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Branhamella catarrhalis: Epidemiology, Surface Antigenic Structure, and Immune Response

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INTRODUCTION

The recognition in the past decade of *Branhamella catarrhalis* as an important human respiratory tract pathogen has generated much interest in research on this bacterium. Knowledge of its epidemiology and antigenic structure is expanding rapidly. The purpose of this article is to review the role of *B. catarrhalis* as a human pathogen, its epidemiology and surface antigenic structure, and the human immune response to *B. catarrhalis*. Emphasis is placed on research performed in the last 5 years.

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BRANHAMELLA CATARRHALIS AS A HUMAN PATHOGEN

B. catarrhalis is a common inhabitant of the human upper respiratory tract. It is an important cause of respiratory tract infections in selected settings, including otitis media in infants and children and lower respiratory tract infections in adults with chronic obstructive pulmonary disease (COPD). More recently, *B. catarrhalis* has been recognized as a nosocomial respiratory tract pathogen. Finally, invasive disease is a less common manifestation of infection by the bacterium. Each of these clinical manifestations of infection is considered briefly.

Otitis Media

Approximately 80% of all children will experience at least one episode of otitis media (middle ear infection) by the age of 3 years (100). Recurrent otitis media is associated with substantial morbidity and results in enormous health care costs

TABLE 1. Recent series of otitis media: results of tympanocentesis

Reference	Yr	Location	% of samples containing:		
			S. pneumoniae	*H. influenzae*	*B. catarrhalis*
159	1990	Norway and Finland	28	16	16
63	1991	Dallas, Tex.	29	30	15
92	1991	Cleveland, Ohio	25	27	11
56	1992	Buffalo, N.Y.	19	35	18
43	1992	Philadelphia, Pa.	48	20	23
33	1992	Galveston, Tex.	39	34	18
133	1993	Galveston, Tex.	29	37	15
148	1993	Turku, Finland	29	19	15
142	1993	Finland (4 sites)	33	33	14
153	1993	Cleveland, Ohio	33	19	11
10	1994	USA (5 sites)	39	27	14
64	1994	France (4 sites)	53	29	13

(163). Careful studies from many centers have defined the etiology of otitis media by performing viral and bacterial cultures of middle ear fluid obtained by tympanocentesis. Culture of this fluid represents the "gold standard" in identifying the etiology of otitis media, because tympanocentesis allows recovery of middle ear fluid, which is free of contamination by nasopharyngeal flora.

Table 1 summarizes the results of bacterial cultures of middle ear fluids obtained by tympanocentesis in 12 studies published since 1990. While some differences are observed, the results of studies from centers in the United States and Europe are remarkably consistent with regard to the relative importance of nontypeable *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *B. catarrhalis* as the predominant bacterial pathogens in otitis media. On the basis of cultures of middle ear fluids, *B. catarrhalis* causes approximately 15% of all episodes of otitis media in infants and children. Approximately 24.5 million office visits are made to physicians annually for the treatment of otitis media (100). Therefore, more than 3.5 million episodes of otitis media caused by *B. catarrhalis* occur each year in the United States.

Studies which rely on cultures of middle ear fluid reveal that no bacteria are recovered from 10 to 40% of samples (Table 1). Respiratory viruses, alone or in combination with bacteria, have been recovered from approximately 20% of middle ear fluids (100). This observation indicates that viruses account for some of the episodes of otitis media in which middle ear fluids are sterile in bacterial culture.

More recently, middle ear fluids have been analyzed for the presence of DNA from nontypeable *H. influenzae*, *S. pneumoniae*, and *B. catarrhalis* by PCR with primers corresponding to the P6 gene, the pneumolysin gene, and *B. catarrhalis* sequences, respectively (139, 166, 178). PCR was substantially more sensitive than culture in detecting all three bacteria in middle ear fluid. These studies suggest that the presence of bacteria in middle ear fluid is more common than is revealed by culture. Such studies which use sensitive methods for detecting bacteria will help to further define the etiology of otitis media, particularly in cases with negative middle ear fluid cultures.

Lower Respiratory Tract Infection

The recognition of *B. catarrhalis* as a human lower respiratory tract pathogen has been delayed for several reasons. The organism colonizes the upper airways of children and adults in the absence of clinical infection. Therefore, the presence of the bacterium in the sputum of a patient with symptoms and signs of respiratory tract infection does not necessarily establish *B. catarrhalis* as etiologic in an individual. Another factor accounting for the difficulty in establishing this organism as a respiratory tract pathogen is the observation that *B. catarrhalis* has a colony morphology which is indistinguishable from commensal *Neisseria* species which colonize the human respiratory tract. Therefore, unless the technician in the clinical microbiology laboratory specifically tests for the presence of *B. catarrhalis*, the organism will be overlooked. Finally, *B. catarrhalis* causes relatively noninvasive infections. As a result, the organism is seldom recovered from blood or pleural fluid cultures.

Most patients who experience lower respiratory tract infections due to *B. catarrhalis* have a predisposing condition. The most common is underlying cardiopulmonary illness. *B. catarrhalis* is an important cause of purulent exacerbations and pneumonia in patients with COPD and other chronic lung diseases. Five principal lines of evidence implicate *B. catarrhalis* in this setting (126). (i) By using strict criteria to evaluate the quality of sputum samples, a subset of patients with exacerbations of COPD have sputum smears which show a predominance of gram-negative diplococci on Gram strain and virtually pure cultures of *B. catarrhalis* (32, 119, 128, 158, 179). (ii) Pure cultures of *B. catarrhalis* have been obtained in transtracheal aspirates from patients experiencing exacerbations of COPD and pneumonia (7, 47, 80, 130, 184). (iii) Clinical improvement is seen in patients with *B. catarrhalis* infections following the administration of specific antibiotic therapy. Many penicillins are not active against *B. catarrhalis*, because most strains produce β-lactamase. Patients with β-lactamase-positive strains who do not respond to therapy with β-lactam antibiotics show clinical improvement following administration of an antibiotic active against *B. catarrhalis* (119, 128). (iv) The organism is occasionally recovered from the blood or pleural fluid of patients with evidence of lower respiratory tract infection (34, 37, 80, 90, 158, 181). Recovering the organism from blood or pleural fluid represents definitive evidence of an etiologic role of the organism. Such patients are unusual; they represent the most invasive end of the spectrum of disease caused by *B. catarrhalis*. (v) Patients with chronic bronchitis who experience exacerbations associated with clinical and laboratory evidence of *B. catarrhalis* infection develop a bactericidal antibody response to the homologous strain (32). The observation of a specific immune response to the organism following clinical infection provides evidence that the bacterium caused the infection.

