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New Therapies for Pneumonia

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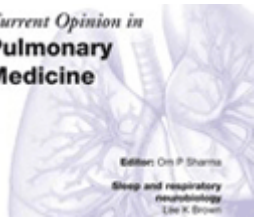

Abstract and Introduction

Abstract

Purpose of review Acute respiratory tract infections are a key public health problem, and represent a major cause of death worldwide. The dramatic shortage of new antibiotics combined with the increasing number of antibiotic-resistant bacteria constitutes a worrisome threat for the global population and a critical challenge for healthcare institutions. Over recent years, a better understanding of bacterial growth, metabolism, and virulence has offered several potential targets for nonantibiotic antimicrobial therapies.
Recent findings Several leads have been investigated, targeting adhesion, communication, toxins, virulence factors, direct bacterial killing by bacteriophages, and vaccine strategies. Promising results have been obtained with these different targets, including inhibition of quorum sensing, use of pilicide compounds to inhibit bacterial adhesion, prevention and treatment of *Pseudomonas* aeruginosa pneumonia by bacteriophages, effective protection against *P. aeruginosa* lung infection with mucosal vaccination, use of anti-PcrV antibodies in *P. aeruginosa*-induced sepsis.
Summary Expectations are high regarding the translation of these experimental results into true clinical benefits for the patients. Importantly, clinical studies are ongoing in some areas, and promising preliminary results have already been obtained in some instances.

Introduction

Acute respiratory tract infections are a key public health problem, and represent a major cause of death worldwide. The dramatic shortage of new antibiotics combined with the increasing number of antibiotic-resistant bacteria constitutes a worrisome threat for the global population and a critical challenge for healthcare institutions. Over recent years, a better understanding of bacterial growth, metabolism, and virulence has offered several potential targets for nonantibiotic antimicrobial therapies. These approaches have several potential advantages: they expand the repertoire of bacterial targets, they preserve the host's endogenous microbiome, and they exert less selective pressure for the development of antibiotic resistance relative to current antibiotics. This study will review the more recent developments of antivirulence and nonantibiotic strategies in the field of lung infections.

Use of Bacteriophages

Bacteriophages are viruses that infect only bacteria. A protein or lipoprotein capsid protects their usually double-stranded DNA core nucleic acid. Most phages are tailed, enabling a specific interaction with various bacterial surface receptors. Upon specific recognition, bacteriophages inject their genetic material into their host.[1] Their genome is then transcribed using the host cell RNA polymerase. Most bacteriophages then replicate their genetic material and synthesized viral proteins, which are assembled and encapsided to form new virions that are released upon cell lysis. Bacteriophages that follow this infectious cycle are called virulent or lytic. Other bacteriophages will follow a lysogenic life cycle, inserting their genome into the host genome, and remaining quiescent. These bacteriophages are called temperate or prophage. Humans are constantly exposed to hundreds of bacteriophages,[2] as underscored by a 4-year survey of drinking water in the Netherlands revealing that the amount of bacteriophages infecting a reference *Escherichia coli* strain was around 10^{-3} to 10^{-4} per liter.[3]

The first bacteriophages discovered by d'Herelle[4] in 1917 were isolated from stools of patients recovering from

dysenteric diseases. Rapidly d'Herelle demonstrated that bacteriophages could be used to treat enteric bacterial infections. The discovery of antibiotics lessened the interest in bacteriophages in western Europe and the United states, contrary to eastern Europe where medical use of bacteriophages was further developed after the Second World War.[5] For example, chronic lung infections of cystic fibrosis patients have been treated with phage preparations in Tbilisi in Georgia for numerous years.[6] The recent increase in antibiotic-resistant strains has renewed the interest in bacteriophages, not only for chronic but also for acute infections. In the respiratory field, Debarbieux *et al*.[7••] have very elegantly shown the preventive and curative beneficial effects of phage therapy. In a lethal mouse model of *Pseudomonas aeruginosa* pneumonia, they showed that mice treated with phages specific for the *P. aeruginosa* strain used in their study survived the bacterial challenge, whereas controls all died by 48 h. When bacteriophages were instilled 4 h after *P. aeruginosa*, 75% of mice survived. This rate dropped to 25% when mice were treated 6h after bacterial challenge. Because authors observed that phages persisted in reasonable amounts 24h after instillation in lungs of uninfected mice, they investigated the effect of a preventive administration of phages. In mice instilled with phages 24 h before *P. aeruginosa* inoculation, survival rate was 100%, whereas it was 0% in controls. This team recently confirmed and expanded its initial successful results using a clinical *P. aeruginosa* strain isolated from a cystic fibrosis patient.[8••] They were able to show that preventive treatment of *P. aeruginosa* pneumonia with bacteriophages was efficient up to 4 days before bacterial challenge, that is, bacteriophages instilled 4 days before *P. aeruginosa* inoculation were in sufficient amount to control and eradicate *P. aeruginosa*.[8• •] Lung infection is not the sole domain in which phage therapy has been successful. Intragastric and intraperitoneal administrations of phages have proved to be efficient in treating *Klebsiella pneumoniae* liver abscesses and bacteremia.[9]

