Microbial Pathogenesis in Cystic Fibrosis: Mucoid Pseudomonas aeruginosa and Burkholderia cepacia

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INTRODUCTION

"Understanding the genetic defect underlying cystic fibrosis is only half the battle. Identifying the specific bacterium infecting CF patients is just as important" (177). This recent statement comes at a time when the opinion has also been expressed that although a cure for cystic fibrosis (CF) may be possible through gene therapy, this is unlikely in the near future (457).

Chronic microbial colonization of the major airways leading to debilitating exacerbations of pulmonary infection is the major cause of morbidity and mortality in patients with CF. The CF lung presents a unique environment to potential microbial pathogens. In healthy individuals, inhaled bacteria are directed onto a mucus blanket lining the major airways, moved upward by mucociliary clearance, and then swallowed and destroyed by stomach acids. Opportunistic pathogens able to overcome mucociliary clearance are faced with phagocytic cells aided by immunological mechanisms including specific opsonizing antibodies. As a consequence of the CF gene defect, mucociliary clearance of bacteria from the lungs is impaired by the viscid
TABLE 1. Some recent landmark developments in the genetics and microbiology of CF

| Yr | Event                                                                 |
|----|----------------------------------------------------------------------|
| 1989 | The gene encoding a protein designated CFTR and the most common mutation (ΔF-508) responsible for CF identified |
| 1990 | CFTR shown to be a chloride channel                                    |
| 1990 | Opsinophagocytic antibodies against mucoid exopolysaccharide alginate demonstrated in older noncolonized CF patients |
| 1991 | Replacement of defective CF gene successfully achieved in laboratory cell cultures |
| 1992 | Normal human gene introduced into laboratory rodents; CF gene defect created in laboratory mouse |
| 1993 | First human CF gene trials performed with viral vectors and liposomal DNA mists |
| 1993 | Nature of the genes and mutations causing conversion to mucoidy in P. aeruginosa isolates from CF reported |
| 1993 | Correlation between CFTR mutation type and risk of acquiring P. aeruginosa infection shown |
| 1995 | Transgenic CF mice shown to have reduced ability to clear respiratory pathogens |
| 1995 | Relationship between CFTR mutations, decreased sialylation of cell surface glycoconjugates, and increased binding of bacterial Staphylococcus aureus to epithelial cells established |
| 1996 | Evidence presented suggesting a role for respiratory epithelia in P. aeruginosa killing and internalization and a link between CFTR defect and reduced clearance |

The dehydrated nature of airway secretions. Bacterial colonization in the CF lung resembles a biological jigsaw puzzle comprising the combined pathophysiological effects of the CF gene defect and pulmonary damage from previous infections with the ability of a relatively narrow spectrum of opportunistic pathogens to overcome normally highly effective lung defenses. This review will provide a brief introduction to the nature of CF and CF microbiology before focusing on mucoid Pseudomonas aeruginosa, the most common pathogen infecting CF patients, and on Burkholderia cepacia, arguably the most important and controversial new opportunistic pathogen to challenge microbiologists and the CF community. In a previous comprehensive review of the microbiology of pulmonary disease in patients with CF, Gilligan (141) discussed the virulence and laboratory investigation of the major nonpseudomonal CF pathogens, Staphylococcus aureus and Haemophilus influenzae and other less common pathogens including Stenotrophomonas maltophilia, Legionella species, various glucose nonfermenters, mycobacteria, and fungal agents, as well as the role of viruses, in particular that of respiratory syncytial virus, in predisposing to Pseudomonas aeruginosa colonization. In the last decade, some CF centers have reported an increasing incidence of Stenotrophomonas maltophilia (previously Xanthomonas maltophilia) infections. The clinical significance of S. maltophilia for CF patients is unclear, but in an extensive retrospective study, Karpati et al. (213) found that prolonged colonization was generally associated with poorer lung function, suggesting that this opportunistic pathogen has the potential to contribute to the slow, relentless damage in CF lungs.

The CF Host

CF is the most common inherited lethal disorder of Caucasian populations, with an incidence of approximately 1 in 2,500 live births and a carrier frequency of 1 in 25 (51). Throughout the world, more than 50,000 individuals are affected and many more carry the damaged gene that results in CF. The CF gene has been identified, cloned, and sequenced, and the structure of its protein product has been analyzed (218, 363, 367). The gene, on the long arm of chromosome 7, is 250 kb long, contains 27 exons, and encodes a transmembrane protein of 1,480 amino acids termed the cystic fibrosis transmembrane regulator (CFTR). CFTR functions as a chloride ion channel protein (108) and belongs to a large family of transmembrane proteins that also include P glycoprotein, the multidrug resistance gene product which reduces the efficacy of cancer chemotherapy by abnormal excretion of chemotherapeutic agents (464). Persons homozygous for mutant alleles of the CF gene have severe defects in chloride ion transport and have a characteristically salty sweat. The same imbalance in the cells lining internal organs leads to a buildup of sticky dehydrated mucus in male sex ducts, ducts of the pancreas, and the airways of the lungs. The glands behind the ducts continue to secrete, causing them to swell and form cysts. In the lungs, mucociliary clearance is impaired, resulting in persistent microbial colonization with intermittent episodes of debilitating and ultimately fatal infection. In most CF populations, approximately 70% of individuals have a deletion of a single codon in one of the nucleotide-binding folds of CFTR, which leads to the deletion of phenylalanine at position 508 (ΔF-508); by 1995, over 400 point mutations within the CF gene were described.

Following the discovery of CFTR, progress in CF research has been unusually rapid, and understanding of the microbiology of the CF lung has also benefited from these recent advances (Table 1). By 1992, mutant CF mice had been developed by targeted insertional mutagenesis (104, 109), and additional refinements have been added in more recent years (490). Shortly after the first CF mice were generated, the mutant gene was corrected in the mouse, and the first human gene therapy trials were performed, with adenovirus- and liposome-mediated CFTR gene transfer (54, 70, 469). Research with CF mice also began to provide a long-awaited clue to why the lethal CF gene should have survived in heterozygote carriers. Logically, any selective advantage conferred by carriage of a single copy of the gene should involve electrolyte transport across cell membranes in young Caucasians, perhaps by conferring increased protection against classic life-threatening diseases, such as tuberculosis and cholera (172, 212). Studies of CF mice have provided evidence that CF heterozygote mice have increased resistance to cholera, an infection of the intestinal mucosa in which profuse and potentially fatal diarrhea results from deregulation of electrolyte transport across the gut mucosa by the action of Vibrio cholerae toxin; if true for humans, this would indicate that the advantage of the carrier status outweighs the consequences of CF (136).

The development of a model of respiratory infection in transgenic CF mice (79) has also begun to address important and previously unanswered questions concerning lung disease. For example, experiments with repeated aerosol exposure to typical CF respiratory pathogens suggest that in the “leaky” CF mice, inflammation-mediated lung disease may result from an abnormal host response to such challenges rather than from CFTR dysfunction per se (79). In this context, it is perhaps important to note that the question whether bacterial pathogens cause inflammation or just aggravate the underlying problem has not been completely settled. In a recent study, Khan et al. (222) studied mediators of inflammation in bronchoalveolar...
lavage fluids in a group of 16 CF infants that included subjects as young as 4 weeks postpartum (mean age, 1 year). These authors reported increased interleukin-8 (IL-8) and neutrophil levels in CF infants compared with the control group although the cultures were negative for the usual CF-associated bacterial, viral, and fungal pathogens. Interestingly, airway macrophages from bronchoalveolar lavages of the CF infants were found to contain elevated IL-8 mRNA levels, suggesting that in addition to other cell types such as epithelial cells, the macrophage may be a contributing source of this neutrophil-recruiting chemokine in CF (362). Since CFTR is expressed in macrophages, albeit at low levels (486), the possibility that another consequence of a CFTR defect is altered macrophage function, including abnormalities in expression of chemokines such as IL-8, cannot be excluded at present. Regardless of whether the inflammation is initiated by a CFTR defect or provoked by infections with various pathogens, the latter process further aggravates the condition, resulting in the establishment of a vicious circle of chronic respiratory infection and inflammation. As a result, recurring episodes of pulmonary exacerbations cause irreversible tissue damage facilitated by protease-antiprotease imbalance (e.g., because of the excess of neutrophil elastase) (28). This leads to typical complications clinically manifested as bronchiolitis, atelectasis, hemoptysis, pneumothorax, fibrosis, and, over the course of many years during which this condition persists, respiratory failure and death (29, 469a).

**Microbiology of CF**

The susceptibility of patients with CF to pulmonary infection has been recognized since the earliest descriptions of the disease in the 1940s. Until the advent of antibiotic therapy, most CF patients died in infancy from staphylococcal infection. Today, most CF patients eventually succumb to the overwhelming sequelae of repeated pulmonary exacerbations arising from persistent *P. aeruginosa* colonization, typically over a period of 10 to 20 years. In the first bacteriological study of CF lung disease, postmortem cultures of lung tissue from 14 patients aged 1 to 40 months yielded *S. aureus* from 12 patients and single isolations of *H. influenzae* and *P. aeruginosa* (then called *Bacillus pyocyaneus*) (99). Today, the prognosis for newly diagnosed individuals has improved to the extent that most patients can expect to survive into early adulthood and the population of adults with CF almost equals that of children with CF (125).

However, there is a caveat to this progress. Aggressive antibiotic therapy with an increasing choice of antimicrobial agents has undoubtedly contributed to the control of infections due to *S. aureus* and *H. influenzae*. In contrast, although the development of antipseudomonal agents since the 1960s has been striking, anti-pseudomonal therapy remains palliative and seldom results in eradication. By early adulthood, most CF patients will have become chronically colonized by mucoid variants of *P. aeruginosa*. The precise mechanisms underlying the characteristic susceptibility of the CF lung to pseudomonal colonization is unclear, but accumulated evidence indicates a role for basic defects associated with abnormal CFTR function, including enhanced asialylated pseudomonal receptors on respiratory epithelia and impaired mucociliary clearance as a result of the dehydrating effects of electrolyte dysfunction combined with the tissue-damaging sequelae of previous bacterial infections.

In 1987, Stutman and Marks (434) reflected the opinion of many microbiologists and clinicians when they wrote, “The microbiology of lung disease in patients with CF is a sub-specialty unto itself.” Unequivocal statements on the pathogenesis, epidemiology, and treatment of infectious exacerbations in CF patients are difficult, but four general statements can be made.

(i) Infections are localized in the lungs, in particular the major and minor airways, rather than the alveoli. Mucociliary clearance is impaired by the viscid dehydrated nature of CF respiratory secretions, and localized infections at nonpulmonary sites or systemic infections are rare.

(ii) Pulmonary infections are associated with insidious and eventually chronic colonization by bacterial pathogens and episodes of acute debilitating exacerbations as a result of both bacterial and viral infections superimposed upon progressive lung disease.

(iii) The spectrum of bacteria, viruses, and fungi associated with CF respiratory infection is comparatively restricted. The microbial pathogens most commonly reported include *S. aureus*, *H. influenzae*, and *P. aeruginosa*. Susceptibility to a particular pathogen tends to be age related, with *S. aureus* appearing in infancy followed by *H. influenzae* in the early years and *P. aeruginosa* in adolescence.

(iv) Colonization by *P. aeruginosa*, in particular the emergence of mucoid alginate-producing variants of the colonizing strain, is recognized as a poor prognostic indicator; once established, these bacteria are seldom eradicated.

The evolving epidemiology of CF was clearly illustrated in 1993 during a comprehensive analysis of 17,857 patients. In her report, FitzSimmons (123) revealed that the median survival age of individuals with CF had doubled between 1969 and 1990, from 14 to 28 years. The most frequently reported respiratory pathogen was *P. aeruginosa*, cultured in specimens from 61% of all patients, ranging from 21% of those less than 1 year of age to more than 80% of those aged 26 years or older. Although it has long been recognized that the incidence of *P. aeruginosa* increases with age and also varies in individual CF centers (161), the figure of 21% for infants seems unexpectedly high and emphasizes the importance and rationale of strategies to prevent chronic *P. aeruginosa* infection by early antibiotic treatment (157, 161, 263, 455).

During the 1980s, additional concern arose in the CF community as a result of the emergence of other opportunistic microbial pathogens. By the early 1990s, multidrug-resistant, transmissible strains of *Burkholderia cepacia* (basonym *Pseudomonas cepacia*), an organism previously best known as a phytopathogen and only distantly related to *P. aeruginosa*, had emerged as a cause of considerable anxiety to the increasing population of CF adults and a major infection control challenge for CF carers (7, 141). In addition, the potential threat of *Stenotrophomonas maltophilia* (basonym *Xanthomonas maltophilia*) was questioned (143). Ironically, contemporary in vitro evidence of antifungal activity by *P. aeruginosa* and *B. cepacia* led to speculation that colonization by these bacterial pathogens may inhibit subsequent colonization by *Candida* and other fungal species (220).

An extensive literature on CF microbiology describes the spectrum of CF pathogens, their laboratory investigation, antibiotic therapy, vaccine development, and the major role played by host inflammatory reactions against mucoid *P. aeruginosa* in progressive lung disease. In this respect, the reader is directed to the early seminal review by Høiby (186) and later publications (141, 169, 187, 329, 337, 416, 468). This review will first focus on mucoid *P. aeruginosa*, the major CF pathogen, and in particular on the pathogenesis, genetics, and the regulation of the biosynthesis of pseudomonal alginate, arguably the most important microbial virulence determinant in CF lung infections; second, it will address the transmissibil-
ity and pathogenicity of *B. cepacia*, a formidable new challenge which has held the CF community to ransom during the last decade.

**MUCOID PSEUDOMONAS AERUGINOSA**

**Association with the CF Lung: an Historical Overview**

In microbial pathogenesis, there are few more striking examples of in vivo microbial adaptation than the asymptomatic colonization of CF lungs by typical nonmucoid *P. aeruginosa* strains and the subsequent emergence of mucoid forms during chronic debilitating pulmonary infection. Mucoid strains of *P. aeruginosa* were first cultured in 1927 from a patient with a fistulating abscess of the gallbladder (414). Subsequent reports of these unusual pseudomonads were sporadic and superficial (41, 57, 71, 180). In the first detailed study, Elston and Hoffman (113) noted an incidence of 1.7% of mucoid forms and reported several characteristics of the mucoid phenotype that were to become familiar in later years; these characteristics included reversion to the nonmucoid phenotype during in vitro culture and reduction or absence of the blue-green pigment pyocyanin, a classic diagnostic feature of most nonmucoid *P. aeruginosa* strains. It is interesting that at the time of these studies, mucoid forms of *P. aeruginosa* were not listed in Bergey’s Manual.

The first descriptions of an association between mucoid *P. aeruginosa* strains and chronic pulmonary colonization in patients with CF appeared in the 1960s (100–102, 195). In their seminal studies, Doggett and colleagues reported that such strains could be isolated in up to 90% of CF patients colonized with *P. aeruginosa*; in contrast, they were rarely cultured from other infections in humans, plants, or animals or from the wide range of environmental habitats associated with this ubiquitous saprophyte. At this time, evidence suggested that in patients with CF, primary asymptomatic colonization occurred with nonmucoid strains but that pulmonary deterioration followed later with the conversion to mucoidy and the development of antipseudomonal antibodies (46, 98).

Although mucoid forms of *P. aeruginosa* are characterized associated with and almost diagnostic of CF, it would appear that the mechanisms underlying the emergence and establishment of mucoid strains are not specific to CF but reflect the molecular regulation and biological properties of alginate and microbial adaptation to a parasitic mode associated with chronic infection. Mucoid *P. aeruginosa* can be isolated in up to 40% of *Pseudomonas*-positive sputa from non-CF patients with chronic bronchitis and other obstructive lung diseases (157), occasionally (up to 10%) from non-CF patients with chronic urinary tract infections (285), and more rarely from other sites in these patients (5, 100). Transition to mucoidy has also been demonstrated in a rat lung model during chronic colonization (477). Chemical analysis has confirmed that non-CF mucoid strains produce alginate with a structure similar to that produced by strains from patients with CF (5, 285, 375, 444).

All nonmucoid wild-type *P. aeruginosa* strains appear to have the genetic potential to give rise to spontaneous alginate-producing mucoid variants (162). This process becomes apparent when mutant strains that overproduce alginate are selected under appropriate environmental conditions, such as those present in the CF lung or in experiments carried out under growth-limiting conditions (420, 446). Interestingly, however, despite the large numbers of environmental *P. aeruginosa* strains isolated and studied during the last 50 years, to our knowledge no natural ecological niche for the overtly mucoid form of *P. aeruginosa* is known. Indeed, mucoid *P. aeruginosa* is so strikingly associated with CF that its culture from respiratory secretions has been suggested to be almost pathognomonic for the disease (361).

