20	11/14
Underlying conditions (no. of patients)		
Chronic bronchitis/bronchial asthma	34	21
Miscellaneous	4	4
Clinical presentation (no. of patients)		
Bronchopulmonary infection[a]	25	15
Pneumonia[b]	6[c]	4
No bronchopulmonary infection	7	6

[a] Diagnostic criteria: rectal temperature, ≥38.5°C; increasing amounts of cough and purulent expectoration; absence of infiltrates on X ray.
[b] Bronchopulmonary infection was present together with infiltrates on X ray.
[c] In three patients *M. catarrhalis* was found in pure culture, while in three patients *M. catarrhalis* was found in an admixture with *S. pneumoniae* (n = 2) and *H. influenzae* (n = 1).

moved by solid-phase absorption with human plasma proteins, and specificity was checked by crossed-immunoelectrophoresis and ELISA), diluted 1:500 in blocking solution, were added for 1 h. New washings followed, and finally, horseradish peroxidase color development reagent (Bio-Rad) containing 4-chloro-1-naphthol was added. Control with no patient serum and with serum from a patient with *M. catarrhalis* isolated from blood cultures were also performed.

Immunoadsorption experiments. To test for cross-reactions between *M. catarrhalis* OMPs and surface epitopes from other species (species either taxonomically related to *M. catarrhalis* or representing other respiratory tract pathogens), serum from a patient from whose blood *M. catarrhalis* had been isolated was adsorbed with the bacterial species prior to Western blot analysis. The serum was diluted 1:1,000 in blocking solution. The various bacteria were grown on horse blood agar plates overnight, washed two times in saline (by centrifugation at 4,000 × g for 5 min), and resuspended to 10% (wt/wt) in either saline (for immediate use) or 2% formalin-NaCl (used after 2 h of incubation at 37°C). A bacterial suspension of 5 ml was centrifuged, the sediment was washed twice and resuspended in 5 ml of the diluted serum, and the mixture was rotated overnight at 4°C. After centrifugation (4,000 × g for 5 min) the supernatant was used in the Western blot analysis with rabbit antibodies to human IgG. Adsorption experiments were done twice with identical results.

Patient sera. Acute- and convalescent-phase sera (convalescent-phase sera were obtained 1 to 2 weeks after the acute-phase sera were taken) from the following two patient groups were examined: (i) 38 patients suspected of bronchopulmonary infection from whom *M. catarrhalis* alone (n = 29) or together with *S. pneumoniae* or *H. influenzae*, or both (n = 9), was isolated from the lower respiratory tract and (ii) 25 patients suspected of bronchopulmonary infection from whom *S. pneumoniae* (n = 5), *H. influenzae* (n = 10), or *N. meningitidis* (n = 8) alone or in combination (n = 2) was isolated from the lower respiratory tract. Only specimens from patients whose lower respiratory tract specimens revealed granulocytes and/or cylindrical epithelium with few or no squamous epithelial cells on microscopy were included. Demographic and clinical data for the patients are presented in Table 1 and have been reported previously (6, 7). Serum from a 74-year-old woman from whose blood cultures *M. catarrhalis* was isolated was used as control, because it contained IgM-, IgG-, and IgA-class antibodies against most OMPs recognized by the tested sera from patients and blood donors. Serum samples from 40 adult blood donors were also included (median age, 58 years; age range, 50 to 64 years).

Statistical method. Fisher's exact test was used for statistical analysis.

RESULTS

OMP patterns. The OMP patterns obtained by SDS-PAGE of the seven *M. catarrhalis* strains studied were similar, but minor differences were found (Fig. 1). Approximately 25 bands with molecular masses of between 140 and 16 kDa could be identified, with six to eight of these being the major bands A to H, with molecular masses of 98, 84, 72, 69, 56, 43, 28, and 21 kDa described by Bartos and Murphy (1). The SDS-PAGE patterns of the mixed OMP preparation included all bands recognized for individual strains. Strain F 48 produced nearly all OMPs present in the mixed OMP preparation, and the results obtained with strain F 48 were similar to previous results of Murphy and Loeb (19). The intensities of the bands and the bands that were present varied slightly between strains.