To summarize, treatment with bacteriophage therapy as an alternative or a supplement to antibiotics is associated with numerous advantages: it is economic and effective against multidrug-resistant bacteria because the mechanisms by which it induces bacteriolysis differ completely from those of antibiotics; it is well tolerated because it has high specificity for its target and neither disturbs the microflora of the body nor affects eukaryotic cells; and administration requires only a single dose of treatment, owing to its self-replicating nature. Obviously, further studies are required to assess the effectiveness of phage therapy to treat lung infection, but interestingly clinical studies are already ongoing in other fields of infectious diseases. Oral administration of bacteriophages to humans was shown to be well tolerated and is currently being investigated in infants with diarrhea caused by *E. coli* strains,[10] and successful results have been reported with bacteriophages given to treat chronic otitis caused by antibiotic-resistant *P. aeruginosa* strains.[11] Significant progress in this field now depends on the results of two very different challenges that face researchers: to obtain regulatory authorizations for the large-scale clinical evaluation and use of bacteriophages in various clinical situations, and to establish rapid determination of specific bacteriophages.

Targeting Adhesion

Adhesion to host cells is a necessary and vital step for bacteria to carry out their infectious process. This fundamental early phase of almost any bacterial disease requires physical contact between host cell surface ligand and bacteria mediated by specific proteins called adhesins. To prevent, counter, or hamper this adhesion is, therefore, a very attractive strategy to control or prevent infection. Gram-negative but also Gram-positive bacteria express a multitude of adhesins, either incorporated in heteropolymeric extracellular fimbrae also called pili, or expressed at the cell surface as monomeric proteins or protein complexes, termed nonfimbrial adhesion. *E. coli* adhesion to the urinary tract has, for obvious public health reasons, yielded numerous studies to elicit the mechanisms by which *E. coli* adhere to urothelial cells. Therapeutic strategies aim at targeting type 1 pili, expressed by practically all uropathogenic *E. coli* clinical strains, either by counteracting contact between pili and cell ligand by administering carbohydrate derivatives of these ligands, or by inhibiting pilus biogenesis (pilicide effect). Pilus assembling is obtained through the chaperone–usher system. Ubiquitous conserved regions on chaperones in the chaperone–usher machinery are targets for new pilicides such as bicyclic 2-pyridone scaffold. They have been shown to inhibit both pili (type 1 and P) assembly and biofilm development. Adhesion to bladder carcinoma cells of different *E. coli* strains is inhibited by these compounds.[12,13] Because other pathogens responsible for lung infections such as *Pseudomonas* spp., *Haemophilus* spp., and *Klebsiella*

spp. also use chaperones and the chaperone–usher pathway, these pilicides offer an interesting broad-spectrum activity and potential to treat or prevent pneumonia.

Quorum Sensing

In many bacterial species, population density-dependent regulation of many functions such as virulence factor expression or biofilm formation is mediated by cell-to-cell chemical communication known as quorum sensing. The bacterial community is, thus, regulated by the release and the recognition of small diffusible molecules, whose concentrations increase with the bacterial population. Virulence factor expression is ignited when a decisive bacterial density is attained, sufficient to trigger infection. For obvious reasons, interfering with quorum sensing is a very attractive strategy to control virulence and biofilm formation. One may speculate that disruption of bacterial communication in a given population by inhibiting quorum sensing may alter the expression of virulence factors. In *P. aeruginosa*, quorum-sensing molecules, also called autoinducers, are namely homoserine lactones (3OC(12)-HSL and C4-HSL). These small diffusible molecules modulate gene expression through the modification of transcriptional activators (LasR, RhlR, and QscR).[14] Many *P. aeruginosa* virulence factors have been shown to be regulated by quorum sensing. These include many secreted factors (elastase, phospholipase C, lecithinase, and rhamnolipids) as well as secondary metabolites (pyocyanin and cyanide) and biofilm development.

