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Serum Antibodies to Outer Membrane Proteins (OMPs) of *Moraxella* (*Branhamella*) *catarrhalis* in Patients with Bronchiectasis: Identification of OMP B1 as an Important Antigen

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***Moraxella* (*Branhamella*) *catarrhalis* is a common cause of lower respiratory tract infections in adults and of otitis media in children. Little is known about the human immune response to this bacterium. In this study, immunoblot assays were performed to detect serum immunoglobulin G antibodies directed at purified outer membrane of *M. catarrhalis*. Twelve serum samples, two each from six patients with bronchiectasis who were persistently colonized with this organism, were tested with their homologous *M. catarrhalis* sputum isolates. In all the sera, the most prominent and consistent antibody response was to a minor 84-kDa outer membrane protein, OMP B1. Immunoblot adsorption assays show that these antibodies recognize surface exposed epitopes on OMP B1. Further analysis of human serum antibodies eluted from the surface of intact bacterial cells shows that these surface-exposed epitopes on OMP B1 are heterogeneous among strains of *M. catarrhalis*. OMP B1 is therefore an important OMP antigen on the surface of *M. catarrhalis* for the human immune response to infection by this bacterium.**

Moraxella (*Branhamella*) *catarrhalis* has become widely recognized as an important human pathogen (2, 5). In recent series, it is the third most common bacterial cause of otitis media in children and of acute bronchitic exacerbations among adults with chronic obstructive pulmonary disease (6, 9, 24–26, 30). It also occasionally causes pneumonia, sinusitis, tracheitis, and invasive infections in children and adults (7, 29, 30). Its pathogenic significance is enhanced because 75% of clinical strains produce β-lactamase, which complicates therapy of these common mucosal infections (17).

The bacterial outer membrane forms the interface between a host and a gram-negative bacterial pathogen. Consequently, outer membrane proteins (OMPs) can play important pathogenic roles such as the expression of virulence factors, adhesins, and surface antigens. The host humoral immune response to a bacterial pathogen is directed toward multiple antigens, many of which are buried beneath the surface of the bacterium and unlikely to play a protective role. Antibodies to surface-exposed epitopes of the OMPs of bacterial pathogens are more likely to be protective against infection. Identifying these epitopes, therefore, becomes important in understanding host-pathogen interactions and in the development of protective vaccines.

Basic characteristics of the OMPs of *M. catarrhalis* are well described (1, 21, 22). However, little is known about which OMPs are important antigens in the human immune response to *M. catarrhalis*. In this study, we have analyzed the serum antibody response to the OMPs of *M. catarrhalis* in patients with bronchiectasis who are persistently colonized with this bacterium. The immunoassays used are specific for antibodies directed toward surface-exposed epitopes of the OMPs, which are potentially the relevant immune targets in vivo.

We found that the predominant humoral (immunoglobulin G [IgG]) immune response in these patients was to a minor 84-kda OMP. This OMP is distinct from a major 80-kDa OMP which we had previously designated OMP B (1). OMP B is identical to the protein named CopB by Helminen et al. (15). To maintain consistency in the literature, we have designated the newly identified 84-kDa OMP as OMP B1 and renamed the previously identified 80-kDa OMP B (CopB) as OMP B2.

MATERIALS AND METHODS

Bacterial strains and sera. Twenty-eight patients (age range, 40 to 71 years; seven males) with bronchiectasis were enrolled into a longitudinal study and monitored in a specialist outpatient clinic for a period of 2 years. All patient studies had the approval of the West Midland health authority research ethical committee, Birmingham, England. These patients were seen on a monthly basis for the first year and every 2 months thereafter. Patients were also seen at the time of infective exacerbations of their bronchiectasis, before antibiotic therapy was initiated, and at the end of therapy. At each study visit, a sputum sample was obtained for bacterial culture; a blood sample was also taken, and the serum was obtained by low-speed centrifugation. The results of quantitative cultures and restriction fragment length polymorphisms (RFLPs) of the strains collected are published elsewhere (18). Twelve (42.9%) of these 28 patients had *M. catarrhalis* isolated from their sputum on at least one occasion. Six (21.4%) patients had *M. catarrhalis* isolated repeatedly (from 2 to 12 times) over the 2-year study period.

