R. Ramphal, S. Vishwanath

Why is Pseudomonas the Colonizer and Why Does It Persist?

Summary: *Pseudomonas aeruginosa* is currently the major cause of morbidity and mortality in cystic fibrosis. Studies to understand why this particular organism is a problem and why host defenses fail to clear it, are beginning to provide some answers. Implicit in any working hypothesis are the prerequisites that: (i) *P. aeruginosa* should have a tropism for the respiratory tract; (ii) there should be a physical clearance defect; and (iii) there should be an acquired immune clearance defect. Studies from many laboratories support these contentions. This organism exhibits its tropism by adhering to tracheal cells and to tracheobronchial mucins by means of pili or the mucoid exopolysaccharide of mucoid strains. The receptors on both cells and mucins contain sialic acid as the dominant sugar moiety. Many factors contribute to its persistence, chief among which is the failure of phagocytic defenses caused by microbial or host enzymes and even by mucins which inhibit the opsonophagocytosis of *P. aeruginosa*. Injury to the mucociliary system, again caused by microbial or host factors, is also a prominent factor in the persistence of *P. aeruginosa*. We hypothesize that this organism is the dominant pathogen because of the existence of receptors in the respiratory tract for it and that it persists because bacteria in stagnant mucus cannot be cleared physically or immunologically. We are doubtful that conventional vaccination approaches will yield a solution to this problem.

Zusammenfassung: *Ursachen für die Besiedelung durch Pseudomonas und seine Persistenz. Pseudomonas aeruginosa* ist zur Zeit die wichtigste Krankheits- und Todesursache bei Patienten mit zystischer Fibrose. Studien, die sich mit den Problemen befassen, die dieser spezielle Erreger verursacht, und mit der Frage, warum er durch Abwehrmechanismen des Wirtes nicht eliminiert wird, geben die ersten Antworten zu diesem Thema. Die Arbeitshypothesen gehen davon aus, daß 1. *P. aeruginosa* eine besondere Neigung hat, sich im Respirationstrakt anzusiedeln; 2. ein Defekt in der physikalischen Klärfunktion besteht und es 3. einen erworbenen Defekt in der immunologischen Komponente der Erregeradikation gibt. Diese Annahmen werden durch Laboruntersuchungen gestützt. Die Adhärenz des Erregers an Trachealzellen und an tracheobronchialen Muzinen mittels Pili oder dem mukoiden Exopolysaccharid mukoider Stämme ist die Grundlage für seinen Tropismus zum Tracheobronchialbaum. Die Rezeptoren beider Zellen und Muzine enthalten Sialinsäure als dominierende Zuckerverbindung. Zahlreiche Faktoren begünstigen die Persistenz des Erregers; am bedeutsamsten ist das Versagen der phagozytären Abwehr unter dem Einfluß von Enzymen, die vom Mikroorganismus oder dem Wirt selbst sezerniert werden, und sogar auch der Muzine, die die Opsonophagozytose von *P. aeruginosa* hemmen. Eine Schädigung des mukoziliaren Systems, die wiederum durch mikrobielle oder Wirtsfaktoren verursacht sein kann, ist ebenfalls für die Erregerpersistenz von Bedeutung. Wir stellen die Hypothese auf, daß dieser Mikroorganismus deshalb der dominierende Erreger ist, weil der Respirationstrakt Rezeptoren für ihn besitzt; die Ursache für seine Persistenz liegt darin, daß die Bakterien im stagnierenden Schleim weder physikalisch noch immunologisch beseitigt werden können. Wir bezweifeln, daß sich mit konventionellen Versuchen einer Vakzination eine Lösung für dieses Problem finden läßt.

Introduction

Pulmonary infection is the major determinant of mortality in cystic fibrosis (CF). The predominant pathogen involved is *Pseudomonas aeruginosa*. Two unanswered questions concerning this infection have been: (i) why is *P. aeruginosa* the dominant colonizer; and (ii) why is the host unable to get rid of it? These questions can be best answered by considering three implications of this phenomenon: (i) *P. aeruginosa* must have a tropism for the respiratory tract; (ii) there must be a defect in physical clearance and (iii) there must be a defect in immune clearance. The following perspective on these questions is based on work done principally in our laboratory. Some events are without a doubt multifactorial and our hypotheses will complement those postulated by others, particularly in regard to persistence.