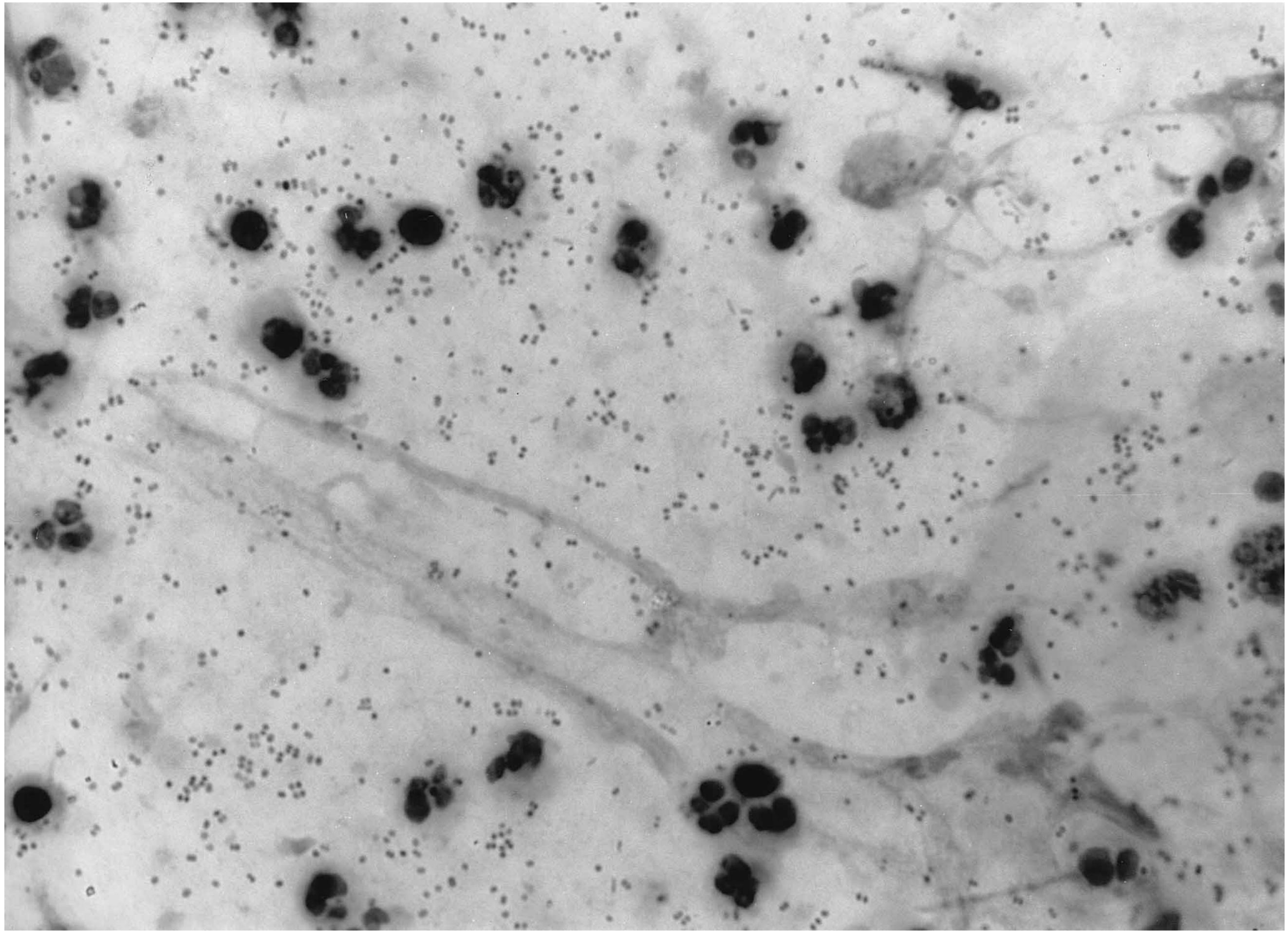

FIG. 1. Photomicrograph (magnification, ×1,000) of a Gram-stained sputum sample from a patient with chronic bronchitis experiencing a purulent exacerbation caused by *B. catarrhalis*. Note the abundance of leukocytes, the presence of large numbers of gram-negative diplococci as the exclusive bacterial form, and the presence of intracellular bacteria in leukocytes.

Taken together, these five lines of evidence indicate that *B. catarrhalis* causes lower respiratory tract infection in adults, particularly in the setting of COPD. It is difficult to estimate the proportion of exacerbations which are due to *B. catarrhalis*. However, one study performed in a Veterans Administration facility found that 30% of exacerbations were caused by *B. catarrhalis* (175).

When *B. catarrhalis* is found in the sputum of a patient with lower respiratory tract infection, it is generally present in large numbers (170). A Gram-stained sputum sample which shows a predominance of gram-negative diplococci is highly predictive for the presence of *B. catarrhalis* (80, 128, 170, 175, 185). Indeed, this is the single most useful diagnostic test in establishing *B. catarrhalis* lower respiratory tract infection. Figure 1 shows a characteristic Gram stain of sputum from an adult with exacerbation of COPD as a result of *B. catarrhalis*. Typical features are the abundance of leukocytes, the presence of large numbers of gram-negative diplococci as the exclusive bacterial form, and the presence of intracellular bacteria in leukocytes.