A major factor in epidemiological studies of mucoid isolates has been the availability and development of reliable typing methods, in particular techniques that could cope with the viscous nature of the bacterial alginate and loss of O-antigenic components of bacterial lipopolysaccharide (LPS) (447). In 1994, an extensive multicenter study reported restriction fragment length polymorphism (RFLP) profiles of rRNA regions to be the most appropriate method for typing CF isolates of *P. aeruginosa*.

Few studies have attempted to identify the clinical or environmental sources from which individuals with CF acquire *P. aeruginosa* and the primary site of colonization in the patient (31, 366, 454). Tredget et al. (454) reported that the incidence of unusual pyocyanin types of *P. aeruginosa* from CF patients is significantly higher (P < 0.001) than that observed in non-CF isolates, suggesting that a subpopulation of *P. aeruginosa* may have a predilection for CF lungs. Subsequently, genomic RFLP analysis of environmental and clinical isolates has shown for the first time that clones of *P. aeruginosa* are prevalent in a broad geographic area and that potentially infectious *P. aeruginosa* strains for individuals with CF are present almost everywhere (366). Most recently clustering analysis of ribotyping data has shown that CF isolates present a lower degree of heterogeneity of rRNA operons than that of *P. aeruginosa* isolates from non-CF infections (275). Interestingly, a comparative study of *P. aeruginosa* PAO1 and other strains showed that the highest mucinophilic and chemotactic responses were in the “hydrotherapy pool strain,” supporting the hypothesis that these properties play a role in the initial stages of pulmonary colonization (314). With respect to the anatomical site of initial colonization in patients with CF, evidence suggests that early colonization is in the respiratory tract, including sinuses, and not the gastrointestinal tract; the latter, however, may become contaminated with mucoid forms following respiratory colonization (1, 106, 418).

The nature of the putative colonizing factors for nonmucoid *P. aeruginosa* in the CF lung is not completely clear. Early observations by Woods et al. (476) suggested (i) that nonmucoid strains adhered significantly better to buccal cells from CF patients than to cells from non-CF patients, (ii) that increased bacterial adherence varied directly with the loss of protease-sensitive fibronection from the cell surface, as well as increased levels of salivary proteases in CF patients, and (iii) that adhesion of nonmucoid *P. aeruginosa* to non-CF buccal cells could be promoted by prior exposure to trypsin. These results indicated that CF patients might be compromised by dietary factors, particularly as pancreatic supplements might provide a source of proteolytic activity. However, the hypothesis of a buccal cell reservoir was not supported by the results of a prospective in vivo study in patients with CF, which failed to demonstrate buccal cell colonization even in patients with high respiratory populations of the organism (167). In addition to bacterial colonizing factors, it has always seemed likely that factors associated with the CF host might help to explain the predilection of *P. aeruginosa* for the CF lung. Recent evidence from studies of the CFTR mutation genotype and the age of onset of chronic colonization with *P. aeruginosa* suggests that the risk of *P. aeruginosa* colonization is highest in patients who are compound homozygotes, when one mutation is ΔF-508 while the other is another severe CFTR allele (238). A variety of mechanisms for initial colonization had been considered in the past: adhesion to tracheobronchial mucin (357), inactiva-
tion of ciliary beat by pyocyanin and rhamnolipid (210, 211, 472), the role of sialic acid in saliva-mediated aggregation of P. aeruginosa (229), etc. More recently, the effects of CFTR mutations on the surface receptors in the context of adhesion or internalization of the pathogens by epithelial cells and their ability to clear or kill P. aeruginosa have received more attention (see the section on the relationship between conversion to mucoidy and chronic colonization of the CF lung, below).

For many years, the transition within the CF lung of the original P. aeruginosa colonizer to the mucoid phenotype was generally accepted in the absence of documented scientific evidence to eliminate the alternative explanation of de novo colonization with mucoid forms. One of the factors hindering scientific confirmation of the transformation is the time interval between regular bacteriological assessments in CF patients, which in some CF centers may occur at 3-monthly intervals. If the first P. aeruginosa cultured from a patient’s sputum is mucoid, it is difficult to determine the time interval for conversion; indeed, such a result could be taken as evidence that primary colonization occurs with already mucoid strains. In the absence of an environmental reservoir for mucoid P. aeruginosa, primary colonization would require cross-infection between CF patients. This mode of acquisition undoubtedly occurs but is generally limited to close and frequent contacts such as occurs between siblings (27). Recent epidemiological data obtained from pyocyanin typing and RFLP analysis have provided convincing evidence that unrelated CF patients are usually colonized with different strains and that once colonized, individual patients harbor the same strain for long periods (145, 171, 365). Bacterial fingerprinting has also provided evidence that hydrotherapy pools can provide at least one environmental source of acquisition of P. aeruginosa and has provided evidence for transition of the initially colonizing nonmucoid form to mucoid mutants within individual CF patients within 3 months (131, 168).

The consequences of alginate biosynthesis by mucoid P. aeruginosa in the CF lung have been extensively reviewed by Pedersen (329); they include the establishment and intractability of bacterial colonization and the progressive pulmonary inflammatory disease which follows. The pathogenic roles of alginate will be systematically covered in the section on alginate and P. aeruginosa pathogenesis in CF, below, but some mention of more traditional views will be made here. One theory states that a major function of alginate may be in the formation of biofilm microcolonies (Fig. 1) that can be readily demonstrated in the respiratory tract of CF patients (243) and in vitro (66, 167). Mucoid-microcolony formation may have profound effects on the ability of the host to cope with the pathogen, and a concept of frustrated phagocytosis which is not capable of clearing the organism has been put forward (157, 167, 404). As a result, the large numbers of neutrophils are attracted by inflammatory chemotaxis, with the ultimate consequence of immune system-mediated tissue damage resulting indirectly from immune complexes and directly from excessive neutrophil elastase activity (52, 105, 157, 165, 187); the latter may also be responsible for degradation of immunoglobulins, complement components, and their cognate receptors on phagocytic cells (416). Alginate biofilm may also present a polyamionic barrier to antimicrobial agents (9, 315), and the slow iron-limited growth of bacteria within the biofilm may reduce their susceptibility to antibiotics (37). It has been shown that alginate enhances the attachment of mucoid strains to cilia of respiratory epithelial cells and to the glycoprotein-rich mucociliary blanket (13, 14, 358). However, the available evidence indicates that alginate probably does not act as a major adhesion ligand (351) and that adhesion alone cannot explain the persistence of mucoid P. aeruginosa in the CF lung. Some individuals with CF appear to be protected against pseudomonal colonization by harboring opsonophagocytic killing antibodies directed against the microbial alginate, sometimes referred to as mucoid exopolysaccharide (343). Most individuals with CF, however, do not appear to benefit from such protection, and a more typical scenario is that opsonins deposited on the bacterial surface or within the alginate biofilm may not interact efficiently with receptors on phagocytic cells (176, 344).

At this stage of our review, we have discussed the historical background of the association of P. aeruginosa and the CF lung and introduced recent considerations attempting to explain a host-dependent susceptibility to colonization with P. aeruginosa in individuals with CF. Initial colonization, however, is only the first stage in a complex host-pathogen relationship in which an opportunistic pathogen eventually converts to a pulmonary parasite aided by unusual bacterial adaptation. Before discussing in detail the major phenotypic change, alginate biosynthesis or mucoidy, it is also important to stress that CF isolates of P. aeruginosa often exhibit other phenotypic changes.

FIG. 1. Electron micrographs of postmortem alveolar samples from a CF patient infected with P. aeruginosa. B, bacterial cells; S, fibrous mass of bacterial exopolysaccharide; T, alveolar tissue surface. Bars, 0.1 μm. Reproduced with permission from reference 243.
including increased serum sensitivity, loss of O-antigen components of LPS, and a most unusual hypersusceptibility (at least in vitro) to a range of antibiotics. The reader is referred to several recent reviews for discussion of this subject (94, 141, 157, 329, 416).

**Relationship between Conversion to Mucoidy and Chronic Colonization of the CF Lung**

*P. aeruginosa* colonizes the CF lung only after a succession of other pathogens, as mentioned in previous sections. The relevance of a history of previous bacterial colonization, antibiotic therapy, and viral infections is not completely understood, but a typical pattern can be discerned (46, 333). For example, according to Pedersen et al. (333), in the age group under 4 years, over 70% of CF patients included in the analysis had a staphylococcal infection while only slightly over 10% of the patients had *P. aeruginosa* colonization. This proportion begins to reverse in the age group of 5 to 9 years, and in young adolescents the prevalence of *P. aeruginosa* colonization is 80% compared with 30% for staphylococcal isolates (333). A comprehensive report by Fitzsimmons (123) is in agreement with these data; in that study, 21% of the patients under 1 year of age were colonized with *P. aeruginosa* whereas the age group over 26 years showed 80% incidence of infection with this pathogen.

The host and bacterial factors leading to colonization with *P. aeruginosa* and the relationship between conversion to mucoidy and the establishment of chronic infection are still not completely understood. The available data and experimental results are mostly empirical, making it difficult to draw firm conclusions. Nevertheless, these issues must be considered before the molecular mechanism of conversion to mucoidy is discussed, since it is difficult to understand this phenomenon outside the context of the lung disease in CF. Below, we describe a model in which the host and pathogen "conspire" (84) to bring about the chronic colonization with *P. aeruginosa*.

Recent studies and some long-believed notions indicate that the following phenomena may be of particular significance for microbial colonization and prevalence of *P. aeruginosa* in CF: (i) the effects of *CFTR* mutations on the movement of electrolytes and water across the surface epithelium in airways and submucosal glands (228, 471); (ii) the collateral abnormalities due to the pleiotropic effects of *CFTR*, which may affect the glycosylation of surface receptors (glycolipids and glycoproteins which appear to be undersialylated) on the apical membranes of epithelial cells (2, 17, 107, 379); (iii) the ability of *P. aeruginosa* to adhere to the undersialylated receptors (196, 235, 236, 379, 380); (iv) the recent observations that epithelia in CF patients may be impaired in direct killing of *P. aeruginosa* (407a) or may be inefficient in its internalization and removal via desquamation (341a), thus reducing the clearance of the pathogen; (v) the innate resistance of *P. aeruginosa* to antibiotics (46, 192, 249, 250, 329, 342, 349); and (vi) the genetic capacity (133, 277, 279) of this organism to convert into the mucoid, alginate-overproducing form. This last phenomenon is believed to promote transition to the chronic phase of infection (228, 329).

The first aspect of the effects of *CFTR* mutations is probably applicable to all pathogens in CF, including those antecedent to *P. aeruginosa*. The ineffective chloride transport due to *CFTR* lesions causes hyposecretion of electrolytes and water in lower airways and hyperabsorption of electrolytes and water in central airways. Under conditions of reduced hydration levels (471), the mucociliary escalation may not be as efficient as normal in removing the bacteria and other pathogens trapped in the mucous blanket (228). Since *P. aeruginosa* usually follows other pathogens, including viral infections (206), it is possible that preexisting inflammation (222) and the inability to increase mucociliary clearance in response to stimulation (471), as demonstrated in a study with β-adrenergic agonists (305), brings about progressive colonization with various organisms including *P. aeruginosa*. However, these views, expressed by the majority of investigators and summarized by Koch and Hoiby (228), remain to be reconciled with the surprising finding that *CFTR* is only marginally expressed in the epithelial cells of airways, including the bronchioles, where the disease usually starts (115). The predominant site for *CFTR* expression is in the submucosal glands (115), which are not present in bronchioles. However, it has been shown in a transgenic mouse model (*CFTR*<sup>ΔF508HGU</sup>) of respiratory infection with *S. aureus* and *B. cepacia*, that the CF mice are more susceptible to bacterial colonization than are their heterozygous littermates (79) despite the relative paucity of submucosal glands in the mouse. Considering this observation and the finding that the tracheal epithelium in *CFTR*<sup>ΔF508HGU</sup> mice is not hyperabsorbing, in contrast to human CF airways (408), it appears that the relative contributions to infection of hydration and other local factors remain to be precisely determined.

The next issue of relevance for *P. aeruginosa* and perhaps also for *S. aureus* infections is the presence of altered glycoconjugates on the surface of CF epithelial cells that may act as receptors for these bacteria. It has been shown that *CFTR* mutations cause undersialylation of glycolipids and glycoproteins on the apical membranes (17, 107, 379), which provide efficient receptors for *P. aeruginosa* adhesins (196, 235, 236, 351, 379, 489). *P. aeruginosa* pilin, the major adhesin of this organism (379, 478), and whole *P. aeruginosa* cells (196) bind to asialoganglioside 1 (aGM1) but not to the sialylated ganglioside GM1, which is not a good receptor for *P. aeruginosa* (235, 236). The amount of aGM1 is increased on respiratory epithelial cells from CF patients (approximately fourfold) (379). Although the precise molecular link between the genetic defect in CF and undersialylation of glycolipids is not known, proposals have been made for the role of the *CFTR* chloride channel in acidification of intracellular organelles including the trans-Golgi network. According to Al-Awqati et al. (2), chloride channels may facilitate acidification of endosomal and other relevant intracellular compartments by supplying counterions (Cl<sup>−</sup>) to balance the H<sup>+</sup> influx into the intracellular vesicles. According to this model, which takes into account a somewhat acidic pH optimum (6.0) of sialyltransferase in the trans-Golgi network, reduction in acidification (2) or some other effects of *CFTR* on a relevant intracellular compartment may cause decreased sialylation of surface glycolipids and proteins. Interestingly, Dosenjih et al. (107) have reported that while they too could detect reduced sialylation of membrane glycoconjugates of ΔF-508-expressing cells, a complete absence of *CFTR* expression did not cause this effect. This suggests that peculiarities of ΔF-508 trafficking and possibly that of some other mutants may interfere with glycosylation of proteins in such cells. Thus, this consequence of *CFTR* mutations combined with the effects on the mucociliary escalator provide a reasonable working model for the initial colonization and tropism of *P. aeruginosa* for CF.

In more recent developments, Smith et al. have demonstrated that primary cultures of airway epithelial cells from CF patients were unable to kill 30 to 300 CFU of *P. aeruginosa* applied in small volumes (20 nl) to the surface of the continuous polarized sheet of cells grown on permeable filter supports with air on the apical surface (407a). In contrast, normal epithelia were able to kill the bacteria. In a different but in-
triguing approach, Pier et al. (341a) reported that CFT1, an airway epithelial cell line derived from a CF patient homogygous for Δ508 CFTR, displayed inefficient internalization of P. aeruginosa compared with the same cell line expressing a functional wild-type human CFTR. These authors proposed that this deficiency could cause reduced uptake of the pathogen by the epithelial cells, thus impairing its removal via desquamation, fashioning their model after innate clearance mechanisms important in protection against bladder infections.

While some of the proposed models may appear contradictory at first, it is possible that all mentioned processes and additional phenomena contribute to the initial colonization. In the case of P. aeruginosa, the recurring antibiotic treatments may also contribute to the ability of this organism to efficiently supplant the pathogens causing antecedent infections because of the innate resistance of this bacterium to antibiotics (249, 250, 342, 349).

The final phenomenon to be discussed represents perhaps the most peculiar aspect of Pseudomonas-CF host interactions. The establishment of chronic infection with P. aeruginosa indicates a poor outlook for patients (329, 332) and is the stage in which conversion to mucoidy may play a particularly important role. Some investigators discern two phases in the colonization with P. aeruginosa (206, 228, 329). The first stage is an insidious infection with the intermittent presence of P. aeruginosa (228, 329). Johansen and Hoiby (206) maintain that not every initial episode of P. aeruginosa infection results in the establishment of chronic condition. These authors define the initial phase of intermittent colonization as a period characterized by only sporadic isolation of the bacterium and detection of antibodies to no more than two P. aeruginosa precipitins in the serum. This phase does not cause significant decline in pulmonary function (219) and can continue for variable periods (0 to 5.5 years) (206). The next stage in the natural progression of the disease is the establishment of chronic P. aeruginosa infection. Johansen and Hoiby define this phase as the condition in which bacteria are cultured continuously for at least 6 months and there is an increased antibody response to P. aeruginosa precipitins (206). The establishment of chronic infection coincides with the conversion to mucoid phenotype (228, 329). Pedersen argues (329) that overwhelming (but nevertheless circumstantial) evidence exists that mucoid P. aeruginosa is associated with the transition to the chronic phase of infection (185, 188). In the majority of CF patients, this condition remains permanent (329). Once the chronic infection is established, the initially acquired P. aeruginosa strain remains present at all times, as, for example, has been shown in a longitudinal study with patients monitored over 8 years by Dau1-Spe1 macrorestriction analysis of sequential isolates (365). This has been corroborated in studies involving arbitrarily primed PCR in addition to macrorestriction typing of CF isolates (221).