Early Colonization

Historically, *Staphylococcus aureus* has been the early colonizer in this disease (1). This is not surprising, since this organism is one of the most common pathogens that infect children. There is nothing unique or unexpected about staphylococcal infections in the early years of life,

Prof. *R. Ramphal*, M.D., Department of Medicine, University of Florida, Gainesville, Florida, U.S.A. 32 610;
S. Vishwanath, M.D., Laboratory of Microbial Structure and Function, Rocky Mountain Laboratories, NIAID, Hamilton, MT U.S.A. 32610;
Correspondence and reprint requests to Prof. *R. Ramphal*.

however, the persistence of this organism in the lower respiratory tract after acute infection is unique to CF children. A priori, we interpret this to mean that there is a primary clearance defect in the CF respiratory tract early in childhood, since *S. aureus* does not persist in the respiratory tract in non CF children. Some reports indicate that the CF respiratory tract is normal at birth (2), however, by four months of age profound anatomic changes have occurred (3) thus one wonders whether it was really "normal". Regardless of the final truth in anatomical terms, the persistence of this organism implies a functional clearance defect in these children, either physical or immunological, or both. As these children grow older, *S. aureus* disappears and is replaced by nonmucoid *P. aeruginosa* which eventually become mucoid in phenotype (4). There is no wholly satisfactory explanation for the disappearance of *S. aureus* but once the switch has occurred *P. aeruginosa* becomes the major organism. It has been suggested, in view of the fact that *P. aeruginosa* has become the predominant pathogen in the antibiotic era, that antibiotic pressure is responsible for this switch in pathogens (5). Critically speaking, this contention is unproven cince the majority of children probably did not live long enough in the preantibiotic era for us to be certain that the switch would not have occurred with age. In addition, there are now cases reported where *P. aeruginosa* was the first documented pathogen to be isolated from patients who had not received antistaphylococcal treatment (6). It may therefore be fruitful to look for other reasons to explain the switch to *P. aeruginosa*. Some reasons that have not been explored include (a) whether antistaphylococcal immunity does indeed develop and functions effectively to clear this organism later in life and (b) whether the respiratory tract changes chemically with maturation, such that receptors for staphylococci become relatively fewer in number and thus colonization is diminished.

Pseudomonas Colonization

There have been several suggestions to explain the evolution of *P. aeruginosa* as the predominating pathogen. The earliest one was the postulation that antibiotic therapy is responsible for the selection of this relatively antibiotic-resistant organism (6). As pointed out earlier, there are objections to this explanation, although it may play a role in hastening colonization. Another factor that has not been explored is the role of maternal immunity. Since *P. aeruginosa* appears at the age when *H. influenzae* is a problem in children, one cannot help but speculate whether loss of maternal immunity with the concomitant clearance defect may play a role. We, however, believe that recent work from our laboratory may provide the best explanation of why *P. aeruginosa* is the most successful colonizer.

Pseudomonas and Respiratory Tract Compromise

Pseudomonas infection of the respiratory tree is seen only when the bronchial mucosa is diseased or injured e.g. endotracheal intubation (7), bronchiectasis (8) and recently in the ciliary dyskinesia syndromes (9), situations where there is some abnormality of the mucosal surface. Therefore, we postulate that in CF there must also be a mucosal or other clearance defect. However, this cannot be the whole story, since one could reason that if a clearance defect alone were present then any organism inhaled or aspirated from the environment would colonize the airways. There must therefore be a selective tropism exhibited by *P. aeruginosa* for the respiratory tract.

Basis of Colonization and Infection

Current microbiological dogma which is well supported experimentally, points out that the basis of selective colonization is a specific interaction on a surface between a microbial adhesin and a specific receptor for that organism (10). There are many examples of these interactions and there are clear examples of specific organisms adhering to cells in anatomic sites where they cause infections (10). The tracheobronchial tree is no exception, e.g. influenza virus, *Mycoplasma pneumoniae* and *Bordetella pertussis* are well recognized tracheobronchial pathogens which demonstrate adhesin receptor interactions with respiratory epithelial cells (11–13). One could therefore predict that such a mechanism exists for *P. aeruginosa* and the tracheobronchial mucosa; however, since infection and colonization by *P. aeruginosa* occurs only when the tracheobronchial tree is injured or compromised, this mechanism may be evident only under certain circumstances.

