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Review

Pneumocystis as a Co-Factor in Pulmonary Diseases

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Abstract

Pneumocystis causes life-threatening pneumonia in immunocompromised populations. More recently it has been implicated as a co-factor in a number of chronic lung diseases including chronic obstructive pulmonary disease (COPD), severe asthma, and cystic fibrosis (CF). In this review, we will examine the current literature regarding *Pneumocystis* and lung diseases in the HIV-infected patients and non-HIV immunocompromised populations, and the barriers to prophylaxis and treatment in these patients. Trimethoprim sulfamethoxazole (TMP-SMX) is an effective therapeutic against *Pneumocystis* but this approach remains problematic due to drug interactions, treatment-limiting adverse events, and break-through *Pneumocystis* pneumonia (PCP) despite prophylaxis. This review summarizes the shortcomings of current prophylaxis and treatment strategies, and the advances that have been made toward the development of novel diagnostics and therapeutics, with a focus on vaccine development.

Keywords

Pneumocystis; lung disease; HIV; COPD; severe asthma; cystic fibrosis; vaccine; immunosuppression; kexin

1. Lung Disease in the HIV-Infected Population

1.1 HIV Infection and PCP

Pneumocystis jirovecii is an opportunistic extracellular fungal pathogen that causes life-threatening pneumonia in immunocompromised individuals. Persistent *Pneumocystis* colonization and acute pneumonia are associated with permanent obstructive lung damage [1]. Recent reports suggest that this organism may also play a role in the development of severe asthma [2, 3] and cystic fibrosis [4] and potentially other lung diseases. The clinical relevance of *Pneumocystis* emerged in the 1980s as a result of the AIDS epidemic [5]. For many years, *Pneumocystis* pneumonia (PCP) was the leading cause of morbidity and mortality in HIV-infected patients. Despite improvements in trimethoprim sulfamethoxazole (TMP-SMX) prophylaxis and antiretroviral therapy (ART), PCP remains the most common serious opportunistic infection among those with AIDS [6, 7]. The overall rate of PCP in the United States is 4.7 cases per 100 person-years and may be significantly higher in those who are either not using or not responding to ART or are undiagnosed [8]. Even where treatment is initiated, mortality in this population remains high at 10-40%, but may be as high as 60-80% in patients who have progressive lung damage leading to respiratory failure [9, 10]. Due to the high rate of mortality with the current standard of care, PCP remains a significant co-morbidity among HIV-infected patients.

1.2 HIV Infection and COPD

In addition to causing PCP, several laboratories have reported an association between *Pneumocystis* colonization and chronic obstructive pulmonary disease (COPD) in both HIV-infected and non-HIV infected populations [1, 10-23]. The pathogenesis of HIV-related COPD is poorly understood, but it is hypothesized that persistent colonization with *Pneumocystis* or other microbes may amplify inflammatory responses and tissue damage [11, 14, 19, 24, 25]. Increasing evidence suggests that *Pneumocystis* colonization is associated with the development and exacerbation of COPD in both HIV-infected and non-HIV infected populations, independent of smoking [1, 10-23]. In HIV-infected persons, *Pneumocystis* colonization is frequent even among individuals receiving anti-*Pneumocystis* prophylaxis and those with high CD4 cell counts who are receiving ART [19, 20]. COPD-like changes have been found in HIV-infected patients following PCP [10, 26, 27], and we have demonstrated that even low levels of *Pneumocystis* correlate with COPD [13]. *Pneumocystis* colonization in HIV-infected patients is associated with worse pulmonary airway obstruction and emphysema [1], as well as *Pneumocystis*-related pulmonary inflammation that may contribute to the pathogenesis of COPD [16, 21]. Our laboratory was the first to demonstrate that in a non-human primate (NHP) model of HIV infection, persistent *Pneumocystis* colonization leads to the development of airway obstruction and emphysema [15]. We found that the decline in pulmonary function occurs early after *Pneumocystis* colonization and these *Pneumocystis*-induced obstructive changes are not reversible following treatment with TMP-SMX or albuterol [28]. Our results, along with mounting clinical evidence, support the concept that *Pneumocystis* colonization contributes to the development of COPD in HIV-infected individuals.

