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Pneumolysin as a vaccine and drug target in the prevention and treatment of invasive pneumococcal disease

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Summary

Streptococcus pneumoniae (the pneumococcus) remains one of the major human pathogens and one of the most common causes of community-acquired pneumonia, otitis media, sinusitis, and meningitis. Aside from the threats posed by emerging antibiotic resistance and infection with the human immunodeficiency virus, the mortality rate among those patients with severe pneumococcal disease who receive seemingly appropriate antimicrobial chemotherapy remains unacceptably high. Because of its involvement in the pathogenesis of invasive disease, pneumolysin, one of the best-characterized virulence factors of the pneumococcus, represents not only a potential vaccine target, but also a target for adjunctive therapy to antibiotics in patients with acute pneumococcal disease. In this paper we review the cytolytic and pro-inflammatory properties of pneumolysin and their involvement in subversion of host defenses and extra-pulmonary dissemination of the pneumococcus, as well as strategies, both immunological and pharmacological, which may counter these harmful activities of the toxin.

Key words:

antibiotics • anti-inflammatory agents • community acquired pneumonia • conjugate vaccines • invasive pneumococcal disease • pneumolysin

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INTRODUCTION

Streptococcus pneumoniae (the pneumococcus) infections continue to be associated with significant morbidity and mortality in both the developing and developed world⁵⁷. The pneumococcus remains one of the most common bacterial causes of community-acquired pneumonia, meningitis, sinusitis, otitis media, and bacteremia. This is particularly true in patients who are concomitantly infected with the human immunodeficiency virus (HIV), in whom *S. pneumoniae* is the most frequent cause of bacterial pneumonia¹⁰.

The outcome of community-acquired pneumonia appears to be substantially improved by the early institution of antimicrobial agents to which the causative organism is susceptible, and adversely affected by delayed or inappropriate antibiotic treatment⁵³. However, despite the availability of potent antimicrobial treatment, as well as various advances in medical therapy, including the establishment of intensive care unit facilities, some patients with pneumococcal pneumonia will still die from their infection⁴⁹. The problem is further compounded by emerging resistance of the pneumococcus to many of the first-line antibiotics commonly used to treat pneumonia²⁴.

RISK FACTORS FOR PNEUMOCOCCAL INFECTION

Risk factors for community-acquired pneumonia are summarized in Table 1. These are numerous and include, among many others, extremes of age, underlying comorbid illness (especially underlying cardiovascular disorders, chronic obstructive pulmonary disease and, more recently, concomitant HIV infection), lifestyle factors such as alcohol consumption, cigarette smoking, and body mass index, various treatments, and low socioeconomic status^{1, 6, 19, 20, 21, 22, 23, 29, 46, 51, 55, 65, 74}. Exactly how these risk factors predispose to pneumonia is uncertain, but in general terms they may be associated with increased bacterial colonization of the airways, may facilitate direct access of the microorganisms into the lungs, and may be associated with impaired clearance mechanisms.

Table 1. Major risk factors for community-acquired pneumonia

- Extremes of age (very young or elderly)
- Underlying comorbid illnesses (especially chronic obstructive pulmonary disease and chronic cardiovascular disorders)
- Concomitant infection with the HIV
- Lifestyle factors (cigarette smoking, excessive alcohol consumption, intravenous drug abuse, poor nutrition, high body mass index)
- Low socioeconomic status

Extremes of age are associated with an increased risk of pneumonia. In the case of the elderly²² it has been suggested that chronological age itself may not be the important factor, but rather that the underlying comorbid conditions that are often present in these patients, and their treatment, may predispose to these infections. Nevertheless, there are several risk factors in the elderly that may predispose to pneumonia; these include age greater than 65 years, macro- or micro-aspiration, swallowing difficulties, impaired mucociliary clearance, poor nutrition, low serum albumin, institutionalization, recent hospitalization, endotracheal/nasogastric intubation, general worsening of health, and a lack of influenza vaccination.

