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Human Antibody Response to Outer Membrane Protein G1a, a Lipoprotein of *Moraxella catarrhalis*

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***Moraxella catarrhalis* is an important cause of respiratory infections in adults with chronic obstructive pulmonary disease (COPD) and of otitis media in children. Outer membrane protein (OMP) G1a is an ~29-kDa surface lipoprotein and is a potential vaccine candidate. The gene that encodes OMP G1a was expressed and purified using a novel plasmid vector. [^{3}H]palmitic acid labeling demonstrated that both native and recombinant OMP G1a contain covalently bound palmitic acid. To assess the expression of OMP G1a during human infection, paired sera and sputum supernatants from adults with COPD followed prospectively were studied by enzyme-linked immunosorbent assays with recombinant lipidated OMP G1a to detect antibodies made specifically during carriage of *M. catarrhalis*. Overall, 23% of patients developed either a serum immunoglobulin G (IgG) response (9%) or sputum IgA response (21%) to OMP G1a, following 100 episodes of acquisition and clearance of *M. catarrhalis*. Patients developed antibody responses at similar rates following episodes of clinical exacerbation compared to asymptomatic colonization. Serum IgG antibodies following natural infection were directed predominantly at OMP G1a epitopes that are not exposed on the bacterial surface. These data show that OMP G1a is expressed during infection of the human respiratory tract and is a target of systemic and mucosal antibodies. These observations indicate that OMP G1a, a highly conserved surface protein, should be evaluated further as a vaccine candidate.**

Over the past 2 decades, *Moraxella catarrhalis* has emerged as an important human respiratory pathogen. Acquisition of a new strain of *M. catarrhalis* is associated with the development of an exacerbation in patients with chronic obstructive pulmonary disease (COPD) (43). Indeed, *M. catarrhalis* is the second most common bacterial cause of exacerbations in adults with COPD after nontypeable *Haemophilus influenzae* (32, 38, 44). COPD is the fourth-leading cause of death in the United States and is projected to rank fifth in the world by 2020 (2, 26, 28, 39). *M. catarrhalis* is also the third-most-common bacterial cause of otitis media in children after *Streptococcus pneumoniae* and nontypeable *H. influenza*e (25). Approximately 25 million episodes of otitis media occur annually in the United States, and $2 billion is spent annually on treatment (25). It is estimated that *M. catarrhalis* causes 10% to 20% of cases of acute otitis media. Recurrent otitis media in infants and young children affects speech development and cognitive abilities (25, 42). Finally, *M. catarrhalis* is also a common cause of sinusitis in children and adults (32). Since *M. catarrhalis* is an important human pathogen for infants and adults with COPD, there is a need for a vaccine. Infants could be immunized to prevent otitis media and adults with COPD could be immunized to prevent exacerbations caused by *M. catarrhalis*.

One approach to vaccine development for gram-negative bacteria has been to use outer membrane proteins (OMPs) as vaccine antigens. Effective vaccines containing OMPs have been developed for Lyme disease and pertussis (7, 17, 23, 47, 49). Several characteristics should be considered when evaluating an OMP as a vaccine candidate, including expression of the protein on the bacterial surface during human infection, conservation of the protein among strains, and the ability to induce a protective immune response (9, 18). A potential vaccine candidate for *M. catarrhalis* is the 29-kDa OMP G1a. Adsorption assays have demonstrated that OMP G1a contains epitopes exposed on the bacterial surface (34). OMP G1a is present in all strains of *M. catarrhalis* studied thus far. In addition, sequence analysis of *ompG1a* from 25 clinical isolates of *M. catarrhalis* identifies the gene as being highly conserved among strains (1). These features indicate that OMP G1a is a candidate for further evaluation as a vaccine antigen. OMP G1a contains a prokaryotic membrane lipoprotein attachment site on the carboxy terminus of the leader peptide, predicting that the protein has a covalently attached lipid. *ompG1a* has strong homology with the gene that encodes the copper tolerance protein NlpE (CutF) in *Escherichia coli* and in several other gram-negative bacteria (21).

The goal of the present study was to elucidate the role of OMP G1a as a target of the systemic and mucosal immune responses in patients with COPD. Recombinant lipidated OMP G1a was expressed and purified. Enzyme-linked immunosorbent assays (ELISAs) were performed, which were designed to measure the development of systemic and mucosal antibodies in adults with COPD who have acquired and cleared *M. catarrhalis* from the respiratory tract. Adsorption assays were performed to elucidate the extent to which human

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antibodies were directed toward surface-exposed epitopes of OMP G1a.