Immunoadsorption experiments. IgG against 120-, 94-, 84-, 60-, and 56-kDa OMPs of *M. catarrhalis* was present when serum from a patient from whose blood *M. catarrhalis* was isolated was diluted 1:1,000. Adsorption with *M. catarrhalis* resulted in the disappearance of IgG against all *M. catarrhalis* OMPs except for the 94-kDa OMP, for which the intensity of the band was weakened considerably. IgG antibodies against the *M. catarrhalis* OMPs were still present after adsorption with *M. caviae*, *M. cuniculi*, *M. ovis*, *M. bovis*, *M. lacunata*, *M. nonliquefaciens*, *M. osloensis*, *M. phenylpyruvica*, *N. cinerea*, *N. gonorrhoeae*, *N. lactamica*, *N. meningitidis* group A, *N. flavescens*, *N. mucosa*, *H. influenzae*, and *S. pneumoniae* type 23 F. However, for sera tested with *M. ovis*, *M. bovis*, and *M. lacunata*, IgG against the 120-, 60-, and 56-kDa OMPs was adsorbed, and for *M. lacunata* the 94-kDa OMP was also adsorbed. This indicates no antigenic cross-reaction between *M. catarrhalis* OMPs and epitopes on the surfaces of these 17 species except for *M. ovis*, *M. bovis*, and *M. lacunata.*

Examinations of serum samples. Examples of the results of Western blot analysis are presented in Fig. 2.

IgM-class antibodies against OMPs of *M. catarrhalis* were found in acute- and/or convalescent-phase sera from 22 of 38 (58%) patients (Table 2) with *M. catarrhalis* in their lower respiratory tract specimens; antibodies against one to seven (median, three) OMPs were found in each positive serum. IgM-class antibodies were found in 8 of 25 (32%) patients without *M. catarrhalis* in their lower respiratory specimens; antibodies against two to six (median, three) OMPs were found in each positive serum specimen. The sera of 6 of 40 (15%) blood donors had IgM-class antibodies against OMPs of *M. catarrhalis*; antibodies against one to two (median, one) OMPs were found in each positive serum specimen. IgM-class antibodies were found more often in patients with *M. catarrhalis* than in blood donors (P = 0.0002), whereas IgM-class antibodies were found equally often in patients with the other respiratory pathogens and in blood donors (P = 0.19).

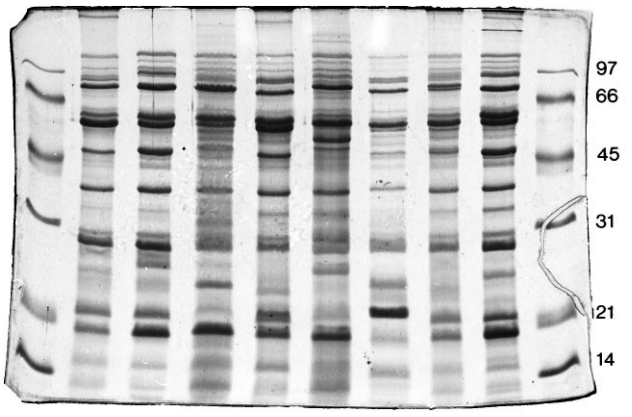

FIG. 1. Coomassie blue-stained SDS-polyacrylamide gels of OMP preparations from strains of *M. catarrhalis*. The outside lanes contain molecular mass markers. Molecular masses (in kilodaltons) are given on the right. Lane 1, type strain; lanes 2 and 3, respiratory tract isolates from Sweden and Denmark, respectively; lanes 4 and 5, blood culture isolates from the United States and Denmark, respectively; lane 6, eye isolate from the United States; lane 7, mixture of OMPs from six strains of *M. catarrhalis* (lanes 1 to 6); lane 8, strain F 48 (see Materials and Methods).