HIV infection is itself a significant risk for community-acquired pneumonia, and in particular pneumococcal infection, although the exact mechanisms are uncertain and are the subject of ongoing investigation^{19, 51, 65, 74}. One study suggested that impaired natural immunity to the pneumococcal toxin, pneumolysin, may be an important predisposing factor to bacteremic pneumococcal pneumonia². Another clinical study conducted in Uganda demonstrated impaired opsonic activity of type-specific immunoglobulin (Ig) G in the serum of HIV-1-infected adults, which may predispose these individuals to invasive pneumococcal infections⁷³. Community-acquired pneumonia occurs at all levels of the CD4 count in HIV-seropositive patients, but there is an inverse relationship between the incidence of pneumonia and the CD4 count. Pneumonia occurs most frequently when the CD4 count falls below 200/mm³. In the case of pneumonia due to *S. pneumoniae*, the patients are more frequently infected with isolates demonstrating increased resistance to penicillin and various other antibiotics.

Many of the risk factors for pneumonia in HIV-seronegative patients are also additional risk factors in HIV-seropositive patients^{19, 51, 65, 74}, including underlying comorbid illness, cigarette smoking, and a history of a previous episode of pneumonia. Interestingly, an important risk factor for pneumonia in these patients is intravenous drug abuse, and there is evidence that the incidence of pneumonia in patients who are HIV-seropositive by way of intravenous drug abuse is higher than in those who are HIV-seropositive by way of heterosexual or homosexual spread⁶⁵. Additional risk factors in HIV-seropositive patients include alcoholism, cirrhosis, neutropenia, and low serum albumin. Factors associated with a decreased risk of pneumonia in HIV-seropositive patients include use of antiretroviral therapy, pneumococcal vaccination, and co-trimoxazole prophylaxis.

It is well-recognized that cigarette smoking predisposes patients to community-acquired pneumonia^{1, 55}. Cigarette smoking impacts negatively on a number of innate and specific host defense mechanisms. Among the humoral immune changes associated with smoking are decreases in some of the Ig levels and an impaired antibody response to antigens. Changes in cellular immunity include effects on macrophages, polymorphonuclear leukocytes, and lymphocytes. In the case of mechanical clearance mechanisms, such as the mucociliary escalator, cigarette smoking decreases ciliary beating and function and increases mucus production, and possibly impairs its quality. Cigarette smoking is a risk factor for community-acquired pneumonia in general, but also pneumococcal infection in particular⁵⁵. In the case of pneumococcal disease, cigarette smoking has been documented to be the most important independent risk factor for the invasive infection⁵⁵. Moreover, a positive trend for risk of community-acquired pneumonia is associated with duration of smoking, the number of cigarettes smoked, and cumulative consumption. The risk of pneumonia occurring in cigarette smokers is apparent even in cases in which there is minimal lung injury. HIV-seropositive patients who smoke cigarettes and use drugs are additionally at risk of pneumonia. There appears to be little direct evidence that passive smoking is associated with a significant increased risk of community-acquired pneumonia.

Several studies have documented significant alcohol consumption to be an important risk factor for community-acquired pneumonia^{23, 29}. This is believed to be especially important in middle-aged individuals. Patients with chronic alcoholism have been shown to be at particular risk of pneumonia caused by Gram-negative bacilli. The mechanisms of increased susceptibility of alcoholics to pneumonia are numerous and include enhanced oropharyngeal colonization, increased risk of aspiration, impaired mucociliary clearance, defective surfactant production, effects on pulmonary function, and negative effects on both cell-mediated and humoral immunity. Alcohol has adverse effects on various components of the immune system, including suppression of the protective functions of alveolar macrophages, polymorphonuclear leukocytes, and lymphocytes and inhibition of cytokine production by various cell types.

Some studies have documented an increased risk of pneumonia in patients who are malnourished or have a low body mass index. A study undertaken in the United States to investigate the relationship of lifestyle factors and community-acquired pneumonia

noted that the body mass index was directly related to the occurrence of pneumonia in women⁶. In both men and women, an increase in weight of 40 pounds or more (≥ 18 kg) was associated with a 2-fold increased risk of pneumonia relative to persons who maintained their weight.