Nosocomial Infection

Nosocomial outbreaks of infections caused by *B. catarrhalis* have been recognized since the mid-1980s. Reports from several centers indicate that clusters of *B. catarrhalis* infections occur in hospitals (1, 13, 39, 73, 79, 86, 89, 99, 118, 121, 134–136, 144, 146). Most nosocomial infections involve the respiratory tract, and several outbreaks have occurred in respiratory units (118, 121, 135, 136, 144). These observations suggest that the presence of a susceptible population contributed to the clusters. Preliminary observations involving typing of strains indicate that some of the clusters involved infections with several distinct strains of *B. catarrhalis* (13, 121). Other studies implicate person-to-person transmission and suggest that hospital personnel may be involved in transmission (135, 144). Analysis of strains from outbreaks by newly developed typing methods will be important in elucidating the factors responsible for nosocomial outbreaks so that rational strategies for prevention can be implemented.

Other Infections

B. catarrhalis causes sinusitis in adults and children (21, 95, 180). The organism is recovered alone or in combination with other bacteria from direct sinus aspirates obtained from patients with clinical and radiographic evidence of acute bacterial sinusitis. Serological data also provide evidence for *B. catarrhalis* as a cause of sinusitis (21).

In addition to the relatively common clinical manifestations noted above, *B. catarrhalis* is a rare cause of invasive infection.

TABLE 2. Typing methods for *B. catarrhalis*

Method	Procedure	Basis of strain differentiation	Reference(s)
Phenotypic			
SDS-PAGE and immunoblot assay	Immunoblot of bacterial lysates with normal human serum	Antigenic and mol wt differences of cellular proteins	118, 121, 144
Esterase electrophoretic polymorphism	Detection of specific esterases by electrophoresis	Relative electrophoretic mobility of esterases	44, 136
LOS typing	Inhibition ELISA with typing antisera	Antigenic differences in LOS	169
Genotypic			
Restriction enzyme analysis of genomic DNA			
Agarose gel electrophoresis	Electrophoretic separation of small fragments of DNA	DNA sequence differences detected by restriction enzymes	44, 45, 58, 89, 118, 121, 134, 135, 144
Pulsed-field gel electrophoresis	Electrophoretic separation of large fragments of DNA	DNA sequence differences detected by restriction enzymes	99, 102, 146
DNA probe	Southern blot probed with labeled random genomic fragments	DNA sequence differences in selected regions	13
Ribotyping	Southern blot probed with labeled rRNA	DNA sequence differences detected by restriction enzymes and hybridization with rRNA	44

These infections are documented predominantly by case reports. Invasive infections caused by *B. catarrhalis* include meningitis (127), endocarditis (48, 149), bacteremia (8, 36, 76, 90, 181), septic arthritis (41, 120), osteomyelitis (140), epiglottitis (177), cellulitis (147), shunt-associated ventriculitis (40), peritonitis (38), pericarditis (104), wound infection (73), and serious lower respiratory tract infection in neonates and infants (50, 79). *B. catarrhalis* is an unusual cause of acute urethritis (46, 154) and can also cause conjunctivitis (98, 111).

TAXONOMY AND CLASSIFICATION

The taxonomic relationship of *B. catarrhalis* to *Moraxella* and other related genera remains controversial, accounting for the frequent changes in nomenclature. The changing and unstable nomenclature of this bacterium has been frustrating for practicing physicians and investigators working on the organism. Ghon and Pfeiffer first described the organism and called it *Micrococcus catarrhalis* (65). The bacterium was subsequently called *Neisseria catarrhalis* because of its similarities in phenotype and ecological niche to *Neisseria* species.

B. catarrhalis was transferred from *Neisseria* to the new genus, *Branhamella*, in 1970 on the basis of differences in fatty acid content and DNA hybridization studies compared with other members of the family *Neisseriaceae* (29). An alternative scheme has *Branhamella* as a subgenus of *Moraxella* (19). Experimental data to support both schemes exist (31). The name *Branhamella catarrhalis* is most rational at this time for two reasons. (i) This nomenclature serves to place rod-shaped organisms (*Moraxella*) in one species and cocci (*Branhamella*) in a separate species (30). (ii) *B. catarrhalis* is an important and common human respiratory tract pathogen, whereas *Moraxella* species are unusual human pathogens. This distinction is highlighted by classifying them as separate species. The nomenclature of these bacteria continues to be a matter of debate, and additional changes may occur.

TYPING SYSTEMS

Reliable methods to type bacteria are critical tools in elucidating the epidemiology and pathogenesis of infection. An ideal system for typing strains of *B. catarrhalis* has not yet been established. A variety of approaches to typing strains of *B. catarrhalis* have been used and are summarized in Table 2.

Serotyping Systems

Serotyping systems based on antigenic differences in surface molecules have been particularly useful in the study of bacterial infections. In addition to providing a means of strain discrimination, such serotyping systems provide other insights. Serotyping systems have identified pathogenic strains within a species; for example, a serotyping system based on capsular polysaccharide specifically identified type b strains of *H. influenzae* as invasive human pathogens (122, 137). Furthermore, serotyping systems can provide insight into the immune response of bacteria to infection. Group A beta-hemolytic streptococci are typed on the basis of the surface M protein (17, 105, 106). The observation that type-specific protection is seen following pharyngitis indicates that a protective immune response in humans is directed at the M protein.