All six patients were colonized with at least two different strains of *M. catarrhalis* as determined by RFLP analysis (18). Six such pairs of strains from the six patients and their homologous sera were studied. Three such pairs of strains from three different patients and their homologous sera were analyzed further and are the focus of this study (Table 1). Strain 25240 is from the American Type Culture Collection.

Purification of outer membrane. Bacterial outer membrane from each of the clinical strains was purified by collecting EDTA-heat-induced vesicles (23). Briefly, 100 ml of brain heart infusion broth was inoculated with several bacterial colonies and grown overnight at 37°C. The culture was centrifuged at 10,000 × *g* for 15 min at 4°C. The bacterial pellet was resuspended in 50 ml of EDTA buffer (0.05 M Na_2HPO_4, 0.15 M NaCl, 0.01M EDTA [pH 7.4]) and incubated at 56°C for 30 min. The suspension was then centrifuged as described above. The supernatant was collected and centrifuged at 100,000 × *g* for 90 min at 4°C. The

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TABLE 1. Sputum isolates of *M. catarrhalis* from three patients with bronchiectasis

Patient	Serum sample[a]	Isolate[b]	Date collected (mo/day/yr)
C	CS1	CM1	11/27/91
	CS2	CM2	01/14/92
F	FS1	FM1	08/05/91
	FS2	FM2	12/02/91
D	DS1	DM1	03/02/92
	DS2	DM2	04/30/92

[a] Serum specimens were collected simultaneously (homologous sera).
[b] Bacterial strains were originally designated as follows: CM1 as 621/KC, CM2 as 690/KC, FM1 as 447/SK, FM2 as 633/SK, DM1 as 958/MD, and DM2 as 1084/MD.

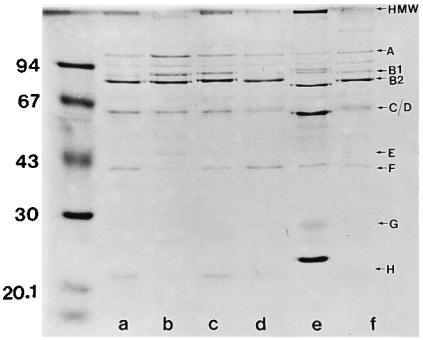

FIG. 1. Coomassie blue-stained SDS-polyacrylamide gel of purified outer membrane of *M. catarrhalis* sputum isolates from patients with bronchiectasis. A to H designate the major OMPs. Lanes a to f contain purified outer membrane of strains CM1, CM2, FM1, FM2, DM1, and DM2, respectively. Molecular mass standards are shown on the left (in kilodaltons). HMW, high molecular weight.

resulting purified outer membrane pellet was suspended in phosphate-buffered saline (PBS).

SDS-PAGE. Purified outer membrane was subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) in 11% separating gels. Samples were mixed with an equal volume of sample buffer (0.06 M Tris, 1.2% SDS, 1% mercaptoethanol, 11.9% glycerol, 0.003% bromophenol blue), loaded onto gels with a single well, and electrophoresed at 100 V until the bromophenol blue dye reached the bottom of the gel.

Immunoblot assay. Gels were placed on a nitrocellulose sheet which had been previously soaked in distilled water. Electrophoretic transfer was carried out with a Transphor electrophoresis unit (Hoefer Scientific Instruments, San Francisco, Calif.) at 60 V for 90 min. The transfer buffer consisted of 0.025 M Tris (pH 8.3), 0.192 M glycine, and 20% methanol. After transfer, the nitrocellulose sheet was blocked with 1% nonfat dried milk in PBS for 1 h and cut into 3-mm-wide strips. These strips were incubated with the homologous sera diluted in PBS overnight at room temperature. They were rinsed three times with PBS and incubated with peroxidase-labeled goat anti-human IgG at a dilution of 1:2,000 for 1 h at room temperature. The strips were rinsed and developed with horseradish peroxidase color development solution (Bio-Rad).

Double-staining immunoblot assay. Antibody 2.9F is a mouse monoclonal antibody which recognizes a surface-exposed epitope on OMP B2 of *M. catarrhalis* (28). A nitrocellulose strip on which purified outer membrane of strain FM1 had been transferred was blocked with 1% nonfat dry milk for 1 h. The strip was incubated overnight at room temperature with PBS containing FS1 (homologous) serum diluted 1:1,000 and tissue culture supernatant of 2.9F at a 1:10 dilution. The strip was rinsed with PBS three times and then incubated with PBS containing 1:2,000 dilutions of peroxidase-labeled goat anti-human IgG and alkaline phosphatase-labeled goat anti-mouse IgG for 1 h at room temperature. The strip was thoroughly rinsed with PBS and immersed in alkaline phosphatase color development solution followed by horseradish peroxidase color development solution. In this way, the human antibodies stain blue and the mouse monoclonal antibody stains red, permitting clear differentiation of their antigenic targets on the blot.