Pneumonia most commonly occurs following aspiration of colonizing microorganisms from the upper respiratory tract, this representing the major route for organisms to reach the lower respiratory tract. For this reason it has long been suggested that the oral cavity may represent a reservoir for respiratory pathogens. Although this appears to be particularly likely in frail and debilitated elderly patients, these cases have additional risk factors for pneumonia, such that the exact contribution of oral hygiene and periodontal pathogens as independent risk factors is difficult to evaluate⁴⁶. However, there is a small number of interventional studies, conducted in both children and in the frail elderly, that have suggested that improving oral hygiene may be associated with a decrease in respiratory tract infections.

Clinical and pathogenetic studies in pneumococcal infections

A number of recent clinical studies of pneumococcal pneumonia have focused on determining the most appropriate antibiotic treatment, particularly for severe, hospitalized cases, with associated pneumococcal bacteremia^{5, 44, 49, 76}. These studies have consistently confirmed that combination antibiotic treatment, most commonly that of a macrolide together with a standard β -lactam agent, is associated with a better outcome in this subset of patients than therapy with a single antibiotic. Although further research is needed to ascertain the mechanism by which combination therapy achieves a better outcome, the beneficial effects appear to be independent of the presence of antibiotic resistance, concomitant infection with “atypical pathogens”, or synergistic antibiotic effects, and may be related to the anti-inflammatory, immunomodulatory effects of antibiotics, such as the macrolides⁷⁶.

Additional studies have focused on the virulence factors of the pneumococcus, with the particular aim of improved understanding of their biological effects and their potential roles in the pathogenesis of pneumococcal infections⁵⁹. One of the most studied virulence factors is the pore-forming, cytolytic, protein toxin pneumolysin, which is known to be produced by all clinically significant pneumococcal isolates⁵⁹. This toxin is thought to play an important role in the pathogenesis of pneumococcal infections, enhancing

initial colonization of the respiratory tract and subsequent invasion of the epithelium^{26, 28, 60, 71}. Importantly, it has been suggested that strategies aimed at targeting pneumolysin may be valuable as adjunctive therapy to antibiotics for pneumococcal infections, while the toxin is also recognized as an ideal protein candidate for a pneumococcal conjugate vaccine¹².

STRUCTURE OF PNEUMOLYSIN

Pneumolysin, a member of the thiol-activated family of cytolysins, is a 52.7-kDa protein which consists of 471 amino acids and is predominantly monomeric in solution⁶⁹. Following binding to cholesterol, the lytic action of pneumolysin involves a complex combination of events, including pore formation within the membrane, extraction of the lipid into free oligomeric complexes, aggregation and fusion of the membranes, and membrane destabilization⁸. The interaction between pneumolysin and cholesterol is very specific, since structurally similar sterols such as water-soluble cholesterol, ergosterol, and lanosterol are not effective antagonists of pneumolysin⁵⁴.

In addition to pore-forming activity, pneumolysin also possesses complement-activating properties, with the former being associated with domain 4 in the C-terminal region of the toxin and the latter with domain 1 in the N-terminal region^{3, 4}.

PNEUMOLYSIN IN MICROBIAL VIRULENCE

Although colonization of the nasopharynx of humans by *S. pneumoniae* is usually uneventful, translocation from this site to the lungs, central nervous system, or circulation, as a consequence of mechanical stress and/or inadequate host defenses, results in serious, often life-threatening infection. The transition from being an unobtrusive, albeit unwelcome, colonist to an aggressive invader is associated with the expression of an array of virulence factors, such as capsular polysaccharides, and virulence proteins, including surface proteins (PspA) A and C, choline-binding protein A (CbpA), surface adhesin A (PsaA), hyaluronate lyase, neuraminidases, caseinolytic protease P (ClpP) and, especially, pneumolysin^{36, 40}.