MATERIALS AND METHODS

Bacteria and growth conditions. *M. catarrhalis* strain 25240 was obtained from the American Type Culture Collection. Chemically competent *Escherichia coli* strains TOP10 and Rosetta (DE3)pLysS were obtained from Invitrogen and Novagen, respectively. *M. catarrhalis* was grown on brain heart infusion (BHI) plates or in BHI broth. *E. coli* strains were grown on Luria-Bertani (LB) plates and in LB broth or Terrific broth with the appropriate antibiotics. *Streptococcus pneumoniae* strain RX1-19F was grown on chocolate agar plates and in Todd-Hewitt broth.

Construction of the pCATCH lipoprotein expression vector. Two oligonucleotides were synthesized that when annealed together would encode a hexahistidine tag and a thrombin cleavage site (5′-GATCCCTGGTGCCGCGCGGCAG CAGCAGCGGCCACCATCATCACCATCACTGA-3′ and 5′-GGACCACGG CGCGCCGTCGTCGTCGCCGGTGGTAGTAGTGGTAGTGACTCTAG-3′). The oligonucleotide construct incorporated restriction enzyme sites to allow cloning of the construct into the BamHI site of the pDUMP lipoprotein expression vector (13). The plasmid resulting from the cloning of the oligonucleotide construct into pDUMP was named pCATCH. Cloning of genes into pCATCH results in the production of lipidated recombinant protein that possesses a thrombin-cleavable C-terminal hexahistidine tag allowing subsequent purification.

Cloning of *ompG1a* gene into pCATCH. Oligonucleotide primers corresponding to the 5′ end, starting after the predicted cysteine, and the 3′ end of *ompG1a* were designed with NcoI and BamHI restriction sites (13). The *ompG1a* was amplified by PCR from genomic DNA of strain 25240 as the template with Platinum *Taq* (Invitrogen, San Diego, CA). The resultant PCR product was ligated into pCATCH and transformed into *E. coli* strain TOP10 (Invitrogen, San Diego, CA). Colonies were picked and grown in broth, and plasmids were purified. PCR and sequencing confirmed the insertion of *ompG1a*. The resultant plasmid of *ompG1a* in pCATCH was called pCATCH6.

Expression and purification of OMP G1a. The pCATCH6 plasmid was transformed into Rosetta (DE3)pLysS (Novagen, Madison, WI) for expression. To express recombinant OMP G1a, 10 ml of LB broth containing 34 μg/ml chloramphenicol and 30 μg/ml kanamycin was inoculated and grown overnight with shaking at 37°C. The next morning, 150 ml of Terrific broth containing 34 μg/ml chloramphenicol and 30 μg/ml kanamycin was seeded with the overnight culture and grown to an optical density at 600 nm (OD_{600}) of 0.6. Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a concentration of 3 mM; after 4 h, the bacteria were harvested by centrifugation at 4,000 × *g* for 15 min at 4°C. The pellet was suspended in 6 ml of 0.05 M NaH_2PO_4, 0.01 M Tris, 6 M guanidine, 0.1 M NaCl, 0.04 M Pefabloc (Roche, Indianapolis, IN) (pH 8.0) (lysis buffer) and mixed with a nutator for 25 min at room temperature to lyse bacteria. The suspension was centrifuged at 10,000 × *g* for 10 min at 4°C, and the supernatant was saved (bacterial lysate).

BD Talon resin (BD Biosciences, Palo Alto, CA) was used in the purification as was described by Bhushan et al. (6). An aliquot of 2 ml of Talon resin was centrifuged at 750 × *g* for 5 min at 4°C. The pellet was suspended in 10 ml of lysis buffer and centrifuged, and the supernatant was discarded. The resin was suspended in the bacterial lysate and mixed with a nutator for 20 min at room temperature. The suspension was centrifuged at 750 × *g* for 5 min at 4°C and the resin was saved. The resin was washed three times with lysis buffer and once with 0.02 M Tris (pH 8), 1% β-octylglucoside, and 0.5 M NaCl (TON buffer). To elute the recombinant OMP G1a, the washed resin was suspended with 1 column volume of TON buffer containing 0.05 M EDTA. The resin was removed by centrifugation, and the supernatant containing the purified recombinant OMP G1a was collected. Centricon YM-10 centrifugal filter devices (Millipore) were used to remove EDTA and exchange into TON buffer. The protein concentration was determined by bicinchoninic acid assay (Pierce, Rockford, IL) and Lowry assay (Sigma Diagnostics, St. Louis, MO).