Several paths have been explored to date including inhibition of quorum sensing molecules synthesis, their increased degradation, or the interference with their recognition. Halogenated furanones, a class of structural analogs of homoserine lactones, have been shown to have quorum sensing inhibitory properties. Exotoxin production and quorum sensing-regulated gene expression in *P. aeruginosa* have been respectively inhibited and repressed by halogenated furanones.[15] These compounds also increased susceptibility of *P. aeruginosa* biofilms to tobramycin.[15] In a mouse model of *P. aeruginosa* pneumonia, administration of synthetic halogenated furanones was found to promote the clearance of *P. aeruginosa*, thereby increasing the survival time of mice.[16] However, it has been shown that to be functional under physiological conditions in mammalian tissue fluids, N-acylhomoserine lactones (AHLs) require an N-acyl side chain of at least four carbons in length and that the longer the acyl side chain the more stable the N-AHL signal molecule.[17] In this study, AHL turnover was discovered to be due to pHdependent lactonolysis.[17] These results obviously hamper the enthusiasm surrounding the use of AHLs. However, a structurally unrelated triphenyl mimic of 3OC12-HSL that is base-insensitive and PON-resistant has been identified, which make it an excellent scaffold for developing quorum sensing inhibitors, and its stability and potency make it ideal for biotechnology uses.[18] In the clinical field, a very recent study highlighted the importance of quorum sensing in ventilator-associated pneumonia (VAP). Kohler *et al*.[19] studied over 300 *P. aeruginosa* isolates from mechanically ventilated patients, of whom 20% developed VAP. Isolates were evaluated for quorum sensing-dependent (production of elastase and rhamnolipids) and quorum sensing independent virulence traits. Interestingly, patients colonized by quorum sensing-proficient isolates more readily developed VAP than those colonized by quorum sensing-deficient isolates. Of all virulence traits tested in colonizing isolates, only rhamnolipids production was statistically associated with VAP development.[19] Of note, numbers in this study were small and results must be confirmed on a larger scale, and with other potent VAP pathogens. Nonetheless, they confirm experimental findings of the early disruption of primary human epithelia by rhamnolipids produced by *P. aeruginosa*.[20] The central role for rhamnolipids was reinforced with the observation that purified rhamnolipids when applied on the epithelial surface were able to alter cell tight junctions, enabling paracellular invasion of rhamnolipid-deficient *P. aeruginosa*.[20] Studies have suggested that quorum sensing inhibition is possible through quorum sensing interference by macrolide antibiotics such as azithromycin. The hypothesis that azithromycin, by inhibiting quorum sensing, could prevent *P. aeruginosa* VAP when given to ventilated patients was tested in a clinical study.[21,22••] Results indicate that biologically, azithromycin was able to significantly inhibit the expression of quorum sensing-dependent genes measured directly in tracheal aspirates.[22••] A decreasing trend in the incidence of *P. aeruginosa* VAP was observed in the treated patients. Moreover, when infections with quorum sensing-deficient *P. aeruginosa* were excluded in the analysis, the incidence of P. aeruginosa VAP was reduced fivefold in the azithromycin-treated group ($P = 0.015$).[21]

Immunotherapy

Active and passive immunotherapy has fuelled a considerable amount of research in the last decade, especially in the field of lung infection and VAP.

Poly-*N*-Acetylglucosamine

Numerous bacteria express a surface polysaccharide poly-*N*-β-(1–6) glucosamine, termed poly-N-acetyl-glucosamine (PNAG), which plays a critical role in biofilm formation, thus making this antigen an attractive candidate for immunotherapies.[23] Several lung pathogens produce PNAG (*Staphylococcus aureus*, *Acinetobacter baumannii*, and *E. coli*). A pivotal study by G. Pier's group showed that protection against *E. coli* infection could be conferred by antibodies directed against *S. aureus* PNAG.[24] In this study, 30 clinical isolates of *E. coli* were studied for the expression of PNAG. Susceptibility to opsonic killing and protection from lethal infection by antibody to PNAG was also assessed. Most *E. coli* strains carried the *pga* locus, expressed immunologically detectable PNAG, and two thirds of strains could be killed by rabbit immunoglobulin G for the deacetylated PNAG. These results were confirmed in a lethal mouse model of intraperitoneal infection that showed that 55–88%of mice given anti-dPNAG goat antibody were protected. Exhaustive study of the immune response made to PNAG further determined that immunogenicity was enhanced with the deacetylated form of PNAG. Importantly, fully nonacetylated synthetic conjugate vaccines generated high levels of opsonic and protective antibodies that were capable of binding to highly acetylated PNAG and were shownto be protective in experimental *S. aureus* skin infection and lethal *E. coli* peritonitis.[25] Recently, the same group reported PNAG could also constitute a target for protective immunity against *A. baumannii* infections using mice pneumonia and bloodstream infection models.[26•] Taken together, these results suggest that active and passive immunization using deacetylated glycoforms of PNAG as antigens is effective in conferring protection against pathogens producing this conserved surface polysaccharide.