There is no clearly defined interval between the initial intermittent colonization and transition to mucoid forms. In one of the first comprehensive studies describing the association of mucoid P. aeruginosa and CF (101), Doggett et al. reported that in the majority of patients monitored, it took 3 to 10 months between the first culture positive for P. aeruginosa and the detection of mucoid forms. However, in 3 of 16 patients monitored in that study, the period was less than 1 month, while in some patients, it took years to observe mucoid strains. More recent studies concur with these early observations. Hoiby and colleagues report periods averaging 1 year for the transition from intermittent to chronic infection, but in 10% of the patients the first isolate was mucoid (329, 332) while in some cases the whole process took 5 years (206). Mahenthiralingam et al. have reported a mean duration of infection until isolation of mucoid P. aeruginosa of 3.37 (±1.76) years (270). Govan and Nelson report conversion in as little as 3 months (168).

The variable timing of the emergence of mucoid forms is consistent with the random mutations causing conversion to the mucoid phenotype followed by the selection of mucoid strains in the CF lung environment. Govan and Fyfe (162) determined that spontaneous mutations causing mucoidy occur at a frequency of 10⁻¹⁴, compatible with the recent definition of such alterations as point mutations (frameshift and nonsense) (278). Considering the low frequency of spontaneous conversion to mucoidy, the selective advantages of mucoid forms in the CF lung must be substantial. The clonal relationship between the initially colonizing strains and their mucoid derivatives has been recently established by classical typing and modern fingerprinting techniques (168).

Once the mucoid forms emerge, it is common to detect the presence of both mucoid and nonmucoid forms in the patient sputa (448). Furthermore, not all mucoid strains are identical, and heterogeneity of their phenotypic appearance on different media has been noted (353). For example, of the CF isolates analyzed in this early study, one-third were mucoid on all six media tested while two-thirds were nonmucoid on one or more of the media used (353). This points out another commonly ignored caveat, i.e., that not all CF isolates that appear nonmucoid are truly nonmucoid but, instead, may belong to the medium-dependent category of mucoid mutants. We have observed over the years that if a strain is nonmucoid on Pseudomonas isolation agar (Difco), it will not be mucoid on any other media tested, and we use this criterion to decide whether a given isolate is nonmucoid. This is in keeping with the observations reported by Pugashetti et al. (353). The molecular basis for variability in expression of mucoid phenotype and related phenomena will be addressed below in sections concerning the molecular mechanism of conversion to mucoidy.

**Alginate and P. aeruginosa Pathogenesis in CF**

Mucoid P. aeruginosa strains owe their appearance to the profuse production and secretion of the exopolysaccharide alginate (Fig. 1 and 2). The emergence of mucoid P. aeruginosa often correlates with a poor patient outlook (185, 188, 228, 329, 330). Poor lung function in CF patients is associated with increased antibody titers to alginate (330). Alginate is present in expectorated sputum and, according to one study, averages 35.5 µg/ml (range, 4 to 101 µg/ml) in patients with P. aeruginosa infection (334). Antibodies to alginate can be detected in all CF patients with chronic P. aeruginosa infection (330) independent of whether mucoid P. aeruginosa forms can be isolated from the lungs of these patients at the time of analysis. Alginate has been ascribed a multitude of functions in the context of the pathogenesis of the respiratory tract in CF, and there have been several meritorious reviews on this subject (141, 157, 329, 416). A compilation of the proposed pathogenic properties of this exopolysaccharide in the context of CF is given in Table 2.

The known or proposed pathogenic roles of alginate, in the form of a mucoid bacterial coating or as a free substance, can be classified in three general categories: (i) the mucoid capsule-like material serves as a direct barrier against phagocytic cells and effective opsonization; (ii) alginate may function as an immunomodulatory molecule; and (iii) alginate production may play a role in biofilm-related phenomena, including contribution to adhesion and antibiotic resistance.

P. aeruginosa grows in the CF lung in microcolonies (Fig. 1)
(243), which represent bacterial biofilm consisting of cells embedded in the exopolysaccharide alginate (419). This mode of growth has numerous repercussions for the course of the respiratory disease in CF. Alginate most certainly interferes with the phagocytosis of *P. aeruginosa*. The interactions of *P. aeruginosa* with phagocytic cells are complex and, in the absence of opsonins (complement or antibodies), involve several attachment factors (pilus and nonpilus adhesins) on the bacterial side, while stimulation of the phagocytic cells by fibronectin and glucose and modulation of receptors on the phagocyte side by LPS (217, 227, 270, 304, 421, 422) have been reported. Alginate interferes with both nonopsonic (52, 234, 319) and opsonic (15, 16, 293, 392, 404) phagocytosis. This property is further enhanced by the ability of pseudomonal alginate to quench reactive oxygen intermediates and scavenge hypochlorite generated by phagocytic cells (245, 405). Curiously, while alginate is consumed during in vitro reactions with chemically generated reactive oxygen species, as monitored by viscosity reduction (245), its degradation could not be demonstrated in direct reactions with phagocytic cells (406), despite the generation of free radicals by the macrophages and neutrophils used in that study. Thus, the copious amounts of alginate produced by mucoid mutant strains may exceed the capacity of the phagocyte degradative systems, overpowering the defense systems.

The effects of alginate on phagocytic cells are not limited to the above phenomena. Alginate most probably interferes with the opsonization process. Opsonic antibodies directed against alginate appear to afford protection, as seen in experimental models and as suggested by observations that protective antibodies may be a factor contributing to the healthy status of the rare CF patients who escape chronic colonization with *P. aeruginosa* (4, 307, 343, 344). However, opsonins such as complement deposited on the bacterial surface and antibodies against other immunodominant antigens may be masked by the alginate coating and may thus become inaccessible for interactions with cognate receptors on phagocytic cells. Although alginate is a bacterial product, it does not stimulate neutrophil chemotaxis; instead, it inhibits complement-driven (426) and N-FMLP-induced (334) chemotaxis. In some instances, alginate affects the oxidative burst in phagocytic cells, as monitored by chemiluminescence and oxygen consumption (23, 234, 405). In low doses (40 μg/ml, which is close to the average amounts detected in CF sputa [35.5 μg/ml] [334]), alginate slightly increases free radical production in mouse peritoneal macrophages stimulated by viable *P. aeruginosa* (405). In keeping with the mild stimulatory effects of the physiological doses of alginate, others have reported that mucoid strains stimulate neutrophils more than nonmucoid strains do (23) whereas CF sputa in general cause enhanced oxidative burst (223). However, in very high doses (e.g., 0.5 or 1 mg/ml), alginate has strong inhibitory effects (405). According to some investigators (234), mucoid strains are inferior inducers of oxidative burst compared with the nonmucoid bacteria. Perhaps differences in alginate doses, cell types, or growth conditions and preparation of bacterial cells for the assay may explain the discrepancies in the interpretation of the observed effects of mucoid on the respiratory burst in phagocytic cells (223, 234). It is also worth noting that biofilm-grown *P. aeruginosa* induces a lower oxidative burst in neutrophils than does free planktonic *P. aeruginosa* (202); this may be more relevant for the in vivo situation (243) than are the results of investigations with cells cultured by conventional bacteriological techniques.

Alginate suppresses lymphocyte functions (272). When alginate is used in high doses (but not in low doses) for immunization in mice, it elicits CD3<sup>+</sup> CD8<sup>+</sup> major histocompatibility complex-unrestricted cytotoxic T cells that selectively kill hybridomas producing opsonic but not nonopsonic antibodies (345). This cytotoxic activity, which is dependent on the presence of immune complexes, may explain the finding that the dose and size of alginate polymer used for immunization studies in mice or human volunteers may be critical parameters determining whether an immunization protocol will elicit opsonic or nonopsonic antibodies (138, 339). Furthermore, mitogen activity of alginate (73), in combination with a similar activity of other *Pseudomonas* antigens, e.g., LPS, may contribute to the hypergammaglobulinemia associated with clinical deterioration in CF (340, 470). In this context, it is significant that alginate-specific immunoglobulin A (IgA) and IgG antibodies are a correlate of poor lung function in the majority of CF patients (330).

Alginate has no appreciable activity in stimulating the alternative complement pathway (334); successful opsonization is dependent on antibodies (4, 343) and activation of complement by the classical pathway (16). Alginate elicits production of proinflammatory cytokines, IL-1 and tumor necrosis factor...
TABLE 2. Proposed roles for alginate in the pathogenesis of the cystic fibrosis lung

| Function or property                          | Comments                                      | Reference(s) |
|----------------------------------------------|-----------------------------------------------|--------------|
| Inhibition of phagocytosis by neutrophils and macrophages | Nonopsonic                                     | 52, 234, 319 |
| Hypochlorite scavenging                       | Opsonic                                        | 15, 16, 293, 392, 404 |
| Quenching of reactive oxygen intermediates   | Acetyl groups important                        | 245          |
| Interference with effective opsonization      | Opsonic antibodies against alginate are protective | 405          |
| Suppression of oxidative burst in neutrophils | In biofilms; free alginate in low doses inhibits | 274, 307, 343, 344, 463 |
| Suppression of neutrophil chemotaxis         | Complement or bacterial products driven chemotaxis | 334, 426 |
| Suppression of lymphocyte functions          | Acetyl groups important                        | 272          |
| Suppression of opsonic antibody production   | Immune complex-dependent cytotoxic T lymphocytes | 138, 345 |
| Mitogen activity and polyclonal B-cell       | Possibly related to hypergammaglobulinemia; associated with clinical deterioration | 73, 340, 470 |
| stimulation                                   | Antibodies also detectable in patients infected with nonmucoid strains only | 330          |
| Correlation of IgG and IgA against alginate  | Mannuronate-rich polymer more active           | 73, 322, 323, 415 |
| and poor lung function                       |                                               |              |
| No activation of alternative complement      |                                               |              |
| pathway                                       |                                               |              |
| IL-1 and tumor necrosis factor alpha         | See Fig. 1                                    | 243, 419     |
| induction                                    |                                               |              |
| Biofilm formation/microcolony mode of growth | Mannuronate-rich polymer more active           | 243, 419     |
| in vivo                                      |                                               |              |
| Resistance to antibiotics                     | Innate and biofilm mode of growth related     | 9, 20, 162, 315, 316 |
| Adhesion                                     | Variable; possibly not a major ligand; indirectly as biofilm | 14, 56, 103, 273, 286, 351, 358 |
| Other                                        | Nutrient scavenging; protection from dehydration; viscosity of sputum and gelling with Ca2+; interaction with phosphorylcholine | 137, 233 |

For alginate, the role in CF is only now beginning to be explored (36, 237, 317, 336, 396, 424, 437). Alginate also stimulates the release of IL-8 from epithelial cells (22), possibly contributing to the recruitment of neutrophils which dominate inflammation of the airways in CF (18, 205, 222).

Other roles of alginate include variable observations regarding adhesion (14, 56, 103, 286, 351, 358), recently reviewed by Prince (351), and antibiotic resistance (9, 20, 162, 315, 316), extensively covered in a review by Pedersen (329). It is likely that alginate contributes to the “stickiness” of Pseudomonas biofilms, but, according to some recent reports, it may not play a role as a significant primary adhesin (351). Its role in antibiotic resistance is somewhat controversial, but it is likely that the slow bacterial growth or some other characteristics of aged biofilms (9) and interference of exopolysaccharide barrier with antibiotic penetration (240) contribute to the inability to eradicate P. aeruginosa by even the most aggressive antibiotic treatments.

An additional, potentially significant characteristic of alginate is its transition from sol to gel states in its interactions with divalent actions and organic molecules. For example, alginate can form a gel (Fig. 2) in the presence of Ca2+ (137, 167). Pederson (329) and Ertesvig et al. (118) have recently discussed the effects of the homopolymeric guluronate blocks on the gelling properties of alginate in the presence of divalent cations such as Ca2++. The buckled chains of polyguluronic blocks, such as those found in abundance in the exine coat layer of the dormant cysts in Azotobacter vinelandii (325, 376), are better chelators of Ca2+ than is the ribbon structure of the polymannuronic chains. Since pseudomonal alginites lack polyguluronic blocks, this is expected to reduce its gelling capacity. Pederson questions (329) whether P. aeruginosa alginate, which, according to some sources, starts to precipitate at Ca2+ concentrations of 3 mM, contributes in this context to the viscosity of CF secretions. Although P. aeruginosa alginate forms clearly visible precipitates at 3 mM Ca2+, while the estimated concentration of Ca2+ in the CF sputum may be below this value (ranging from 0.5 mM to 1.8 mM) (225), microcospically visible microgels can be detected at 1 mM Ca2+ with P. aeruginosa alginate (159a). It is perhaps noteworthy that in one early report, patients with P. aeruginosa infections showed lower concentration of Ca2+ in their sputum (225) than did those colonized with S. aureus, which could be related to chelation of Ca2+ by alginate or some other physiological processes.

In a similar example of interaction between alginate and the local environment in the CF lung, phosphorylcholine, which is a phospholipase C (PLC) digestion product of the major lung surfactant phosphatidylycholine, interacts with alginate in a specific fashion, causing gelling and gradual formation of capsule-like structures (233). Alginate plays a role in protection against environmental stress in A. vinelandii (280, 325), and a similar role for alginate in P. aeruginosa in noninfectious sites is likely. The latest uncovering of the genetic elements which control conversion to mucoidy in P. aeruginosa (278, 279) indicates that the alginate system is an integral part of a broader defense system protecting bacterial cells against environmental stress in natural environments. The mutants overproducing alginate are most probably selected in the CF lung on the basis of their multiple properties enhancing P. aeruginosa survival in this hostile environment (93, 94).

The list of the proposed roles for alginate in the P. aeruginosa pathogenesis in CF continues to grow. This underscores several relevant points. (i) On the basis of the available information, a decisive function for alginate cannot be positively singled out at this stage, and it appears that the role of alginate production in pathogenesis may be a combination of multiple
effects. (ii) There may still be room for uncovering a more subtle but potentially critical function that has not been appreciated or has escaped detection. (iii) It is likely that overproduction of alginate may be only one manifestation of the upregulation of the stress response system (see later sections) involved in the defense of *P. aeruginosa* from environmental insults. These putative defense functions coregulated with alginate are only now beginning to be explored (32, 279, 486a, 488). The possibility that there are additional properties of mucoid mutants relevant for the pathogenesis in CF and related to the upregulation of the stress response system controlling conversion to mucoidy (93, 94) warrants future investigations. (iv) There is also a need for studies with defined isogenic mutants aimed at reexamining the role of alginate and, separately, testing the possibly alginate-independent roles of the stress response systems coregulated with mucoidy in a relevant animal model. Nonetheless, it is important to emphasize that mucoid mutants are selected in the CF lung (278) and that the fact that these strains have upregulated stress response systems (see further sections for details) underscores the importance of these changes for the long-term survival of the bacterium in the CF lung. The recent demonstration of the isolation of mucoid *P. aeruginosa* from a chronically infected rat (412, 477) is in keeping with the existence of selective pressures in the chronically colonized lung that favor either mucoid phenotype or overexpression of specific stress response systems.

**Role of *P. aeruginosa* Alginate in Other Ecological Niches**

The ability of *P. aeruginosa* to synthesize the exopolysaccharide alginate has received attention primarily because of its connection with CF. However, all wild-type *P. aeruginosa* strains have the genetic capacity to synthesize alginate but normally produce only very small amounts of this polymer (5, 243, 338). The antibody response to alginate detected in patients apparently infected with only nonmucoid *P. aeruginosa* (330) supports this notion but cannot be taken as a proof, since a history of conversion to mucoidy with subsequent reversion to nonmucoidy cannot be excluded in such cases. The role of alginate in natural environments other than CF has not been studied as extensively as in the context of the CF lung, except that it is believed to participate in the formation of biofilms (33, 80, 243), which are the preferred mode of *Pseudomonas* growth in natural environments (67). In light of the latest developments in understanding the genetic systems controlling alginate production (93, 94, 276, 279), this polymer probably plays important protective and homeostatic roles. Alginate synthesis is not restricted to *P. aeruginosa*, and the ability to produce similar or identical exopolysaccharides has been demonstrated in a variety of pseudomonads (121, 148, 163, 224, 321). In *A. vinelandii*, protection against environmental stress involves alginate production, as manifested by the process of encystment, which involves the formation of dormant cells (cysts) with outside layers (intine and extine) containing alginate (325, 376, 435).