[^{3}H]Palmitic acid labeling of OMP G1a. The sequence of OMP G1a contains homology to a prokaryotic membrane lipoprotein attachment site. To investigate experimentally whether OMP G1a is lipidated, cultures were grown in the presence of [^{3}H]palmitic acid (palmitic acid; [9,10-^{3}H]). Cultures of Rosetta(DE3) pLysS with pCATCH6, Rosetta(DE3)pLysS without pCATCH6, and *M. catarrhalis* were grown in LB broth and BHI broth, respectively. The cultures were grown to an optical density at 600 nm of 0.6 for *E. coli* and 0.3 for *M. catarrhalis*. The cultures were then supplemented with 5 μCi per ml of culture of [^{3}H]palmitic acid (specific activity, 45.0 Ci/mmol) and 3 mM IPTG for the *E. coli* samples. After 3 h, 1 ml of culture was removed, centrifuged, suspended in sample buffer, and heated at 100°C for 15 min. The lysates were then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The gel was soaked in Enhance 3 solution (Perkin-Elmer) for 1 h, followed by a 15-min soak in cold distilled H_2O. The gel was dried on a gel drier for 1 h, placed onto x-ray film, and exposed at −80°C for 8 days, and then the film was developed.

COPD study clinic. All serum and sputum samples were obtained from patients enrolled in a prospective study of COPD at the Buffalo VA Medical Center (43). Inclusion criteria for this study were chronic bronchitis, as defined by the American Thoracic Society (2) and a willingness to attend clinic monthly. Patients with asthma, known bronchiectasis, malignancies, or other immunocompromising illnesses were excluded. Patients were seen monthly in the clinic and at the time of suspected exacerbations. A study nurse performed a clinical evaluation at every clinic visit and a study physician (S.S. or T.F.M.) evaluated patients who were suspected of having an exacerbation. A clinical evaluation determined whether the patient was having an exacerbation or had clinically stable disease as described previously (43).

Serum and expectorated sputum samples were collected at each clinic visit. Sputum samples were subjected to quantitative culture by the addition of an equal volume of 6.5 mM dithiothreitol in phosphate-buffered saline (PBS), mixed by vortexing, and incubated at 37°C for 15 min. Aliquots were plated on blood, chocolate, and MacConkey plates. Standard techniques were used to identify the bacteria. Isolates of *M. catarrhalis* were subjected to pulsed-field gel electrophoresis to identify the duration of carriage of each strain. Sputum supernatants were prepared by centrifugation at 27,000 × *g* for 30 min at 4°C. The sputum supernatants and serum samples were stored at −80°C (43).

Enzyme-linked immunosorbent assay. Development of new immunoglobulin G (IgG) in human serum and IgA in sputum supernatants to OMP G1a were measured by ELISA for each new acquisition of *M. catarrhalis*. The wells of a 96-well microtiter Immunolon 4 plate (Thermo Labsystems, Franklin, MA) were coated overnight at 4°C with 10 μg/ml of OMP G1a in 0.1 M sodium carbonate–0.1 M sodium bicarbonate (pH 9.6). Wells were washed three times with PBS containing 0.5% Tween-20 (PBS-Tween) after each step. Wells were blocked with 2% nonfat dry milk in PBS-Tween and incubated at room temperature for 1 h. After the wells were washed, serum or sputum supernatants diluted in PBS-Tween containing 2% nonfat dry milk were added to the wells and incubated at 37°C for 2 h. After the wash, horseradish peroxidase-conjugated rabbit anti-human IgG (Kirkegaard & Perry Laboratories, Gaithersburg, MD) or horseradish peroxidase-conjugated rabbit anti-human IgA (Dako, Denmark) diluted at 1:3,000 in PBS-Tween containing 2% nonfat dry milk was added to the wells and incubated for 1 h at room temperature. Wells were washed, and color was developed by the addition of 0.1 mg of 3,3′,5,5′-tetramethylbenzidine-dimethyl sulfoxide and 0.02% hydrogen peroxide/ml in 0.1 M sodium acetate adjusted to a pH of 4.5 with citric acid. After 15 min, the color development was stopped by the addition of 4 N H_2SO_4 and the OD_{450} value was read.