Evidence derived from murine models of experimental pneumococcal pneumonia, as well as from limited clinical studies in humans, has convincingly underscored the involvement of pneumolysin in both lung colonization and extra-pulmonary dissemination of the pneumococcus. Initial evidence was provided by Paton et al.⁵⁹, who reported that immunization of

mice with pneumolysin conferred partial protection against subsequent challenge with virulent pneumococci. This same group also reported that inactivation of the gene encoding the toxin resulted in attenuation of pneumococcal virulence in a murine model of experimental lung disease, with significant reductions in bacterial replication in the lung, as well as delayed bacteremia⁵⁹. Recently, García-Suárez et al.³² reported that passive administration of IgG monoclonal antibodies to pneumolysin protected mice against subsequent intravenous challenge with a lethal dose of the toxin. These monoclonal antibodies, which block the binding of pneumolysin to eukaryotic cells, also significantly decreased the frequencies of bacterial lung colonization and bacteremia and increased the survival time of mice infected via the intranasal route with virulent *S. pneumoniae*³². These observations confirm and extend an earlier report by Musher et al.⁵⁰, who found that passive administration of purified human IgG antibodies to pneumolysin protected mice against subsequent intraperitoneal challenge with *S. pneumoniae* by preventing dissemination of the bacteria.

All of these studies, together with earlier observations that administration of recombinant pneumolysin into the airways of rats induces the salient histological features of pneumococcal infection in the rat lung²⁷, provide compelling support, albeit from experimental animal studies, for the involvement of pneumolysin in pneumococcal colonization of the airways, the development of pneumonia, and the extra-pulmonary dissemination of this microbial pathogen. This contention is supported by observations in humans that circulating antibodies to pneumolysin measured in adult patients with pneumococcal pneumonia at the time of hospital admission were highest and lowest in those patients with non-bacteremic and bacteremic disease, respectively⁵⁰.

More recently, Orihuela et al.⁵⁶ infected mice nasopharyngeally with a series of virulence gene knockout mutants of the pneumococcus and monitored microbial translocation to other anatomical sites. They found that neuraminidase, pyruvate oxidase (which generates hydrogen peroxide) and, to a lesser extent, CbpA contributed to prolonged nasopharyngeal colonization, while CbpA and neuraminidase contributed to translocation to the lungs. Pneumolysin, pyruvate oxidase, and autolysin were the major contributors to bacterial replication in the lungs and dissemination to the bloodstream, while only pneumolysin and autolysin were required for high-titer replication, with CbpA required for invasion of the cerebrospinal fluid⁵⁶.

MECHANISMS BY WHICH PNEUMOLYSIN PROMOTES MICROBIAL COLONIZATION AND DISSEMINATION

Pneumolysin possesses both cytotoxic and pro-inflammatory properties, both of which are secondary to the pore-forming actions of the toxin. The cytotoxic effects of pneumolysin on ciliated respiratory epithelium, alveolar epithelial cells, and pulmonary endothelial cells are thought to be the major mechanism by which the toxin promotes both colonization of the airways by the pneumococcus and invasive disease^{27, 45}. Pneumolysin is a potent inhibitor of the ciliary beat frequency of ciliated human respiratory epithelium, an activity which favors microbial colonization and dissemination^{25, 26}. In the case of alveolar epithelial cells and pulmonary endothelial cells, the toxin disrupts the alveolar capillary boundary, producing alveolar flooding, which not only provides nutrients for bacterial growth, but also favors extra-pulmonary dissemination of the pneumococcus^{36, 45, 63, 64}. Endothelial and epithelial cell death results from both apoptotic and necrotic mechanisms consequent to pneumolysin-mediated damage to the plasma membrane.

Although its precise contribution to the pathogenesis of severe pneumococcal disease remains to be established, the pro-inflammatory activity of pneumolysin has also been linked to the invasiveness of the pneumococcus. At sub-cytolytic concentrations, considerably less than those which are cytotoxic for respiratory epithelium and which are comparable to those measured at sites of acute pneumococcal infection in humans⁷⁰, pneumolysin augments the pro-inflammatory activities of neutrophils and monocytes/macrophages^{13, 14, 17, 35, 43, 61, 62}, which is due at least in part to toxin-mediated influx of extracellular Ca^{2+} into these cells¹⁷. These pro-inflammatory interactions of pneumolysin with neutrophils and monocytes/macrophages are summarized in Table 2. Rather than contributing to eradication of the pneumococcus, however, these mechanisms may favor microbial persis-