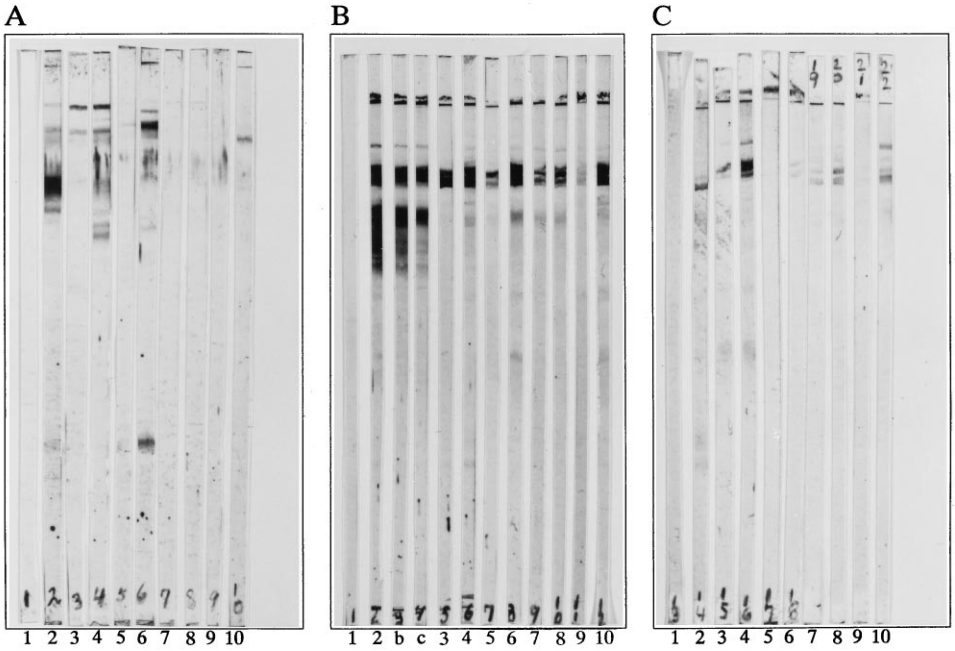

FIG. 2. Results of Western blot analysis examinations in which OMPs of *M. catarrhalis* were tested with sera from four patients suspected of having bronchopulmonary infection caused by *M. catarrhalis*. Sera were tested for the presence of IgM (A), IgG (B), and IgA (C) against OMPs of *M. catarrhalis*. Lanes 1 and 2, negative and positive controls, respectively (i.e., no addition of serum and addition of serum from a patient whose blood yielded growth of *M. catarrhalis* on culture); lanes 2b and 2c, the positive control was diluted 1:2 and 1:4, respectively; lanes 3 and 4 (sera from patient 1), 5 and 6 (sera from patient 2), 7 and 8 (sera from patient 3), and 9 and 10 (sera from patient 4) provide results for acute- and convalescent-phase sera (for each pair of lanes, respectively) from four patients with different antibody response patterns; IgM-class antibody responses are seen for patients 1, 2, and 4; increased IgG-class antibody responses in convalescent-phase sera are seen for all patients; increased IgA-class antibody responses in convalescent-phase sera are seen for patients 1, 2, and 4.

IgG-class antibodies against *M. catarrhalis* OMPs were found in acute-phase sera from all except two patients with *M. catarrhalis* and from all blood donors (Table 2). In acute-phase sera, there were no significant differences with regard to the number of serum IgG-class antibodies directed against different OMPs between patients with *M. catarrhalis* (median, 6; range, 0 to 14), patients without *M. catarrhalis* (median, 8; range, 2 to 17), and blood donors (median, 7; range, 2 to 15). An increase in the number of different IgG-class antibodies, from acute- to convalescent-phase serum, against OMPs of *M. catarrhalis* was found for 19 of 38 (50%) patients with *M. catarrhalis* and 4 of 25 (16%) patients without *M. catarrhalis* (Table 3).