Pseudomonas aeruginosa and Injured Cells

In order to study whether *P. aeruginosa* has a specific tropism for tracheal cells we have examined the ability of this organism to adhere to the tracheal surfaces of different animal species. Studies with intact mouse and ferret

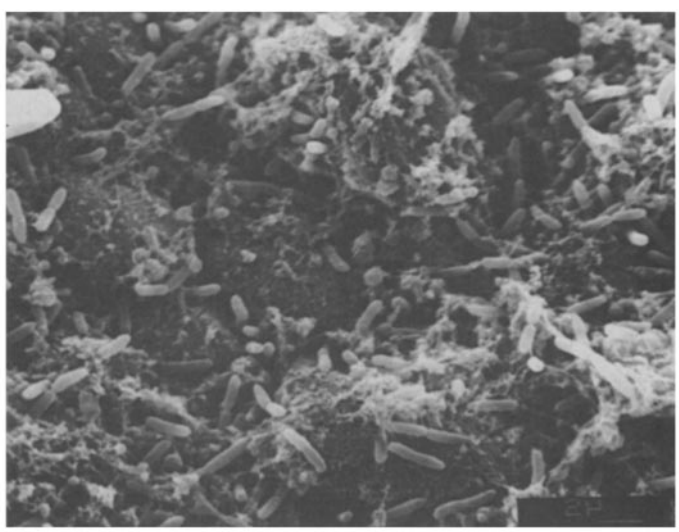

Figure 1: *Pseudomonas aeruginosa* adhering to acid injured tracheal cells.

Infection 15 (1987) Nr. 4

tracheas yielded negative results. However, we discovered that this organism adhered to cells that had been injured by a variety of methods, e.g. influenza virus (14), endotracheal intubation (14) or by mild acid treatment (15) (Figure 1). In fact, other bacteria such as *Escherichia coli* and *Klebsiella pneumoniae* did not bind very well to the injured cells (15). This binding of *P. aeruginosa* to injured cells was mediated by a specific receptor adhesin mechanism. In the case of the mucoid strains, the mucoid exopolysaccharide (alginic acid like exopolysaccharide) mediated adherence to the cells (16). Pili or fimbriae mediated the adherence of the nonmucoid strains (17). The receptor for these adhesins was found to be a glycolipid (18) with sialic acid as the major functional group at the binding site (19). This would suggest that the receptor on the cell surface for *P. aeruginosa* is a ganglioside.

While this specific interaction between injured tracheal cells and *P. aeruginosa* is apparent, it is not clear that this is necessarily relevant to chronic colonization in CF. To date, there has been no demonstration that *P. aeruginosa* actually colonizes tracheal cells in CF. One may speculate that cells injured by viruses or mycoplasmas during exacerbations could become colonized, or perhaps cells injured by enzymes and exotoxins from staphylococci or pseudomonas could also be colonized, but this would probably be a secondary phenomenon after the organisms are established in the respiratory tree, contributing to persistence since injury to the cells would result in abnormal mucociliary clearance.

Pseudomonas aeruginosa and Mucins

The hypothesis we favor to answer the question "why is *P. aeruginosa* the colonizer" is based on some of our experimental observations as well as the clinical observations made in cystic fibrosis. While performing studies with mice, we observed that *P. aeruginosa* attaches to mucin strands (Figure 2) on the uninjured trachea (15). In addition, we also noted that incubating bovine submaxillary mucin and rat tracheal mucin with *P. aeruginosa* prevented the bacteria from adhering to injured tracheal cells (19).

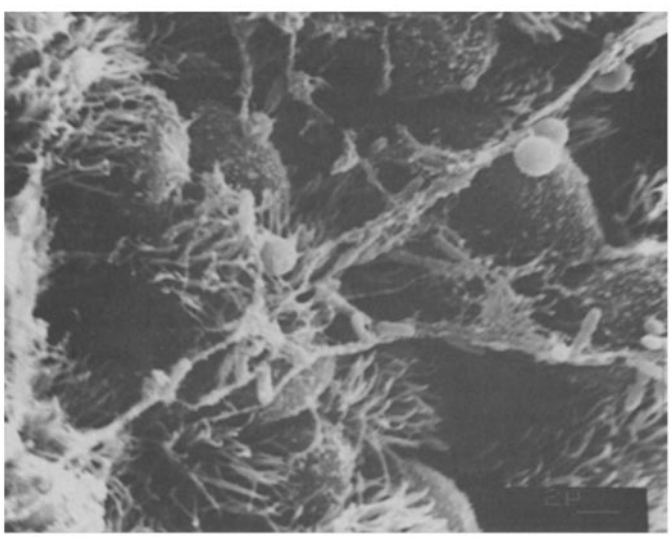

Figure 2: Normal uninjured trachea with almost all bacteria associated with or adherent to mucin strands.