**Alginate Biosynthesis**

Alginate is a linear copolymer of (1→4)-linked β-1-mannuronic acid and its C-5 epimer α-L-guluronic acid (253, 254). There have been numerous studies on its chemical composition, and many of these properties have been recently reviewed (137). Pseudomonal alginites are similar to the polymer extracted from seaweeds but are different because of the presence of O-acetyl groups (254). There are no detectable poly-L-guluronate blocks in *P. aeruginosa* alginate, which may be of significance regarding the question whether guluronate is incorporated into alginate or is generated by epimerization of some residues at the polymannuronic acid level. The mannan-to-guluronic acid (M/G) ratio affects the gelling and physiological properties of alginate (138, 281, 428). Moreover, it has been reported that alginites rich in mannanuronic acid induce the inflammation cytokines IL-1 and tumor necrosis factor alpha at a higher rate than do guluronate-rich polymers (415). In one report, it was found that among alginites from nine different CF isolates, the average uronic acid content was 72%, acetylation was 16%, and the average M/G ratio was 4.7 (331). In a recent report, Tatnell et al. showed that the defined mucA mucoid mutants (see below) make similar alginites with M/G ratios of 1.4 to 2.6 (444). However, another report (353) showed a wide range of M/G ratios, which varied from 99 to 0.8. Acetylation (O-2, O-3, or O-2,3 on mannanuronic acid residues) also determines various biological properties of alginate, ranging from its role in scavenging of reactive oxygen intermediates to its effects on lymphocyte and neutrophil activity (245, 275).

The alginate biosynthetic pathway (Fig. 3) closely resembles that proposed for *A. vinelandii* (347). The corresponding enzymatic activities have been demonstrated in *P. aeruginosa*, initially by Piggot et al. (346), followed by in-depth analyses by others using cloned genes and purified enzymes (recently reviewed in references 283 and 284). The flux of sugar precursors
Many of the enzymes in the alginate-biosynthetic pathway have been characterized (Table 3), and since several recent reviews (283, 284) cover their biochemical and physicochemical properties in significant detail, that information will not be repeated here. The majority of enzymes involved in the synthesis and modifications of the alginate polymer are encoded by the large cluster of genes at 34 min of the P. aeruginosa chromosomal map (77) (Fig. 4). This chromosomal region used to be referred to as “the alginate biosynthetic gene cluster” or “Pseudomonas aeruginosa alginate gene region II” (77, 467). However, because of the recent demonstration (68, 150, 484) that many of the enzymes involved in alginate synthesis also participate in LPS biosynthesis (see the next section), this chromosomal region can be no longer considered entirely alginate specific. Notwithstanding these nomenclature refinements, the cluster at 34 min contains several genes essential for alginate synthesis and its modification (listed in the order of transcription): algD, encoding GDPmannose dehydrogenase, which catalyzes the final step in the precursor biosynthesis (86, 87, 354, 371); algG, encoding the epimerase which converts mannuronic residues into guluronate (60, 128); algX, encoding a 53-kDa polypeptide whose function is unknown but which appears to be necessary for alginate production (303); algL, encoding an enzyme termed alginate lyase for its ability to depolymerize alginate but which may also be required for alginate polymerization (34, 303, 385); algF and algD, two newly identified genes required for alginate acetylation (129a) (interestingly, the predicted gene product of algD is 30% identical (69% overall similarity) to AlgX [129a]); algF, encoding an acetylase (129, 400); and algA, encoding phosphomannomannose isomerase, which converts fructose-6-phosphate into mannose-6-phosphate (75, 76, 140, 399), and also has a pyrophosphorylase activity implicated in GDPmannose synthesis (399). The genes within the cluster at 34 min account for the majority of the known enzymatic and modification activities, except for the unlinked gene algC, encoding an enzyme with phosphomannomannose activity capable of converting mannose-6-phosphate into mannose-1-phosphate (491). Several other genes have been identified and sequenced in the same cluster with algD (Table 3; Fig. 4), but their functions have not been determined with certainty. It has been proposed that the gene product of algE (62) is an outer membrane protein (170), and Maharaj et al. suggest that the genes corresponding to the mutations alg-8 and alg-44 might be involved in the polymerization of mannuronic acid residues (269).

Overlaps between Alginate and LPS Biogenesis

It has recently been shown that two enzymes, the bifunctional enzyme phosphomannose isomerase/GDPmannose pyrophosphorylase (the gene product of algA) and phosphomannomannose (the gene product of algC), which also has a phosphoglucomutase activity [hence the alternative name pmm], previously thought to be specific for alginate synthesis (Fig. 3), participate in the synthesis of LPS core (68, 150, 484). Goldberg and colleagues (68, 150) have demonstrated that
some parts of the alginate biosynthetic pathway also participate in the synthesis of various LPS components. For example, phosphomannomutase (encoded by algC) has a strong, initially unappreciated phosphoglucomutase activity (68, 484). Mutations in this gene abrogate incorporation of glucose into the LPS core (68, 484), and algC knockout strains are less virulent in the burned-mouse model of infection, most probably because of the effects on LPS (146). Complementation of algC mutations restores the synthesis of both A-band (alternative names, common antigen and D-rhamnan polysaccharide side chain) and B-band (antigenically dominant O side chain) LPS (68, 150, 484), which can be explained by the fact that A-band and B-band polysaccharides in a given strain most probably have the same core (175). Furthermore, other enzymes and intermediates of the pathway appear to be shared. GDPmannose is most probably the precursor for incorporation of D-rhamnose into the A-band LPS (251). This provides another overlap with alginate production, since GDPmannose is a critical intermediate in alginate synthesis. When oxidized into GDPmannuronic acid, it becomes the direct precursor for alginate polymerization (Fig. 3). Thus, with the possible exception of the last step, catalyzed by GDPmannose dehydrogenase, the entire precursor pathway appears to be shared with LPS.

Proposals have been made suggesting that the conversion to mucoidy may cause the loss of the LPS O side chains, another frequently encountered feature of CF isolates (174), which renders these strains less resistant to serum, perhaps because of the increased deposition of C3b on O side chain-deficient CF isolates (384). However, given the fact that the intracellular concentration of GDPmannose exceeds that of GDPmannuronate even in mucoid mutants (443), it is unlikely that changes in LPS may be explained solely by depletion of some critical sugar intermediate in cells overproducing alginate. Furthermore, from the studies of Goldberg et al., it appears that mutations causing changes in LPS O side chains may occur in the rfb locus (149), which is apparently unrelated to alginate synthesis. When a plasmid carrying the rfb locus from PA103 is introduced into CF isolates with a rough LPS, these clinical strains express PA103 antigen (IATS O11) and in addition
Gene Alternative designation Function(s) and comment(s) Map location (min) Reference(s)

algU algT, Pa αβσ

algD, algR(Pprox), algU(P1, P3); sensitivity to ROF; suppressor mutations a,b,c,d,e 67.5 93, 97, 182, 276, 279, 488

algW HtrA(DegP) homolog (see mucD) 69 32

mucA Regulator of αβσ (algU); site of mutations that cause conversion to mucoidy in CF isolates 67.5 278, 390a

mucB algN Regulator of Pa αβσ (algU); experimental inactivation results in mucoidy 67.5 148, 277, 390a

mucC Putative regulator 67.5 32

mucD HtrA(DegP) homolog; experimental inactivation causes mucoidy 67.5 32

a AlgU is 66% identical to (93, 264, 276, 279) and functionally interchangeable with (488) the extreme heat shock sigma factor σg (RpoE) from enteric bacteria. When no longer controlled by MucA and other Muc factors (e.g., in mucA mutants; see footnotes c to e and g), AlgU appears to be toxic to bacterial cells. This contributes to the instability of the mucoid phenotype. Suppressor mutations inactivate algU, causing loss of mucoidy (97, 388).

b AlgU is also involved in resistance to reactive oxygen intermediates (ROI) independently of the mucoid phenotype (279, 468a, 488).

c Site of second-site suppressor mutations causing pseudoreversion to nonmucoidy in a subset of mucoid strains carrying muc4 mutations (97, 388).

d AlgW encodes a homolog of the serine protease HtrA involved in resistance to ROI and virulence in several bacterial pathogens (32, 207, 368, 255). Mutations in algW render P. aeruginosa more sensitive to ROI (32). Alginate synthesis and mucoidy can be induced in algW mutants in the presence of redox cycling compounds (32). See footnote e.

e Negative regulator of AlgU (anti-σ factor [390a]). Nonsense and frameshift mutations in this gene are known to cause a mucoid phenotype in CF isolates (Fig. 5).

f Negative regulator of AlgU activity (periplasmic location [390a]). Experimental inactivation by gene disruption causes a mucoid phenotype.

g Similar to ORF4 in the gene cluster containing algU, mucA, mucB, and mucC homologs in Photobacterium sp. (59). ORF4 is required for adaptation to extreme conditions in this deep-sea bacterium.

h Another homolog of HrA (see footnote c). Inactivation of mucD results in a mucoid phenotype on standard media without additional stimulation.

The first alleles identified in this process have been muc-2 and muc-22 alleles, which represent frameshift mutations (an 8-bp

strains was correctly localized by several groups (133, 268) and more recently was investigated by using recombinant DNA tools (125, 126), the precise genetic alterations underlying the molecular mechanism of conversion and the function of the genes controlling these processes were not characterized until recently. Following renewed interest in the muc mutations, Martin et al. (276–279) pursued their identification and molecular characterization. These authors have reported a number of different cosmid clones that can suppress mucoidy and algD transcription in a wide range of clinical isolates from CF patients and in laboratory strains (PAO568, PAO578, PAM425, and PAO579) with mapped muc mutations (133, 268). The cosmids have been isolated from a reference genomic library of the nonmucoid standard genetic strain PAO1 (359), constructed by B. W. Holloway and collaborators. The initial reports have focused on two cosmids, pMO011809 and pMO012046, that complement a significant number of CF isolates to nonmucoidy (276). Since the cosmid DNA came from a wild-type nonmucoid strain, one possible explanation was that the cloned genes directly complemented or suppressed a mutation(s) (muc) on the chromosome of mucoid strains. In particular, pMO012046 complemented the “classical” muc mutations muc-2 and muc-22 (133) and was subjected to a thorough molecular analysis (88, 276–278). Physical and genetic mapping of the genes cloned on pMO012046 has established that they are transmissible with pplaB, to the extent matching that reported for muc-2 and muc-22. Deletion subcloning experiments have delimited the complementation activity to a 1.9-kb segment of DNA, containing three genes, algU, mucA, and mucB (Table 4 and Fig. 4), which encode polypeptides with predicted molecular masses of 22, 20, and 33 kDa, respectively (276, 277). The mucB gene was independently described by Goldberg et al. and termed algN (148). In the subsequent studies, it turned out that mutations causing mucoidy occur in the second gene of the cluster, mucA, and this was demonstrated by identification of the exact nucleotide changes followed by gene replacements with mutant alleles to show that they are indeed responsible for the mucoid phenotype (278).

The first alleles identified in this process have been muc-2 and muc-22, which represent frameshift mutations (an 8-bp

Mucoidy in Cystic Fibrosis

The work identifying chromosomal mutations as the underlying mechanism for conversion to mucoidy has been initiated by Fyfe and Govan (133). Using methods of traditional genetics (F-factor crosses and phage transduction analyses), these authors have mapped several mutations, which they termed muc (for mucoidy), as the cause of mucoid phenotype in P. aeruginosa. It has also been observed that muc mutations can occur in several chromosomal loci (133, 134, 157). One group of mutations, represented by the muc-2 and muc-22 alleles, has been found to be cotransducible with the pplaB marker located at 67.5 min (133, 134). Another site, represented by the muc-23 mutation, has also been mapped (134) within the late region of P. aeruginosa but with the location between the hisI (69 min) and proB markers (71 min). These studies have been further corroborated by MacGeorge et al., who independently mapped muc loci (e.g., the muc-3739 mutation) as tightly linked to pplaB (268), and by Flynn and Ohman, who cloned a locus, termed algST, also mapping in the late region on the chromosome (126). The latter authors also postulated that this locus functions as an on/off programmed genetic switch (126). The relationship of algST to muc is discussed in a later section.

Mutations Causing Mucoidy

Although the general chromosomal position of the site for mutation causing conversion to mucoidy in a subset of CF
duplication and a deletion of a single C within a string of 5 C residues) inactivating mucA (Fig. 5). The key to the identification of these mutations was the approach in which isogenic nonmucoid parental strains and their mucoid mutants were compared. By using this strategy, it was possible to identify the wild-type (functional) genes first and then compare them with mutant alleles in mucoid derivatives (278). Once the use of laboratory strains unequivocally demonstrated the nature and effects of muc mutations, it was important to examine whether CF isolates carry similar mutations. Through extensive verification of this concept in clinical strains (278), identical mutations to the mucA22 allele and additional frameshift or nonsense mutations (Fig. 5) in the mucA gene have been characterized in several mucoid isolates from CF patients (32, 97, 278).

It should be emphasized, however, that not all mucoid strains that have been investigated carry mutations in mucA (278), suggesting the participation of additional sites. This is in keeping with the earlier mapping data, e.g., muc-23, which is not in the same region on the chromosome as mucA (134), and the existence of additional cosmids clones affecting mucoidy, such as the algW locus (32) from pMO011809 (276, 359). Furthermore, although no mutant mucB allele has yet been identified in a CF isolate, when this gene is insertionally inactivated on the PAO chromosome by genetic manipulation, this also results in conversion to mucoidy (277). Potentially, mucB could be a site for spontaneous muc mutations, but the reasons why mucoid mutants with alterations in this gene are not selected in CF or are at least underrepresented in the sample studied thus far are currently not known. There are two additional genes, termed mucC and mucD (32) (Fig. 4), in the cluster located immediately downstream of mucB. These could be the sites of additional mutations affecting mucoidy. For example, experimental inactivation of mucD also causes conversion to mucoidy (32). Moreover, the role of the genes on the remaining eight cosmids affecting mucoidy (276) and whether mutations causing mucoidy may occur in any of these sites need to be investigated. Another perhaps helpful should be to characterize muc mutations in various strains used in the past by different investigators, since physiological studies had been performed in uncharacterized muc backgrounds. This would facilitate comparisons and perhaps resolve sometimes conflicting results from different laboratories.

**AlgU Is an Alternative Sigma Factor Controlled by Accessory Elements**

In previous sections, it has been mentioned that AlgU is an alternative sigma factor required for initiation of algD transcription. When algU is insertionally inactivated, this abrogates algD transcription and mucoidy (276). The first report on AlgU characterization (276) indicated its similarity to the alternative sigma factor Spo0H (109), which controls sporulation and other post-exponential-phase processes in *Bacillus* spp. However, the reported similarities between Spo0H and AlgU, although statistically significant, did not extend to the rest of the superfamilies of $\sigma^{70}$ proteins (265) at a sufficiently high level to be directly detected in global homology searches (276). The subsequent substantial growth of sequenced databases helped to explain this apparent paradox (93, 264, 279). On the basis of several more recent comparisons, it became evident that AlgU is a founding member of a novel family of alternative sigma factors which are only distantly related to the $\sigma^{70}$ class of sigma factors (93, 264). Spo0H shares similarities with both groups of proteins and is placed in between these two superfamilies (264). Of even greater significance is the fact that these recent developments (93, 97, 264, 279) have uncovered the presence of close homologs of AlgU in *Escherichia coli* and *Salmonella*.
typhimurium (66% identity and 91% overall similarity) (93, 264, 279). Several independent observations have indicated that the E. coli homolog of AlgU is the alternative sigma factor σ^E (Fig. 6), which has been previously characterized at the biochemical level (116, 117, 466). The E. coli and S. typhimurium genes encoding σ^E have been named rpoE (93, 183, 264, 279, 356, 370, 484). These relationships have been confirmed by functional analyses (183, 356, 370, 488).

**Functional Equivalence of AlgU and σ^E (RpoE)**

Mutations in rpoE have been recently generated in E. coli, and the results of these experiments matched the predicted phenotypic consequences of rpoE inactivation (183, 356, 370). σ^E has been shown to transcribe the major heat shock sigma factor gene rpoH in E. coli from its P3 promoter under conditions of extreme heat shock (116, 466). It is also the only sigma factor transcribing htrA (degP) (116, 255–257, 430), a gene required for resistance to heat killing in E. coli (255–257, 430) and for resistance to reactive oxygen intermediates and full virulence of S. typhimurium (207).