2. Pneumocystis-Related Lung Disease in Non-HIV-Infected Populations

2.1 PCP in Non-HIV-Infected Patients

In contrast to the HIV-infected population, PCP is of increasing concern in non-HIV infected persons receiving immunosuppressive therapies. These at-risk populations include cancer patients, transplant recipients, individuals treated for inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease, and persons experiencing natural immunosuppression to due aging, congenital or acquired immunosuppressive states [29]. In the non-HIV immunosuppressed population, approximately 15-20% are at risk for PCP, with mortality rates as high as 60% [24, 30, 31]. Systemic immunosuppressive steroid therapy is the treatment most commonly associated with the development of PCP, and combination cytotoxic therapies (e.g. cyclophosphamide, methotrexate, CHOP regimen (cyclophosphamide, hydroxydaunorubicin, oncovin, prednisone)) that have a synergistic T cell suppressive effect are associated with increased risk. Patients treated with monotherapies, even at low doses, as well corticosteroid-sparing regimens, are also at risk of PCP [32]. As immunologic control of *Pneumocystis* infection is dependent upon T and B cells, there is an increased incidence of PCP among patients treated with immune-therapeutics (*e.g.* B cell therapeutics, TNF inhibitors). As the number of cancer cases worldwide is expected to increase to approximately 21 million by 2030, along with increases in patients receiving organ transplants and immunosuppressive therapies, over two million patients are estimated to be at risk of development of PCP. Of increasing significance is a widespread lack of PCP prophylaxis guidelines in many of the non-HIV immunocompromised patient populations and there is a growing list of immunosuppressive agents associated with the development of PCP [32]

2.2 COPD in Non-HIV-Infected Patients

Several groups, including our own, have identified an association between *Pneumocystis* colonization and chronic obstructive pulmonary disease (COPD) in the non-HIV population [13, 33-36]. Smoking is still considered to be the primary cause of COPD, but there is increasing evidence that *Pneumocystis* colonization is a co-factor that maintains or increases an inflammatory response, accelerating the development of severe COPD [21, 37, 38]. Although non-immunosuppressed patients rarely develop PCP [39], persistent low levels of *Pneumocystis* DNA have been documented in patients with chronic bronchial disease [40]. Our study found that *Pneumocystis* colonization is increased in smokers with COPD (non-HIV-infected) compared to smokers without COPD and correlates with disease severity [13]. These data were further supported by later clinical studies which found that *Pneumocystis*-colonized patients have higher levels of systemic pro-inflammatory cytokines than non-colonized patients [16, 21], suggesting that *Pneumocystis* may play a role in disease progression.

Clinical studies profiling the cytokine environment in non-HIV-infected COPD patients have revealed that *Pneumocystis* colonization is associated with the expression of TNF-α, IL-6 and IL-18 [21], and Th1-associated genes such as IFN-γ and chemokine ligands CXCL9, CXCL10, and CXCL11 [16]. Likewise, fungal β-1,3-glucans (BG) found in *Pneumocystis* can act as potent inducers of alveolar macrophage activation that initiate inflammatory responses by lung parenchymal cells, characterized by the secretion of TNF-α, IL-6, IL-8, MIP-2, eicosanoid metabolites, and reactive oxidant species [41, 42]. Thus, inflammatory responses that occur in response to chronic

Pneumocystis colonization resemble the inflammatory environment observed in the lungs non-HIV-infected COPD patients [16, 21], supporting the hypothesis that *Pneumocystis* exacerbates COPD.

2.3 Severe Asthma

Patients with severe asthma exhibit sustained symptoms such as coughing, wheezing, and shortness of breath despite the use of high levels of inhaled corticosteroids or oral corticosteroids [3]. Clinical studies have documented the presence of *Pneumocystis* in individuals with severe asthma patients [43]; however, there has been a lack of data to discern whether *Pneumocystis* drives pathogenesis in this disease or if *Pneumocystis* colonization is due to high corticosteroid use within this population. Supporting this hypothesis, a murine model of *Pneumocystis* infection has been shown to promote several pathologic features associated with asthma, including increased Th2 inflammation, increased mucus production, airway remodeling, and eosinophil recruitment [2]. Two recent studies support the correlation between *Pneumocystis* colonization and the development of severe asthma [2, 44]. Eddens et al., reported that higher levels of sera IgG and IgE titers against whole *Pneumocystis* lysate (PC-hi) were associated with worse symptoms and lung functions in patients with severe asthma when compared to patients with lower levels of *Pneumocystis* antibodies (PC-low), despite similar levels of inhaled corticosteroids or oral steroids [2]. Moreover, when comparing the lung microbiomes of cohorts with pediatric lung disease, children with severe asthma had significantly higher levels of several fungal species including *Pneumocystis* in bronchoalveolar lavage fluid than children with cystic fibrosis [44], demonstrating a connection between *Pneumocystis* and severe asthma independent of pulmonary disease. Together these studies support the hypothesis that *Pneumocystis* may be a co-factor in the development of severe asthma.