IgA-class antibodies were found in acute-phase serum from 25 of 38 (66%) patients with *M. catarrhalis*, in sera from 21 of 25 (84%) patients without *M. catarrhalis*, and in sera from 30 of 40 (75%) blood donors (Table 2). In acute-phase sera there were no significant differences with regard to the number of serum IgA-class antibodies directed against different OMPs between patients with *M. catarrhalis* (median, four; range, one to eight), patients without *M. catarrhalis* (median, three; range, one to eight), and blood donors (median, two; range, one to five). An increased number of IgA-class antibodies directed against different OMPs, from acute- to convalescent-phase serum, was found in sera from 13 of 38 (34%) (median, three; range, one to nine) patients with *M. catarrhalis* and the serum of 1 of 25 (4%) (one IgA-class antibody band) patients without *M. catarrhalis*. The sera of eight of these latter patients had

TABLE 2. Immunoglobulin response in serum against OMPs of *M. catarrhalis* in the different groups examined

Immunoglobulin response	No. of responding serum samples		
	Patients with *M. catarrhalis* (n = 38)	Patients without *M. catarrhalis* (n = 25)	Blood donors (n = 40)[a]
IgM in acute- and/or convalescent-phase serum	22	8	6
IgG in acute-phase serum	36	25	40
Increased IgG titers in convalescent-phase serum	19[b]	4	ND[c]
IgA in acute-phase serum	25	21	30
Increased IgA titers in convalescent-phase serum	13[d]	1	ND
IgM and/or increased IgG titers in convalescent-phase serum	30[e]	10	ND

[a] The terms acute- and convalescent-phase sera are not relevant to the blood donor sera.
[b] P = 0.008 for the ratios 19/38 versus 4/25.
[c] ND, not determined.
[d] P = 0.03 for the ratios 13/38 versus 1/25.
[e] P = 0.004 for the ratios 30/38 versus 10/25.

TABLE 3. Serum IgG response, from acute- to convalescent-phase sera, against OMPs of *M. catarrhalis* in patients showing an increase in the number of different IgG-class antibodies

Patient group and no.	Molecular masses (kDa) of *M. catarrhalis* OMPs (kDa) in the following sera with IgG responses[a]:		No. of changes[b]
	Acute-phase serum	Convalescent-phase serum (in addition to those found in acute-phase serum)	
Patients with *M. catarrhalis*			
1	140, 94, 87, 84	56, 50, 38	3
2	0	72	1
3	87, 84, 78, 72, 60	140, 94	2
4	140, 98, 94, 84, 78, 72, 64, 58, 56, 54	69, 50, 47, 30, 28	5
5	140, 94, 84, 78, 72, 64, 58, 56, 54, 51, 47, 46, 43	98, 60, 35	3
6	140, 98, 84, 78, 72, 69, 64, 60, 58, 56	94, 60	2
7	84	140, 120, 94, 78, 72	5
8	140, 120, 84, 78, 72, 64, 58, 47, 38, 28	94, 69, 56, 21	4
9	140, 120, 94, 84, 78, 72, 69, 60, 58, 50, 47, 35, 28	56, 54	2
10	140, 98, 84, 78, 72, 64, 60, 58, 54, 50, 47, 38	56	1
11	84, 78, 72, 60, 28, 21	38	1
12	94, 84, 78, 72, 60, 28, 21	140, 98, 64, 54, 50, 47	6
13	98, 84, 78, 28, 21	140, 94, 72, 64, 60, 47, 38, 28, 21	9
14	84, 78, 72, 58	140, 120, 110, 98, 94, 60, 43, 38, 35	9
15	84, 78, 72	140, 120, 110, 98, 94, 69, 60, 58, 35, 28	10
16	84	120, 110, 78, 72	4
17	94, 84, 78	140, 120, 110, 98, 60	5
18	140, 110, 98, 94, 84, 78, 69, 60, 35, 28	120, 50, 38	3
19	84, 64, 43	140, 120, 98, 94, 47, 35	6
Patients without *M. catarrhalis*			
1	140, 120, 98, 94, 84, 78, 60, 35	56	1
2	140, 84, 78	98, 87	2
3	140, 120, 94, 84, 78, 72, 69, 64, 60, 58, 38, 28	43	1
4	140, 94, 84, 78, 72, 60, 47	58, 56, 54, 28	4

