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***Pneumocystis*: A Novel Pathogen in Chronic Obstructive Pulmonary Disease?**

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Abstract

Chronic obstructive pulmonary disease (COPD) results in significant morbidity and mortality. Smoking has long been recognized as the primary risk factor for development of COPD, but factors determining the severity or pattern of disease in smokers are largely unknown. Recent interest has focused on the potential role of infectious agents and the associated host response in accelerating progression of airway obstruction or in perpetuating its progression following discontinuation of tobacco exposure. *Pneumocystis jirovecii* is a fungal pathogen that causes pneumonia in immunocompromised individuals. Recent evidence has linked this organism with COPD. Using sensitive molecular techniques, low levels of *Pneumocystis* have been detected in the respiratory tract of certain individuals and termed colonization. Several findings support the theory that colonization with *Pneumocystis* is involved in the “vicious circle” hypothesis of COPD in which colonization with organisms perpetuates an inflammatory and lung remodeling response. *Pneumocystis* colonization is more prevalent in smokers and in those with severe COPD. The presence of *Pneumocystis* in the lungs, even at low levels, produces inflammatory changes similar to those seen in COPD, with increases in numbers of neutrophils and CD8⁺ lymphocytes. HIV-infected subjects who have had PCP develop permanent airway obstruction, and HIV-infected patients have a high prevalence of both emphysema and *Pneumocystis* colonization. In addition, a non-human primate model of colonization shows development of airway obstruction and radiographic emphysema. Additional studies are needed to confirm the role of *Pneumocystis* in the pathogenesis of COPD, given that this agent might be a treatable co-factor in disease progression.

Keywords

Pneumocystis; colonization; COPD; lung function

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death in the United States (1) and is expected to rank third in the world by 2020 (2). In the year 2000, 2.7 million people died of the disease worldwide (3). Twenty-four million adults in the United States have evidence of impaired lung function and experience 9.5 million office and emergency room visits, 726,000 hospitalizations, and 119,000 deaths each year (4).

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Despite efforts aimed at smoking cessation, little impact has been made on COPD incidence, and current treatments have a marginal impact in slowing progression of the disease. The enormous health burden and associated medical costs of COPD have prompted renewed interest in determining factors that alter disease progression. Although smoking is clearly the leading risk factor for COPD, the severity and patterns of disease expression vary widely (5). In addition to the influences of genetics and the environment, there has been increased interest in the role of infections in the pathogenesis and progression of COPD (3).

Recent evidence supports the hypothesis that infectious agents are critical in triggering and perpetuating the inflammatory response in COPD. Tissue inflammation in COPD is characterized by a predominant neutrophil, CD8+ lymphocyte, and macrophage infiltration (6–9). It has been proposed that the mechanism of tissue damage involves the recruitment and activation of neutrophils, macrophages, and CD8+ T cells with concomitant upregulation of several cellular proteases and inflammatory cytokines. While smoking can stimulate inflammation in the lungs, smokers with COPD have an increased inflammatory response compared to smokers without COPD, and numbers of inflammatory cells, particularly neutrophils and CD8+ lymphocytes, correlate directly with degree of airflow obstruction (6–9). In addition, such inflammation can persist despite smoking cessation and has been found to involve increases in cell types including B cells, CD4+ lymphocytes and eosinophils (10, 11). Furthermore, recent studies have documented the presence of inflammatory mucous in peripheral airways and lymphoid follicles associated with peripheral airways and in the parenchyma of patients with advanced COPD (12,13). These observations suggest that an ongoing adaptive immune response exists, even in former smokers with advanced disease, and that microbial pathogens are agents that perpetuate the chronic inflammatory response in COPD.

Many pathogens such as adenovirus and *Haemophilus influenzae* have historically been associated with development and progression of COPD (10,14–16). Over the past several years, increasing evidence has linked the pathogen *Pneumocystis jirovecii* (formerly *Pneumocystis carinii*) with COPD. This pathogen has not traditionally been associated with COPD, but several studies in humans and in a non-human primate model suggest that *Pneumocystis* is another pathogen that is responsible for differential progression of COPD in smokers. This finding is potentially important as *Pneumocystis* is treatable with inexpensive, common antibiotics. This review will briefly discuss the role of infections in general in COPD and will present the data supporting the role of *Pneumocystis* specifically.