All samples were run in duplicate. To control for background, serum and sputum supernatant samples were assayed in sham coated wells (no antigen) with each dilution. The OD value of the sham coated wells was subtracted from the OD value of each sample well of the corresponding dilution. The percent change of OD between the preacquisition and postclearance sample for each dilution was calculated by the following formula: [(OD of postclearance sample − OD of preacquisition sample)/OD of preacquisition sample] × 100.

To determine the cutoff for a significant increase in OD between the preacquisition and postclearance samples, ELISAs were performed with 10 pairs of serum samples and 10 pairs of sputum supernatant samples (2-month interval between samples in each pair) from patients in the COPD study clinic whose sputum was negative in culture for *M. catarrhalis*. These control samples were used to calculate the cutoff for a significant percent change between the paired samples, as described in Results.

Adsorption assays. To determine the extent to which serum IgG antibodies to OMP G1a were directed at epitopes on the bacterial surface, adsorption assays were conducted as previously described (37). The bacteria were grown in broth and centrifuged at 12,000 × *g* for 10 min at 4°C. After the pellet was washed with the same volume of PBS supplemented with 1.25 mM $CaCl_2$–0.5 mM $MgCl_2$ (designated PCM), bacteria were centrifuged and suspended in 0.5 ml of serum diluted 1:100 in PCM. Following incubation for 30 min with rotating at 4°C, bacteria were removed by centrifugation at 16,000 × *g* for 15 min. The supernatant was stored at 4°C and used in ELISA within 1 week. The percent adsorption was calculated using the following formula: [(OD of unadsorbed serum − OD of adsorbed serum)/OD of unadsorbed serum] × 100. The cutoff for significant change in adsorption was determined by performing adsorption assays with *S. pneumoniae* as a negative control as described below.

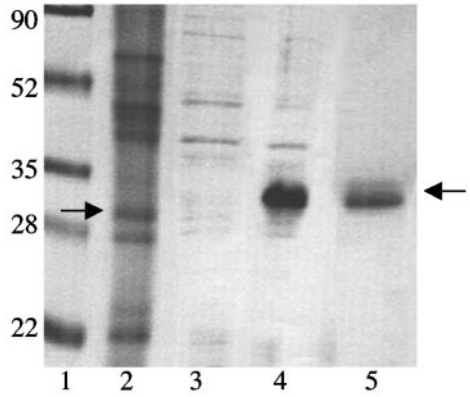

FIG. 1. Coomassie-stained SDS-PAGE gel. Lane 1, molecular mass standards in kilodaltons; lane 2, outer membrane preparation of *M. catarrhalis* 25240; lane 3, whole-cell lysate of *E. coli* containing pCATCH6 preinduction; lane 4, whole-cell lysate of *E. coli* containing pCATCH6 after 4-h induction with IPTG; lane 5, purified recombinant OMP G1a. The arrow on the left indicates the native OMP G1 band in lane 2. The arrow on the right indicates the recombinant OMP G1a in lanes 4 and 5.

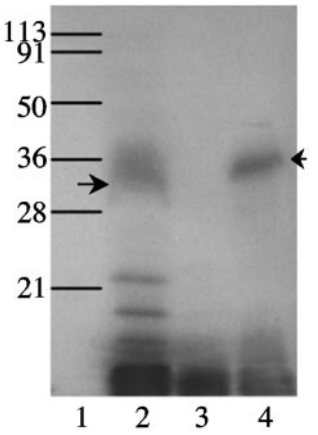

FIG. 2. Autoradiograph of an SDS-PAGE gel of whole-cell lysates grown in the presence of [^{3}H]palmitic acid. Lane 1, molecular mass standards in kilodaltons; lane 2, *M. catarrhalis*; lane 3, *E. coli* with no plasmid (negative control); lane 4, *E. coli* with the pCATCH6 plasmid containing *ompG1a*. The arrows indicate the position of OMP G1a.

Statistical analysis. Comparison of dichotomous variables was performed by chi-square analysis or Fisher's exact test, as appropriate. P values of <0.05 were considered significant. All statistical analysis was performed with Statview 5.0 software.