The sole currently available typing system based on antigenic differences in surface molecules for *B. catarrhalis* is lipooligosaccharide (LOS) typing, which was developed by Vaneechoutte et al. (169). This system involves inhibition enzyme-linked immunosorbent assay (ELISA) and distinguishes three LOS types on the basis of antigenic differences in the LOS molecule. A total of 94% of strains can be serotyped. The method is limited by the small number of serotypes and the observation that 60% of clinical isolates belong to a single serotype. This work suggests that the relatively limited antigenic diversity in LOS will not provide adequate discriminatory power to use LOS as the basis of a serotyping system. Outer membrane proteins (OMPs) may exhibit the antigenic heterogeneity necessary to form the basis of a serotyping system, and this approach should be pursued. It is important to develop a serotyping system for *B. catarrhalis*. Such a system has the potential to provide insights into the epidemiology of *B. catarrhalis* infection, identify pathogenic strains of the species, and reveal protective immune responses to infection.

Genotype-Based Typing Systems

The most widely used method to type strains of *B. catarrhalis* to date has been restriction endonuclease analysis of genomic DNA (44, 45, 58, 89, 118, 121, 134, 135, 144). More recently, a more refined form of restriction endonuclease analysis involving pulsed-field gel electrophoresis of large fragments of genomic DNA has been developed (99, 102). These methods have been useful in studying nosocomial outbreaks and are

providing important observations about the epidemiology of *B. catarrhalis* colonization and infection. Restriction endonuclease analysis is limited by the difficulty of interpreting complex profiles and by the relatively laborious procedure (115). A convenient, reproducible, and reliable typing method for *B. catarrhalis* is needed. PCR-based systems have been highly effective for other bacterial species, and these methods hold promise as a means for typing strains of *B. catarrhalis* as well (115).

EPIDEMIOLOGY AND RESPIRATORY TRACT COLONIZATION

B. catarrhalis has been recovered exclusively from humans. The bacterium colonizes primarily the human respiratory tract, although it has occasionally been recovered from the genital tract (46, 154). Studies from several centers have assessed the prevalence of colonization of the upper respiratory tract by *B. catarrhalis* in various populations. There is a strong relationship between age and colonization rates.

Prevalence of Colonization in Adults

Approximately 1 to 5% of healthy adults are colonized by *B. catarrhalis* (52, 95, 138, 170). Adults with chronic lung diseases appear to have a higher rate of respiratory tract colonization than healthy adults. Several studies have surveyed the results of cultures of sputum samples and examined the clinical status of patients from whom *B. catarrhalis* was isolated (20, 138, 155). These studies show that sputum samples which contain *B. catarrhalis* are more likely to be recovered from patients with chronic lung disease than from healthy adults. *B. catarrhalis* was recovered from 28% of patients with bronchiectasis monitored prospectively in Birmingham, United Kingdom (102). In a prospective study of patients with chronic bronchitis in Buffalo, N.Y., *B. catarrhalis* was recovered from approximately 10% of sputum samples (121a). These observations suggest that adults with chronic lung disease are colonized with *B. catarrhalis* at a higher rate than are healthy adults. However, this comparison has not yet been studied rigorously.

Prevalence of Colonization in Children

Upper respiratory tract colonization with *B. catarrhalis* is common throughout infancy (9, 58, 112, 170, 171). Several authors have demonstrated a strong seasonal variation, with higher rates of colonization in winter months (20, 42, 138, 171). Analysis of carefully performed prospective studies reveals substantial regional variation in rates of colonization of infants by *B. catarrhalis*. A prospective study in Buffalo, N.Y., in which nasopharyngeal cultures were obtained monthly from birth showed that 66% of infants were colonized with *B. catarrhalis* at some time during the first year of life and 78% were colonized by the age of 2 years (58). A similar study performed in Göteborg, Sweden, demonstrated a colonization rate approximately half that of the Buffalo study (9). A third study of infants in a rural Aboriginal community near Darwin, Australia, revealed that 100% of infants were colonized by 3 months of age (107). All three studies used similar culture methods, indicating that the different results truly reflect different rates of colonization in the populations. Several factors may be operative in accounting for the different rates; these may include living conditions, hygiene, environmental factors (e.g., smoking among parents), genetic characteristics of the populations, host factors, and others. The precise factors which account for these differences in colonization rates are not yet clearly defined.

Association of Colonization with Otitis Media

Studies from several centers have established that nasopharyngeal colonization with middle ear pathogens, including *B. catarrhalis*, is associated with otitis media (57, 58, 107, 141, 160, 161). Although the presence of the bacterium in the nasopharynx of an individual has no diagnostic value with regard to the presence or absence of otitis media, the epidemiological association between colonization and otitis media is strong. Otitis-prone children are colonized with *B. catarrhalis* at a higher rate than are healthy children (57, 58, 107).

The precise relationship between upper respiratory tract colonization and otitis media has yet to be elucidated. Strains of *B. catarrhalis* recovered from the middle ear are present in the nasopharynx, indicating that the middle ear isolate comes from the nasopharynx via the eustachian tube (45). Collectively, the current data suggest that colonization of the upper respiratory tract with middle ear pathogens including *B. catarrhalis* is a necessary first step in the pathogenesis of otitis media; however, colonization alone is not sufficient to cause otitis media. Another triggering event which occurs in a colonized child is probably necessary to cause otitis media. The reason for increased colonization in otitis-prone children is still not clear. A selective abnormality in the immune response, involving a reduced ability to clear pathogens from the upper respiratory tract has been suggested (58). An alternative explanation is that environmental factors cause some children to be colonized at a higher rate and that these children become otitis prone in part as a result of being colonized with middle ear pathogens for a longer period than are non-otitis-prone children. It will be important to elucidate the role of colonization by *B. catarrhalis* in the pathogenesis of otitis media to devise rational strategies for preventing this disease.

Dynamics of Colonization

Two prospective studies have examined the dynamics of respiratory tract colonization by *B. catarrhalis* (58, 102). Faden et al. (58) monitored 120 children from birth to 2 years of age. Restriction endonuclease analysis of genomic DNA from the longitudinally collected strains revealed that children eliminated and acquired new strains of *B. catarrhalis* frequently. Similarly, Klingman et al. (102) studied strains of *B. catarrhalis* recovered prospectively from adults with bronchiectasis. Analysis of strains by pulsed-field gel electrophoresis of large fragments of genomic DNA demonstrated that the mean duration of colonization by individual strains was only 2.3 months.