Table 2. Pro-inflammatory properties of pneumolysin

Property	Cell type affected	Reference
Increased chemoattractant-activated production of superoxide, eicosanoids and prostanoids, release of granule enzymes, and CR3 expression	N	14, 17
Activation of NF κ B	M	43
Inactivation of α -1-proteinase inhibitor	N	15
Increased activity of phospholipase A_2	N, EC	14, 63, 64
Synthesis of pro-inflammatory cytokines/chemokines/nitric oxide synthase	M, N	13, 61, 62

Abbreviations: EC – endothelial cells, M – monocytes/macrophages, N – neutrophils.

tence and dissemination as a consequence of inflammation-mediated damage to epithelium.

Recently we identified an additional mechanism by which pneumolysin may exacerbate inflammation-mediated epithelial damage. This mechanism involves pneumolysin-mediated augmentation of the oxidative inactivation of α -1-protease inhibitor by chemoattractant-activated human neutrophils¹⁵. This in turn leads to uncontrolled activity of neutrophil-derived elastase, a potent epithelial toxin, while oxidized α -1-protease inhibitor *per se* possesses pro-inflammatory properties which may intensify the inflammatory cascade^{48, 72}. Pneumococcal disease is therefore likely to be accompanied by a multi-pronged assault on respiratory epithelium mediated directly by pneumolysin and other pneumococcal cytotoxins, such as hydrogen peroxide²⁵, acting in concert with excessive amounts of phagocyte-derived elastase and reactive oxidants generated during pneumolysin-orchestrated, over-exuberant inflammatory responses. These events are summarized in Fig. 1.

Researchers using animal models of experimental

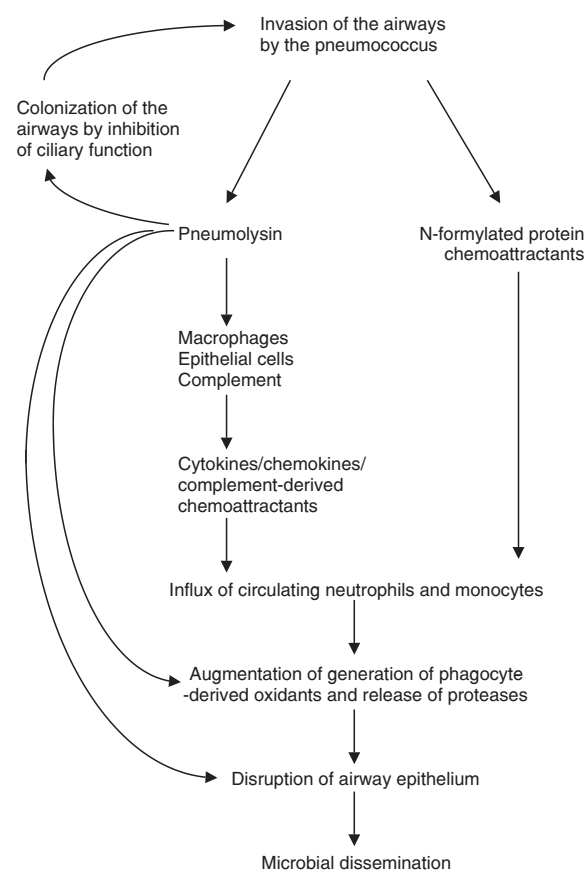


Figure 1. Schematic representation of the cytotoxic and pro-inflammatory mechanisms by which pneumolysin contributes to the pathogenesis of pneumonia, and disrupts respiratory epithelium, resulting in extra-pulmonary dissemination of the pneumococcus.

pneumococcal disease have convincingly demonstrated that the pro-inflammatory activities of pneumolysin are operative *in vivo*^{27, 38, 45, 52} and appear to contribute to microbial dissemination^{38, 50}, possibly as a consequence of inflammation-mediated damage to bystander epithelium as mentioned above. Feldman et al.²⁷, using a rat lung model, reported that the pore-forming activity of the toxin appeared to be the major contributor to pulmonary inflammation, with a lesser, albeit significant contribution, attributable to complement activation. More recently it has been confirmed that both the pore-forming and complement-activating properties of the toxin contribute to lung pathology and timing of onset of bacteremia in experimental murine bronchopneumonia, promoting neutrophil and T lymphocyte recruitment/activation, respectively³⁸.