RESULTS

Cloning the gene and characterization of purified recombinant OMP G1a. The plasmid pDUMP was developed from pET9c (Novagen, Madison, WI) and includes the *E. coli* major outer membrane lipoprotein (Lpp) signal sequence, lipoprotein signal peptidase recognition site, and the +2 outer membrane-sorting signal at the amino terminus (13). pCATCH differs from pDUMP only by the addition of a C-terminal hexahistidine tag and thrombin cleavage site. *ompG1a* from ATCC strain 25240 was cloned into pCATCH to form plasmid pCATCH6. The protein was expressed in the Rosetta (DE3)pLysS strain of *E. coli*. The Rosetta strain contains a plasmid that encodes codons that are rare in *E. coli*, as *ompG1a* contains three of these rare codons (24, 46). Purification was accomplished by affinity chromatography on a Talon column containing cobalt ions to which the carboxy-terminal hexahistidine tag bound. Purified OMP G1a migrates as a single band by SDS-PAGE (Fig. 1, lane 5). Recombinant purified OMP G1a migrated at a slightly higher molecular mass compared to native OMP G1a (Fig. 1, lane 2), due to the addition of the hexahistidine tag on the recombinant protein.

Sequence analysis of *ompG1a* predicts that OMP G1a is a lipoprotein based on the presence of a lipid attachment site on the amino terminus. To confirm that native and recombinant OMP G1a are lipidated, cultures were grown in the presence of [^{3}H]palmitic acid. *M. catarrhalis* strain 25240, *E. coli* with the pCATCH6 plasmid, and *E. coli* without the plasmid were utilized in this study. Bacteria were harvested, and whole-bacterial-cell lysates were subjected to SDS-PAGE; radiolabeling was assessed by autoradiography. Both native OMP G1a (Fig. 2, lane 2) and recombinant OMP G1a expressed in *E. coli* (Fig. 2, lane 4) were lipidated. The band was absent in the *E. coli* control (Fig. 2, lane 3). *M. catarrhalis* also contained three smaller lipoproteins of approximately 22 kDa and smaller.

COPD study clinic samples. From March 1994 through December 2000, 120 episodes of acquisition and clearance of *M. catarrhalis* occurred based on molecular typing of isolates of *M. catarrhalis* recovered from monthly sputum cultures (36). Of the 120 episodes, 100 paired serum samples and 86 paired sputum supernatant samples were available for analysis. Preacquisition sera and sputum supernatants were collected approximately 1 month prior to acquisition of *M. catarrhalis*. Postclearance sera and sputum supernatants were collected approximately 1 to 2 months following clearance of *M. catarrhalis* from the respiratory tract.

Human systemic antibody response to OMP G1a. To characterize the serum IgG response to OMP G1a, ELISAs were performed to measure IgG to OMP G1a in serum samples from adults with COPD. To measure the antibody response made specifically during the episode of carriage of *M. catarrhalis*, the change in OD from before the acquisition to after the clearance of the strain was calculated using the following formula: [(OD of postclearance serum − OD of preacquisition serum)/OD preacquisition serum] × 100.

To determine the cutoff value for a significant percent change in OD between preacquisition and postclearance samples, 10 pairs of control serum samples collected 2 months apart from subjects in the COPD study clinic who were never colonized with *M. catarrhalis* were subjected to ELISAs with purified recombinant OMP G1a. The mean ± standard deviation (SD) of the control serum pairs was 6.85 ± 7.70. These paired control serum samples demonstrated an upper limit of the 99% confidence interval of 26.7%. Therefore, any change in OD of >26.7% between preacquisition and postclearance sera was regarded as significant. Of the 100 serum pairs available for testing, 55 were episodes of exacerbation and 45 were episodes of asymptomatic colonization with *M. catarrhalis*. A total of 9 (9.0%) of 100 pairs of serum showed an increase in serum IgG (33.3% to 3,346.2% increase) (Fig. 3). Five of 55 (9.1%) episodes of exacerbation were followed by a serum IgG

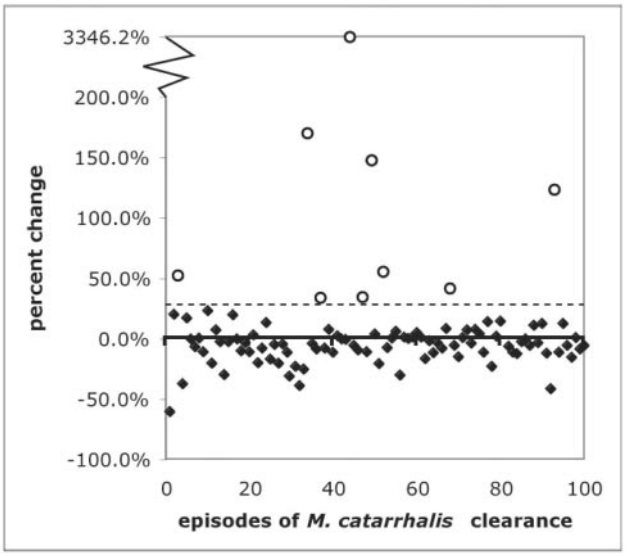

FIG. 3. Results of serum IgG ELISA for 100 episodes of carriage of *M. catarrhalis*. The *y* axis is the percent change in OD between the preacquisition and postclearance samples. The dotted line indicates the cutoff (26.7%) for a significant percent change in serum IgG. The nine open circles indicate the samples with a significant increase in percent change from preacquisition to postclearance, while the solid diamonds indicate the negative samples.