Inactivation of algU decreases the P. aeruginosa survival rate upon exposure to high temperatures and diminishes its resistance to superoxide-generating redox cycling compounds (279, 488). This effect is independent of the mucoid or nonmucoid status of the parental strain in which algU has been inactivated (279). Furthermore, algU transcription is induced by environmental stress such as heat shock (390). These observations suggest that algU may play a physiological role similar to that of rpoE in enteric bacteria and that alginate production is only a subset of systems regulated by algU in P. aeruginosa. In a recent work, the interchangeability of rpoE from E. coli and algU from P. aeruginosa has been demonstrated (488). These findings, along with the strong sequence conservation of AlgU and RpoE (93, 264, 279), confirm that algU encodes the σ^E equivalent of P. aeruginosa. In keeping with these observations are the similarities of the canonical sequences of rpoE- and algU-dependent promoters (Fig. 7) (93, 94, 116, 256, 390). Since σ^E has been biochemically characterized as an alternative sigma subunit of RNA polymerase in E. coli (116, 117, 183, 356, 370, 466), it is possible to conclude that AlgU is the analogous sigma factor of P. aeruginosa. Consistent with this gene, plasmid-borne rpoE complements algU mutations and induces mucoidy in nonmucoid wild-type P. aeruginosa strains by activating the chromosomally encoded algD (488).

That AlgU can stimulate transcription in vitro was recently reported by Hershberger et al. (182) who cloned the algU gene by PCR on the basis of its published sequence (276) and tested it on the previously mapped P1 promoter (97, 279) of algU. Schurr et al. have shown that AlgU, in addition to initiating transcription from the P1 promoter, associates with RNA polymerase (391), thus fulfilling the second biochemical criterion for an RNA polymerase σ subunit.

**Relationship of algT and algN to algU and mucB**

Considering the complex history of the studies on the conversion to mucoidy in P. aeruginosa, it may be useful to review the ultimately convergent development of research on the algU mucABCDo region and the algT algS algN loci, which turned out to be equivalent (97, 148). The initial reports describing these genes suggested that they may be different. For example, algT was reported not to be cotransducible with pruAB (126) whereas algU was demonstrated to be linked to this locus (276) to an extent identical to that shown for the previously mapped muc mutations (133, 134). Also, some of the initial reports on the products of genes in the algT region indicated that this gene encoded a 34-kDa polypeptide (474) which differed from the experimentally determined and predicted values for AlgU (27.5 and 22 kDa, respectively) (276). However, a recent report by DeVries and Ohman showed that algT has an identical sequence to that of algU (97). Moreover, the sequences of algN (148) and mucB (277) are identical. In addition, Goldberg et al. have demonstrated that algN inhibits alginate production when present on a plasmid (148). These results are in keeping with the report by Martin et al. that inactivation of mucB on the chromosome can result in conversion to mucoidy (277). In the study with algN (148), a partial sequence of the region upstream of this gene was presented, although no genes were assigned to that region. Martin et al. (278) noted a mutation in this sequence which corresponded to a partial sequence of the mucA gene. The mucA mutation in strain FRD, used by Gold-
berg et al. (148) and frequently employed in studies by Ohman and colleagues, is similar to the one characterized as mucA22 in PAO578 and in the CF isolates CF14 and CF23 (278). A complete sequence of mucA from FRD has been recently presented, indicating this lesion (97). On the basis of the published genetic data (126), the mucA mutation in strain FRD corresponds to the previously reported location for algS, and thus algS and mucA should be considered equivalent.

Instability of Mucoid Phenotype

Once the mutations causing mucoidy were identified, it was possible to address the question of the instability of mucoidy. The “metastable” character of alginate overproduction varies from strain to strain (74, 156, 164, 268, 353) and can now be at least partially explained by the toxicity of the uncontrolled AlgU (97, 388). Thus, the published reports that considered the on/off switch model were hampered by focusing on the putative “reversion” events. Recent analyses of the mechanisms of reversion to nonmucoidy have indicated that this process is not a true reversion of the original mutation, e.g., in mucA, but can be attributed to second-site suppressor mutations (97, 388). Some of these compensatory mutations (reviewed in reference 487) appear to be in algU (algT) (97, 388) and represent mutations which inactivate algU (388) or cause missense alterations in this gene (97). While there are additional pathways (that still need to be defined) of suppression of mucoidy (32, 276), it is safe to conclude that a variety of compensatory mutations, in conjunction with selective pressures exerted upon cells containing runaway AlgU, can explain the high frequency of the pseudoreversion to nonmucoid phenotype.

Regulation of AlgU Activity

The nature of the systems induced by $\sigma^E$ (AlgU) and the consequences of mutations in rpoE and algU suggest that this sigma factor contributes to bacterial defense against extreme environmental stress (279, 356, 370, 488). A recent study has demonstrated that transcription of algU from its two AlgU-dependent promoters, P1 and P3 (Fig. 7), can be induced by heat shock (390). Moreover, another AlgU-dependent promoter (the proximal promoter of algR) is inducible by osmolarity changes in addition to heat shock (391). These findings are in keeping with the reports that mutations affecting outer membrane proteins in E. coli, normally regulated by osmolarity changes, affect $\sigma^E$ activity (291). Taken together, these observations may help explain the reports that agents such as ethanol (96), high osmolarity (25), starvation (420, 446), and oxygen availability (19, 232) affect alginate production. These phenomena, collectively referred to as “environmental regulation of mucoidy,” were described as early as in 1973 by the discoverers of the chemical nature of the P. aeruginosa mucoid exopolysaccharide (120), who reported that high osmolarity and inadequate protein supply enhanced alginate production by mucoid P. aeruginosa. However, since these numerous studies were performed with genetically uncharacterized mucoid mutants and since the nature of mutations causing mucoidy is now defined in several strains, it may be necessary to revisit the environmental regulation of alginate production in the wild-type nonmucoid background or in defined muc mutants. In relation to the derepression of alginate production upon inactivation of mucA or mucB (277, 278), it is also necessary to examine how AlgU and its negative regulators, MucA and MucB, interact and which environmental signals bring about physiological induction of the system in the absence of mutations.

The mechanism of the negative regulation of AlgU by MucA and MucB has been recently addressed (390a). It has been proposed that MucA may act as an anti-sigma factor (91, 278), as has been shown to be the mode of regulation with several other alternative sigma factors (24, 35, 39, 110, 173, 194, 296, 318). In a recent study (390a), a direct interaction between AlgU and MucA has been demonstrated. AlgU and MucA, when coexpressed in vivo, could be cross-linked, forming apparent 1:1 complexes. Furthermore, purified MucA inhibited AlgU-dependent initiation of transcription (390a). In the same study, it was shown that MucB exerts its function from the periplasmic compartment. A deletion of the leader peptide-recoding region precluded localization of MucB to the periplasm, and the MucB trapped in the cytoplasm lost its negative effect on alginate synthesis. Thus, while MucA directly interacts with AlgU and inhibits its activity, consistent with an anti-$\sigma$ activity, MucB may transduce stress signals from the periplasm or play some other modulatory role. It will also be of interest to investigate how the external stimuli (e.g., heat shock, osmolarity changes, presence of reactive oxygen species or other oxidants, and possibly other denaturing agents) are recognized and transduced to permit activation of AlgU and $\sigma^E$-dependent genes. Furthermore, the role of the recently identified genes (32) mucD and algW, both encoding polypeptides homologous to the serine protease HtrA (255, 257), in the control of AlgU remains to be examined. Interestingly, knockout mutations in algW facilitate the induction of mucoidy by exposure to reactive oxygen intermediates, while inactivation of mucD renders cells constitutively mucoid (32). According to some proposals (32, 487), these putative protease may affect AlgU activity either by removing the signals generated by stress (e.g., denatured or otherwise damaged proteins) or by acting directly on AlgU or its putative positive regulators. In either model, inactivation of algW or mucD should cause increased alginate production in keeping with the experimental observations (32).

Ancillary Factors in Regulation of Mucoidy

Prior to the uncovering of the mechanism of conversion to mucoidy, the algD and algC promoters have served as major tools to investigate the transcriptional regulation of mucoidy. As a product of efforts by different investigators, numerous factors have been demonstrated or suggested to control or structurally support algD and algC expression. These regulatory and bacterial nucleoid structural elements are listed in Table 5. Several recent reviews (83, 92, 283, 284) have extensively covered this topic.

Among the ancillary regulatory elements of algD and algC, the best-studied factor is algR (83, 85, 132, 214, 297, 299–302). It belongs to the superfamily of response regulators from two-component signal transduction systems (83, 326, 327, 427). AlgR has been purified, its predicted sequence has been confirmed by protein sequencing at the N terminus and several internal positions (214, 299), and its binding to the algD and algC promoters has been demonstrated (132, 214, 299, 492). The binding sites for AlgR in algD form an unusual set of direct and inverted repeats (Fig. 5) (300, 389). The significance of this peculiar arrangement needs to be clarified, but it appears that all three sites (termed RB1, RB2 and RB3 [RB, for AlgR binding] [299–301]) contribute to the high expression levels of algD. In all AlgR-binding sites, a consensus sequence termed core (ACCGTGTGTC) is invariably present and is centered within approximately 20 bp protected by the AlgR footprint (214, 299–301). The core sequence has been shown by
site-directed mutagenesis (300) to represent the AlgR recognition site.

The putative AlgR cognate sensor, AlgZ, has recently been identified (486b). The AlgZ gene is located immediately upstream of algR, and its predicted gene product shares limited homology with a small subset of sensors-kinases from the superfAMILY of two-component systems. Interestingly, the only known phenotypic effect of algZ inactivation is an increase in alginate production in mucA cells (486b). This may appear paradoxical and suggests a negative regulatory role for algZ in alginate production. It is not known at present how AlgZ exerts its function and whether it acts as a kinase, since it lacks several typical motifs found in the majority of histidine protein kinases. An intriguing possibility exists that AlgZ may act as a phosphatase or otherwise block or negatively modulate AlgR activity. However, a positive regulatory role for AlgZ in wild-type cells (mucA−) cannot be ruled out at present.

A gene termed algQ (90, 230) (also known as algR2 [215]) was initially suggested to play this role. This paper was followed by a significant number of publications that seemed to have supported this idea (215, 372–374). This concept has been recently scrutinized by the same research group. In a recent publication, these authors stated that “AlgR2 itself is not a kinase, but rather that it regulates a kinase of similar size” (386). Regardless of the conflicting reports, some relationship to alginate production is likely to exist, since defined mutations in algQ affect the mucoid phenotype. In another recent study, the same authors (387) have shown that algR2 is insertionally inactivated in a mucoid P. aeruginosa isolate, this affects the capacity of the mutant strains to produce alginate. Following this line of research, the same investigators have explored the role of this factor in the regulation of energy metabolism and tricarboxylic acid cycle in P. aeruginosa, with specific emphasis on the enzymes nucleoside diphosphate kinase and coenzyme A synthetase (386, 387). The exact relationship of these issues to alginate production is currently not clear, except that proposals have been made that this system may affect the levels of GTP, which in turn may influence the production of GDPmannose. However, such suggestions must be reconciled with the observations that the concentration of GDPmannose is always higher than that of GDPmannuronate (443) (see the section on genetics of alginate biosynthesis, above). Nevertheless, these investigations have recently increased the number of genes proposed to participate in alginate production, by adding a new locus termed algH, which has a complicated and possibly synergistic relationship with algR2 (387). Furthermore, analyses of algR2 in the context of its similarities to pfrA, a siderophore regulator in Pseudomonas putida recently reported by Venturi et al. (460–462), are under way (387).

Several other factors have been reported as possibly affecting algD transcription. For example, algB (151, 152), another response regulator (147, 480) with similarities to the NtrC-type factors, has been reported to affect algD expression. Elements like AlgB usually interact with σ54 (RpoN), an alternative sigma factor which plays a major role in the expression of adhesins and nitrogen assimilation in P. aeruginosa (198, 203, 401, 452). This introduced a paradox, since AlgU (σ5) and apparently not σ54 activates algD (301, 452). Furthermore, integration host factor from P. aeruginosa has been purified and shown to bind to the algD promoter (453). Similar results have been reported by others (298, 479). Wozniak has shown that mutations in the integration host factor-binding sites within algD decrease its expression (479), suggesting that integration host factor may have an effect on structural features of the algD promoter perhaps related to the interaction of activators and RNA polymerase. Studies by DeVault et al. with purified proteins from E. coli have suggested that factors such as E. coli cyclic AMP receptor protein (95) bind to algD, but the relevance of these studies for the situation in P. aeruginosa remains to be shown. Likewise, the role of the histone-like protein, AlgP (alternative names, Hp1 and AlgR3) remains to be determined (216, 230, 292), although some nonspecific DNA-binding activity has been reported (292).

Functional Equivalents of AlgU in Other Bacteria

Although σ5 is well characterized at the biochemical level, the gene for this sigma factor was not identified until recently. The recognition of the rpoE gene in E. coli has been facilitated by the strong sequence conservation of its predicted gene product with AlgU (93, 264, 279). Moreover, it appears that the close homologs of AlgU (σ5) are widely spread among various groups of bacteria (Fig. 6). The rpoE gene of Salmonella typhimurium has been isolated and sequenced (279). A gene analogous to algU has been identified in A. vinelandii (280). Another recently described system is the rpoE gene in the barophilic and psychrophilic bacterium Photobacterium sp., which may regulate the expression of the outer membrane proteins and possibly other functions related to the extreme conditions of growth under high pressure and low temperatures in this deep-sea bacterium (59). As a result of the determination of the nucleotide sequence of the complete genome of H. influenzae (124), two additional rpoE/algU homologs have been identified. On the basis of these findings and the high conservation between algU in P. aeruginosa and its rpoE ho-
FIG. 8. Conservation of gene clusters encoding AlgU-RpoE sigma factors and their regulators and different organizational types. (i) Four genes, algU mucA-B mucD, seen in P. aeruginosa (P.a.) and A. vinelandii (A.v.). (ii) Three genes, rpoE mclA orf2 orf3, immediately followed by lepA, but there is no mucD (htrA) equivalent within the cluster; seen in E. coli (E.c.) and Photobacterium spp. (Ph). S. typhimurium most probably belongs to this group. (iii) Three genes, rpoE mclA mucB; seen in H. influenzae (H.i.). This organism has two genes encoding MucC homologs elsewhere on the chromosome. (iv) The rpoE htrA locus from M. leprae (M.l.) is reminiscent of the two termini of the algUmucABCD cluster, but whether this reflects functional overlaps with algU and mucD is not known. The gene product of M. leprae rpoE shows only limited similarity to the AlgU-RpoE sigma factors from gram-negative organisms. This similarity is typical of the levels (20 to 30% identity) observed with other members of a large family of \( \sigma^H \)-like factors (93, 94), also known as the ECF family of alternative \( \sigma \) factors (264).

In addition to the AlgU-RpoE factors, which display a high degree of homology, additional sigma factors and putative \( \sigma \)-like elements have been noted in other species. These factors represent a more divergent group but nevertheless share some homology to AlgU and have been termed \( \sigma^H \)-like factors (94).

In addition to the AlgU-RpoE factors, which display a high degree of homology, additional sigma factors and putative \( \sigma \)-like elements have been noted in other species. These factors represent a more divergent group but nevertheless share some homology to AlgU and have been termed \( \sigma^H \)-like factors (94).

For example, HrpL controls Pseudomonas syringae pathogenicity and hypersensitivity response (482). SigE in Streptomyces coelicolor controls an enzyme that degrades agar (264), while CarQ (287) controls the expression of carotenoids that scavenge oxygen radicals during aerobic growth. A gene termed rpoE has been sequenced in Mycobacterium leprae as a part of the genomic sequencing project in this organism (GenBank accession number U15180), and although its function is not known, it and a downstream gene homologous to htrA and mucD have organizational features suggestive of its relatedness to the bona fide AlgU-RpoE factors (Fig. 8). These and several other more distant homologs of AlgU-RpoE most probably represent a functionally heterogeneous group of minor sigma factors. According to one classification, all such factors including \( \sigma^H \) have been grouped in the ECF (extracytoplasmic function) family of proteins (264). Although this term may seem appealing, considering the observation that some of the properties regulated by such factors involve secretion or the periplasmic space, this concept should be applied with caution. For example, \( \sigma^H \) in E. coli controls the main heat shock sigma factor (\( \sigma^{H} \), RpoH) which enhances the expression of many intracellular heat shock proteins. Therefore, the term “ECF” does not appear adequate when factors such as AlgU and RpoE from the gram-negative bacteria are considered, and perhaps it is not applicable in as broad a sense as was originally intended (264) or as the alternative name “\( \sigma^H \)-like family” offers.