These observations implied that there were receptors in mucins for *P. aeruginosa*. They also suggested that tracheobronchial mucins probably served a defensive function to protect respiratory cells from colonization by binding bacteria; the bound bacteria would then be removed by ciliary action. On the other hand if mucociliary clearance was defective, the situation would not be beneficial to the host. The bacteria bound to mucins would persist in stagnant mucus, and produce a state of chronic colonization. Clinical observation in cystic fibrosis suggest that this is indeed the case, since these patients are unable to clear their mucus.

Hypothesis

From the foregoing observations we propose that *P. aeruginosa* chronically colonizes the respiratory tract because (i) only this organism among the constellation of pathogenic bacteria finds a specific receptor in tracheobronchial mucins and (ii) the mucins with adherent bacteria are not cleared. We believe that these two factors are the primary reasons for *P. aeruginosa* as the major colonizer (Figure 3). The argument can, however, be made that any bacterium which encounters stagnant mucus should be able to colonize it; e.g. *E. coli* or Klebsiella strains, which, even if they do not bind to the mucin molecules, should encounter enough nutrient to survive in the liquid milieu of tracheobronchial mucus. We, however, believe that there are special consequences of bacterial binding to mucins which allow them to persist and not be removed by host immune defenses, as opposed to organisms which do not bind to mucin. These will be discussed under reasons for persistence.

Table 1: Comparison of adherence of *Pseudomonas aeruginosa* with other gram-negative bacteria. Ratio of pseudomonas to other bacteria[a].

Non mucoid *Pseudomonas aeruginosa*/Klebsiella 8	46:1
Non mucoid *Pseudomonas aeruginosa*/Klebsiella m	15:1
Non mucoid *Pseudomonas aeruginosa*/ *Escherichia coli* (mar)	4:1
Non mucoid *Pseudomonas aeruginosa*/ *Escherichia coli* sw818	49:1
Non mucoid *Pseudomonas aeruginosa*/ mucoid *Pseudomonas aeruginosa* m35	2:1
Non mucoid *Pseudomonas aeruginosa*/ mucoid *Pseudomonas aeruginosa* 2192	2:1.7

a: Modified from *Vishwanath* (23).

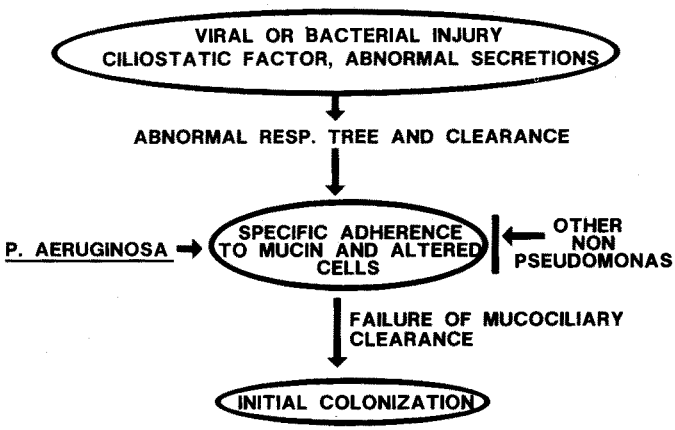

Figure 3: Scheme describing factors responsible for initial colonization.

Adherence of Pseudomonas aeruginosa to Human Mucins

If our hypothesis is correct, then *P. aeruginosa* should adhere to human tracheobronchial mucins and it should be more adherent than the usual strains of *E. coli* and other gram-negative bacteria. Indeed, using purified human mucins in a microtiter plate adherence assay, we have demonstrated that *P. aeruginosa* binds preferentially when compared to other gram-negative organisms (20). While there may be some binding of the occasional non pseudomonad, the extent of binding never reaches that of *P. aeruginosa* (Table 1). We have not tested the mucoid *E. coli* strains which have been found as colonizers in cystic fibrosis (21) but we suspect that these may also adhere quite well because of their uronic acid containing exopolysaccharides. We have also examined the binding of Proteus, Serratia and Acinetobacter strains and find that they too adhere poorly.

Mucin Receptors for Pseudomonas aeruginosa

Since mucins reflect the cell surface carbohydrates to some degree (22), the chief candidates for receptors would more than likely be the sugars of the oligosaccharide chains of the mucin molecules. Pronase digestion of the molecules did not inhibit binding but periodate oxidation of the carbohydrate chains and neuraminidase treatment did. Sialic acid and N-acetylglucosamine inhibited binding of the strains to varying degrees (23). However, one of the most important observations made, was the fact that exposure of mucin to influenza virus inhibited further binding of *P. aeruginosa* (Table 2). This implies that these two organisms may share a common receptor and it has been known for some time that sialic acid is part of the receptor for influenza virus (11). These data therefore point to sialic acid as being part of the Pseudomonas receptor in mucin, hence the mucin receptor is similar to the cellular receptor (19).