Maus et al.⁴⁵ recently reported that intra-tracheal administration of recombinant pneumolysin to mice is accompanied by disruption of the alveolar-capillary barrier and recruitment of neutrophils and monocytes into the alveolar space. Attenuation of neutrophil influx by administration of monoclonal antibodies to neutrophil/endothelial adhesion molecules or clodronate-induced depletion of resident alveolar macrophages did not, however, reduce the increase in lung permeability mediated by intra-tracheal pneumolysin. These authors concluded that pneumolysin-mediated lung injury therefore results exclusively from the direct cytotoxic actions on the alveolar-capillary barrier, independently of the pro-inflammatory activities of the toxin. While this elegant experimental design clearly demonstrates the involvement of the direct cytotoxic actions of pneumolysin on airway epithelium in the pathogenesis of pneumococcal disease, it is not, however, completely representative of the pathophysiological situation with intact, virulent bacteria. In this setting, early influx of neutrophils and monocytes into the airways is critically dependent on the release of bacterial-derived, N-formylated polypeptide chemoattractants^{31, 58}. These chemoattractants in turn not only promote recruitment to the airways of neutrophils and monocytes from the circulation, but also sensitize these cells, as well as resident pulmonary macrophages, to the pro-inflammatory actions of pneumolysin, which is released later in the time course of infection, largely as a consequence of autolysis of the bacteria. The relative contributions of the cytotoxic and pro-inflammatory activities of pneumolysin to microbial colonization remain to be conclusively established.

PNEUMOLYSIN AS A TARGET FOR VACCINES

Given its undoubted involvement in the pathogenesis of invasive disease, pneumolysin is a compelling tar-

get for the development of novel strategies to prevent and treat severe pneumococcal disease. Although no such strategies currently exist, several approaches show promise. Pneumolysin-based vaccines appear to be a viable option in disease prevention, while administration of toxin-neutralizing antibodies to patients with acute pneumococcal disease is a potentially beneficial strategy to counter development of bacteremia.

Active immunization

The capsular polysaccharides of *S. pneumoniae* form the basis of the 23-valent polysaccharide vaccine. However, due to its weak immunogenicity, this vaccine has had a rather limited impact on the morbidity and mortality of pneumococcal infections, especially in high-risk cases⁹. Attempts at improving immunogenicity have resulted in the development of conjugate vaccines in which the capsular polysaccharides are coupled to T lymphocyte-dependent protein carriers, such as tetanus and diphtheria toxoids¹¹. Although these conjugate vaccines have proven to be highly immunogenic and efficacious in disease prevention in young children, including those infected with HIV^{39, 67}, they have proved to be disappointing in immunizing the elderly against pneumococcal infections⁹. Other limitations include the potential for pneumococcal strain replacement in the airways of vaccine recipients due to the limited number of pneumococcal serotypes represented in the vaccine (usually the 7–9 prevalent types in a given geographic region), resulting in vaccine failure, as well as the prohibitive costs of immunization with conjugate vaccines for developing countries^{7, 9}.

The development of conjugate vaccines based on pneumolysin as the protein carrier, ideally as a stable toxoid⁴², represents an attractive alternative to the protein carriers currently used in conjugate vaccines for the following reasons: 1) pneumolysin is produced by all clinically-relevant strains of the pneumococcus; 2) the toxin induces T lymphocyte-dependent immune responses resulting in the production of circulatory IgG and secretory IgA antibodies^{37, 66}; 3) inactivated pneumolysin has been demonstrated to function as an effective immunogenic carrier protein in a murine model of conjugate vaccine efficacy, being equivalent or superior to conventional carriers⁴²; and 4) although unlikely to prevent infection with non-vaccine serotypes, vaccine-mediated immunity to pneumolysin may result in less severe disease. Nevertheless, the development of pneumolysin-based vaccines is clearly dependent on the convincing demonstration of protective efficacy in humans, which is superior to that of existing conjugate vaccines.