Conservation of the Regulatory Systems Controlling AlgU/RpoE Activity

In addition to the highly similar sigma factors, the AlgU/RpoE-encoding gene clusters have conserved regulatory elements downstream of the genes encoding the sigma factors. These corresponding gene clusters show conserved organization. Several types of clusters have been discerned (487), as illustrated in Fig. 8. The first type is represented by the P. aeruginosa and A. vinelandii algUmucABCD clusters. Similarly grouped genes are seen in Photobacterium spp. (59). The genes orf2 to orf4 encode polypeptides homologous to MucA, MucB, and MucC. However, Photobacterium spp. do not have a linked mucD equivalent but instead have the lepA gene. The lepA gene is involved in the control of signal peptidase (lepB) activity. Only one gene, mclA, the mucA homolog, has been characterized this far downstream of rpoE in E. coli (488). It is likely that enteric bacteria have a similar cluster organization to that of Photobacterium spp. This prediction is based on the close genetic linkage of rpoE and lepA lepB genes in E. coli.

The third organizational type is seen in H. influenzae. This is an even shorter version of the cluster, with only the first three genes (termed rpoE, mclA, and mucB) present (124). Immediately downstream of mucB in H. influenzae is a divergently transcribed gene, with no obvious relationship to the cluster. To be sure, H. influenzae has mucD (htrA) homologs elsewhere on the chromosome (124), and two unlinked homologs of mucC have been recognized. H. influenzae also has another homolog of algU, but this gene has only a limited similarity with algU and should be categorized in the class of \( \sigma^H \)-like factors along with CarQ, HrpL, etc. At the far extreme of the systems presented in Fig. 8 is the rpoE htrA cluster in M. leprae. In this organism, a distant homolog of AlgU, termed rpoE, is followed by an htrA homolog, in what amounts to a compressed version of the algU-mucABCD cluster with only the first and the last genes represented.

AlgU (\( \sigma^H \))-Regulated Systems and Pathogenesis: Future Perspectives and Concluding Remarks

Future examinations of genes controlled by AlgU, \( \sigma^H \), and their equivalents may uncover important aspects of bacterial pathogenesis. For example, in Salmonella spp., one of the known genes (htrA) regulated by \( \sigma^H \) is absolutely required for survival in macrophages and virulence in mice (207). In Brucella abortus, an htrA homolog encodes a major immunoreactive protein during infection (114, 368, 445). Moreover, the htrA gene of B. abortus has a recognizable \( \sigma^H \) promoter and is inducible by heat shock (114). In addition, a Rochalimaea henselae antigen that shares similarities with htrA (GenBank accession number L20127) may be significant in disease caused by this pathogen, while a putative serine protease showing homology to HtrA is an immunoreactive protein in Mycobacterium avium (53). Since several traits controlled by \( \sigma^H \) (AlgU) have already been defined as virulence or persistence factors (93, 114, 207), it is likely that further investigations of the regulons controlled by AlgU-RpoE factors in different organisms may unveil pathogenic determinants or important antigens. For AlgU, the recently initiated analyses of stress defense systems controlled by this factor and its effects on virulence in animal models (486a) may uncover additional antigens that

P.a. algU mucA mucB mucC mucD

A.v. algU mucA mucB mucC mucD

Ph rpoE orf2 orf3 orf4 lepA

E.c. rpoE mclA orf2 orf3

S.t. rpoE mclA orf1 orf2

H.i. rpoE mclA mucB

M.l. rpoE htrA


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could potentially contribute to the inflammation and hypersensitivity in CF.

As a final remark, nearly 30 years after Doggett et al. suggested that mucoid mutants of *P. aeruginosa* are being selected in the CF lung (101, 102), we are beginning to define the genes and mutations participating in these processes. Hopefully, the detailed information regarding the molecular mechanism of conversion to mucoidy that is currently emerging will improve our understanding of the critical host-pathogen interactions in CF. One can also hope that better diagnostic tools, improved management of inflammation, or realistic targets for drug development will result from these studies.

**BURKHOLDERIA CEPACIA**

Until the early 1980s, *B. cepacia* (previously *Pseudomonas cepacia*, *Pseudomonas multivorans*, *Pseudomonas kinki* and eugonic-oxidizer group) was best known as the phytopathogen first described by Burkholder (43) as a cause of soft rot of onion bulbs (*Latin cepia*). In 1992, on the basis of 16S rRNA sequences, DNA-DNA homology values, cellular lipid, fatty acid composition, and phenotypic characteristics, Yabuuchi et al. (483) proposed that *P. cepacia* and other members of RNA homology group II be transferred to the new genus *Burkholderia*, with *B. cepacia* as the type species; this proposal was subsequently validated (262). At present, the genus *Burkholderia* comprises *B. cepacia*, *B. gladioli*, *B. mallei*, *B. pseudomallei*, *B. carophylli*, and, recently added to the group, *B. plantarii*, *B. glumae*, *B. vandii*, *B. cocovenenans*, and *B. vietnamiensis*.

During the 1980s, *B. cepacia* emerged as a multi-drug-resistant nosocomial pathogen in immunocompromised patients. Although, in some cases, culture was thought to reflect mere colonization or contamination rather than infection, clinically significant *B. cepacia* infections were confirmed during extensive studies (190, 201, 282). For example, Jarvis et al. (201) analyzed the 1980 to 1985 U.S. database of nosocomial infections and found the *B. cepacia* infection rate to be 2.4/100,000 patient discharges, with a significant increase in infections during this period. The study did not specifically identify cultures from CF patients; nevertheless, it is interesting and disturbing to observe that the most frequently reported site of *B. cepacia* infection was the lower respiratory tract (31%) and that such infections were often associated with mortality. The apparent predilection of *B. cepacia* for lungs is further emphasized in patients with chronic granulomatous disease, in whom *B. cepacia* pneumonia and septicemia are life-threatening (30, 72, 242, 320). More recently, it has also been of concern that cases of community-acquired bacteremic pneumonia due to *B. cepacia* and brain abscesses secondary to chronic supplicative otitis media have been found in immunocompetent hosts (184, 355).

The first report of *B. cepacia* pulmonary colonization in patients with CF appeared in the early 1970s (111), followed a decade later by a report of clinical complications associated with *B. cepacia* pneumonia and septicemia in a 17-year-old patient (369). The report of septicemia is noteworthy, since systemic spread of other pathogens from infected lungs is rare in patients with CF.

In the early 1980s, the seminal papers of Isles et al. (199) and Thomassen et al. (450) drew attention to an increased culture rate for *B. cepacia* in two North American CF centers which forecast the emergence of *B. cepacia* as a major threat to the CF community. Anxiety among patients with CF and their families, clinicians, and caregivers concerning *B. cepacia* colonization arises from three major concerns not previously associated with *P. aeruginosa*.

First, approximately 20% of *B. cepacia*-colonized patients succumb to “*B. cepacia* syndrome,” a necrotizing pneumonia with fever, bacteremia, elevation of erythrocyte sedimentation rate, and leukocytosis, which culminates in rapid and fatal clinical deterioration (199). It should be emphasized that the prognosis for most patients colonized by *B. cepacia* resembles that for those with *P. aeruginosa* infection, namely, asymptomatic colonization associated with steadily increasing humoral immune responses, interspersed with episodes of acute pulmonary exacerbation and increasing lung disease. *B. cepacia* does not invariably colonize patients with CF. Colonization may be transient and has never reached the 80% prevalence levels of *P. aeruginosa* colonization (123). In most clinics, *B. cepacia* is cultured from fewer than 10% of patients; however, the prevalence in some regional centers may reach 40% (381).

Second, in addition to its potential virulence, compelling epidemiological evidence for patient-to-patient transmission both in hospital and through social contacts has led to cohorting of *B. cepacia*-positive individuals. Approval of such a draconian strategy is not unanimous. Segregation creates difficulties in the management of CF clinics and devastating disruption of social contacts (7, 12a, 169, 465).

Third, the characteristic multi-drug-resistance of *B. cepacia* to potent antipseudomonal agents has led to a lack of effective antimicrobial therapy (248, 350, 402). Even when susceptibility to antimicrobial agents can be shown in vitro, aggressive therapy seldom results in significant clinical improvement or even reduction in the numbers of bacteria cultured from sputum specimens.

**Questions and Issues**

The emergence of *B. cepacia* as a major pathogen raises questions concerning the evolving microbiology of lung disease in patients with CF and important practical and ethical issues (7, 168, 423). The reasons for the increased prevalence of *B. cepacia* during the 1980s are the subject of much debate. One explanation relies on the consequences of aggressive antibiotic therapy and the selection of multi-drug-resistant opportunists in an increasing population of young CF adults with highly interactive social lifestyles and various degrees of preexisting lung damage. There is no doubt that although the spectrum of CF pathogens remains relatively limited, during the last four decades it has expanded from *S. aureus* and, later, *H. influenzae* and *P. aeruginosa* to include other opportunistic microbial pathogens, including fungi and viruses. With the possible exception of *P. aeruginosa* (238, 489), we cannot explain the peculiar propensity of individual pathogens for the lungs of individuals with CF. Experience of CF microbiology suggests that it would be naïve to expect a simple explanation for the emergence of *B. cepacia*. Nevertheless, accumulating data have led to the following speculations.

**Regional Differences in Prevalence**

Culture and identification of *B. cepacia* can present difficulties (142, 190, 260, 348). Thus, an explanation for regional differences in prevalence during the 1980s could be laboratory variation in microbiological expertise. For example, in a multicenter study of sputum seeded with *B. cepacia* published in 1987, only 36 (32%) of 115 laboratories detected the organism (439). Subsequently, although the development and use of *B. cepacia* selective media (141, 142) improved the reliability of bacteriological surveillance, it did not explain continuing regional differences in prevalence. The use of selective media requires care. Growth of *B. cepacia* may require incubation for 72 h or more, and since some strains of *S. maltophilia* and *Comamonas acidovorans* will also grow on selective media, a
presumptive *B. cepacia* isolate needs to be identified by an appropriate multitest system such as API 20NE (168, 348). Despite these caveats, the prevalence of *B. cepacia* during the late 1980s continued to show not only regional variation but disturbing increases in some centers. Between 1986 and 1989, surveillance studies in the United Kingdom indicated a maximum prevalence of 7%; by 1992, however, while prevalence remained low or even absent in some centers in the United Kingdom, colonization rates in one large regional clinic had reached almost the 40% prevalence experienced in a major North American CF center (7, 160, 382).

It seems reasonable to speculate that the characteristic multi-drug resistance of *B. cepacia* to antimicrobial agents might have played a role in the increased prevalence during the 1980s. One of the most striking features of this resistance is that the organism includes penicillin *G* in its repertoire of utilisable substrates (21). The intrinsic resistance of *B. cepacia* to β-lactams is based on a highly inducible *penA* gene encoding a β-lactamase-like enzyme (209, 352), whereas the low permeability of the *B. cepacia* outer membrane, approximately 10 times less permeable than that of *Escherichia coli* (328), may contribute to resistance to a range of structurally unrelated antimicrobial agents and disinfectants (141, 350). Unfortunately, there is little scientific evidence to associate the increased prevalence of *B. cepacia* with antimicrobial therapy, including the reasonable speculation that the emergence of *B. cepacia* followed the increased use of nebulized colomycin (263, 455), to which *B. cepacia* isolates are invariably resistant.

**Transmission between CF Patients**

An alternative explanation for the increased incidence of *B. cepacia* during the 1980s and for regional differences in prevalence arose from evidence for transmission of *B. cepacia* between individuals with CF. Initially, case-control studies showed that increasing age, underlying severe lung disease, use of aminoglycosides, having a CF sibling colonized with *B. cepacia*, and previous hospitalization were significant risk factors for acquisition (439, 440). Subsequently, clustering of cases in individual CF centers and reduction in the incidence of new acquisitions following segregation of colonized and noncolonized patients suggested epidemic spread of the organism (449). These early epidemiological studies, however, provided only circumstantial evidence for transmission; attempts to provide unequivocal scientific evidence for transmission were hindered by the lack of suitable typing systems to fingerprint individual strains. Thus, the sources and modes of *B. cepacia* transmission remained unclear.

By the early 1990s, the development and use of phenotypic and DNA-based genomic typing systems for *B. cepacia* made a significant contribution to our understanding of the epidemiology of this organism, including confirmation of epidemic strains (63, 65, 78, 166, 179, 208, 231, 308, 433) and confirmation that most chronically colonized patients harbor a single strain of *B. cepacia* for prolonged periods (259). In 1990, LiPuma et al. (258) reported the use of ribotyping, a method of strain identification based on analysis of bacterial genomic RFLPs, to investigate the acquisition of *B. cepacia* by a CF patient during attendance at an educational camp. Analysis of isolates recovered from the index patient and his contacts led the authors to conclude that the mode of transmission was person to person. This important study produced the first scientific evidence for *B. cepacia* transmission but left several questions unanswered. For example, LiPuma et al. did not clarify whether transmission had occurred via direct transfer of body fluids from the index patient (e.g., droplet spread) or whether the immediate environment or a contaminated fomite (e.g., a shared drinking container) was responsible; an environmental source for this saprophytic opportunists was also possible. LiPuma et al. cited previous failures to recover *B. cepacia* from environmental surfaces as evidence that direct person-to-person transmission was the primary mode of nosocomial transmission. Subsequent studies, however, showed that colonized patients could contaminate their environment with *B. cepacia*, indicating the potential for indirect transmission following patient or environmental contamination of drinking containers, respiratory equipment, disinfectants, and injection devices (42, 63, 313, 324); *B. cepacia* respiratory infection in immunocompromised non-CF patients has also been associated with contaminated nebulizer devices (441). Of relevance to the issue of indirect transmission is the ability of *B. cepacia* to survive for long periods on environmental surfaces. When *B. cepacia*-positive sputum is spread on environmental surfaces, some strains, including epidemic strains, survive for more than a week (167a).

In another study of *B. cepacia* transmission between six children, leading to two fatalities, the evidence suggested a chain of cross-infection originating from the index patient and associated with hospital admissions over a 5-year period (295). In another CF center, despite a strict segregation policy involving separation of colonized and noncolonized patients as both inpatients and outpatients, new cases of colonization suggested the possibility of person-to-person transmission of *B. cepacia* through social contact outside the hospital (407). Person-to-person contact was also considered to be a possible mode of *B. cepacia* acquisition at summer camps (335). In 1993, Govan et al. (160) reported compelling evidence for the transmissibility of *B. cepacia* based on the use of bacteriocin typing, ribotyping, RFLPs detected by pulsed-field gel electrophoresis, and a detailed epidemiological investigation of social contacts. Analysis of isolates from 210 patients from the United Kingdom, attending regional clinics in Edinburgh and Manchester, showed that the increased incidence of *B. cepacia* infection between 1986 and 1992 that culminated in an epidemic of 54 cases and 12 deaths was primarily due to a highly transmissible strain (*B. cepacia* CF5610/2315) that had spread between patients in both clinics and through social contacts with patients attending other regional centers. Detailed epidemiological investigations of social and hospital contacts provided clues to the degree of risk of transfer from colonized patients during social contacts including regular attendance at fitness classes and social kissing and the high risk associated with more intimate contact within sexual relationships. The study also emphasized other aspects of *B. cepacia* colonization, transmission, and pathogenesis, including the role of host factors. First, although transient colonization had been experienced with some strains of *B. cepacia*, individuals who acquired the epidemic strain invariably remained chronically colonized. Second, speculation that transmissibility was strain dependent was strengthened when a patient who harbored two strains of *B. cepacia* transmitted only the epidemic strain to his girlfriend. Third, clinical outcome was strongly associated with individual hosts and could not be predicted in patients colonized by the same strain. Although the epidemic resulted in a fatality rate of almost 50%, some individuals, including the index patients, did not show a decline in condition during the period of the epidemic and have remained stable for 6 years (our unpublished data). Colonization did not correlate with poor pulmonary status, but the latter tended to be associated with reduced survival (38).

In 1992, at the request of The Association of CF Adults (United Kingdom), a working party organized by the Cystic...
Fibrosis Trust (United Kingdom) published interim guidelines to reduce the risk of *B. cepacia* acquisition which were updated in 1993 (12a) to take account of new data. Similar guidelines were produced in Europe, Canada, and the United States. In January 1993, the U.S. Cystic Fibrosis Foundation, after consultation with legal counsel and infectious-diseases experts at the Communicable Disease Center, Atlanta, Ga., recommended that all centers accredited to the U.S. Cystic Fibrosis Foundation should discontinue sponsorship, endorsement, and support of CF summer camps. In March 1993, at an international workshop organized by the French CF Association, there was consensus on the status of *B. cepacia* as a transmissible and potentially fatal pulmonary pathogen and on the need for further studies to clarify the bacterial and host interactions responsible for the pathogenicity of this organism (27). The consequences of these stringent infection control policies on the CF community have been emotionally devastating and contentious, with *B. cepacia*-colonized individuals finding themselves to be social pariahs within the CF community (160, 169, 465).

