On the Complexity of the Pulmonary Microbiology in Cystic Fibrosis: Thoughts of a Clinician

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Abstract

The pulmonary microbiology is a dominant element in cystic fibrosis and the main cause of death. Contemporary consensus accords an exclusive role in this to a single microorganism, *Pseudomonas aeruginosa*. The evidence convincingly shows that the microbiology consists of a multiplicity of species living in perpetual interaction and in a variety of forms – planktonic, sessile, anaerobic – and in organized communities as microcosms, biofilms and ecosystem. This compound microbiology, the essence of the pulmonary disease, is of necessity exposed to constant influence both from without (the air) and within (via the blood), leading to a perpetual state of flux with consequent impact on the clinical course. It is perhaps significant that to date, most or all microbiologic studies were probably conducted, classically, with inert instruments (glass? plastic?), whereas in real life the CF microbiology lives in "test-tubes" of live mucosa with which it maintains a permanent "cross-talk." The difference to microbial life between these two media may well be very important. It therefore justifies study and may be far-reaching in its effect. There is persuasive argument to strive for a novel holistic view of the totality of the complex microbiology of CF, and to initiate fresh concepts, strategies and methods.

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The lower respiratory tract is normally free of infection due to nature's excellent defense mechanisms. "Healthy organs clear themselves" seems to be a law of nature, hence interference in these mechanisms in any way, such as by the inevitably cystic fibrosis-altered bronchial pathophysiology [1,2], makes infection mandatory. Bacteria enter unselectively with the inspired air and by minor aspirations from the mouth and pharynx, but their colonization must follow prior interaction with the intra-bronchial "milieu interieur." This step will necessarily also be influenced by qualities inherent in the microorganism and relevant to colonization and is therefore likely to be selective, favoring some bacteria over others. Until recently and by standard laboratory methods, more than 20 species have been found in CF, the more frequent being *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Proteus* spp., *Escherichia coli*, *Haemophilus influenzae*, atypical mycobacteria, and fungi such as *Candida albicans* and *Aspergillus fumigatus* – frequently or usually several species at the same time [3]. New technology, however, reveals a multiplicity of simultaneously present species including anaerobes, many of which have not previously been reported in CF [4].

The presence of different microorganisms in close proximity affords, indeed compels interaction in the form of competition, but it also facilitates inter- and intraspecies exchange of various factors, including for example the sensitivity to antibiotics [5]. Bacteria resident in the bronchi probably behave much as they do in their natural habitats, which is to live in communities of multiple species, each responsible for certain functions, providing a division of labor and permitting the collective to thrive as an organized interacting community – a microcosm [6]. Some bacteria, like *P. aeruginosa*, have the propensity to secrete an extracellular polysaccharide matrix that envelopes the microcolonies, thus creating biofilms of single or mixed species. These biofilms, too, are organized communities of differentiated structured groups of cells with communal properties and exhibit functional heterogeneity [7]. Such a conglomerate mosaic of planktonic bacteria, microcosms and biofilms, in being exposed to irregular influences from without (via bronchi) including repeated bacterial invasions and from within (via blood), can understandably be in a permanent state of flux and change.

P. aeruginosa and less so *Staph. aureus* may be almost permanent, if not frequently concomitant, residents. Other bacteria tend to reside for shorter periods, some transiently others recurrently. The above-mentioned unstable flux may well be instrumental in these periodic changes in the flora. Presumably some microorganisms may occasionally so diminish in number as to become undetectable on culture, seeming to have disappeared [8]. Later, with change in balances, they may again proliferate and resurface as "new infections." It would be interesting to test genetically whether these re-emerging bacteria may not really or occasionally be of the original strains.

The natural history of CF leads to irregular haphazard structural and obstructive changes in the bronchi, resulting in a patchy inequality in their internal micro-environments [1] and consequently inequality in phenotypic characters, secondarily acquired by the bacteria [9]. Among these may also be sensitivity to antibiotics. Such phenotypic differences lead to divergence from the original strains so that organisms initially identical become "strangers," at least partially, to one another [10]; and sputum originating in one area of the lung may, in this respect, differ from that from another. This aspect of phenotypic divergence has been demonstrated by genetic studies of *P. aeruginosa* isolates from sputa, which in any one patient (although fre-

CF = cystic fibrosis

quently and persistently of a single strain representing the initial or early invaders) may over the years have become of multiple phenotypes [10]. Such singular prolonged domicile as reported for P. *aeruginosa* is intriguing since, in view of its ubiquity, invasions from the environment by fresh strains of P. *aeruginosa* must certainly have occurred repeatedly but apparently always failed colonization. The probable "barrier" being the inevitable interaction with the bronchial "milieu interieur" confronting every invader. This latter phenomenon seems in effect to guard against cross-infection, at least for this species. This alone should stimulate deeper study. In fact it has been noted that cross-infection by P. *aeruginosa* is rare [11] but has occurred.