In addition to pneumolysin, several other pneumo-

coccal virulence proteins have been identified as potential vaccine candidates *per se*, as well as carriers in conjugate vaccines. These include CbpA, ClpP protease, PspA and C, and PsaA^{7, 9, 36, 40}. Non-conjugate protein-based vaccines containing cocktails of these virulence factors designed to achieve maximum protective efficacy represents an alternative immunization strategy, as does the use of combinations of these virulence proteins as carriers for conjugate vaccines.

Passive immunization

The use of pneumolysin-targeted monoclonal antibodies as a therapeutic strategy in acute pneumococcal disease is underscored by the recent study of García-Suárez et al.³² alluded to above. Ideally, these monoclonal antibodies should neutralize both the pore-forming and complement-activating actions of the toxin. However, several factors, such as expense, timing of administration (likely to be most effective early in the course of infection), targeting to sites of microbial colonization, and possible amplification of harmful inflammatory responses, may restrict the clinical applications of this approach⁷⁵.

PNEUMOLYSIN-TARGETED PHARMACOLOGICAL STRATEGIES

These fall into three categories. First, inhibitors of the synthesis of pneumolysin; these are exclusively antimicrobial agents. Secondly, agents which interfere with the binding of the toxin to eukaryotic cells, and thirdly, agents which antagonize the pro-inflammatory and pro-apoptotic actions of the toxin.

Antimicrobial agents

β -Lactam antibiotics are the agents of choice in the treatment of severe pneumococcal disease and are generally highly effective. However, even in the setting of seemingly appropriate antimicrobial chemotherapy and notwithstanding the impact of emerging antibiotic resistance and coexistent HIV infection, the rate of treatment failure in severe disease remains unacceptably high. Recently it has been proposed that combining a β -lactam with a macrolide antimicrobial agent leads to a reduction in the mortality associated with bacteremic pneumococcal pneumonia^{30, 44, 49, 76}. Although the precise mechanisms which underpin the therapeutic efficacy of the β -lactam/macrolide combination remain to be established, the added benefits of the macrolide may be related to inhibition of the synthesis of protein virulence factors, particularly pneumolysin, which may accompany β -lactam-mediated disintegration of the pneumococcus³⁴.

This contention is supported by the study of Spreer et al.⁷⁰ who, using a rabbit model of pneumococcal meningitis, observed that treatment with a non-bacteriolytic antimicrobial agent (rifampicin or clindamycin) resulted in significantly lower concentrations of pneumolysin in the cerebrospinal fluid relative to those observed following administration of ceftriaxone. These observations are in agreement with the findings of an earlier study conducted by Lagrou et al.⁴¹, who reported that erythromycin treatment of *S. pneumoniae* is associated with a marked reduction in the synthesis/release of pneumolysin. Combining the β -lactam with a macrolide may therefore reduce the potential risk of excessive release of pneumolysin during the early stages of antimicrobial chemotherapy of severe pneumococcal infection. Nevertheless, the potential clinical benefits of combination therapy await confirmation in large-scale prospective clinical trials.

Antagonists of pneumolysin

Cholesterol, the putative pneumolysin-binding molecule in eukaryotic cell membranes, antagonizes the pore-forming actions of the toxin³. In a recent series of experiments we investigated the effects of the non-toxic phytosterol β -sitosterol (24 α -ethylcholesterol) on the pore-forming, pro-inflammatory interactions of sub-cytolytic concentrations (8.37 ng/ml) of pneumolysin with human neutrophils. The results of typical experiments in which we measured the effects of pre-treatment of isolated human blood neutrophils with β -sitosterol (5 μ g/ml) on pneumolysin-mediated influx of extracellular calcium, an indicator of the pore-forming activity of the toxin, are shown in Fig. 2