The rigid stance taken on the issue of CF summer camps, a much favored institution, has not been universally welcomed, particularly by CF centers in which the incidence of *B. cepacia* colonization has remained low or in which cross-infection has not been demonstrated (425). It has to be stressed, however, that transmission is probably strain dependent and that a subset of highly transmissible bacterial lineages are responsible for the clustering of cases within regional clinics and for intercontinental spread (159, 261, 436). Although epidemiological evidence clearly points to individual strains with enhanced transmissibility, possibly associated with phylogenetic exchange (154, 312, 381), it is not possible at present to unequivocally identify all epidemic strains. There is little doubt that the incidence of *B. cepacia* has fallen following the introduction of segregation and hygiene guidelines. Until the role of *B. cepacia* colonizing factors can be confirmed and transmissible strains can be clearly identified, it would seem wise to advocate the relaxation of segregation policies and hence potentially compromise the safety of individuals with CF.

In conclusion, an evolving network of social and therapeutic care and support for individuals with CF, which previously had been encouraged, has been greatly disturbed by the addition of *B. cepacia* to the spectrum of CF pathogens. The long-term consequences and efficacy of surveillance and segregation require careful analysis. For the present, to conclude that fear of acquisition of *B. cepacia* has had a major impact on the CF community of patients and caregivers is to risk an understatement.

**Laboratory Identification**

The social and psychological consequences of segregation of *B. cepacia*-positive patients places a considerable burden on microbiological laboratories to identify this organism accurately. Species other than *B. cepacia*, in particular *S. maltophilia*, *A. caligenes* spp., *C. acidovorans*, and some strains of *P. aeruginosa* grow on selective media. Therefore, the presumptive identification of sputum isolates must be confirmed by standard multistest identification systems, such as API 20NE or RapID NF Plus. Large numbers of isolates may be screened economically by observing biochemical characteristics on arginine-glucose medium (158). However, even when multistest systems are used, unequivocal identification of some isolates is difficult (226a, 402). For example, comprehensive biochemical tests and fatty acid analysis of multi-drug-resistant strains (402) previously identified as *B. cepacia* indicated that these isolates possessed characteristics atypical of *B. cepacia* and closely resembled *Burkholderia gladioli*, an organism previously regarded solely as a plant pathogen and of little clinical relevance in individuals with CF (61). In view of the potential clinical and social problems posed for CF patients by these multiresistant strains and the comparable endotoxicity of LPS extracts from both species (see the section on pathogenesis and immune response, below), it would be prudent to consider *B. cepacia* and *B. gladioli* as of equal significance. Difficulties in identification and speculation on the taxonomic and evolutionary status of highly transmissible *B. cepacia* lineages (410) have been supported by recent molecular phylogenetic analyses (167a, 456). Isolates which had been identified as *B. cepacia* by API 20NE were further characterized by analysis of cellular proteins and fatty acid components and clustered by computer-assisted numerical comparison of the profiles. Representative isolates from individual clusters were then selected to determine genotype relatedness within and between clusters by means of DNA-DNA and DNA-tRNA hybridization assays. The results reveal that organisms presently identified by conventional tests as *B. cepacia* represent several new *Burkholderia* species (167a, 456) or, more correctly, genomovars.

**Pathogenesis and Immune Response**

In contrast to the extensive information on the virulence determinants of *P. aeruginosa*, including the regulation and biosynthesis of alginate, knowledge about the virulence factors and pathogenesis of *B. cepacia* is scanty (153, 312). *B. cepacia* appears to produce few recognized virulence factors, and animal models of infection indicate that it is less virulent than *P. aeruginosa* (429). This section of the review will focus on the most recent data which might clarify the mechanisms involved in *B. cepacia* colonization of the CF lung and the subsequent accelerated pulmonary deterioration in some patients. When appropriate, the reader’s attention will be drawn to the properties identified in isolate CF5610 (J2315), which represents arguably the most highly transmissible strain associated with colonization and numerous fatalities in individuals with CF in the United Kingdom and North American (160, 208, 348a, 436) and pragmatically referred to as the Edinburgh/Toronto/ET12 lineage (167a).

**Colonization and Adherence**

Adherence of potential pathogens to host mucosal or epithelial surfaces is an important step in the establishment of infection. Pili (fimbriae) are major adhesins for many bacterial pathogens and thus seem logical candidates to explain the initial stages of *B. cepacia* colonization in the CF lung. Electron microscopy has shown that approximately 60% of *B. cepacia* strains express peritrichous pili (49, 239); polar pilation previously demonstrated by Saiman et al. (378) is relatively uncommon and may reflect the loss of peritrichous fibers during preparation (383). Kuehn et al. (239) reported that a 16-kDa *B. cepacia* subunit showed homology with PAK pili of *P. aeruginosa*; in contrast, other studies reported little cross-reactivity between *B. cepacia* and *P. aeruginosa* anti-pilin monoclonal antibodies and no homology between *P. aeruginosa* pilin gene probes and *B. cepacia* genomic DNA (380). Recent studies have shown *B. cepacia* pilin peptides to be more characteristic in homology to *E. coli* pili than to those of *P. aeruginosa* (383). A wide spectrum of hemagglutination reactions for animal and human erythrocytes also supports heterogeneity in *B. cepacia* pili (47, 49). Binding experiments with *B. cepacia* and *P. aeruginosa* (235, 236) demonstrated that both species adhere to the same GalNAcβ1-4Gal sequence present in many asia-
logglycolipids. However, competition for epithelial receptors was not observed, suggesting that the two species may use different epithelial receptors (378).

The observation that B. cepacia binding increased in the presence of P. aeruginosa has led to speculation of a synergistic relationship whereby P. aeruginosa exoproducts modify epithelial cell surfaces, exposing receptors and facilitating increased B. cepacia attachment (378). On the basis of the characteristically high prevalence of P. aeruginosa among young adults with CF, synergistic interaction between the two species appears a reasonable explanation for the increased incidence of B. cepacia in this age group. In many CF centers, a characteristically high prevalence of P. aeruginosa in young adults would ensure that most new cases of B. cepacia occurred in patients already colonized with P. aeruginosa. However, not all CF patients harbor P. aeruginosa prior to acquisition of B. cepacia; for example, in an epidemic outbreak of B. cepacia in the United Kingdom, almost 40% of patients colonized with B. cepacia were not co-colonized with P. aeruginosa (160). CF microbiology is never simple, and, not unexpectedly, microbial synergy cannot be the only explanation to account for the acquisition of B. cepacia.

The respiratory mucosal blanket provides an important primary barrier to microbial pathogens, and attachment to respiratory mucin has been shown to be an important factor in P. aeruginosa colonization (314, 351, 357). Thus, we should consider the ability of B. cepacia to adhere to respiratory mucins. Sajjan et al. (381) reported that binding to mucin from CF and non-CF individuals was common in B. cepacia and was present in 28 of the 30 isolates tested. A 22-kDa pilin protein distributed along the length of pilin fibers was identified as a mucin-binding adhesin specific to piliated strains (382). B. cepacia isolates exhibiting the highest mucin-binding values tended to correlate with patients with severe illness, leading the authors to speculate that variability in the binding may be associated with morbidity and mortality and may explain why some CF patients are transiently colonized whereas strains in other patients are never lost. Deglycosylation treatment indicated that the mucin receptors for B. cepacia included N-acetylgalactosamine and N-acetylgalactosamine, molecules which can act as chemoattractants but not adhesins for P. aeruginosa (314).

Another study, involving similar mucin overlay assays, reported mucin binding in only 6 of 38 B. cepacia isolates and concluded that both the degree and incidence of mucin binding in B. cepacia are less than with P. aeruginosa (49). An explanation for these apparent contradictions may lie in the choice of isolates used and, in particular, the transmissibility of B. cepacia. The isolates used by Butler et al. (49) represented strains from different geographical locations whose lack of clonal relationship had been confirmed by phenotypic and genomic fingerprinting. In contrast, Sajjan et al. (381) used a collection of strains from a single clinic and no typing data were provided to exclude the possibility of clonal relationships between the isolates investigated. Of interest, among the six B. cepacia isolates shown by Butler et al. to possess mucinophilic properties, the epidemic isolate CF5610 (J2315) showed binding sixfold greater than that of any of the other five adherent strains.

Recent molecular and electron-microscopic studies have clearly demonstrated the phylogenetic diversity of B. cepacia isolates from clinical and environmental sources, confirmed at least five different structural classes of pilin fibers (154), and implicated pilin adhesins in the enhanced transmissibility of at least one epidemic lineage. Epidemiological analyses for phenotypic expression and genotypic studies based on the use of the pilin-specific gene probes suggest that four of the five structurally distinct classes of pili correlate with the source from which strains expressing the appendage were isolated. Synthesis of peripherally arranged pili with unique cable-like morphology, cable (cbl) pili, assembled from a major 17-kDa pilin subunit, was observed in all isolates associated with an epidemic outbreak in a large North American CF center (154, 383). The authors speculated that the novel cbl pilus had evolved for colonization-based adhesion to mucus-glycoprotein-carbohydrate molecules present within the mucosal blanket of the CF lung, with the tendency of the cbl pili to tangle with similar fibers from neighboring bacteria, enhancing the survival of bacterial microcolonies. Preliminary results also implied that the cbl pili may interfere with mucociliary transport by binding both to CF mucin and to the airway epithelial cells and their cilia (154, 436). Goldstein et al. (154) included the important caveat that similar analysis was required of other highly transmissible B. cepacia lineages. Subsequent DNA sequence analysis of cblA in a range of isolates revealed that the United Kingdom isolate CF5610 and Toronto isolates represented a unique and divergent highly transmissible evolutionary lineage of B. cepacia (436).

It is interesting that Goldstein et al. (154) combined the properties of virulence and transmissibility in their speculations. Similarly, the term “high virulence” was used to describe CF5610 and other isolates sharing the same multilocus enzyme fingerprint, designated ET12, (208). Other studies, however, have indicated a strong host involvement in B. cepacia pathogenesis, suggesting that enhanced transmissibility is not inevitably associated with more acute pulmonary deterioration. For example, although there were compelling epidemiological data to suggest that enhanced transmissibility was strain dependent in the United Kingdom outbreaks involving the CF5610/ET12 clone, longitudinal studies showed that the clinical outcome in patients colonized by the same strain could not be predicted and ranged from rapidly fatal deterioration within a few months to asymptomatic colonization for over 6 years (160; unpublished data). Similarly, rapidly fatal pulmonary deterioration has been associated with strains of B. cepacia for which no evidence of transmissibility was found and at a time prior to the introduction of the segregation of B. cepacia-positive patients (144).

Extracellular Colonization and Virulence Factors

Some isolates of B. cepacia produce proteases, lipases, hemolysins, and exopolysaccharide, but there is little evidence that any of these factors play major roles in CF lung disease, nor is there convincing evidence that B. cepacia isolates produce any of the major P. aeruginosa virulence factors including exotoxin A, cytotoxins, and alginate (312). Vasil et al. (458) reported that Southern hybridization experiments with an exotoxin A gene probe failed to produce a positive signal with any of eight B. cepacia strains tested, even under low-stringency conditions. Similar experiments conducted in the authors’ laboratories failed to demonstrate the presence of the exotoxin A gene in environmental isolates of B. cepacia, although, interestingly, a positive band was obtained with the epidemic isolate CF5610. However, despite growth of CF5610 under conditions of iron limitation, polyacrylamide gel electrophoresis of the cell-free culture supernatant and immunoblotting with anti-exotoxin A serum failed to confirm production of the 66-kDa exotoxin A protein (312). A B. cepacia 34-kDa proteinase with antigenic properties similar to P. aeruginosa elastase and the ability to cleave gelatin and collagen but not human immunoglobulins has been described (288). Intratracheal instillation of the purified protein-
ase into rat lungs produced bronchopneumonia, polymorphonuclear cell infiltration, and proteinaceous exudate into large airways. Active immunization of rats with protease elicited an immunological response, which was not protective when the animal were subsequently challenged with *B. cepacia*.

Hemolysin and phospholipase C (PLC; lecinthinase) expression in *B. cepacia* appears to be strain dependent and complex. Some strains produce a heat-labile hemolysin which has both PLC and sphingomyelinase activities (459). The study of Nakazawa et al. (309) found that only 4% of clinical isolates were capable of clear hemolysis whereas 67% of the same isolates produced lecinthinase activity. Another study showed hemolytic activity in 40% of clinical isolates when a range of animal erythrocytes was used as the substrate (459). PLC cleaves phosphatidylethanolamine, a major lung surfactant, and has been associated with cytopathology of lung tissue; antibodies directed against PLC can be detected in the early stages of colonization by *P. aeruginosa*. Unlike *P. aeruginosa* PLC, the PLC activity of *B. cepacia* does not correlate with hemolytic activity (459).

However, all hemolytic strains produce lecinthinase activity (309, 459). Lipolytic activity can be detected in approximately 60% of *B. cepacia* strains (55, 266, 289), but the purified enzyme with a molecular weight of 25,000 is not cytotoxic for mice or HeLa cells (266). Other studies have demonstrated that rat pulmonary alveolar macrophages exposed to *B. cepacia* lipase have fewer pseudopodia and microvilli and exhibit reduced phagocytic activity against *B. cepacia* (431). As in the case of elastase, the role of PLC in *B. cepacia* colonization is equivocal. On the basis of the above evidence and the fact that many CF isolates of *B. cepacia* show no lipolytic, proteolytic, or hemolytic activity, it seems reasonable to conclude that these putative extracellular factors do not play a major role in *B. cepacia* infection of the CF lung.

*B. cepacia* produces at least three distinct iron-binding siderophores, cedapactin, azurechelin, and pyochelin, the last chemically unrelated to the pyochelin siderophore of *P. aeruginosa* (40, 294, 410, 411). Morbidity and mortality in *B. cepacia*-colonized patients with CF has been reported to correlate with production of pyochelin (410). Although many of the *B. cepacia* isolates examined were pyochelin negative, azurechelin was identified in 88% of all clinical isolates and exogenously administered pyochelin enhanced the virulence of non-pyochelin-producing *B. cepacia* strains for rat lungs (413). Production of siderophores enables bacteria to compete for iron with the host iron-binding proteins, transferrin and lactoferrin, and is an important virulence factor in many bacterial infections. Although the role of siderophore production in *B. cepacia* is inconclusive, it seems worthy of more detailed investigation.

Gessner and Mortensen (139) investigated an extended range of other putative pathogenic factors for *B. cepacia* and reported the following characteristics to be more frequent in CF isolates: production of catalase, ornithine decarboxylase, valine aminopeptidase, C₁₄ lipase, alginate, and trypsin; reduction of nitrate to nitrite; hydrolysis of urea and xanthine; and hemolysis of bovine erythrocytes. With the exception of catalase, which will be discussed in a later section in association with the potential intracellular survival of *B. cepacia*, the role of any of these factors in the CF lungs is unclear.

Although attempts to identify virulence factors in *B. cepacia* have proved relatively unsuccessful, they have revealed interesting differences in the genetic organization of *P. aeruginosa* and *B. cepacia*. In contrast to the consistent patterns observed in the PLC gene of *P. aeruginosa*, the genetic organization of the PLC gene of *B. cepacia* is highly variable (459). The variable manner in which a *B. cepacia* PLC-specific probe hybridized with restricted *B. cepacia* DNA, the variability in expression of hemolytic and PLC activities of different strains, and the association of DNA arrangements with temperature-dependent conversion of a hemolysin-positive to a hemolysin-negative variant may be associated with the relatively large number of insertion sequences (more than 25) identified in *B. cepacia* (394, 459) and the presence of multiple replicons (58, 364). The insertion sequences which can be found in multiple copies and can both activate and inactivate gene expression in *B. cepacia* have not been found in *P. aeruginosa* despite the extensive genetic knowledge of the species.

**Cell Surface Properties**

Production of copious amounts of alginate by mucoid variants of *P. aeruginosa*, leading to the formation of biofilms, is the major virulence determinant associated with *P. aeruginosa* colonization in CF patients (141, 169, 329). Alginate biosynthetic potential has also been shown in other pseudomonads belonging to RNA group I, including *Pseudomonas fluorescens*, *Pseudomonas putida*, and *Pseudomonas mendocina*, by using a carbencillin selection technique (163). Ironically, in contrast to their major role in CF lung disease, there is much commercial interest in the biosynthesis and structure of alginate because of their potential exploitation as food stabilizers and gelling agents (64, 395, 438).