Pseudomonas Adhesins for Mucin

We then performed studies similar to those carried out with tracheal cells to find out whether the Pseudomonas adhesins for mucins were the same as those for cells. In fact, we found that pili mediated binding of the nonmucoid strains to mucins and the mucoid exopolysaccharide mediated the binding of the mucoid strains (24). Other useful pieces of information, gathered from these later studies, included the observation that the ability of antipilus antibody to inhibit bacterial adherence was strain specific but antibodies against the mucoid exopolysaccharide reacted broadly against all mucoid strains. Thus we again found that the interactions of Pseudomonas with mucins was similar to its interactions with cell surfaces.

Why Does Pseudomonas aeruginosa Persist?

Persistence of any organism in the respiratory tract must depend on the failure of host defenses to clear the organisms from the airways. Host defenses in the respiratory tract consist of (i) the mucociliary system which functions by entrapping invading bacteria in mucus and expelling them by ciliary motion, and (ii) immune defenses, which consist of complement, antibodies and phagocytes. In the nonimmune host, the defenses against invading bacteria consist of the mucociliary system, complement-mediated killing and nonopsonic phagocytosis by polymorphonuclear leukocytes (PMN) and alveolar macrophages. In the immune host (who has developed specific antibodies to the invading microorganism), the defenses again involve the mucociliary system, but, in addition, secretory IgA may block bacterial adherence; IgM antibodies may, along with complement, kill bacteria; and opsonic IgG, with or without complement, may opsonize bacteria for subsequent phagocytic destruction.

Failure of the physical clearance system alone would allow the serum and phagocyte-mediated killing of colonizing bacteria and conversely, even if defects occurred in the humoral immune and phagocytic defense mechanisms, a normally functioning mucociliary system should still clear colonizing bacteria. Thus, in order for bacteria to chronically colonize the respiratory tract, defects in both the mucociliary system and the immune system should be present. The cystic fibrosis patient should therefore have defects in both the physical and immunological clearance systems that allow *P. aeruginosa* to persist in the airways.

Defective Mucociliary Clearance in CF

Stagnation of mucus in CF implies a defect in the mucociliary system. The following observations have been proposed to explain this defect: (i) The presence of a ciliary dyskinetic factor that occurs uniquely in cystic fibrosis (25); (ii) the increased viscosity of mucus in cystic fibrosis which could plug the airways (26); (iii) intercurrent viral

Infection 15 (1987) Nr. 4

Table 2: Effect of heat-inactivated influenza A virus on adherence of *Pseudomonas aeruginosa* to mucin[a].

Strain	Dose of virus[b]	Adherent bacteria per well (mean ± SD)	% reduction	p value
Pseudomonas aeruginosa R_a	0	15,067 ± 4180	–	–
non mucoid	1,000	4,850 ± 2151	68	< 0.001
	10,000	1,650 ± 957	89	< 0.001
Pseudomonas aeruginosa M_{35}	0	36,933 ± 759	–	–
mucoid	1,000	13,660 ± 10,773	63	< 0.02
	10,000	500 ± 141	99	< 0.001

a: Modified from *Vishwanath* (23);
b: Hemagglutinating units added per well.

and mycoplasmal infections which could damage the mucociliary system: however this does not explain the persistent failure of clearance, since such infections are common in the population at large; (iiii) injury to cells by enzymes produced by *P. aeruginosa.* Therefore, the unanswered question is not whether there is a clearance defect in CF, but what causes it.

Immune Defects in CF

The presence of an adequate and often hyperimmune response to *P. aeruginosa* and the lack of consistent defects in phagocytic function in cystic fibrosis (27, 28) implies that the organism somehow evades the opsonophagocytic host defenses. *Schiller* and *Millard* found that sputum from cystic fibrosis inhibited the serum killing of *P. aeruginosa,* but not of *Escherichia coli* (29). However, whether complement-mediated killing is a normal host defense in sputum is unknown, since the levels of complement found in sputum are low. *Fick* et al. have found Fab and F(ab')2 fragments of IgG in cystic fibrosis sputum (30). These fragments inhibit opsonophagocytosis. They also found that elastase elaborated by *P. aeruginosa* (31) could cleave IgG into these fragments. Thus elastase could cleave IgG molecules, enable the organisms to evade opsonization and persist in the airways if the elastase was present in significant quantities in the sputum. However, *Döring* et al. have found that pseudomonas elastase is absent in the sputum of cystic fibrosis patients who have anti-elastase antibodies in their sera and sputa, and that most CF patients who are colonized with *P. aeruginosa* have anti-elastase antibodies (32). Thus the role of pseudomonal elastase in causing antibody dysfunction is not clear.