These studies in two distinct patient populations indicate that colonization of the upper respiratory tract by *B. catarrhalis* is a dynamic process, with frequent elimination and acquisition of new strains. This observation suggests that an immune response is mediating the high rate of turnover of strains. More prospective studies of colonization by *B. catarrhalis* in various populations are needed. Analysis of the mucosal and systemic immune response to *B. catarrhalis* is needed to understand the role of the immune response in the elimination of the organism from the respiratory tract. Such work could lead to identification of a potentially protective immune response to *B. catarrhalis.*

SURFACE ANTIGENS

Rapid progress has been made in the last decade in the identification and characterization of surface antigens of *B. catarrhalis*. Surface structures include OMPs, pili, and LOS. Preliminary studies have suggested the possibility of a capsule,

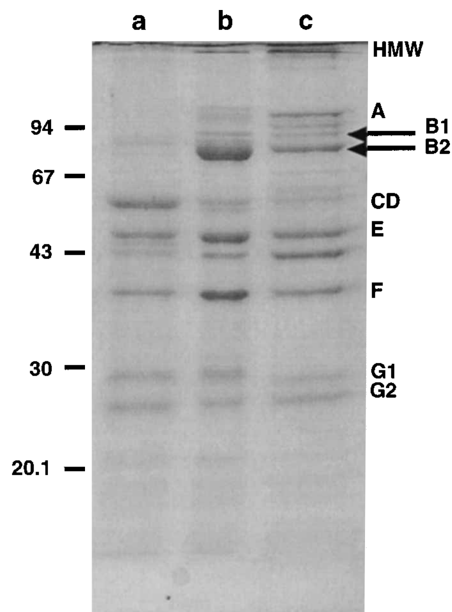

FIG. 2. SDS-PAGE of a gel stained with Coomassie blue. All lanes contain outer membrane preparations from *B. catarrhalis* 25240. Lanes: a, Zwittergent extract of cells grown on chocolate agar; b, Zwittergent extract of cells grown in iron-deficient medium (sample provided by A. Campagnari); c, EDTA heat-induced vesicles of cells grown in brain heart infusion broth. OMPs are labeled on the right. Molecular mass markers are noted in kilodaltons on the left.

but the presence of a capsule has yet to be definitively proven (4, 82).

Outer Membrane Proteins

In the late 1980s, methods for purifying the outer membrane of *B. catarrhalis* were identified and OMPs of various strains were first studied by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (12, 125). OMP patterns observed by SDS-PAGE showed a high degree of similarity among strains from diverse geographic and clinical sources (12). Several major OMPs have been identified, and these are shown in Fig. 2. Experience with various methods of purification of the outer membrane has revealed that the relative proportions of various OMPs differ depending on the method used to purify the outer membrane (Fig. 2). The current state of our knowledge about five major OMPs is summarized briefly (Table 3).

High-molecular-weight outer membrane protein (UspA). A protein band with a molecular mass varying from 350 to 700 kDa (called high-molecular-weight OMP [HMW-OMP] or UspA) is present in all or most strains of *B. catarrhalis* (101). Incubation of HMW-OMP in formic acid produces a single band with a molecular mass of 120 to 140 kDa, suggesting that the HMW-OMP is an oligomer composed of several monomers (101). Analysis with monoclonal antibodies has established that the HMW-OMP contains epitopes on the bacterial surface (84, 101). Furthermore, passive immunization of mice with a monoclonal antibody to the protein enhanced pulmonary clearance of *B. catarrhalis* (84).

The function of HMW-OMP has not been defined. However, one might speculate that the protein is involved in adherence of the bacterium to host cells, because the presence of a high-molecular-weight surface structure is reminiscent of large protein molecules functioning as adhesins in other gram-negative human pathogens (131, 143, 162, 165, 168). Furthermore, the HMW-OMP appears to be a vitronectin-binding protein and is involved in complement resistance (172). Further characterization of the role of the HMW-OMP in the interaction of *B. catarrhalis* with the human host will be important in elucidating mechanisms of pathogenesis and the human immune response to infection.

Outer membrane protein B1. Analysis of serum samples from patients with bronchiectasis who were colonized with *B. catarrhalis* produced a surprising result, which led to the identification of OMP B1 (151). The most prominent and consistent antibody response in these patients was directed to a minor, 84-kDa OMP. This OMP is distinct from a major, 80-kDa OMP which was previously designated OMP B (12, 125). To maintain consistency in the literature, we designated the newly identified 84-kDa protein OMP B1 and renamed the

TABLE 3. Characteristics of selected OMPs of *B. catarrhalis*

OMP	Mol mass (kDa)	Proposed function[a]	Antigenic conservation among strains	Additional observation(s)	Interaction with immune system	References
HMW-OMP (UspA)	350–700	Adherence	Preliminary data suggest it is conserved	Probably an oligomer	Vitronectin-binding protein mediates complement resistance; antibodies enhance clearance in mouse model	84, 101, 172
OMP B1	84	Iron acquisition	Moderately heterogeneous	Iron repressible	Major target of human antibody in bronchiectasis	24, 26, 151
OMP B2 (Cop B)	80	Iron acquisition	Moderately heterogeneous	Iron repressible	Antibodies enhance clearance in mouse model; involved in serum resistance	26, 83, 85
OMP CD	46 (60)	Porin	Highly conserved	Heat modifiable; migrates as a doublet; homologous with OprF of *Pseudomonas* spp.		12, 88, 124, 150
OMP E	50	Porin	Highly conserved	Borderline homology with porins of *E. coli*		15, 16, 123

[a] Functions of OMPs have yet to be definitively established.

previously identified 80-kDa protein as OMP B2 (also called CopB).