2.4 Cystic Fibrosis

Cystic fibrosis (CF) is an inherited chronic lung disease caused by a defect in the CFTR gene that controls fluid and electrolyte homeostasis of epithelial cells [45, 46]. This deficiency results in unusually thick and viscous mucus that accumulates in small airways, leading to airway obstruction, frequent bacterial infections, and inflammatory lung disease. Although specific fungal agents such as *Aspergillus* have well documented roles in disease exacerbation in mouse models of CF [47-49], *Pneumocystis* has been thought to be an unrelated side effect of pre-existing lung disease. Of note, recent evidence suggests that *Pneumocystis* may play a more an active role in CF than previously thought [50-56]. Several studies have reported *Pneumocystis* colonization in CF patients that support this hypothesis. *Pneumocystis* colonization in this population is highly variable but is estimated to occur in 7.4-32.8% of CF patients [4, 57, 58], significantly higher than in healthy populations, and may be dependent upon geographical location [59, 60]. Despite the paucity of *Pneumocystis* colonization within CF patients, a prospective study found that the rate of colonization was five times higher in CF patients undergoing acute respiratory attacks (9.2%) than in stable CF patients (2%), implicating colonization incidence with the severity of disease [61]. Moreover, it has been suggested that these patients may serve as a reservoir for human transmission that put other susceptible populations at risk during outpatient treatment. More

research is necessary to investigate the pathogenic relationship between CF and *Pneumocystis* to determine if *Pneumocystis* intervention ameliorates disease severity.

3. Critical Barriers and Challenges to Prevention and Treatment of PCP

The preferred therapeutic for the prevention and treatment of *Pneumocystis* is trimethoprim-sulfamethoxaxone (TMP-SMX, Bactrim). Prophylactic use of TMP-SMX is effective in reducing the incidence of PCP in immunocompromised individuals but this approach remains problematic due to drug interactions, treatment-limiting adverse events, and break-through PCP despite prophylaxis [62-69]. Even when alternative antibiotics are used, such as clindamycin-primaquine, pentamide [70] or atovaquone [71], there are increasing concerns for antibiotic resistance and break-through PCP. PCP infection rates have increased in incidence from 2002 to 2010 [72] with approximately 15,000 hospitalization per year in the US. Despite low frequency of PCP in the non-HIV immunosuppressed population (5-15%), there is a high mortality rate with the current standard of care (10-40%) [24, 30, 31].

In addition to the challenges of TMP-SMX use, many barriers to treatment and prevention of *Pneumocystis*-related sequalae may stem from an inability to identify highly susceptible individuals within the immunosuppressed and control hospital-acquired infections during outpatient visits. It has been well documented that *Pneumocystis*-colonized patients have served as a source of infection during outbreaks [73, 74]. There have been numerous reports which have attempted to identify risk factors for PCP among the non-HIV immunosuppressed by correlating traits such as immunologic disorders, graft rejection in transplantation recipients, cytomegalovirus, and corticosteroid use [75, 76]. Despite these studies, there remains no reliable diagnostic method to identify the highest risk patients within the growing number of patients receiving immunotherapy. As a result, transplantation centers have been pressed to adopt TMP-SMX prophylaxis from 3-12 months for many transplant recipients, especially in renal transplant patients [77, 78]; however, the length and uniformity of prophylaxis has been debated.

Lapses in prophylaxis can lead to the development of PCP, even in patients with a successful history of long-term graft control and chronic disease maintenance. One case report even identified a renal transplant patient that succumbed to PCP following withdrawal of 24 years of TMP-SMX prophylaxis [79]. The difficulty in adopting uniform PCP prophylaxis guidelines is further exemplified by a recent report of serial outbreaks of PCP over a 10-year period in a renal transplant unit, despite *Pneumocystis* prophylaxis in some individuals [74]. In this study, intermittent use of TMP-SMX prophylaxis failed to prevent serial outbreaks among patients that were spread via human-to-human transmission during visits to the outpatient clinic [74]. These authors conclude that life-long PCP prophylaxis may be necessary in renal transplant recipients [74, 79]. With extensive use of TMP-SMX for both prophylaxis and treatment, it is not surprising that the rising prevalence of drug resistance increasingly complicates treatment options. Finally, as with all antibiotics, treatment of PCP with TMP-SMX does not prevent subsequent infections. These challenges to PCP treatment and prophylaxis, along with the inability to identify highly susceptible patients, emphasize the need for alternative strategies for disease prevention and treatment, such as vaccines and neutralizing antibodies, to supplement the current standard of care.