Since the airways in CF are chronically inflamed, chemical mediators of the inflammatory reaction may damage the immune system. Earlier studies have shown that elastase produced by human PMN cleaves human IgG (33). Recent work suggests a role for PMN elastase in CF. *Suter* et al. reported that PMN elastase activity was increased in bronchial secretions of children with CF who were colonized with *P. aeruginosa* (34). They also presented evidence suggesting that PMN elastase cleaved the complement component, C3. Thus, by inactivating two important opsonins, PMN elastase could cause a defect in immune clearance and contribute to bacterial persistence.

Bacterial Adherence to Mucin – The Link between Physical and Immune Clearance Defects

Mucins have been generally thought to have a defensive role in the respiratory tract, but they also lower resistance to bacterial infections in animal models when given with bacteria. The mechanism by which resistance is lowered is not fully known, however, *Olitzki* showed that mucins could inhibit the agglutination of bacteria by antibodies (35) and, more recently. *Edwards* et al. have shown that the sialic acid in the capsule of type III group B Streptococcus inactivates the alternative pathway of complement (36). Since sialic acid is a component of mucin, it seems likely that mucins may also have this anticomplementary activity. In addition, mucin-coated bacteria may mimic "self" and thus evade the immune system. We therefore hypothesize that *P. aeruginosa* is able to evade opsonophagocytosis by binding to mucin. On the other hand, those bacteria that do not bind to mucin, although not cleared physically in CF, would be cleared by opsonophagocytosis.

Our preliminary studies have shown that this is most likely the case (37). We have found that respiratory mucin protected *P. aeruginosa* from opsonophagocytosis, whereas an *E. coli* strain which binds poorly to mucin was not protected from opsonophagocytosis. Preincubating the PMN with mucin did not inhibit phagocytosis, but preincubating the bacteria with mucin did, showing that adherence to mucin was a requirement for protection from phagocytosis. The mechanisms by which mucin protects the bacteria from opsonophagocytosis is not fully understood but the degree of protection is dependent on the concentration of mucin. This phenomenon would be responsible in part, not only for bacterial persistence, but also for the selection of *P. aeruginosa* as the predominant colonizer over other bacteria.

In summary, three events enable *P. aeruginosa* to persist

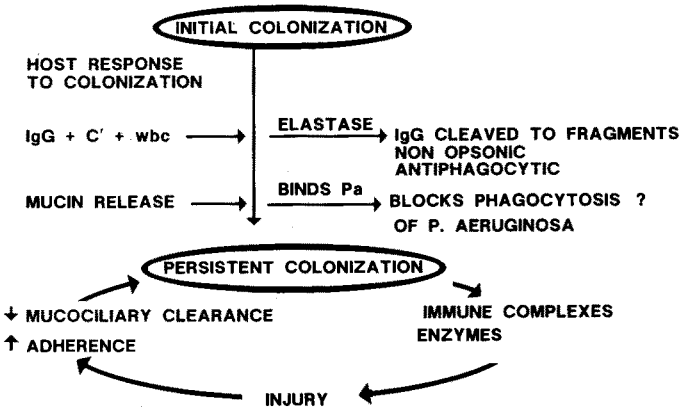

Figure 4: Factors responsible for persistent colonization.

in the airways, i.e. (i) adherence of the bacteria to mucin, (ii) a defect in mucociliary clearance, and (iii) a defect in immune clearance caused by adherence to respiratory mucin and by PMN elastase. Fulfillment of these requirements explains the persistence of *P. aeruginosa* in CF (Figure 4).

Conclusions

If our hypotheses are true, one cannot help but marvel at the perfect adaptation of *P. aeruginosa* for the CF host. To possess such a finely tuned mechanism that exploits a defect in the most basic lung defense of all-physical clearance by mucins, and then to exploit this to protect itself from the host's immune response is the perfect strategy. In addition, this organism's ability to elaborate injurious enzymes and to evoke host responses which are deleterious to the host make the problem of colonization seemingly insurmountable.