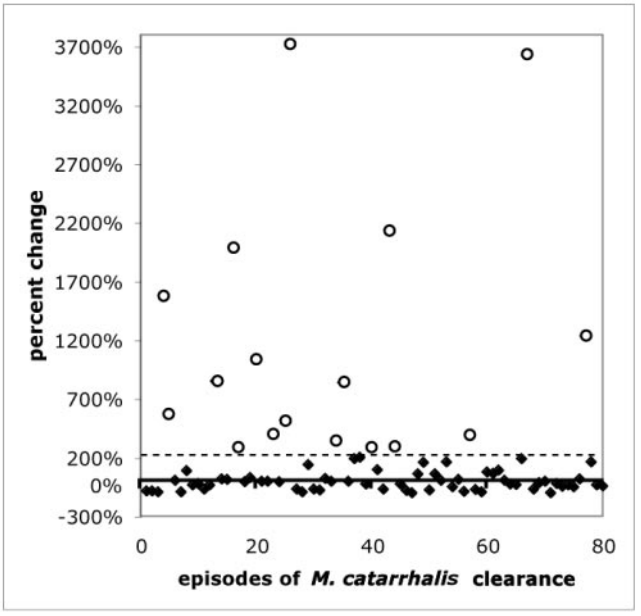

FIG. 4. Results of sputum supernatant IgA ELISAs for 80 episodes of carriage of *M. catarrhalis*. The *y* axis is the percent change in OD between the preacquisition and postclearance samples. The dotted line indicates the cutoff (223.1%) for a significant percent change in sputum supernatant IgA. The 17 open circles are the samples that showed a significant increase in sputum supernatant IgA from preacquisition to postclearance samples. The solid diamonds indicate the samples that did not show a significant increase in IgA to OMP G1a.

antibody response compared to 4 of 45 (8.9%) episodes of asymptomatic colonization.

To assess the possible effect of antibiotic administration on the likelihood of developing a serum IgG antibody response, we compared the frequency of antibody response in patients who received antibiotics compared to those who did not. Five of 56 episodes of carriage associated with antibiotic administration had an antibody response, compared to 4 of 44 episodes in which antibiotics were not administered. Therefore, although the numbers are small, no apparent effect of antibiotic administration on serum IgG response was observed.

Human mucosal antibody response to OMP G1a. ELISAs were conducted to measure the IgA response to OMP G1a in sputum supernatants. A total of 86 paired sputum supernatants were available for analysis. Of these, six yielded unacceptably high nonspecific binding in the control wells that contained no antigen. Therefore, a total of 80 samples yielded interpretable results.

To determine the cutoff value for a significant increase in OD from preacquisition to postclearance samples, 10 paired sputum supernatants from patients in the COPD study clinic who were never colonized with *M. catarrhalis* were subjected to ELISA with purified OMP G1a. The mean ± SD was 68.28% ± 60.02%. The higher variability in the sputum supernatants was consistent with our previous observations and is likely a result of the heterogeneity among sputum supernatant samples and the viscous nature of sputum. The paired control sputum supernatant samples demonstrated an upper limit of the 99% confidence interval of 223.1% when tested with purified OMP G1a. Therefore, any increase in OD of >223.1% from preacquisition to postclearance samples was regarded as significant. A total of 17 (21.3%) of 80 pairs of sputum supernatants contained a significant increase in sputum supernatant IgA following clearance of *M. catarrhalis* from the lower respiratory tract (Fig. 4). Eleven of 41 (26.8%) exacerbations were associated with the development of a sputum supernatant IgA response, while 6 of 39 (15.4%) episodes of asymptomatic colonization developed a sputum supernatant IgA response ($P = 0.27$; Fisher's exact test). Therefore, a sputum IgA response occurred at a similar frequency following exacerbations and asymptomatic colonizations.