In contradistinction to this is a seemingly ongoing epidemic at present, by a few new strains of P. *aeruginosa* that are highly transmissible (i.e., easily cross-infect), lead to severer disease, to superinfection, to displacing the resident unique P. *aeruginosa* and even to infecting non-CF contacts [12]. These characteristics place these mutants in a group of their own, outside that of the usual run of the mill P. *aeruginosa*. They almost fulfill Koch's postulates and are recognized as the dominant cause of the epidemic. A rather similar situation, that of a virulent newcomer, shook the CF world two decades ago when B*urkholderia* (then P*seudomonas*) *cepacia* entered the scene [13]. And again even earlier, in the 1960s, when the then reigning offender, S*taphylococcus aureus*, was displaced by the newcomer P. *aeruginosa* [14].

The continuity and contiguity of the microcosms throughout the broad expanse of the bronchi presumably and conveniently encourage continuing interaction, thereby creating an ecosystem. Conceptually, this perhaps resembles the multispecies ecosystem naturally developing in another hollow viscus, the neonatal intestines, following initial bacterial invasion [15].

Since the sputum is the main source of information relating to the microbiology, it is important to ascertain the degree to which it reflects the situation(s) in the bronchi. This has been done by comparing, in the same patients, the microorganisms found in the sputum with those obtained directly at bronchoscopy or operation. Conflicting results were obtained – some concordant with the same bacteria and antibiotic sensitivities present in sputum as in bronchi [16], others discordant with microorganisms and phenotypes in the sputum that were absent in the bronchi and vice versa [17–19]. On reflection, the discordant results may not be so surprising. The sputum has its beginning in the smaller bronchi as small drops of mucus merging with other such drops in its course outward. The bacteria transported in the sputum-to-be come in contact and presumably mix with a diversity of phenotypes from neighboring bronchi and in the process possibly change somewhat from their original selves, i.e., the organisms reaching the "final" sputum become discordant to what they had been at source. Hence, when matching microorganisms in the sputum with those *in situ* in the bronchi, the likelihood of encountering identical phenotypes in both locations is probably not great, although perhaps not impossible. In view of this it is the concordant results that may be somewhat surprising, not least because when multiple cultures are taken from single sputum specimens the bacteria isolated, even those of the same species, frequently differ in their antibiotic phenotypes [20]. Apparently, but not unexpectedly, a single sputum specimen may, or perhaps usually does, harbor microorganisms that come from more than one microcosm or location. At the same time it is unlikely to represent *all* the microcosms, particularly not those in mucus-obstructed bronchi, these latter being desirable if not always achievable targets to reach with antibiotics. Hence, single sputum samples can at best give an incomplete view of the microbiology and it is therefore doubtful that they can provide the information necessary for the rational choice of antibiotics for treatment.

The role of being the "main pathogen" in CF generally attributed to P. *aeruginosa* [21] apparently derives from its impressive growth on culture in the laboratory. This need not mean that bacteria that are less prolific in the laboratory are irrelevant in the bronchi. Their actual presence can be revealed by sophisticated newer techniques [4], or with the aid of selective media that suppress the accompanying laboratory-dominant P. *aeruginosa*, permitting the less prolific to grow [22]. This convincingly shows that within the bronchi, i.e., even without the aid of selective media, these "less prolific" bacteria do in fact thrive and are not overgrown to extinction by the P. *aeruginosa* and thereby provide the source from which they appear in the sputum. Thus in the bronchi, the seemingly domineering potential of P. *aeruginosa*, so prominent in the laboratory, is curtailed, possibly by microcosmal interactions. A comparison of this point, in principle but not in character, namely the part exemplified by less-prolific organisms, may be borrowed from the clinical setting of a patient with bacillary dysentery where the S*higella bacillus* is of course the real cause of the disease, but this can become evident only with the aid of selective media. The concentration or amount of one species in relation to another is probably no indication of the degree of its activity and effect in interacting with the microcosms and ecosystem [4].

Studies of such cryptic or "hidden" bacteria have shown that standard laboratory methods may meet with the apparent paradox of failing to isolate bacteria that are actually present but demonstrable only by newer techniques [4], including fluorescent *in situ* hybridization [23] i.e., a situation of VBNC (**v**iable **b**ut **n**ot **c**ulturable). Real life in the bronchi, including the microcosms and biofilms, differs considerably from that depicted by the routine laboratory [7,17].

Perhaps it is mainly for technical reasons that attention to anaerobic bacteria is frequently lacking in the routine examination of CF sputa. Conditions within the CF bronchi, with the accent on obstruction by mucus, bronchiectatic and cystic changes, nooks and crannies, tend to create widespread areas of varyingly diminished oxygen tension that provide havens for anaerobic opportunistic invaders [4,16–18]. Also the presence and added influence of microorganisms such as P. *aeruginosa* in these oxygen-restricted environments (in patients harboring this organism) may result in frank anaerobiosis in the bronchial mucus [2]. Moreover, the concomitant presence of aerobes and anaerobes permits their interaction, with possible alteration in their synergistic behavior such as an increase or otherwise of

virulence [24]. Obviously then, anaerobes can and probably do participate in intermicrobial interactions, but little is known of their part in the ecosystem. Have they no place in therapy?