With the exception of a single strain which produced an acetylated alginate-like compound containing 72% guluronic acid (432), no alginate-producing variants of *B. cepacia* have been identified in the extensive culture collections now available, nor have attempts to isolate alginate-producing variants in vitro been successful (163). Further evidence to suggest a lack of alginate-synthesizing capability in *B. cepacia* comes from PCR amplification studies with oligonucleotide primers based on *algD*, the gene encoding GDPmannose dehydrogenase and a pivotal enzyme for alginate biosynthesis, which failed to produce a positive signal in 10 *B. cepacia* strains studied (312). Additional studies in our laboratories and by others (3, 377) have shown that some *B. cepacia* isolates produce an exopolysaccharide comprising galactose, glucose, mannose, glucuronic acid, and rhamnose, with lesser amounts of uronic acid; no mannnuronic or guluronic residues characteristic of microbial alginites were identified. Mucoid colonial morphotypes of *B. cepacia* exist but are relatively rare (<1%) in both environmental and clinical isolates. Experience of many hundreds of *B. cepacia* isolates in our laboratories over the last 15 years have shown that mucoid *B. cepacia* isolates are seldom cultured from patients with CF, nor do nonmucoid forms, including the epidemic isolate CF5610, produce extracellular exopolysaccharide under nitrogen limitation or raised osmolarity conditions, which stimulate alginate biosynthesis in *P. aeruginosa*. Although *B. cepacia* may gain protection from *Pseudomonas* alginate when the two pathogens coexist in CF lungs, *P. aeruginosa* colonization is not a prerequisite for acquisition of *B. cepacia* (160) and the presence of bacterial biofilm or microcolonies has not been reported in patients colonized solely by *B. cepacia*. There is, however, an interesting caveat to this discussion of the putative role of exopolysaccharide in *B. cepacia* colonization of the CF lung. During longitudinal studies of *B. cepacia*-colonized patients with CF, we have recently observed transformation of a *B. cepacia* strain from a nonmucoid to a mucoid colonial morphotype within 1 year of initial colonization; the isolates could not be distinguished by bacteriocin typing or pulsed-field gel electrophoresis, and the nature and properties of the bacterial exopolysaccharide are under investigation.
Host Immune Response

In contrast to the increasing epidemiological data on \textit{B. cepacia} colonization in patients with CF, information on the immune response to \textit{B. cepacia} has been scanty until recently. In 1991, Aronoff et al. reported that IgG antibodies to \textit{P. aeruginosa} outer membrane proteins cross-react with those of \textit{B. cepacia}, and, since \textit{P. aeruginosa} is often the original colonizer, they concluded that \textit{B. cepacia} colonization was not inhibited by the presence of specific serum antibodies to outer membrane proteins (12). Subsequently, Nelson et al. (311) used CF isolates of \textit{B. cepacia} and strains representing 10 O serotypes and, with whole cells or extracted LPS as antigens, demonstrated that \textit{B. cepacia}-colonized patients produced a rising IgA and IgG antibody response. Absorption studies and immunoblot analysis of serum from colonized patients also showed that a component of the anti-\textit{B. cepacia} LPS antibody response is specific for \textit{B. cepacia} and does not react with the core LPS of \textit{P. aeruginosa}. Anti-core LPS antibodies resembled those against outer membrane proteins in not preventing bacterial colonization; however, their detection in the serum of patients can provide useful confirmation of \textit{B. cepacia} colonization. In some patients, a two- to fourfold rise in antibodies preceded the first sputum culture of \textit{B. cepacia}. Since laboratory identification of some \textit{B. cepacia} isolates can be difficult (348, 402) and pulmonary colonization may not be detected by currently recommended culture methods for as long as 2 years after acquisition (260), detection of a specific antibody response may provide an early warning of \textit{B. cepacia} colonization (241, 311). Recently, the presence of IgA antibody against a 30-kDa porin has been associated with better prognosis, suggesting a potential target for immunotherapy (44).

By the early 1990s, there was compelling evidence for person-to-person transmission of \textit{B. cepacia} and a growing consensus on the efficacy of cohorting of colonized patients. There was also circumstantial evidence that colonization increased the rate of pulmonary decline in patients with advanced lung disease and, more ominously, was associated with rapid and fatal pulmonary decline in some relatively healthy patients with CF (38, 160) and fatal community-acquired pneumonia in non-CF patients (355). However, opinions varied greatly, and some individuals remained of the opinion that \textit{B. cepacia} was not a pathogen but merely a marker of deteriorating lung disease or even of CF itself. Recently, remaining doubts on the pathogenic potential of \textit{B. cepacia} have been dispelled by the demonstration that LPS preparations from clinical and environmental isolates and from the closely related phytopathogen \textit{B. gladioli} exhibit a higher endotoxic activity and cytokine stimulation in vitro than do LPS preparations from \textit{E. coli} and from the major CF pathogen, \textit{P. aeruginosa} (Fig. 9) (397). These observations not only confirm the pathogenic potential of \textit{B. cepacia} but also help to explain the increased levels of inflammatory markers observed in patients colonized by \textit{B. cepacia} compared with \textit{P. aeruginosa} (112). Evidence of this pathogenic potential is important for a number of reasons: (i) the rational design of new therapeutic strategies, (ii) uncertainty concerning the clinical significance of \textit{B. gladioli} (61, 402) and (iii) the growing debate on the clinical relevance of environmental \textit{B. cepacia} and its potential as a biocontrol agent for soil decontamination and prevention of plant disease (26, 48).

Is \textit{B. cepacia} an Intracellular Pathogen?

A recent report by Butler et al. (50) provides an interesting immunological anomaly with respect to \textit{B. cepacia} survival in vivo. \textit{B. cepacia} isolates of clinical or environmental origin commonly exhibit smooth colonial morphotype and a range of serotypes based on variable but complete O side chain LPS on polyacrylamide gel electrophoresis analysis. In contrast, epidemic isolates from patients with CF frequently exhibit a rough colonial morphotype and loss of the O side chain portion (B-band) and are serum sensitive (50). Bacterial strains possessing rough LPS are typically sensitive to the bactericidal activity of serum, are generally less virulent than serum-resistant strains, and are seldom associated with bacteremia (69). The fact that CF isolates of \textit{B. cepacia} are serum sensitive but achieve prolonged pulmonary colonization and bacteremia poses an interesting and important problem in respect of their survival in CF lungs.

\textit{B. cepacia} isolates grown under different nutrient conditions show various degrees of susceptibility to killing by human serum and by polymorphonuclear leukocytes (8). The pathogenic potential of \textit{B. cepacia} for non-CF patients is emphasized by the susceptibility of patients with chronic granulomatous disease to \textit{B. cepacia} infection, which, in turn, is associated with the resistance of catalase-positive \textit{B. cepacia} strains to neutrophil-mediated nonoxidative bactericidal killing (417). Accumulated evidence indicates that serum-sensitive \textit{B. cepacia} strains survive a pronounced humoral and cellular immune response in the lungs of patients with CF. Furthermore, even when susceptibility to antibiotics is demonstrated in vitro, there is seldom any demonstrable reduction in the bacterial load within sputum. On the basis of this evidence, it is tempting to agree with speculation that despite their natural role as a colonizers of the soil rhizosphere, some \textit{B. cepacia} strains have adapted to the role of human intracellular pathogens with the capability to invade and survive within respiratory epithelial cells and professional phagocytes (45, 312).

Does Environmental \textit{B. cepacia} Pose a Hazard to CF Patients?

A review of the progress made in clarifying the routes of transmission of \textit{B. cepacia} would be incomplete if consideration were not given to the role of natural or contaminated environments as a source of infection. A first consideration is to review the often quoted statement that \textit{B. cepacia} is a ubiquitous saprophyte with a penchant for onions. This view is probably an exaggeration. Even with the use of selective media, \textit{B. cepacia} is not readily cultured from the homes of patients with CF or from environmental sites (48, 122, 306). For example, a prospective study of 619 environ-
mental samples from homes, soil, water, sink drains, vegetables, food store counters, and salad bars produced a maximum isolation rate of only 4.5% in food stores and salad bars (122). Another prospective study attempted to recover 

B. cepacia from the soil, water, and vegetative sources in a large botanical complex. Butler et al. (48) used strategies for culture of 

B. cepacia which had been previously proved successful in identifying the organism in the immediate environment of colonized CF patients. This microbiological safari included sampling of soils, ponds, and vegetation in a range of microenvironments contained in tropical and temperate greenhouses; 12 

B. cepacia cultures were obtained from 55 samples (17%), with positive cultures obtained primarily from soil samples. The genomic diversity of the species was also demonstrated when pulsed-field gel electrophoresis analysis revealed all but two of the isolates to possess unique patterns when cut with the restriction enzyme SpeI (ACTAGT). There is little evidence to link true environmental 

B. cepacia (as distinct from clinical isolates contaminating hospital equipment) with infection in patients with CF. An epidemiological study of 

B. cepacia acquisition during four CF summer camps (191) revealed that the four environmental isolates (3 cultured from lake water and 1 cultured from iced drinking water out of 58 environmental samples) had ribotypes distinct from any of the 

B. cepacia strains present in or acquired by campers. Analysis of accumulated data has led to speculation that the principal source for 

B. cepacia acquisition by CF patients is the respiratory secretions of colonized patients (348).

A major caveat to the conclusion that the environment is not a major source of 

B. cepacia infection in humans concerns the considerable interest in using 

B. cepacia as a biocontrol agent (189, 252). The nutritional versatility of 

B. cepacia and its biosynthesis of antimicrobial agents are currently being developed in the biological control of a range of plant infections and fungal spoilage of stored fruit (10, 82, 130, 178, 193, 197, 200, 226, 290) and the biodegradation of organic pollutants (81, 398). Large-scale culture of 

B. cepacia is also under development to take advantage of its exceptional metabolic diversity for use in bioremediation of contaminated soils (398, 451).

It is particularly relevant to the issue of the use of 

B. cepacia in biological control to clarify whether clinical and environmental strains represent two distinct groups of organisms and whether environmental strains have the ability to cause human infections, either de novo or through adaptation. With respect to the latter, although phenotypic variability can be observed on the basis of differences in colonial morphology, antibiotic susceptibility, pigment production (244), serum sensitivity and LPS structure (50), the pathogenic significance of such variability is unclear. Longitudinal studies of 

B. cepacia-colonized CF patients have provided little evidence for phenotypic adaptation or selection of variants in vivo, unlike the characteristic transition of 

P. aeruginosa to mucoidy.

Previous comparative studies of clinical and environmental isolates which have suggested that environmental and clinical isolates of 

B. cepacia represent distinct groups, that clinical strains lack the ability to act as phytopathogens, and that environmental isolates are unlikely to be responsible for human infections (26, 155, 208, 485) have been handicapped by inclusion of only a few environmental isolates. For example, on the basis of data from two environmental isolates, it has been suggested that “ 

B. cepacia found in the environment is demonstrably quite different genomically from pulmonary and nosocomial isolates and unlikely to be responsible for infections in CF patients” (208). This distinction is unfounded. Most clinical isolates readily cause soft rot of onions (48). In turn, evidence that environmental isolates have the ability to cause human infection is provided by the macerated, hyperkeratotic foot lesions, known as swap rot, experienced by troops during swim training (442). The distinctive phenotypic properties of multi-drug-resistant epidemic strains of 

B. cepacia (160, 402) suggest that a subpopulation of 

B. cepacia may have a predilection for the CF lung. It has been shown that epidemic strains of 

B. cepacia share cell fatty acid content and other characteristics with the closely related phytopathogen 

B. gladioli (401) and that the G+C content of the 

B. cepacia cbl pilin is intermediate between that of 

E. coli genomic DNA and that of 

B. cepacia genomic DNA (383). Additional genetic evidence that the 

B. cepacia genome in the type strains ATCC 17616 and ATCC 25416 consists of multiple replicons (58, 364) and the identification of transposable elements (247, 394) have led to speculation that the transition of environmental 

B. cepacia from phytopathogen to human pathogen is enhanced by intraspecies adaptation or the acquisition of genes through horizontal gene transfer from other bacterial species (383, 397, 402). A recent study of the genetic structure of an aquatic population of 

B. cepacia by multilocus linkage disequilibrium analysis suggests frequent recombination relative to binary fission in this environmental population (473). Rodley et al. (364) noted that when Johnson et al. (208) used ribotyping and multilocus enzyme electrophoresis to examine phylogenetic linkage in an extensive collection of 

B. cepacia, the data showed that the nearest neighbor to ATCC 25416 was a highly transmissible clone, represented by the epidemic isolate CF5610, responsible for numerous outbreaks and fatalities in the United Kingdom and North American (160, 436). In a recent preliminary screening which included 15 newly isolated environmental 

B. cepacia strains, we observed that as bacterial populations, CF isolates differed positively from environmental isolates (P < 0.05) in various putative virulence factors including ability to grow at 37˚C and in CO2. In contrast, clinical isolates exhibited poor antifungal activity (P < 0.05). Of particular interest, we identified two environmental isolates in which these three characteristics were grouped as a cassette. This cassette was also found with the ATCC 25416 soil isolate and supports the working hypothesis that despite segregation of 

B. cepacia-positive individuals, sporadic cases of colonization occur when CF patients are exposed to a subpopulation of 

B. cepacia or 

B. cepacia-like organisms within the environment which are primed to colonize lungs already compromised by poor mucociliary clearance and epithelial tissue damage (48, 167). Clearly, the existence and identification of such potentially primed bacterial subpopulations or genomovars (167a) and their phenotypic and genomic characteristics require further investigation.

In conclusion, the extent to which the use of 

B. cepacia as a biological control agent presents a hazard to individuals with CF living in the same geographical location remains unclear. Nevertheless, the fact that such an issue should arise strikingly emphasizes the role of 

B. cepacia as a microbial Jekyll and Hyde and the urgent need for collaborative studies by microbiologists from different specialties. At present, unequivocal statements on the potential of environmental 

B. cepacia isolates to cause infections in CF patients and other immunocompromised hosts are frustrated by scanty knowledge of the virulence factors and the mechanisms of pathogenesis involved. The recent development of mutant CF mice (104) and their use to show that 

B. cepacia causes pneumonia in CF mice but not control animals (79) provides a valuable model to investigate the pathogenic potential of environmental 

B. cepacia isolates for CF patients.
CONCLUSION

A striking characteristic of pulmonary infections in individuals with CF is the limited spectrum of pathogens responsible, in particular the limited role of classic pulmonary pathogens such as Strepococcus pneumoniae, Klebsiella pneumoniae, and Mycoplasma species. The unusual and, in the case of alginate biosynthesis, unique phenotypic changes associated with the adaptation of P. aeruginosa from a saprophytic opportunist to a biofilm-embedded parasite in the CF lung might reasonably have led to optimism that P. aeruginosa would represent the last major microbiological challenge in an expanding and maturing CF community. This review has illustrated the major progress made in understanding the regulation of Pseudomonas alginate, arguably the major bacterial virulence factor in the CF lung. In addition, the evolving challenge of CF microbiology has been illustrated by the transmissibility and indeterminate virulence of the pythopathogen B. cepacia.

The aims of future research must be to use the increasing knowledge of the pathophysiology of CFTR, including its influence on the rheology of respiratory secretions and host bacterial receptors, to implement preventative therapeutic strategies in the patients most at risk. The importance of early treatment has recently been emphasized by the first unequivocal demonstration of respiratory infection and inflammatory sequelae in CF infants in the first months of life (11). A detailed understanding of alginate biosynthesis should also lead to the development of strategies to reduce P. aeruginosa colonization. Faced with the quite different challenge of B. cepacia, it is clear that segregation policies have reduced but not eliminated the incidence of new cases, and the cost in social terms is high and not universally accepted. Identification of the host and bacterial factors responsible for enhanced transmissibility and virulence could lead to less stringent segregation and a more scientific assessment of the hazards posed by the environment as a source of B. cepacia. These aims are most likely to be achieved by interdisciplinary research combining molecular epidemiology, phylogenetic analysis, and pathogenic studies, including use of the CF transgenic mouse model for in vivo analysis of mRNA expression during infection. Such research, combined with a greater understanding of the inflammatory effects of B. cepacia colonization and the action of anti-inflammatory mediators, may lead to novel prophylactic therapy and vaccine development, in the absence of effective antibiotic therapy. Finally, although the much-awaited trials of CF gene therapy show promise, this innovative therapy will ultimately be judged by its ability to prevent pulmonary colonization by highly adaptive and challenging microbial opportunists.

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