**Conservation of Outer Membrane Protein E among Strains of Moraxella catarrhalis**

TIMOTHY F. MURPHY,* AIMEE L. BRAUER, NORINE YUSKIW, ERIN R. McNAMARA, AND CHARMAINE KIRKHAM

Division of Infectious Diseases, Department of Medicine, and Department of Microbiology, State University of New York at Buffalo, and Veterans Affairs Western New York Healthcare System, Buffalo, New York 14215

Received 30 October 2000/Returned for modification 18 December 2000/Accepted 25 January 2001

Outer membrane protein E (OMP E) is a 50-kDa protein of Moraxella catarrhalis which has several features that suggest that the protein may be an effective vaccine antigen. To assess the conservation of OMP E among strains of *M. catarrhalis*, 22 isolates were studied with eight monoclonal antibodies which recognize epitopes on different regions of the protein. Eighteen of 22 strains were reactive with all eight antibodies. The sequences of *ompE* from 16 strains of *M. catarrhalis* were determined, including the 4 strains which were nonreactive with selected monoclonal antibodies. Analysis of sequences indicate a high degree of conservation among strains, with sequence differences clustered in limited regions of the gene. To assess the stability of *ompE* during colonization of the human respiratory tract, the sequences of *ompE* of isolates collected from patients colonized with the same strain for 3 to 9 months were determined. The sequences remained unchanged. These results indicate that OMP E is highly conserved among strains of *M. catarrhalis* and preliminary studies indicate that the gene which encodes OMP E remains stable during colonization of the human respiratory tract.

---

*Corresponding author. Mailing address: VA Western New York Healthcare System, Medical Research 151, 3495 Bailey Ave., Buffalo, NY 14215. Phone: (716) 862-7874. Fax: (716) 862-6526. E-mail: murphyt@acsu.buffalo.edu.
ml of 0.01 M HEPES, pH 7.4. Cells were pelleted by centrifugation at 1,000 × g for 15 min at 4°C. The pellet was resuspended in 0.5 ml of HEPES buffer and sonicated three times for 10 s with a Branson Sonifier (small tip, setting 4). After transfer of the entire suspension to a fresh tube, cell envelopes were pelleted by centrifugation at 16,000 × g for 45 min at 4°C. The cell envelopes were suspended in −0.5 ml of HEPES buffer and stored at −20°C until use.

**MAbs.** Seven MAbs which recognize epitopes on OMP E have been described previously (4, 17). MAb 2E11 was developed from a fusion in which mice were immunized subcutaneously with 50 μg of purified recombinant OMP E from *M. catarrhalis* strain ATCC 25240 (4) with incomplete Freund’s adjuvant on days 0 and 14. On day 28 the mice received approximately 10^6 CFU of *M. catarrhalis* strain ATCC 25240 intraperitoneally without adjuvant. On day 31, splenocytes were fused with SP2/0-Ag14 plasma cells by a modification of the procedure of Kennett (10) and another previously described method (21).

**Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblot assay.** Whole-bacterial-cell lysates and cell envelope preparations were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblot assay as previously described (17).

**ELISA.** Levels of immunoglobulin to OMP E were assayed in human serum and sputum supernatants by enzyme-linked immunosorbent assay (ELISA) as previously described (4).

**PFGE.** Strains of *M. catarrhalis* recovered from the sputum of adults monitored in the COPD Study Clinic were subjected to pulsed-field gel electrophoresis (PFGE) as previously described (12). Isolates were determined to be identical to one another when identical PFGE patterns were observed following the restriction of genomic DNA separately with both SpeI and NheI in the pairs of isolates.

**Determination of sequences of ompE.** Primers GCCGCGCGGATCCGGCCAGG CCTGGATCGCTC and ATATATGAACTTCGGTGTATGAAAGCAAAG were used to amplify *ompE* by PCR from genomic DNA prepared using a genomic DNA kit (Promega). The PCR mixture consisted of 10 ng of genomic DNA, 100 ng of each primer, 1 μl of 10 mM deoxynucleoside triphosphate, 5 μl of Thermopol buffer (New England Biolabs), 0.5 μl of Vent polymerase (New England Biolabs), and 40.5 μl of water (total volume, 50 μl). After an initial denaturation for 3 min at 94°C, 30 cycles of the following program were carried out: 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min. This was followed by incubation at 72°C for 3 min.