To determine the possible effect of antibiotic administration on development of a sputum supernatant IgA response, the frequency of antibody response in patients who received antibiotics compared to those who did not was assessed. Eleven of 42 episodes of carriage associated with antibiotic administration had an antibody response, compared to 6 of 38 episodes in which antibiotics were not administered ($P = 0.28$; Fisher's exact test). Therefore, although the numbers are small, no apparent effect of antibiotic administered on sputum IgA response was observed.

Characterization of antibodies to surface-exposed epitopes. Adsorption assays were performed to determine the proportion of serum IgG antibodies that were directed toward surface-exposed epitopes to OMP G1a. Since OMP G1a shows a high degree of sequence conservation among strains of *M. catarrhalis*, adsorptions were carried out with a single strain (ATCC 25240). The nine serum pairs that showed a significant increase in the serum IgG to recombinant OMP G1a were studied in adsorption assays. Aliquots of serum were adsorbed with *M. catarrhalis* strain 25240. An ELISA to detect IgG

antibodies to OMP G1a was performed simultaneously on aliquots of adsorbed and unadsorbed serum. A decrease in OD with the adsorbed sera compared to the unadsorbed sera indicated that antibodies bound to surface-exposed epitopes on *M. catarrhalis*. The percent adsorption was calculated with the following formula: [(OD of unadsorbed serum − OD of adsorbed serum)/OD of unadsorbed serum] × 100.

As a control to determine that the adsorption was specific for *M. catarrhalis* and not an artifact of the assay, an aliquot of serum was adsorbed simultaneously with an irrelevant organism, *S. pneumoniae*. The gram-positive bacterium *S. pneumoniae* was used as a negative control because *E. coli* and other gram-negative bacteria contain NlpE (CutF) homologs, which share homology with OMP G1a. The results of adsorption assays with *S. pneumoniae* were used to determine the cutoff for a significant change between adsorbed and unadsorbed aliquots of serum. Adsorption assays of preacquisition sera with *S. pneumoniae* showed a mean ± SD of 20.5% ± 11.7%; the results with postclearance sera showed a mean ± SD of 15.6% ± 10.5%. A change in adsorption of 50.7% for the preacquisition and 42.7% for the postclearance samples represented the upper limit of the 99% confidence interval. Therefore, adsorption values of >50.7% for the preacquisition and >42.7% for the postclearance sera were considered significant. Of the nine preacquisition samples from the serum pairs that showed a serum IgG response to OMP G1a, none had a significant level of adsorption with *M. catarrhalis* (range, −26.9% to 50.0%), indicating that antibodies were directed toward epitopes that are not exposed on the bacterial surface. One (11.1%) of the nine postclearance serum samples contained IgG antibodies that were directed toward surface-exposed epitopes of OMP G1a (61.6% adsorption). IgG antibodies in the remaining eight postclearance serum pairs were directed at OMP G1a epitopes that were not accessible on the surface of *M. catarrhalis*.

DISCUSSION

Previous work from our laboratory identified a 29-kDa OMP band that was called OMP G1 (3). Subsequent work demonstrated that the protein band was actually two proteins, OMP G1a and OMP G1b, which contain no homology to each other (1). OMP G1a is a lipoprotein and has an isoelectric point of 4. Adsorption assays with *M. catarrhalis* showed that OMP G1a contains surface-exposed epitopes (33, 34). OMP G1a shares homology with copper binding lipoproteins in several gram-negative bacteria, including NlpE (CutF) of *E. coli* (21). NlpE (CutF) is involved in inducing the Cpx pathway during cell-surface interactions (14, 15, 21, 41, 45).

In the present study, we evaluated the human antibody response to OMP G1a using a set of carefully characterized serum and sputum supernatant samples collected from a cohort of adults with COPD followed prospectively. The prospective design along with molecular typing of isolates of *M. catarrhalis* provided the opportunity to study true preacquisition and postclearance samples, allowing us to evaluate whether OMP G1a is expressed during infection of the human respiratory tract and to detect antibodies made specifically during the episode of carriage. A relatively small proportion (9.0%) of patients developed a serum IgG response associated with clearance of the organism from the respiratory tract. Although not statistically significant, there was a trend for a somewhat larger proportion (21.3%) of developing sputum IgA following clearance of *M. catarrhalis* ($P = 0.07$; Fisher's exact test). Overall, 23% of episodes of carriage were associated with either a serum IgG or sputum supernatant IgA response. The assays in this study were designed to measure antibodies made specifically in response to the episode of carriage, excluding the effect of preexisting background antibody. Therefore, these results indicate that OMP G1a is expressed by *M. catarrhalis* during infection of the human respiratory tract.