Immunoblot adsorption. Antibodies to surface-exposed epitopes on outer membrane antigens were identified by using the immunoblot adsorption method of Loeb (20). A 70-ml volume of brain heart infusion broth was inoculated with an overnight culture of *M. catarrhalis* and incubated at 37°C until the optical density at 600 nm was 0.5 (late log phase). The culture was centrifuged at 12,000 × *g* for 10 min at 4°C. The supernatant was discarded, and the bacterial pellet was washed twice with 50 ml of ice-cold PCM (0.01 M Na_2PO_4, 0.15 M NaCl [pH 7.2], 0.5 mM $MgCl_2$, 0.15 mM $CaCl_2$). The bacterial pellet was resuspended in 1 ml of PCM, transferred to an Eppendorf tube, and centrifuged at 16,000 × *g* for 5 min, and the supernatant was discarded. Homologous serum which had been heat inactivated for 30 min at 56°C was diluted 1:1,000 in PCM, and 1 ml of the diluted serum was added to the bacterial pellet. The bacterial pellet was resuspended gently with a Pasteur pipette. The mixture was incubated on ice for 30 min and then centrifuged at 16,000 × *g* for 15 min at 4°C. The supernatant was collected. This is the adsorbed serum and was stored at −20°C. The adsorption was performed concurrently with *Escherichia coli* JM109 to account for dilution by fluid trapped in the bacterial pellet, nonspecific adsorption of antibodies, or adsorption of cross-reactive antibodies.

Antibody elution. Antibodies from the patient sera which had bound to the intact bacterial surface during the immunoblot adsorption assay were eluted by using a modification of the method of Engleberg et al. (8). The bacterial pellet from the adsorption experiment was washed with 1 ml of PCM three times and resuspended in 1 ml of elution buffer (0.2 M NaCl, 0.2 M glycine [pH 2.8]). The mixture was incubated at room temperature for 30 min on an orbital shaker at 300 rpm and then centrifuged at 16,000 × *g* for 10 min. The supernatant was transferred to another Eppendorf tube and titrated to a pH of 7.5 with 2 M Tris. These eluted antibodies were stored at −20°C. The elution was performed concurrently with the pellet of *E. coli* JM109 from the adsorption experiment as a negative control. The unadsorbed serum, adsorbed serum, and eluted antibodies were subjected to immunoblot assay with an outer membrane preparation of the homologous *M. catarrhalis* strain.

Cloning of the OMP B2 gene and expression of recombinant OMP B2. The published sequence of the OMP B2 (CopB) gene of *M. catarrhalis* O35E (15) was used to design primers corresponding to the amino and carboxy termini of the mature protein. These primers contained *Bam*HI sites and were used in a PCR to amplify the OMP B2 gene from genomic DNA of *M. catarrhalis* 25240. One microgram of template DNA was incubated with 300 ng of oligonucleotides, and the OMP B2 gene was amplified by 30 cycles of denaturation and polymerization. An annealing time of 1 min at 45°C, an extension time of 2 min at 72°C, and a denaturation time of 1 min at 94°C were used. DNA fragments of 2.2 kb were precipitated with 2 M ammonium acetate and cloned into the *Bam*HI sites of plasmid vectors pGEM 7Zf- (pGM4) and pGEX 2T (pXE 19) (27). The nucleotide sequences of both strands of the insert in pGM4 were determined, using dideoxy sequencing with Sequenase (U.S. Biochemicals, Cleveland, Ohio) according to the manufacturer's instructions.