Immunoblot adsorption and elution experiments revealed that OMP B1 contains surface-exposed epitopes which are moderately conserved among strains (151). The epitopes are a major target of the human immune response. When *B. catarrhalis* is grown under iron-limited conditions, a striking increase in expression of OMP B1 is observed (26). This observation indicates that OMP B1 is probably involved in iron uptake by *B. catarrhalis* (see below).

Outer membrane protein B2 (CopB). OMP B2 is a major, 80-kDa outer membrane protein. Analysis with monoclonal antibodies shows that the protein contains moderately conserved epitopes on the surface of the intact bacterial cell (83, 121a). Passive immunization of mice with a monoclonal antibody to OMP B2 enhances pulmonary clearance (83). A mutant which does not express OMP B2 is more sensitive to killing by normal human serum and is more readily cleared from the lungs of mice than is the isogenic parent strain (85). These observations indicate that OMP B2 plays a role in the interaction of *B. catarrhalis* with host defense mechanisms. When *B. catarrhalis* is grown under iron-limiting conditions, the expression of OMP B2 is increased (26). Furthermore, OMP B2 shares homology with several iron-regulated proteins from other bacterial species (14). These observations suggest that the protein is involved in iron uptake.

Outer membrane protein CD. Initial analysis of OMPs by SDS-PAGE identified two bands in the 60-kDa range; these were named OMP C and OMP D (12, 125). Subsequent work has established that these bands represent OMP CD, a single gene product which migrates as a doublet in SDS-PAGE (124). OMP CD is a heat-modifiable protein which runs aberrantly in SDS-PAGE. Analysis of its sequence predicts a mature protein of 46 kDa, which differs from its apparent molecular mass as seen in SDS-PAGE (~60 kDa). A proline-rich region of the protein accounts for its aberrant migration in SDS-PAGE (124).

The protein contains epitopes which are abundantly expressed on the bacterial surface (150). Several lines of investigation indicate that OMP CD is highly conserved among strains of *B. catarrhalis*. (i) The protein shows identical migration among strains in SDS-PAGE (12). (ii) Analysis with monoclonal antibodies reveals highly conserved epitopes, including several on the bacterial surface (121a, 150). (iii) Restriction fragment length polymorphism analysis in and around the OMP CD gene from 30 isolates of diverse origins shows identical patterns (124). (iv) Restriction fragment length polymorphism and sequence analysis of the OMP CD gene from strains collected from patients with bronchiectasis and chronic bronchitis show a high degree of conservation among strains (88).

OMP CD shares homology with the OprF protein of *Pseudomonas* species (124). Since OprF is a porin for *Pseudomonas* species, one may postulate that OMP CD is a porin as well. Of interest, OprF is an effective vaccine for the prevention of infection by *Pseudomonas aeruginosa* in several animal models (68, 69, 117). Its abundant expression on the bacterial surface and its high degree of conservation among strains suggest that OMP CD may be an effective vaccine antigen, and this line of investigation should be pursued.

Outer membrane protein E. Immunoblot adsorption experiments indicated that OMP E, a 50-kDa protein, expressed epitopes on the bacterial surface (123). The gene encoding OMP E has been cloned, and its sequence has been determined (15). Borderline homology was observed with FadL of *Escherichia coli*, which is involved in binding and transport of fatty acids, and with the OmpF family of porin proteins. The native size of OMP E indicates that it is arranged as a trimer. These observations suggest that OMP E is a trimeric porin protein, but definite proof of this awaits porin function studies.

PCR-restriction fragment length polymorphism analysis has established that OMP E is highly conserved among strains of *B. catarrhalis* (15). Studies with monoclonal antibodies indicate that the protein contains conserved, surface-exposed epitopes (16). These characteristics suggest that OMP E is a potential vaccine antigen.

Iron-regulated proteins. *B. catarrhalis* expresses specific OMPs in response to iron-limited growth in vitro (26, 186). *B. catarrhalis* uses iron-saturated human transferrin or lactoferrin as the sole source of iron for growth in the absence of siderophores (26). These observations suggest that *B. catarrhalis* competes for iron bound to human transferrin and human lactoferrin in a manner similar to that used by *Neisseria* species. *B. catarrhalis* transferrin receptors show a strong preference for iron-saturated transferrin over apotransferrin, and in this regard they differ from *Neisseria* receptors (186). Two of the iron-repressible proteins are OMPs B1 and B2 (Fig. 2) (26). More recently, Campagnari et al. (24) have demonstrated that OMP B1 binds human transferrin, suggesting that this protein is a receptor, or part of a receptor, for human transferrin. Iron-regulated proteins probably play an important role in pathogenesis, since they allow *B. catarrhalis* to obtain iron, which is necessary for survival and growth in vivo.

Pili (Fimbriae)

Many gram-negative bacteria which colonize mucosal surfaces express pili, which mediate adherence to host cells. Most strains of *B. catarrhalis* have pili, as shown by electron microscopy (2, 3, 5, 6, 114, 145). Expression of pili decreases with in vitro passage (5). A DNA probe consisting of the cloned *Moraxella bovis* type 4 pilin gene hybridizes with genomic DNA of *B. catarrhalis*, suggesting that type 4 pili are present in strains of *B. catarrhalis* (114). Little else is known about the pili of *B. catarrhalis.*

The role of pili in adherence of *B. catarrhalis* to human cells is poorly understood. No correlation is observed between the presence of pili, adherence to human oropharyngeal epithelial cells, and hemagglutination (6, 145). No other putative adhesins have been identified in *B. catarrhalis*. A model system to study the adherence of *B. catarrhalis* is needed to investigate the molecular basis of adherence of *B. catarrhalis* to host cells. In view of the observation that *B. catarrhalis* is an exclusively human pathogen, studies involving human cells and tissue are the most likely to be fruitful.