Decades of clinical experience have shown antibiotics to be beneficial in the treatment of lung infections, especially in exacerbations [1]. However, their choice is always a quandary. Clinicians rely on antibiotic-sensitivity testing of *the* causative organism. This concept is borrowed from and appropriate to unibacterial diseases such as meningitis or bacteremia that fulfill Koch's postulates where only a single microorganism is found in blood or cerebrospinal fluid. The latter media are normally always sterile, both before the illness and again after recovery. But in CF, the microorganisms are of very many species [4], of multiple phenotypes and sensitivities, have been resident in the bronchi almost from birth and remain so before, during and even after therapy. This scenario is clearly more complicated than that in unibacterial disease and therefore casts doubt on both the value and rationale of sensitivity testing as commonly practised. Not surprisingly it is not rare to experience yet another paradox, that of a patient improving under an antibiotic given at the time that the sputum is sent for culture, only later to find that this antibiotic was "inappropriate." This by virtue of the isolated bacterium being found resistant to the antibiotic [25]. The only way that one can presume an antibiotic to have been correct is perhaps post-factum, by witnessing the results.

The complexity of the microbial world within the bronchi makes it difficult to unravel the dynamics of how antibiotics achieve their acknowledged beneficial effects. It is gratifying, albeit surprising and little understood, that for decades the same few antibiotics (e.g., ceftazidime, carbenicillin, piperacillin, gentamycin, tobramycin, amikacin etc.) have almost consistently controlled pulmonary exacerbations [1]. An accepted explanation attributes this to the very significant decrease in the bacterial load accompanying therapy [26]. Yet this explanation is doubtful since the decrease is short-lived and fully reconstituted within 2 weeks, whereas the pulmonary improvement persists, frequently for months and even years with the bacterial load having long returned to what it was prior to therapy, but this time apparently with no concomitant detrimental effect. An alternative explanation may be that antibiotics, by having a broad-spectrum effect, cannot but subdue some or many inevitably present still sensitive bacteria, not necessarily all of the same species. The resulting disruption and reshuffling of balances in the microcosms may understandably be responsible for the clinical improvement, thus resembling the mechanism proposed above in explanation of "transient" infections.

The impressive shifts in the bacterial population following the above therapy deserve comment. The rapidity of restitution of the bacterial load is appropriate to normal bacterial growth in the exponential growth phase, but this obviously does not continue unabatedly for it would then literally plug the bronchi solid with bacteria. This never happens because, appropriately, a stationary phase in bacterial growth follows, obviously due to control, through quorum sensing (?) [27]. Equally interesting is the phenomenon, commonly seen in CF patients between exacerbations, of billions of bacteria in trachea and bronchi, in a steady-state or truce, at peace with the host frequently for months or even years, yet almost never invading parenchyma or blood, and the alveoli only rarely – suggestively a self-disciplined ecosystem. This may hark back to the comparison, suggested above, with the not dissimilar ecosystem in the neonatal intestinal tract.

The comments above regarding P. *aeruginosa* do not detract from the contributions during decades of clinical observation and research concerning this microorganism. One may wonder though that these efforts centered almost solely on P. *aeruginosa*, to the exclusion of the other microorganisms present and what is perhaps no less important, to the exclusion also of the interactions between them. These other bacteria are frequently not saprophytes but pathogens in their own right, bearing similar or identical biologic factors (toxins) as those in recognized pathogens. It is therefore only logical to acknowledge them as such and to realize that, like Mount Everest, they are there! – and to be reckoned with. Moreover, in polymicrobial infections, of which CF is a prominent example, there may well be commensals as well [4]. The essence and significance of this compound biology is that all elements – viruses, fungi, mycobacteria, anaerobes, planktonic bacteria, VBNCs, biofilms and ecosystems – interact and participate in an orchestration that is not fully understood [28]. It is unrealistic to regard the microbiology of CF unidimensionally, as the private domain of single "domineering" pathogens that behave in isolation from the other microorganisms present. Reality demands viewing the situation multidimensionally in its holistic complexity. This probably requires novel concepts, strategies and methodologies, including the construction of models of the real CF world. A strong introduction along these lines to CF microbiology has recently been made [4].

Finally the mucosa – a living tissue enveloping the composite microbiology of CF and in fundamental contrast to the inanimate and inert materials (glass?) classically employed in *in vitro* laboratory studies. However, the mucosa in the bronchi, unlike that in the intestine, is presumably not programmed by nature, *a priori*, to live in contact with the overwhelming, vital, ever-changing microbiology of the magnitude and character seen in CF. This fact alone guarantees interaction between the two, probably permanently. It is too early in the present initial stage of understanding this relationship to grasp its implications, but it certainly is time for clarification. In view of the expanse of the mucosa and its potential for cross-talk with the underlying microbial population, it is likely, as in other hollow viscera [29], to add a weighty and important facet to an already impressively complex situation, deserving study and incorporation into these new methodologies.

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