Although the numbers were small for rigorous comparison, no apparent effect of antibiotic or systemic corticosteroid administration on developing antibody responses to OMP G1a was observed. Of interest, no difference in the frequency of antibody responses between clinical exacerbations and asymptomatic colonization was observed. This observation parallels studies with *S. pneumoniae* that show the development of serum antibody responses to pneumococcal antigens following colonization of the respiratory tract (20, 30, 31, 40).

The leader peptide for OMP G1a contains a consensus lipoprotein signal peptidase II processing site (Leu-Ser-Ala-Cys), predicting that the protein contains a covalently bound lipid (1, 50). The present study confirms experimentally that OMP G1a is a lipoprotein and that the mature protein contains at least one palmitic acid moiety. While the genome contains abundant genes predicted to encode lipoproteins, OMP G1a is the first *M. catarrhalis* OMP proven experimentally to be a lipoprotein.

The immunoassays in the present study were performed with lipidated recombinant OMP G1a. The lipid moieties in lipoproteins are important in allowing the protein to assume its correct conformation. In addition, the lipid confers immunostimulatory and adjuvant properties to the protein and interacts with toll-like receptors (4, 5, 8, 12, 16, 22). The pDUMP/pCATCH plasmids are novel vectors engineered to express recombinant lipoproteins in *E. coli* (13). Recombinant OMP G1a expressed in the pCATCH plasmid system grown in the presence of [^{3}H]palmitic acid was radiolabeled, indicating that recombinant OMP G1a contains covalently bound palmitic acid.

A limitation of the study is the reliance on expectorated sputum cultures, which may not accurately reflect the bacteria in the lower respiratory tract. However, more invasive approaches for repeated sampling in a prospective study are not feasible. Another limitation is that we cannot rule out a simultaneous respiratory viral infection, since viruses were not investigated in the sputum cultures. In spite of these limitations, the prospective design of this study allowed us to measure antibodies made specifically to each episode of acquisition of *M. catarrhalis* because of the availability of prospectively collected preacquisition and postclearance samples.

Christensen et al. demonstrated the presence of serum IgG to a nonheat modifiable 28-kDa protein in patients with lower respiratory tract infections (10, 11). Since OMP G1a is of similar size and not heat modifiable, we speculate that the antibody response identified might be directed at OMP G1a (1). Another study that analyzed serum from children showed an absence of antibodies to the 29-kDa protein range in *M. catarrhalis* (19). Immunoblot assays in other studies of human

antibody responses to *M. catarrhalis* did not include analysis of antigens in the molecular size range of OMP G1a; therefore, little or no data on the immune response to OMP G1a were available prior to the present study (19, 29, 48).

Using an approach similar to that in the present study, 9 of 40 patients developed a serum IgG response to OMP CD and 3 of 28 patients developed a sputum IgA response (37). In a more recent paper, we showed that adults with COPD show a high degree of variability among individuals in the antigens to which antibody responses are directed (35). The present study indicates that OMP G1a does not appear to be an immunodominant antigen, following infection of the respiratory tract by *M. catarrhalis*, based on the observation that a minority of patients made new antibody responses to the protein.

Since *M. catarrhalis* is an important human respiratory tract pathogen, there is interest in developing a vaccine to prevent *M. catarrhalis* infections (25, 36, 44). OMP G1a has several characteristics that indicate that it might be an effective vaccine antigen. OMP G1a is highly conserved among clinically and geographically diverse strains of *M. catarrhalis* (1). OMP G1a has epitopes that are exposed on the surface of the bacterium (34). The present study showed that OMP G1a is expressed during human infection, an important feature for a potential vaccine antigen. These observations suggest that OMP G1a is a potential vaccine antigen. This work also suggests that OMP G1a is not an immunodominant antigen in *M. catarrhalis* because only a small proportion of adults with COPD made new antibody responses upon clearing the organism from the respiratory tract. This feature would not preclude its potential value as an immunoprotective antigen. Furthermore, the serum IgG to OMP G1a recognizes epitopes that are not available for binding on the bacterial surface. Such antibodies, resulting from natural exposure to *M. catarrhalis*, are unlikely to mediate protection and might explain the reacquisition of *M. catarrhalis* that occurs with COPD patients. In evaluating OMP G1a as a potential vaccine antigen, it will be important to define surface-exposed epitopes to assess whether the protein can be presented to the host in a form that will elicit the production of antibodies to surface epitopes and to evaluate the protective capability of those antibodies.

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