Lipooligosaccharide

The outer membrane of *B. catarrhalis* contains LOS, which lacks the long O-polysaccharide side chains observed in enteric gram-negative bacteria (51, 61). In this regard, the LOS of *B. catarrhalis* resembles other nonenteric gram-negative bacteria. In addition, the LOS of *B. catarrhalis* shares at least one epitope with the LOS of other nonenteric gram-negative bacteria (27).

The structure of the oligosaccharide portion of the LOS molecule of one strain of *B. catarrhalis* has been determined by methylation analysis, mass spectrometry, and nuclear magnetic resonance spectroscopy (51). Antigenic differences among strains reside in the oligosaccharide region of the molecule. The observation that 95% of all strains of *B. catarrhalis* belong to just three LOS serotypes indicate that less antigenic heterogeneity is present in the LOS of *B. catarrhalis* than in that of

TABLE 4. Animal models of *B. catarrhalis* infection

Model of infection	Animal	Inoculum (CFU)	Outcome measurement	Observations	Reference(s)
Pulmonary clearance	Mouse	Inoculation into lung, 1×10^5 to 5×10^5	Rate of clearance of bacteria from lungs	*B. catarrhalis* is cleared from lungs by 24 h	83, 85, 113, 132, 167, 174
Otitis media	Chinchilla	Intrabulbar, 3×10^8	Signs of otitis media and clearance of bacteria from middle ear	*B. catarrhalis* is cleared from middle ear by 5 days	35, 49
Systemic infection	SCID mouse	Intranasal, intraperitoneal, or intravenous, 10^2 to 10^7	Clinical and postmortem findings	*B. catarrhalis* is not recovered from blood	81

other nonenteric gram-negative bacteria such as *Haemophilus* and *Neisseria* species, whose LOS show enormous heterogeneity among strains (25, 169, 176, 182).

The structure of the lipid A portion of the LOS molecule has been elucidated by nuclear magnetic resonance spectroscopy and mass spectrometry. Its backbone structure is identical to that of other gram-negative bacteria (116). The lipid A portion exhibits cross-reactivity with the lipid A of members of the family *Enterobacteriaceae* (61). The lipid A portion of the LOS molecule is responsible for the profound biological activities of LOS. The LOS of *B. catarrhalis* is fully biologically active in vivo and in vitro (61).

The LOS of other bacteria is involved in the pathogenesis of infection. Little is known about the role of LOS in the pathogenesis of infection caused by *B. catarrhalis*, and this is an important area of investigation.

ANIMAL MODELS

Since animal models have provided important data regarding the pathogenesis of infection of a wide spectrum of microbial pathogens, there is much interest in developing a reliable animal model of *B. catarrhalis* infection. Unfortunately, all three models which have been described suffer from significant limitations regarding their usefulness in studying human infection.

Table 4 summarizes the features of three animal models of *B. catarrhalis* infection. A pulmonary clearance model in mice has been used by three laboratories (83, 85, 113, 132, 167, 174). *B. catarrhalis* is instilled directly into the lung, and the clearance of viable bacteria from the lung is quantitated. The mice do not develop evidence of pneumonia, and bacteria cannot be recovered from the lungs after 24 h. The prompt clearance of bacteria and the absence of pneumonia in mice represent serious limitations as a model of pulmonary infection in humans.

The chinchilla has been used as a model of otitis media for *S. pneumoniae* and nontypeable *H. influenzae* (11, 66, 67, 74, 164). When *B. catarrhalis* is instilled into the middle ear of the chinchilla, the bacterium is cleared within 5 days (35). The inability to reliably establish otitis media in the chinchilla has thus far precluded the use of this model to study *B. catarrhalis*. The SCID mouse and the SCID/beige mouse have been challenged with *B. catarrhalis* by several routes in an effort to establish infection (81). Although clinical and postmortem findings of infection have been observed, the findings bear no resemblance to human disease associated with *B. catarrhalis* infection and it is difficult to recover the bacterium from blood and organs.

A common observation in the animal model systems which have been studied is that *B. catarrhalis* is readily cleared by the animals and infection does not become established. This is consistent with the observation that *B. catarrhalis* is an exclusively human pathogen. The specificity of *B. catarrhalis* for humans could be a result of a variety of factors including the specificity of adhesin-receptor interactions on human cells and a fundamental difference in the human and animal immune responses to *B. catarrhalis*. A reliable animal model which parallels human infection would be most helpful in studying *B. catarrhalis* infections. However, the specificity of *B. catarrhalis* for humans promises to make this a formidable challenge.

IMMUNE RESPONSE

The literature on the human immune response to *B. catarrhalis* is confusing. Authors have used various immunoassays with clinical samples which have been epidemiologically defined to various degrees. A critical evaluation of the immunoassays used to perform studies is necessary to understand the literature on the human immune response to *B. catarrhalis.*

Immunoassays To Detect Antibodies to Surface Antigens

The sources of the antigens used in immunoassays to study the human antibody response to *B. catarrhalis* are of particular importance in interpreting the literature. Some studies have used a single strain (28, 62, 70, 71), and some have used mixtures of strains (18, 23, 96, 97, 103, 108). Other studies have used antigen preparations from homologous strains to study the immune response to the isolate infecting the patients (32, 59, 60, 151, 161). Different antigen preparations will yield markedly different results.

The importance of the antigen preparation used in immunoassays becomes apparent when one considers new and evolving data on the structure of the gram-negative outer membrane. Molecules on the bacterial surface are the first bacterial antigens to interact with host defenses. OMPs are prominent on the surface of *B. catarrhalis* and are important targets of the human immune response. Common themes in the structures of OMPs have emerged (91, 129, 183). For example, porin proteins consist of a beta sheet with 16 strands that traverse and remain largely buried within the outer membrane. Interspersed within the relatively conserved backbone of the protein are eight peptide loops that are potentially exposed on the bacterial surface. These surface-accessible regions show antigenic diversity among strains. Since OMPs contain surface determinants which vary among strains, it is important to use the homologous strain as the antigen source in immunoassays. The most important immune response to OMPs is often directed at strain-specific epitopes (22, 77, 78).