The 1,300-bp amplicon was either sequenced directly or cloned into pCR Blunt (Invitrogen) using the manufacturer’s directions. For cloning into the plasmid, three separate PCRs were performed and the amplicons were cloned individually into pCR Blunt. The sequences were determined for two of the clones. If these two sequences differed, then the third clone would have been subjected to sequencing. For each isolate, the sequences of the first two clones were identical to one another.

**RESULTS**

**Strain specificity of MAbs.** The 22 isolates (21 clinical isolates and 1 American Type Culture Collection strain) of *M. catarrhalis* were assayed with each of the eight MAbs in an immunoblot assay using either whole-bacterial-cell lysates or cell envelope preparations. Figure 1 shows the results of an immunoblot assay with MAb 1B3 for nine clinical isolates of *M. catarrhalis*. Eight of the clinical isolates were reactive with MAb 1B3, whereas strain 534 (lane b) was nonreactive. The results of the present study in combination with assays of MAbs 1B3, 9G10d, 5B3, and 14E10. Of the four nonreactive isolates, two were sputum isolates from Buffalo, N.Y., one was a sputum isolate from Birmingham, United Kingdom, and one was a nasopharyngeal isolate from Utrecht, The Netherlands. MAb 12D5, which recognizes an epitope in the region of amino acids 160 to 260, was reactive with 21 of 22 isolates. The nonreactive isolate was the same sputum isolate from Birmingham, which was nonreactive with four other MAbs.

**Sequence analysis of ompE.** The nucleotide sequence of the gene which encodes OMP E was determined for 16 strains of *M. catarrhalis*, including the four isolates which were nonreactive with some of the eight MAbs. Table 2 shows that OMP E displays a substantial degree of sequence conservation among strains of *M. catarrhalis*. Analysis of the amino acid sequences reveals that six different sequences of OMP E are represented by the 16 strains. Seven strains which are reactive with all eight MAbs have an identical amino acid sequence (Table 2, top row). Single amino acid differences among strains were identified at five positions, as noted in Table 2. In three additional positions in the protein, differences in two to six amino acids were noted. These include positions 77 and 78 (VQ versus IK), positions 112 to 117 (LAYKS versus FTYRRA) and positions 137 and 138 (IV versus TL). Overall, nucleotide sequences were 95.7 to 100% homologous with *ompE* of strain ATCC 25240. Amino acid sequences were 96.6 to 100% identical to OMP E of strain ATCC 25240.

**Preliminary epitope analysis.** Since the regions of OMP E containing epitopes recognized by MAbs are known, correlating patterns of the reactivity of MAbs with amino acid sequences allows predictions regarding the location of epitopes recognized by the MAbs. MAbs 1B3 and 9G10d recognize the same, or a closely related, epitope on the bacterial surface in the region represented by amino acids 80 to 180 (4, 17). Amino acid sequences of OMP E of strains which were reactive with MAbs 1B3 and 9G10d were compared with sequences of nonreactive strains. Comparison of sequences in the region of amino acids 80 to 180 reveals that amino acid 95 (I), amino acids 112 to 116 (LAYKS), and/or amino acids 137 and 138 (IV) are important for reactivity of MAbs 1B3 and 9G10d.

FIG. 1. Immunoblot assay with MAb 1B3. Lanes contain whole-bacterial-cell lysates of strains 93P3B1 (a), 534 (b), 55P26B1 (c), 47P31B1 (d), 19P7B1 (e), 14P15B1 (f), 12P6B1 (g), 10P28B1 (h), and 3P3B1 (i). Molecular mass standards are noted in kilodaltons on the right.
TABLE 1. Summary of reactivities of eight MAbs to OMP E from 22 strains of *M. catarrhalis*

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive</td>
<td>18</td>
<td>21</td>
<td>22</td>
<td>18</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Nonreactive</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Region of OMP E recognized by MAbs based on amino acid (aa) sequence of mature protein (17).