We are not encouraged by the prospects of preventing this clinical problem through conventional methods such as vaccination. We believe this problem can be solved only by identification of the clearance defect and its reversal by pharmacologic means.

Acknowledgements

We thank *Athena Christ* for typing this manuscript. This work was supported by grants HL-33622 and 1KO4-01479 from the National Institutes of Health.

Literature

1. **Mearns, M. B.:** Natural history of pulmonary infections in cystic fibrosis. In: *Sturgess, J.* (ed.): Perspectives in cystic fibrosis. Proceedings of the 8th International Cystic Fibrosis Conference, 1980, pp. 325–334.
2. **Oppenheimer, E. H.:** Similarity of the tracheobronchial mucous glands and epithelium in infants with and without cystic fibrosis. Hum. Pathol. 12 (1981) 36–48.
3. **Bedrossian, C. W. M., Greenberg, S. D., Singer, D. B., Hansen, J. J., Rosenberg, H. S.:** The lung in cystic fibrosis. Hum. Pathol. 7 (1976) 195–204.
4. **Doggett, R. G., Harrison, G. M., Wallis, E. S.:** Comparison of some properties of *Pseudomonas aeruginosa* isolated from infections of persons with and without cystic fibrosis. J. Bacteriol. 87 (1964) 427–431.
5. **Huang, N. N., Vanloon, E. L., Sheng, K. T.:** The flora of the respiratory tract of patients with cystic fibrosis of the pancreas. J. Pediatr. 59 (1961) 512–521.
6. **Stern, R. C., Boat, T. F., Doershuk, C. F., Tucker, A. S., Miller, R. B., Matthews, L. W.:** Cystic fibrosis diagnosed after age 13. Ann. Intern. Med. 87 (1977) 188–191.
7. **Niederman, M. S., Ferranti, R. D., Zeigler, A., Merrill, W. W., Reynolds, H. Y.:** Respiratory infection complicating long term tracheostomy. Chest 85 (1984) 39–44.
8. **Rivera, M., Nicotra, M. B.:** *Pseudomonas aeruginosa* mucoid strain. Its significance in adult chest diseases. Am. Rev. Respir. Dis. 126 (1982) 833–836.
9. **Pedersen, M., Stafanger, G.:** Bronchopulmonary symptoms in primary ciliary dyskinesia. A study of 27 patients. Eur. J. Respir. Dis. 64 (1983), Suppl. 127, 118–128.
10. **Beachey, E. H.:** Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to muscosal surfaces. J. Infect. Dis. 143 (1981) 325–345.
11. **Schulze, I. T.:** The biologically active proteins of influenza virus: the hemagglutinin. In: *Kelbourne, E. D.* (ed.): The influenza viruses and influenza, Academic Press, New York 1975, pp. 53–82.
12. **Sobeslavsky, O., Prescott, B., Chanock, R. M.:** Adsorption of *Mycoplasma pneumoniae* to neuraminic acid receptors of various cells and possible role in virulence. J. Bacteriol. 96 (1968) 695–705.
13. **Tuomanen, E., Weiss, A.:** Characterization of two adhesins of *Bordetella pertussis* for human ciliated respiratory epithelial cells. J. Infect. Dis. 152 (1985) 118–125.
14. **Ramphal, R., Small, P. M., Shands, J. W., Jr., Fischlschweiger, W., Small, P. A., Jr.:** Adherence of *Pseudomonas aeruginosa* to tracheal cells injured by influenza infection or by endotracheal intubation. Infect. Immun. 27 (1980) 614–619.
15. **Ramphal, R., Pyle, M.:** Adherence of mucoid and non mucoid *Pseudomonas aeruginosa* to acid-injured tracheal epithelium. Infect. Immun. 41 (1983) 345–351.
16. **Ramphal, R., Pier, G. B.:** Role of *Pseudomonas aeruginosa* mucoid exopolysaccharide in adherence to tracheal cells. Infect. Immun. 47 (1985) 1–4.
17. **Ramphal, R., Sadoff, J. C., Pyle, M., Silipigni, J. D.:** Role of pili in the adherence of *Pseudomonas aeruginosa* to injured tracheal epithelium. Infect. Immun. 44 (1984) 38–40.
18. **Ramphal, R., Pyle, M.:** Further characterization of the tracheal receptor for *Pseudomonas aeruginosa.* Eur. J. Clin. Microbiol. 4 (1985) 160–162.
19. **Ramphal, R., Pyle, M.:** Evidence for mucins and sialic acid as receptors for *Pseudomonas aeruginosa* in the lower respiratory tract. Infect. Immun. 41 (1983) 339–344.
20. **Vishwanath, S., Ramphal, R.:** Adherence of *Pseudomonas aeruginosa* to human tracheobronchial mucin. Infect. Immun. 45 (1984) 197–202.
21. **Macone, A. B., Pier, G. B., Pennington, J. E., Matthews, W. J., Goldman, D. A.:** Mucoid *Escherichia coli* in cystic fibrosis. N. Engl. J. Med. 304 (1981) 1445–1449.
22. **Williams, R. C., Gibbons, R. J.:** Inhibition of streptococcal attachment to receptors by antigenically similar salivary glycoproteins. Infect. Immun. 30 (1975) 694–699.
23. **Vishwanath, S., Ramphal, R.:** Tracheobronchial mucin receptor for *Pseudomonas aeruginosa:* Predominance of aminosugars in binding sites. Infect. Immun. 48 (1985) 331–335.
24. **Ramphal, R., Guay, C., Pier, G. B.:** *Pseudomonas aeruginosa* adhesins for tracheobronchial mucin. Infect. Immun. 55 (1987) 600–603.