The pXE 19 clone was used to produce recombinant purified OMP B2 as follows. A colony of pXE 19 was grown overnight in 100 ml of LB-ampicillin. One liter of LB-ampicillin was inoculated with the overnight culture and grown for 1 h at 37°C. Isopropylthiogalactopyranoside (IPTG; 100 mM) was added to 0.1 mM, and incubation continued for an additional 4 h to induce expression of the OMP B2–glutathione *S*-transferase fusion protein. The culture was centrifuged at 5,000 × *g* for 10 min, and the supernatant was discarded. The pellet was resuspended in 10 ml of ice-cold PBS and lightly sonicated in an ultrasonic probe sonicator. The OMP B2 fusion protein was expressed as insoluble inclusion bodies and was concentrated in the precipitate after sonication. The fusion protein was solubilized in 4 M guanidine hydrochloride as described by Sambrook et al. (27). After solubilization, the fusion protein was mixed with a 50% slurry of glutathione-agarose beads to which it bound through the glutathione *S*-transferase moiety. Recombinant OMP B2 was cleaved off the beads with thrombin and subjected to SDS-PAGE and immunoblot assays as described above.

RESULTS

Analysis of OMP patterns by SDS-PAGE. The purified outer membranes of the six *M. catarrhalis* isolates from the three patients were resolved by SDS-PAGE. The OMP patterns of five of the six isolates were very similar to each other (Fig. 1) despite their varied RFLP patterns (18). The OMP pattern of strain DM1 differed from those of the other strains with respect to molecular weights of OMPs B, C/D, and H. The major OMPs seen were in the range of 20 to 98 kDa and correspond to the previously described OMPs A to H (1). OMP B2 (80 kDa) was the most prominent band in the range of 67 to 94 kDa. A band of variable intensity was seen consistently above OMP B2 at approximately 84 kDa.

Serum IgG antibodies to OMPs of homologous *M. catarrhalis*. Serum FS1 was subjected to an immunoblot assay with purified outer membrane of its homologous isolate, FM1. The serum was diluted 1:100, 1:1,000, and 1:10,000 in PBS. At a dilution of 1:100, bands corresponding to several OMPs were

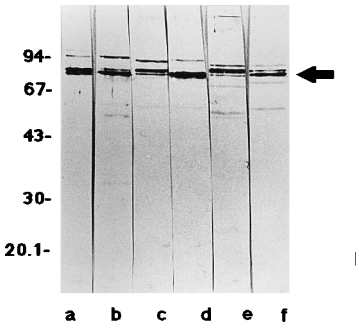

FIG. 2. Composite of immunoblot assays of patient sera with purified outer membranes of their homologous *M. catarrhalis* sputum isolates. All sera were diluted 1:1,000. Lanes: a, serum CS1, strain CM1; b, serum CS2, strain CM2; c, serum FS1, strain FM1; d, serum FS2, strain FM2; e, serum DS1, strain DM1; f, serum DS2, strain DM2. Conjugate antibody in all lanes is peroxidase-labeled goat anti-human IgG. The arrows indicate the prominent 84-kDa band seen in all six blots (OMP B1). Molecular mass standards are noted on the left (in kilodaltons).

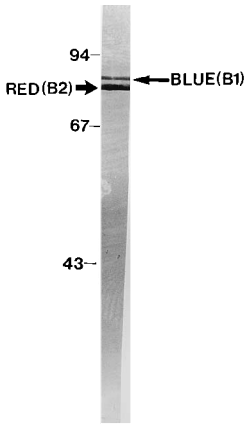

FIG. 3. Double-staining immunoblot assay. Purified outer membrane of *M. catarrhalis* FM1 was incubated with homologous serum FS1 and mouse monoclonal antibody 2.9F. Conjugate antibodies were alkaline phosphatase-labeled goat anti-mouse IgG and peroxidase-labeled goat anti-human IgG. The upper band at approximately 84 kDa (corresponding to OMP B1) was blue, and the lower band at 80 kDa (corresponding to OMP B2) was red. Molecular mass standards are noted on the left (in kilodaltons).

seen. However, at dilutions of 1:1,000 and 1:10,000, the most prominent band seen was in the range of 80 to 85 kDa. Immunoblot assays of the other sera with their homologous strains also showed a prominent band in the same region at a dilution of 1:1,000. This finding indicated that all six patients showed a high-titer antibody response to an OMP in that molecular size range. Further detailed analysis of this antibody response was done with six strains and homologous sera from three patients (Table 1 and Fig. 2). Our subsequent experiments were to identify the OMP seen by the antibodies and to determine if the antibodies were binding to surface-exposed epitopes of this OMP.