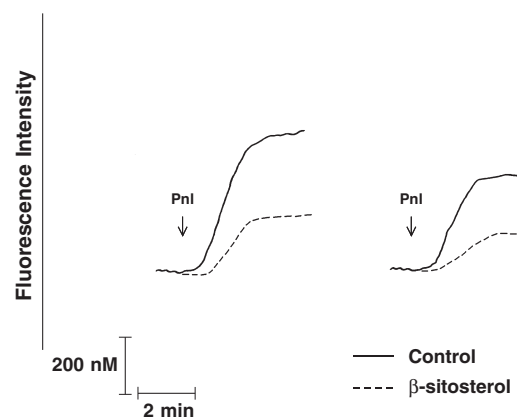


Figure 2. Measurement of the effects of pre-treatment of human neutrophils with β -sitosterol (5 μ g/ml) on pneumolysin (Pnl, 8.37 ng/ml)-mediated influx of Ca^{2+} using a fura-2/AM-based spectrofluorimetric procedure as described previously¹⁷. The results of two representative experiments clearly demonstrate the increase in fura-2 fluorescence due to influx of Ca^{2+} after a lag phase of about 1 min, which accompanies exposure of the neutrophils to pneumolysin, and which is attenuated by β -sitosterol.

and clearly demonstrate the protective activity of the phytosterol.

Agents, such as the ω -3 long chain-polysaturated fatty acids, docosahexaenoic acid, and eicosapentaenoic acid, which lower cholesterol levels in cell membranes³³, also have the potential to antagonize the pore-forming actions of pneumolysin. We have recently reported that a brief exposure of isolated human neutrophils to either of these fatty acids attenuates the pore-forming, pro-inflammatory actions of the toxin¹⁶. In this experimental setting the pneumolysin-neutralizing actions of the fatty acids cannot be attributed to cholesterol-lowering activity, indicating that other, as yet undefined, mechanisms are also operative. The therapeutic efficacy, if any, of β -sitosterol and ω -3 fatty acids, as well as other types of fatty acid, has not yet been investigated in murine models of experimental pneumococcal disease.

The complement-activating region of pneumolysin also represents a potential target for pharmacological antagonists. To our knowledge, however, no such agents currently exist.

Anti-inflammatory agents

These agents, which we have recently reviewed elsewhere¹², have the potential to attenuate the pro-inflammatory actions of pneumolysin which are consequent to toxin-mediated influx of Ca^{2+} into immune and inflammatory cells, particularly macrophages and neutrophils. Foremost among these are corticosteroids and macrolides, which have been reported to reduce mortality in severe pneumococcal disease^{18, 47, 68}. Notwithstanding their inhibitory effects on the synthesis of pneumolysin, macrolides, as well as ketolide antimicrobial agents, possess anti-inflammatory properties, underscoring the potential of these agents as adjuncts to chemotherapy with β -lactams¹².

Table 3. Pharmacological and immunological pneumolysin-neutralizing strategies

Strategy	Phase of development	Reference
Design and development of pneumolysin-based vaccines	pre-clinical	42
Passive immunization with monoclonal antibodies	pre-clinical	32
Adjunctive antimicrobial/anti-inflammatory chemotherapy by combining a β -lactam with a macrolide	undergoing both pre-clinical and clinical evaluation	18, 47, 68
Adjunctive anti-inflammatory chemotherapy with corticosteroids	undergoing clinical evaluation	
Adjunctive anti-inflammatory chemotherapy with cAMP-elevating agents or inhibitors of elastase or membrane-interactive pneumolysin antagonists/anti-inflammatory agents or inhibitors of apoptosis	theoretical; not yet evaluated in animal models of experimental pneumococcal disease	

Other categories of anti-inflammatory agents, albeit of untested potential, include cyclic adenosine 3',5'-monophosphate-elevating agents, especially inhibitors of type 4 phosphodiesterase and agonists of subtype A_{2A} adenosine receptors, pharmacological inhibitors of elastase, and inhibitors of cell death pathways¹².

These various pharmacological pneumolysin-neutralizing strategies are summarized in Table 3.

CONCLUSIONS

As underscored by this review, research into pneumococcal diseases has gained considerable momentum, generating significant new information. While much of this has focused on counteracting ongoing morbidity and mortality by optimizing immunization strategies and antimicrobial chemotherapy, recent insights into disease pathogenesis have identified potential, novel adjunctive and preventive strategies, with pneumolysin representing a particularly attractive target.

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