For an immunoassay to provide meaningful results regarding antibody responses to OMPs, it should detect antibodies to epitopes exposed on the surface of the intact bacterial cell, because these are the regions of the molecule which are accessible to potentially protective antibody. As noted above, the conserved portions of OMPs which are buried within the outer membrane are inaccessible to antibodies in the intact bacte-

rium. Furthermore, these conserved regions share antigenic determinants among many gram-negative bacteria which frequently colonize humans. Therefore, serum and secretions contain "background" antibodies to conserved, inaccessible parts of OMPs, obscuring potentially meaningful antibody responses to surface epitopes on OMPs of *B. catarrhalis* (93). Immunoassays which reliably detect antibodies to surface epitopes include functional assays (bactericidal and opsonophagocytosis assays), flow cytometry (16, 157), adsorption assays (109, 110, 123, 151), elution assays (54, 151), and whole-cell radioimmunoprecipitation assays (75). In addition, assays such as ELISAs, immunofluorescence microscopy, and immunoelectron microscopy can detect antibodies to surface-exposed epitopes when performed such that antibodies are allowed to bind to nondenatured whole bacterial cells before fixation.

In summary, a critical review of the literature on the human antibody response to *B. catarrhalis* demands that these two aspects be considered: (i) bacteria express determinants which are antigenically heterogeneous among strains, and (ii) immunoassays which specifically detect antibodies to surface-exposed determinants are necessary to identify a meaningful immune response to OMPs.

Systemic Antibody Response

Several studies have detected systemic antibody responses to *B. catarrhalis* in humans (18, 53, 71, 84, 96, 108). These observations indicate that an immune response occurs following infection. The Gm allotype of the host influences the antibody response to *B. catarrhalis* antigens (28, 70).

The presence of serum bactericidal antibodies is associated with protection from infection by several gram-negative bacteria, including nontypeable *H. influenzae* and *Neisseria meningitidis* (55, 72, 152). This observation has stimulated interest in studying functional antibody responses to *B. catarrhalis* in an effort to characterize the immune response and identify correlates of protection from infection. Chapman et al. (32) studied adults with lower respiratory tract infection caused by *B. catarrhalis* for the development of bactericidal antibody to their homologous strain. Most adults developed an immunoglobulin G (IgG) antibody response that mediated serum bactericidal activity by the classical complement pathway.

Strains of *B. catarrhalis* vary in their susceptibility to killing by serum (87, 94, 156, 173). Strains which are recovered from patients with infections are more resistant to serum killing than are strains recovered from colonized individuals (87, 94). Therefore, serum resistance may be a virulence factor for the bacterium. It is noteworthy that the results of bactericidal assays are highly dependent on the conditions used in the assays. Each laboratory which has studied serum bactericidal assays uses different methods (32, 94, 173). These differences include growth media, diluting buffers, complement sources, ratio of bacteria to antibodies, and others. Therefore, while observations on the association of serum resistance with virulence and the demonstration of a functional immune response are potentially important, the reproducibility of these observations by comparable methods must be established.

Opsonophagocytosis assays are a second method used to identify a functional antibody response. Children with otitis media develop serum opsonic antibody to their own strain following otitis media due to *B. catarrhalis* (60). Studies of bactericidal and opsonic antibody responses hold promise in identifying an immune response which may correlate with protection from infection caused by *B. catarrhalis.*

Another approach to characterizing the immune response is to identify a human antibody response to surface-exposed epitopes on bacterial molecules. Sethi et al. (151) used adsorption and elution assays to identify the surface-exposed epitopes to which serum antibodies of patients colonized with *B. catarrhalis* are directed. The predominant antibody response in patients with bronchiectasis was to OMP B1, an 84-kDa, iron-regulated protein. Adsorption and elution assays established that patients made antibodies which recognized surface-exposed epitopes on OMP B1 of their own strains. It will be important to characterize the human antibody response to surface epitopes in patients with other infections such as otitis media and exacerbations of COPD. Such studies will detect a meaningful antibody response and serve as a guide to identify potentially protective antigens.

Mucosal Antibody Response

Since infections due to *B. catarrhalis* occur on mucosal surfaces, the mucosal immune response is probably important in a protective immune response. Several studies have established that antibodies to *B. catarrhalis* are present on the upper respiratory tract mucosal surface (59, 96, 97, 161). Analysis of the systemic and local antibody responses to the homologous strains of *B. catarrhalis* following otitis media indicates that young children consistently develop a mucosal antibody response but fail to develop systemic antibody in a uniform manner (59). Children harbor *B. catarrhalis* which is coated with IgG and secretory IgA in the nasopharynx (161). Interestingly, otitis-prone children had significantly fewer secretory IgA-coated bacteria than did non-otitis-prone children. No significant difference was noted between the two groups regarding IgG coating (161). These two studies (59, 161), which measured antibodies in the upper respiratory tract to homologous strains, suggest that the mucosal immune response is important in otitis media caused by *B. catarrhalis*. The human immune response to *B. catarrhalis*, with particular emphasis on the mucosal immune response, is an area of investigation which should receive high priority.

FUTURE DIRECTIONS

The recognition of *B. catarrhalis* as an important human pathogen has stimulated research which has resulted in rapid progress in the past decade. This work will form the basis for future studies, which should include (i) the development of better typing systems for *B. catarrhalis* so that the epidemiology of colonization and infection can be further defined; (ii) elucidation of mechanisms of pathogenesis of infection; (iii) characterization of the mechanisms which mediate the elimination of strains of *B. catarrhalis* from the human respiratory tract; and (iv) identification of a protective immune response. Such work will lead to a basic understanding of *B. catarrhalis* infections and will facilitate the development of new strategies to treat and prevent these infections.

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