Our initial hypothesis was that the predominant antibody response observed in these patients was directed at OMP B2, which is the only major OMP in the 80- to 85-kDa region. This protein is identical to the OMP called CopB by Helminen et al. (15), as established by us by N-terminal sequencing of the protein eluted from an SDS-gel. Recombinant OMP B2 was purified in the form of a fusion protein with glutathione *S*-transferase. Digestion of the fusion protein with thrombin yielded a mixture of the fusion protein (107 kDa) and recombinant OMP B2 (80 kDa).

All six serum samples from the three patients were then tested in an immunoblot assay at a 1:1,000 dilution with the recombinant OMP B2. Only two of the six sera, both from patient D, contained antibodies to OMP B2. This observation was compatible with two hypotheses: first, that the recombinant OMP B2 of ATCC 25240 had limited antigenic cross-reactivity with the OMP B2 of the clinical isolates, and second, that the predominant immune response in these patients was not to OMP B2 but to a minor OMP in the same molecular weight range.

Identification of OMP B1. To test these hypotheses, we performed a double-staining immunoblot assay as described above. In this assay, mouse monoclonal antibody 2.9F, which recognizes OMP B2, will stain red, while antibodies in human serum will stain blue. If the patient antibodies were to OMP B2, the immunoblot would have shown a single band composed of overlapping red and blue bands, as the patient antibodies and the monoclonal antibody would have bound to the same protein. However, the blot revealed two distinct bands in the 80- to 85-kDa region. The band at approximately 84 kDa was blue because of peroxidase conjugated with the human antibodies, while the band at 80 kDa was red because of alkaline phosphatase conjugated with the mouse monoclonal antibody to OMP B2 (Fig. 3). Sera FS2, CS1, and CS2 gave the same results in this assay with their homologous isolates. Sera DS1 and DS2 gave a doublet with a blue band at 84 kDa and a red band at 80 kDa which turned blue with the peroxidase developer, indicating that these sera contained antibodies to both OMP B1 and OMP B2.

This observation confirmed that the OMP to which human antibodies are directed is a minor OMP of 84 kDa. To maintain consistency with the previous nomenclature, we have designated this OMP as OMP B1 and will henceforth refer to the 80-kDa OMP B (CopB) as OMP B2.

Identification of antibodies to surface-exposed epitopes on OMP B1. Straightforward immunoblot assays do not distinguish between antibodies which recognize surface-exposed epitopes from antibodies which recognize epitopes buried beneath the surface of the outer membrane. We performed immunoblot adsorption and antibody elution assays to determine if the human antibodies to OMP B1 recognize surface epitopes.

These assays were first performed with the six sera and the simultaneously recovered homologous isolate (Table 1). All assays were performed with sera diluted 1:1,000 in PBS. In all adsorption assays, the antibodies directed toward OMP B1 described above were strongly adsorbed by the homologous strain (Fig. 4). Aliquots of the sera adsorbed with *E. coli* did not show any difference from the unadsorbed sera. This finding indicates that antibodies to OMP B1 are directed toward surface-exposed epitopes and are specific for *M. catarrhalis*.

As a second method to detect antibodies to surface-exposed epitopes, antibodies which had bound to the bacterial surface in the adsorption assay were eluted and tested in an immunoblot assay. Antibodies to OMP B1 were eluted in four of the six instances, with the exception of strains DM1 and DM2 and their homologous sera. No antibodies were detected on immunoblots of simultaneous elutions from the surface of *E. coli* with any of the sera. These observations confirm the results of the adsorption assays.

Strain specificity of antibodies to OMP B1. RFLP analyses of genomic DNAs of the six strains studied by the immunoassays described above have shown them to be different from