[a] The molecular masses of the major OMPs A to H designated by Murphy and Bartos (18) are ~98, 84, 72, 69, 56, 43, 28, and 21 kDa.
[b] For patients with *M. catarrhalis*, the median is 4 (range, 1 to 10); for patients without *M. catarrhalis*, the median is 2 (range, 1 to 4).

IgM-class antibodies against *M. catarrhalis* OMPs, and there were an increased number of IgG-class antibodies against different *M. catarrhalis* OMPs in their convalescent-phase sera. Furthermore, the convalescent-phase sera of two of these patients demonstrated IgM-class antibodies, and the convalescent-phase sera of four patients demonstrated an increased number of IgG-class antibodies.

Significantly more patients with *M. catarrhalis* than patients without *M. catarrhalis* had IgM-class antibodies and/or increased numbers of IgG-class antibodies in convalescent-phase serum against different *M. catarrhalis* OMPs (Table 2). Of the patients with *M. catarrhalis*, 11 patients had IgM-class antibodies and increased numbers of IgG-class antibodies in their convalescent-phase sera, 11 patients had IgM-class antibodies and 8 patients had an increased number of IgG-class antibodies. Of the patients without *M. catarrhalis,* two had IgM-class antibodies and an increased number of IgG-class antibodies, six had IgM-class antibodies in their convalescent-phase sera, and two had an increased number of IgG-class antibodies in their convalescent-phase sera.

DISCUSSION

Murphy and Loeb (19) have examined five procedures for the isolation of OMPs and compared the results with the results obtained by the sucrose gradient technique (19). Two of the procedures, those that used broth culture supernatants (which was used in the present study) and EDTA-heat-induced vesicles, resulted in OMP SDS-PAGE patterns comparable to the patterns obtained after sucrose density centrifugation, which is considered the "gold standard." The OMPs of *M. catarrhalis* strains of various geographical and anatomical origins demonstrate little variability, as shown by Bartos and Murphy (1) and as also seen in the present study. With regard to OMP production, the F 48 strain described by Murphy and Loeb (19) appeared to be very representative, because it produces nearly all OMPs produced by different *M. catarrhalis* strains. Some OMPs have been shown to be antigenically conserved among different *M. catarrhalis* strains (13, 18, 21), suggesting that, in an immune response to OMP antigens of one strain, a second strain might be recognized (11). In the control serum, from a patient from whose cultured blood *M. catarrhalis* was isolated, antibodies against practically all OMPs were present, thereby being an indirect marker of the sensitivity of the detection method.

When evaluating the significance of antibodies against *M. catarrhalis*, it is important to render it probable that the antibody responses are specific for *M. catarrhalis*. This was demonstrated by the immunoadsorption experiment, in which only *M. ovis*, *M. bovis*, and *M. lacunata* demonstrated epitopes that cross-reacted with four *M. catarrhalis* OMPs; these three species are, however, extremely rarely isolated from humans.

The method used, Western blotting, allows for the recognition of the production of antibodies to OMPs of *M. catarrhalis*, in spite of an already existing antibody response to other OMPs. This is of importance because it has been shown that the sera of many healthy adults already have IgG- and IgA-class antibodies (9, 12) against *M. catarrhalis*, as was also seen in the present study. The major OMPs, A to H, described by Murphy and coworkers (1, 19) were major targets for antibody responses, but other OMPs also often elicited an antibody response.