PcrV

Many Gram-negative pathogens possess a toxin secretion system that enables delivery of toxins or effector proteins from the bacterial cytoplasm directly into the host eukaryotic cell cytosol, called the type III secretion system. Expression of this system is associated with virulence and pathogenicity. In this needle complex, PcrV is an important structural translocation component that facilitates the delivery of cytotoxins.[27] PcrV has limited variations between most *P. aeruginosa* strains worldwide.[28•] Antibodies against PcrV have been shown to preserve the host's phagocyte function, reduce inflammation in acute infection, and reduce mortality in an animal model of *P. aeruginosa*-associated pneumonia.[29,30] Preliminary results from a phase II clinical trial that used a PEGylated recombinant human Fab' antibody fragment designed to bind and to inhibit PcrV (KB001) were recently released.[31•] This elegant study by Chastre *et al*.[31•] aimed to reduce the incidence of *P. aeruginosa* lung infection in patients who were already colonized by *P. aeruginosa* but not infected (≥10^3CFU/ml by endotracheal aspirate or ≥10^2CFU/ml by bronchoalveolar lavage quantitative culture). Thirtynine patients were randomized to receive either a placebo or a single IV infusion of one of two doses of KB001 (3 or 10 mg/kg). Incidence of *P. aeruginosa* VAP was 60% in the placebo group compared with 33 and 31% in the anti-PcrV-treated groups (3 and 10 mg/kg, respectively). The observed half-life of this PEGylated Fab was 8–9 days with endotracheal concentrations at approximately 2–6% of the serum concentration. These promising results must obviously be confirmed in a larger randomized placebocontrolled trial. They represent nonetheless, to date, the most advanced antivirulence strategy explored in ICU patients.

Treating Fungal Colonization

The *Candida* species is frequently retrieved in pulmonary samples of mechanically ventilated ICU patients. Although this fungus is rarely responsible for lung infections in these patients,[32,33] clinical data suggested that *Candida* spp. airway colonization may play a role in the subsequent development of *P. aeruginosa* VAP.[34] Recent experimental data supported this hypothesis by showing that airway colonization with *Candida albicans* induced a Th1 response and favored the development of *P. aeruginosa*-associated pneumonia in rats.[35] Antifungal treatment enhanced the clearance of *C. albicans* from the lungs and reduced the lung inflammatory

response along with a normalization of pulmonary γ-interferon levels. In the colonized animals, antifungal treatment significantly decreased the rate of *P. aeruginosa*-associated pneumonia (to one similar to the rate observed in noncolonized animals) compared with the rate in the untreated animals.[36] These findings are in agreement with the association found between antifungal treatments and a reduced risk of *P. aeruginosa* tracheobronchial colonization or VAP in ventilated patients with *Candida* spp. colonization.[37]

Conclusion

Results presented here show the numerous pathways by which bacterial virulence can be fought efficaciously. Expectations are high for these different strategies to translate into clinical benefits for the patients. However, long-term effects of antivirulence therapy must be carefully considered. Recent data with quorum sensing inhibition have highlighted the risk of diminished selection toward reduced virulence and increased prevalence of more virulent genotypes. The combination of these strategies with traditional antibiotics may perhaps offer the best compromise to combat pathogens.

Sidebar

Key Points

- The dramatic shortage of new antibiotics combined with the increasing number of antibiotic-resistant bacteria constitutes a worrisome threat for the global population and a critical challenge for healthcare institutions.
- This situation has fuelled intense research in the field of antivirulence strategies.
- Use of bacteriophages is a very promising technique not only to treat but also to prevent lung infection.
- Inhibition of quorum sensing by azithromycin has provided encouraging results in a clinical trial on *P. aeruginosa* pneumonia.
- Use of anti-PcrV antibodies to prevent *P. aeruginosa* ventilator-associated pneumonia has been tested in a clinical study with hopeful results.

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Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
• • of outstanding interest

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