4. Development of Pneumocystis Prevention and Treatment Strategies

The development of a vaccine for the prevention of PCP has long been a goal of the field [80]. Several antigenic components have been evaluated in murine models and the roles of CD4 T cells and antibodies have been clearly established [18, 81-91]. These vaccine candidates include the major surface glycoprotein (MSG) [89], SPD1 [88], and kexin [90, 91]. Our laboratory has evaluated host responses to the *Pneumocystis* protein kexin and we have shown that humoral response against components of kexin (KEX1) may provide promising approaches toward the development of preventive and therapeutic vaccines, novel antibody-based therapeutics and improved diagnostic methods. KEX1 is a subtilisin-like serine protease that is highly expressed in *Pneumocystis* and related fungal species [92-94]. From animal and clinical studies, we know that *Pneumocystis* antibodies are important for protection against *Pneumocystis*-related pulmonary sequalae [18, 81-87], but KEX1-specific antibodies in particular play major role in abrogating *Pneumocystis*-related disease [46-52,57].

Most individual are seropositive against *Pneumocystis* antigens by early childhood [95-97] and have pre-existing humoral immunity against KEX1; however, the levels of *Pneumocystis* KEX1-specific immunity can vary between individuals. Based on these clinical studies, our laboratory has explored the utility of the humoral response to KEX1 as a biomarker for predicting susceptibility to PCP and *Pneumocystis*-related sequalae. Indeed, we have shown that low *Pneumocystis* KEX1-specific natural plasma antibodies are associated with increased risk of *Pneumocystis* colonization levels and are an independent predictor of susceptibility to PCP in HIV-infected patients [18] and in an experimental NHP model of HIV-associated PCP [23]. In a prospective study of newly diagnosed HIV-infected individuals, we demonstrated that high antibodies against KEX1, but not the *Pneumocystis* major surface glycoprotein (MSG), correlated with reduced incidence of PCP [18]. The association of low *Pneumocystis* KEX1 antibody levels as a predictor of subsequent PCP development was also demonstrated in a NHP model of HIV and *Pneumocystis* co-infection [23]. SHIV-infected macaques with high baseline KEX1 titers, were able to generate KEX1-specific antibodies in response to natural exposure to *Pneumocystis* and correlated with protection from colonization and preserved lung function[6].

A negative correlation between KEX1 antibody levels and lung disease was also found in HIV-negative smokers and COPD patients. In these populations, low anti-*Pneumocystis* KEX1 antibody titers were independently associated with more severe airway obstruction, suggesting that KEX1 antibodies may contribute to protection from *Pneumocystis* colonization and progressive COPD [12, 98]. These results provide further evidence that low KEX1 IgG titers may be a novel biomarker to predict PCP risk and may be a useful parameter to refine PCP prophylactic protocols, particularly in non-HIV immunocompromised populations where long-term prophylaxis may not be well tolerated by all individuals or necessary for prevention of PCP [32].

Currently, there are no clinically approved vaccines for the prevention of fungal infection. The observation that most individuals have been primed to KEX1, and that higher KEX1 plasma titers inversely correlate with susceptibility to PCP in HIV-infected individuals, suggest that “boosting” immune responses to KEX1 may induce protection against PCP. Using a non-human primate model of HIV and PCP co-infection, we demonstrated that vaccination of healthy macaques with KEX1 prior to SHIV protects against PCP, despite SHIV-associated immunosuppression [90]. These studies in pre-clinical primate models of HIV-associated PCP support the exploration of KEX1 as a

vaccine for the prevention of PCP in the HIV-infected and non-HIV-infected populations. In addition, combined vaccination with other protective *Pneumocystis* antigens such as SPD1 [88] could provide enhanced protection.

In summary, as a ubiquitous organism among human populations, *Pneumocystis* exposure in healthy and immunocompromised individuals elicits a spectrum of immunologic responses that can be protective or alternatively can contribute to immune-mediated lung pathology. Insights into the host responses against *Pneumocystis*-protective antigens, such as KEX1, provide a path toward the anti-*Pneumocystis* vaccine development, improved diagnostic, and the development of novel immune-therapeutics for the treatment of acute PCP.

Author Contributions

All authors made equal contributions to this work.

Competing Interests

The authors have declared that no competing interests exist.

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