TABLE 2. Amino acid differences and MAb reactivities of OMP E from strains of *M. catarrhalis*

<table>
<thead>
<tr>
<th>Strain(s)</th>
<th>Amino acid(s) at position(s)</th>
<th>MAb reactivity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 25240, 7169, 15P4B1, 3614, 3583, 14P15B1, 14P23B1</td>
<td>A I VQ I LAYKS 1V K A</td>
<td>All 8 No. of strains with result with MAb Reactive Nonreactive</td>
</tr>
<tr>
<td>8185</td>
<td>S V VQ I LAYKS 1V K A</td>
<td>All 8 None</td>
</tr>
<tr>
<td>5P7B1, 5P10B1, 15P1B1</td>
<td>S V IK I LAYKS 1V K A</td>
<td>All 8 None</td>
</tr>
<tr>
<td>4608</td>
<td>A V IK V FTYRRA TL K V</td>
<td>9E3, 12D5, 4C11, 2E11 IB3, 9G10d, 5B3, 14E10</td>
</tr>
<tr>
<td>15P9B1, 15P12B1, 3.215</td>
<td>S V IK V FTYRRA TL K V</td>
<td>9E3, 12D5, 4C11, 2E11 IB3, 9G10d, 5B3, 14E10</td>
</tr>
<tr>
<td>534</td>
<td>S V IK V FTYRRA TL R V</td>
<td>9E3, 4C11, 2E11 IB3, 9G10d, 12D5, 5B3, 14E10</td>
</tr>
</tbody>
</table>

* Reactivities with eight MAbs noted in Table 1.
three sites, including amino acids 95, 112 to 117, and 137 and 138 (Table 2). Any or all three of these sites may be part of the epitope recognized by MAbs 1B3 and 9G10d. Fusion proteins corresponding to sequences in and around the region of amino acids 107 to 145 did not react with the MAbs, suggesting either that the epitope is located around amino acid 95 or that a larger peptide is required to reproduce the conformation of the epitope.

Comparison of sequences and patterns of reactivity with MAbs reveals that single amino acid differences account for reactivity with selected MAbs. For example, MAb 12D5 recognizes amino acids 160 to 260 (17). Strain 534 is nonreactive with 12D5 and differs from reactive strains in a single amino acid in that amino acid range, indicating that the lysine at position 202 is critical for the epitope recognized by MAb 12D5. Similarly, MAbs 5B3 and 14E10 recognize amino acids 240 to 340. Reactive and nonreactive strains differ by a single amino acid in this region of OMP E. The valine in place of alanine at position 269, a difference of a single methyl group, accounts for the lack of reactivity with MAbs 5B3 and 14E10.

The gene which encodes protein P2, the major outer membrane protein of nontypeable Haemophilus influenzae, undergoes nonsynonymous point mutations under immune selective pressure and undergoes horizontal transfer between strains in the human respiratory tract (7, 8, 24). These are thought to be mechanisms of immune evasion, thereby facilitating persistent colonization of the respiratory tract. As a preliminary assessment of the stability of OMP E of M. catarrhalis in the human respiratory tract, the sequence of ompe was determined in sets of isolates which colonized the respiratory tracts of adults with COPD continuously for 3 to 9 months. Immunoassays showed that all three patients had serum IgG to OMP E prior to colonization, and no change in the level was observed during colonization (Table 3). The genes showed identical nucleotides at all positions, indicating that the gene did not undergo changes during persistent colonization. A similar observation has been made with OMP CD of M. catarrhalis (9).

OMP E has several characteristics which indicate that it may be an effective vaccine antigen. The protein is present in all strains tested (2, 3). It is abundantly expressed on the bacterial surface based on results of immunofluorescence assays and flow cytometry with MAbs (4). OMP E is immunogenic in animals (4, 17). Some adults with COPD have serum IgG to OMP E, and the majority have mucosal IgA to OMP E (4). The present study establishes that OMP E is highly conserved among strains of M. catarrhalis, an important characteristic for an effective vaccine antigen.

TABLE 3. Levels of immunoglobulin to OMP E in supernatants of serum and sputum from three adults with chronic bronchitis colonized by M. catarrhalis

<table>
<thead>
<tr>
<th>Patient identification no.</th>
<th>Serum pair</th>
<th>Serum IgG (µg/ml)</th>
<th>Sputum IgA (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>5E6-5E11</td>
<td>0.54</td>
<td>0.7</td>
</tr>
<tr>
<td>13</td>
<td>13E2-13E8</td>
<td>0.25</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>14</td>
<td>14E14-14E24</td>
<td>1.88</td>
<td>&lt;0.2</td>
</tr>
</tbody>
</table>

ACKNOWLEDGMENT

This work was supported by grant AI28304 from the National Institute of Allergy and Infectious Diseases and the Department of Veterans Affairs.

REFERENCES