A significantly more frequent occurrence of either IgM-class antibodies and/or an increased number of IgG-class antibodies against different *M. catarrhalis* OMPs from acute- to convalescent-phase sera was found in patients with *M. catarrhalis* than in patients without *M. catarrhalis*. This supports a pathogenic role of the bacterium in patients with signs of bronchopulmonary infection from whom *M. catarrhalis* is isolated from the lower respiratory tract. Other methods that have been used to demonstrate an immune response to *M. catarrhalis* in patients with pulmonary infections are (i) the demonstration of bactericidal activity in convalescent-phase sera (4); (ii) the use of whole-cell antigen to demonstrate rises in the titers of IgG- and/or IgA-class antibodies in convalescent-phase sera (2, 8, 14) by immunofluorescence or ELISA techniques; and (iii) demonstration of rises in IgG-class antibody titers against the P protein (5, 10), an *M. catarrhalis* OMP. Generally, the convalescent-phase sera of approximately 50% of patients suspected of having *M. catarrhalis* infection elicited an increased antibody response to the organism. Using a whole-cell protein suspension as antigen, we previously found (8) IgG-class antibodies against a 28-kDa protein more frequently in patients suspected of *M. catarrhalis* bronchopulmonary infection than in patients suspected of non-*M. catarrhalis* bronchopulmonary infection. However, the supposition that the 28-kDa protein was an OMP could not be verified in the present study, illustrating the difficulties in correlating results when using different serological methods.

Bacteriological findings in relation to acute exacerbations of chronic bronchitis change from episode to episode, which may explain some of the IgM-class antibody responses among patients from whom *M. catarrhalis* was not isolated from the lower respiratory tract. For IgA- and IgG-class antibody responses, the time interval between the times that the two serum samples were obtained may have been inadequate to observe seroconversion. Fourteen patients had an increased number of IgA-class antibodies against different *M. catarrhalis* OMPs in their convalescent-phase sera, and *M. catarrhalis* was not isolated from only 1 of these 14 patients, strongly associating increased numbers of IgA-class antibodies with the presence of *M. catarrhalis.*

As a diagnostic test, the method described here is not useful because antibody production is not restricted only to patients suspected of *M. catarrhalis* bronchopulmonary infection. In relation to the reported increase in the number of acute otitis media episodes alleged to be caused by this bacterium, the OMPs of *M. catarrhalis* have been mentioned as potential components of a vaccine (17). However, no uniform pattern of antibodies elicited in relation to infection occurred; therefore, it is very difficult to comment on the possible protective capabilities of the individual OMPs of *M. catarrhalis.*

The exact role of bacterial infections in patients with chronic bronchitis is not known (20). Bacterial infection does not appear to initiate the disease, but bacteria probably play a major role in the characteristic acute exacerbations (16, 20). The present study indicates that *M. catarrhalis* should be considered one of the bacteria involved in acute exacerbations of chronic bronchitis.