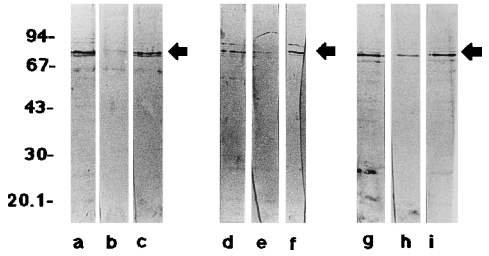

FIG. 4. Immunoblot adsorption assay. Serum CS1 was adsorbed with homologous *M. catarrhalis* sputum isolate CM1 and with *E. coli* JM109. Lanes a to c contain purified outer membrane of strain CM1 incubated with unabsorbed CS1, CS1 after adsorption with CM1, and CS1 after adsorption with *E. coli* JM109 (control), respectively. Serum FS1 was adsorbed with homologous *M. catarrhalis* sputum isolate FM1 and with *E. coli* JM109. Lanes d to f contain purified outer membrane of strain FM1 incubated with unadsorbed FS1, FS1 after adsorption with FM1, and FS1 after adsorption with *E. coli* JM109 (control), respectively. Serum DS2 was adsorbed with homologous *M. catarrhalis* sputum isolate DM2 and with *E. coli* JM109. Lanes g to i contain purified outer membrane of strain DM2 incubated with unadsorbed DS2, DS2 after adsorption with DM2, and DS2 after adsorption with *E. coli* JM109 (control), respectively. All sera were diluted 1:1,000. Conjugate antibody in all lanes is peroxidase-labeled goat anti-human IgG. The arrows indicate OMP B1. Molecular mass standards are noted on the left (in kilodaltons).

each other (18). To characterize the degree of strain specificity among the antibodies to the surface-exposed epitopes of OMP B1, serum CS1 was incubated at a 1:100 dilution with the homologous strain CM1, and antibodies were eluted from the bacterial surface. The eluate was immunoblotted with purified outer membrane of all six strains. Similarly, serum FS1 was incubated with strain FM1, and the antibodies were eluted and assayed. The antibodies eluted from CM1 recognized OMP B1 of three of the six strains, not recognizing strains CM2, FM1, and DM1. The antibodies eluted from FM1 also recognized three of the six strains, not recognizing strains CM1, FM2, and DM2 (Fig. 5). This result indicates that OMP B1 has surface-exposed epitopes which show some heterogeneity among strains. Further, these epitopes are important antigens for the human humoral immune response. Another interesting observation was that the antibodies to OMP B1 of strain CM1 did not recognize OMP B1 of strain CM2, which was isolated from the same patient at a later date. Similarly, antibodies to OMP B1 of strain FM1 did not recognize strain FM2, a strain isolated from the same patient at a later date. This result suggests that antibodies to OMP B1 may have a functional role in vivo

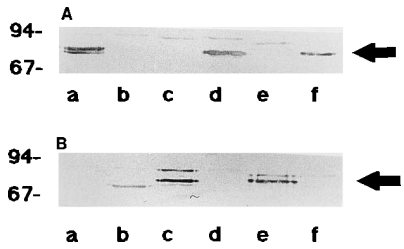

FIG. 5. Strain specificity of eluted antibodies to OMP B1. (A) Serum CS1 was incubated with *M. catarrhalis* CM1 at a 1:100 dilution. Antibodies were eluted off the bacterial pellet and incubated with purified outer membrane of *M. catarrhalis* strains CM1 (lane a), CM2 (lane b), FM1 (c), FM2 (d), DM1 (e), and DM2 (lane f). (B) Serum FS1 was incubated with *M. catarrhalis* strain FM1 at a 1:100 dilution. Antibodies were eluted off the bacterial pellet and incubated with purified outer membrane of *M. catarrhalis* strains CM1 (lane a), CM2 (lane b), FM1 (lane c), FM2 (lane d), DM1 (lane e), DM2 (lane f). Conjugate antibody in all lanes of both panels is peroxidase-labeled goat anti-human IgG. The arrows indicate OMP B1. Molecular mass standards are noted on the left (in kilodaltons).

in clearance of the colonizing strain and acquisition of new strains.

DISCUSSION

It is now widely recognized that *M. catarrhalis* is an important pathogen in patients with underlying chronic respiratory disease, especially chronic obstructive pulmonary disease (24). Its role in bronchiectasis has not been described before, mostly because of lack of systematic study of bacterial epidemiology in this disease. Patients with bronchiectasis are known to be persistently colonized in the lower respiratory tract with a wide variety of bacterial species, resulting in long-term exposure to bacterial antigens. Therefore, these patients are a relevant population in which to study the human immune response to this bacterium. Similar studies have been performed with *Pseudomonas aeruginosa* in patients with cystic fibrosis (11, 14, 19). These studies have yielded important information regarding antigenic determinants on *P. aeruginosa* which elicit a host immune response (11, 14, 19).