25. **Spock, A., Heick, H. M. C., Cress, H., Logan, W. S.:** Abnormal serum factor in patients with cystic fibrosis of the pancreas. Pediatr. Res. 1 (1967) 173–177.
26. **Boat, T. F., Dearborn, D. G.:** Etiology and pathogenesis. In: *Taussig, L. M.* (ed.): Cystic fibrosis. Thieme-Stratton, Inc., New York 1984, pp. 35–36.
27. **Høiby, N., Andersen, V., Bendixen, G.:** *Pseudomonas aeruginosa* infection in cystic fibrosis. Humoral and cellular immune responses against *Pseudomonas aeruginosa*. Acta. Pathol. Microbiol. Scand. (C) 83 (1975) 459–468.
28. **Schiøtz, P. O.:** Systemic and mucosal immunity and non-specific defense mechanisms in cystic fibrosis patients. Acta. Paediatr. Scand., Suppl. 301 (1982) 55–62.
29. **Schiller, N. L., Millard, R. L.:** Pseudomonas-infected cystic fibrosis patient sputum inhibits the bactericidal activity of normal human serum. Pediatr. Res. 17 (1983) 747–752.
30. **Fick, R. B., Naegel, G. P., Squier, S. U., Wood, R. E., Gee, J. B. L., Reynolds, H. Y.:** Proteins of the cystic fibrosis respiratory tract. Fragmented immunoglobulin G opsonic antibody causing defective opsonophagocytosis. J. Clin. Invest. 74 (1984) 236–248.
31. **Fick, R. B., Baltimore, R. S., Squier, S. U., Reynolds, H. Y.:** IgG proteolytic activity of *Pseudomonas aeruginosa* in cystic fibrosis. J. Infect. Dis. 151 (1985) 589–598.
32. **Döring, G., Obernesser, H.-J., Botzenhart, K., Flehmig, B., Høiby, N., Hofmann, A.:** Proteases of *Pseudomonas aeruginosa* in patients with cystic fibrosis. J. Infect. Dis. 147 (1983) 744–750.
33. **Prince, H. E., Folds, J. D., Spitznagel, J. K.:** Proteolysis of human IgG by human polymorphonuclear leucocyte elastase produces an Fc fragment with *in vitro* biological activity. Clin. Exp. Immunol. 37 (1979) 162–168.
34. **Suter, S., Schaad, U. B., Roux, L., Nydegger, U. E., Waldvogel, F. A.:** Granulocyte neutral proteases and pseudomonas elastase as possible causes of airway damage in patients with cystic fibrosis. J. Infect. Dis. 149 (1984) 523–531.
35. **Olitzki, L., Shelubsky, M., Efrati, E.:** Action of certain carbohydrates on the reaction of *Eberthella typhosa* with antibody O. Proc. Soc. Exp. Biol. Med. 64 (1947) 258–259.
36. **Edwards, M. S., Kasper, D. L., Jennings, H. J., Baker, C. J., Nicholson-Weller, A.:** Capsular sialic acid prevents activation of the alternative complement pathway by type III, group B streptococci. J. Immunol. 128 (1982) 1278–1283.
37. **Vishwanath, S., Pier, G. B., Ramphal, R.:** Human tracheobronchial mucin inhibits the phagocytosis of *Pseudomonas aeruginosa*. In: Abstracts of the Annual Meeting of the American Society for Microbiology, 1986, abstract D-126, p. 87.