REFERENCES

1. **Bartos, L. C., and T. F. Murphy.** 1988. Comparison of the outer membrane proteins of 50 strains of *Branhamella catarrhalis*. J. Infect. Dis. **158:**761–765.
2. **Black, A. J., and T. S. Wilson.** 1988. Immunoglobulin G (IgG) serological response to *Branhamella catarrhalis* in patients with acute bronchopulmonary infections. J. Clin. Pathol. **41:**329–333.
3. **Catlin, B. W.** 1990. *Branhamella catarrhalis*: an organism gaining respect as a pathogen. Clin. Microbiol. Rev. **3:**293–320.
4. **Chapman, A. J., Jr., D. M. Musher, S. Jonsson, J. E. Clarridge, and R. J. Wallace, Jr.** 1985. Development of bactericidal antibody during *Branhamella catarrhalis* infection. J. Infect. Dis. **151:**878–882.
5. **Chi, D. S., A. Verghese, C. Moore, F. Hamati, and S. Berk.** 1990. Antibody response to P-protein in patients with *Branhamella catarrhalis* infections. Am. J. Med. **88**(Suppl. 5A)**:**25–27.
6. **Christensen, J. J., O. Gadeberg, and B. Bruun.** 1986. *Branhamella catarrhalis*: significance in pulmonary infections and bacteriological features. Acta Pathol. Microbiol. Immunol. Scand. Sect. B **94:**89–95.
7. **Christensen, J. J., O. Gadeberg, and B. Bruun.** 1988. *Neisseria meningitidis*: occurrence in non-pneumonic pulmonary infections. APMIS **96:**218–222.
8. **Christensen, J. J., J. Renneberg, B. Bruun, and A. Forsgren.** 1995. Serum antibody response to proteins of *Moraxella* (*Branhamella*) *catarrhalis* in patients with lower respiratory tract infection. Clin. Diagn. Lab. Immunol. **2:** 14–17.
9. **Ejlertsen, T., E. Thisted, P. Å. Østergaard, and J. Renneberg.** 1994. Maternal antibodies and acquired serological response to *Moraxella catarrhalis* in children determined by an enzyme-linked immunosorbent assay. Clin. Diagn. Lab. Immunol. **1:**464–468.
10. **Eliasson, I.** 1980. A protein characteristic of *Branhamella catarrhalis*. Serological identification of the genus. Acta Pathol. Microbiol. Scand. Sect. B **88:**281–286.
11. **Faden, H., J. Hong, and T. Murphy.** 1992. Immune response to outer membrane antigens of *Moraxella catarrhalis* in children with otitis media. Infect. Immun. **60:**3824–3829.
12. **Goldblatt, D., M. W. Turner, and R. J. Levinsky.** 1990. *Branhamella catarrhalis*: antigenic determinants and the development of the IgG subclass response in childhood. J. Infect. Dis. **162:**1128–1135.
13. **Helminen, M. E., I. Maciver, J. L. Latimer, L. D. Cope, G. H. McCracken, Jr., and E. J. Hansen.** 1993. A major outer membrane protein of *Moraxella catarrhalis* is a target for antibodies that enhance pulmonary clearance of the pathogen in an animal model. Infect. Immun. **61:**2003–2010.
14. **Kerttula, Y., M. Leinonen, M. Koskela, and P. H. Mäkela.** 1987. The aetiology of pneumonia. Application of bacterial serology and basic laboratory methods. J. Infect. **14:**21–30.
15. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
16. **Leeder, S. R.** 1975. Role of infection in the cause and course of chronic bronchitis and emphysema. J. Infect. Dis. **131:**731–742.
17. **Murphy, T. F.** 1989. The surface of *Branhamella catarrhalis*: a systematic approach to the surface antigens of an emerging pathogen. Pediatr. Infect. Dis. J. **8:**S75–S77.
18. **Murphy, T. F., and L. C. Bartos.** 1989. Surface-exposed conserved determinants of outer membrane proteins of *Branhamella catarrhalis*. Infect. Immun. **57:**2938–2941.
19. **Murphy, T. F., and M. R. Loeb.** 1989. Isolation of the outer membrane of *Branhamella catarrhalis*. Microb. Pathog. **6:**159–174.
20. **Reynolds, H. Y.** 1990. Chronic bronchitis and acute infectious exacerbations, p. 533. *In* G. L. Mandell, R. G. Douglas, and J. E. Bennett (ed.), Principles and practice of infectious diseases, 3rd ed. Churchill Livingstone, New York.
21. **Sarwar, J., A. A. Campagnari, C. Kirkham, and T. F. Murphy.** 1992. Characterization of an antigenically conserved heat-modifiable major outer membrane protein of *Branhamella catarrhalis*. Infect. Immun. **60:**804–809.
22. **Vaneechoutte, M., G. Verschraegen, G. Claeys, B. Weise, and A. M. Vandenabeele.** 1990. Respiratory tract carrier rate of *Moraxella* (*Branhamella*) *catarrhalis* in adults and children and interpretation of the isolation of *M. catarrhalis* from sputum. J. Clin. Microbiol. **28:**2674–2680.