When tested in an enzyme-linked immunosorbent assay (ELISA), normal adult human serum contains high-titer antibodies to OMPs of *M. catarrhalis* (10). Intermittent upper airway colonization since childhood, episodes of otitis media, and antibodies to antigenically cross-reactive OMPs of other gram-negative bacteria probably account for the presence of these antibodies. This antibody response in normal people also illustrates that a bacterium is a very complex antigenic stimulus for a human host, with literally hundreds of antigenic determinants to which an immune response can be detected. The majority of these antibodies are directed at epitopes which are buried beneath the surface of the outer membrane. Antibodies directed at surface-exposed epitopes of OMPs of *M. catarrhalis* are more likely to be meaningful in vivo. Dissection of a relevant protective immune response to a surface antigen from this multitude of largely irrelevant antibodies requires sensitive and specific immunoassays.

Immunoblot adsorption was first described by Loeb (20) as a method to identify surface-exposed membrane components of *Haemophilus influenzae* type b by using rabbit antisera. The present study describes the first successful application of this technique to samples from human subjects. Antibody elution was first described by Engleberg et al. (8) to detect surface exposure of cloned *Legionella pneumophila* antigens in *E. coli*, using rabbit polyclonal antisera. Again, we have applied this method to human sera.

Perhaps the most striking observation in this study was that one minor OMP of *M. catarrhalis*, OMP B1 (84 kDa), was a major target of serum IgG of the bronchiectatic patients. Antibody responses to previously described major OMPs were seen, but none were as consistent or as high titer as the antibody response to OMP B1. This immune response was consistently seen in all six patients and 12 sera which were studied. When purified outer membranes of diverse strains of *M. catarrhalis* are resolved by SDS-PAGE and stained with Coomassie blue, OMP B1 is present consistently, although in relatively small amount. The results of the immunoassays described in this study indicate that OMP B1 is distinct and antigenically different from OMP B2. Goldblatt et al. (12) have studied age-specific and IgG subclass-specific responses in children to *M. catarrhalis* with whole-cell ELISA and immunoblots to OMPs of *M. catarrhalis*. They observed that the most prominent immune response in all IgG subclasses was to an approximately 82-kDa OMP. This is a very provocative observation, as this OMP is in the same molecular weight range as OMP B1 and may be identical to it. Future experiments similar to this

study with sera from children with otitis media and adults with exacerbations of chronic obstructive pulmonary disease due to *M. catarrhalis* will enable us to determine if OMP B1 is as important an antigen in these patient populations as it is in patients with bronchiectasis.

The serum antibodies to OMP B1 were directed at surface-exposed epitopes of this protein and were specific for *M. catarrhalis*. Functional assays with these antibodies will help us to further delineate this important host-pathogen interaction. Heterogeneity in dominant surface antigens (OMPs) is an important mechanism that enables bacterial pathogens to be transmitted between hosts who have had prior exposure to other strains of the same pathogen (3, 13). The strain specificity of the immune response to surface epitopes of OMP B1 indicates that this OMP has heterogeneous surface epitopes. We hypothesize that this heterogeneity of a major surface antigen enables *M. catarrhalis* to colonize patients with prior immune experience with other strains of *M. catarrhalis*. However, this heterogeneity of surface epitopes of OMP B1 is only moderate, as indicated by the cross-reactivity seen in the elution experiments.

In spite of having specific antibodies in their sera directed at the strain of *M. catarrhalis* in their sputa, these patients were colonized in high titer by the same strain. Similar observations have been made for cystic fibrosis patients with *P. aeruginosa* infection (4, 14). Obviously, other factors are important in the clearance of the organism from the sputa of bronchiectatic patients, such as the efficiency of mucociliary clearance, mucosal immune response (IgA and IgG), and penetration and effectiveness of serum antibodies in the milieu of bronchiectatic respiratory mucosa (16). Characterization of the mucosal immune response to *M. catarrhalis* in patients with bronchiectasis should lead to a greater understanding of these factors. The serum immune response may play a greater role in preventing dissemination of the organism than in reducing sputum colonization. Studies of the serum antibody response may help identify antigens likely to be important at a mucosal level.

In summary, patients with bronchiectasis who are chronically colonized with *M. catarrhalis* develop high-titer serum IgG antibodies to an 84-kDa OMP (OMP B1) of this bacterium. These antibodies are directed toward both antigenically conserved and heterogeneous surface-exposed epitopes on this protein. Further studies to characterize this protein and to assess its antigenicity in diverse patient populations are required to define its usefulness as a vaccine antigen. The mucosal antibody response to *M. catarrhalis* in patients with bronchiectasis needs to be studied, as it may be influencing chronic colonization and strain variation in these patients.

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