ROLE OF INFECTIONS IN COPD

The vicious circle hypothesis

While overt bacterial infection has long been associated with symptomatic chronic bronchitis and acute bronchitis, it has recently been emphasized that inflammatory processes may occur in many patients with or without classic symptoms of sputum production. Cigarette smoke is commonly the initial inciting factor in the inflammatory cascade seen in COPD; however, smokers have varying degrees of inflammation, and ex-smokers can have persistence of inflammation and progression of airflow obstruction. These observations suggest that other agents, such as low level infections, likely act to amplify the smoking-induced inflammatory response. The hypothesis that micro-organisms perpetuate inflammation and lung destruction in COPD has been termed the vicious circle hypothesis (16). The theory was originally proposed with bacteria, but could apply to any pathogen. In the vicious circle, cigarette smoke results in impaired mucociliary clearance and defective local immunity and leads to low level infection or colonization with micro-organisms.

The organisms in turn have several effects in the lung. They can further impair the ability of the respiratory system to clear infection, thus resulting in their persistence. The organisms also stimulate chronic inflammation that accelerates pulmonary decline with or without the persistence of tobacco exposure. Accentuation of the complex cellular inflammatory response leads to the release of a variety of cytokines and proteases. This inflammatory response in turn stimulates a variety of tissue responses including fibroblast proliferation with peribronchiolar fibrosis, mucosal hyperplasia, matrix destruction, and cell apoptosis leading to alveolar septal loss.

The role of bacteria in COPD

Several microbiologic agents including bacteria, viruses, and fungi have been proposed to play a role in progression of COPD. Bacteria have been associated with COPD progression, and the most commonly implicated bacteria are *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa* (17). These pathogens can be found in patients with COPD in the stable state and have been detected in as many as 50% of exacerbations (17). Pathogens have typically been identified by routine cultures, but polymerase chain reaction (PCR) is now commonly used. As bacterial load increases, forced expiratory volume in one second (FEV₁) falls (18). Bacterial colonization has been associated with greater sputum purulence, increased sputum neutrophils, and increased levels of interleukin (IL)-8, tumor necrosis factor (TNF)- α , and neutrophil elastase (18–21). Colonization is also associated with a greater degree of airway obstruction, and colonized patients have more exacerbations (21,22).

The role of viruses in COPD

Viruses might also be important in the pathogenesis of COPD. Retamales and colleagues reported a higher rate of detection of adenovirus E1A protein in lung tissue of smokers with emphysema compared to age- and smoking-matched controls without evidence of airway obstruction (10). Based on elevated levels of CD8⁺ lymphocytes and interferon (IFN)- γ in lung samples, the authors speculated that the presence of latent adenovirus in the lungs provokes a heightened inflammatory response that leads to worsening of airway obstruction. Data in animals support this hypothesis, as guinea pigs with adenoviral infections have a more pronounced inflammatory response and accelerated emphysema development compared to non-infected controls (23).

Several other viruses seem to be important in COPD, particularly in exacerbations, half of which are associated with PCR-detected viruses (24). Exacerbations associated with viruses are more severe and last longer than those without a viral trigger (25,26). Because exacerbations are associated with more rapid progression of COPD over time, these viruses might be important in overall disease course as well. Rhinovirus is the most frequent virus identified during exacerbations and has been detected in one quarter to over half of subjects with COPD exacerbations (25). Experimental rhinovirus inoculation of subjects with COPD results in increased respiratory symptoms and airway obstruction (27). Respiratory syncytial virus (RSV) might also play a role in COPD as it has been detected in patients both during stable disease and exacerbations (25).

Using PCR, Influenza A and B have been found in 16% of patients' nasal aspirates during exacerbation (25). Human metapneumovirus is a recently described virus that was found by real time-PCR in nasopharyngeal specimens of 12% of patients hospitalized with COPD exacerbations (28). These viruses likely worsen symptoms and progression of COPD via upregulation of pulmonary inflammation. Influenza leads to increases in IL-6, IL-8, and RANTES in bronchial epithelial cells (29). RSV also results in increased cytokine expression in airway epithelial cells (30).

The role of *Pneumocystis* in COPD

In the past several years, new data have emerged implicating *Pneumocystis* as another pathogen with a potentially important role in COPD progression in certain patients. The evidence linking *Pneumocystis* and COPD includes an increase in *Pneumocystis* colonization in COPD that corresponds to disease severity and is independent of smoking history, the high rates of both emphysema and *Pneumocystis* colonization in HIV-infected smokers, COPD-like changes that occur after *Pneumocystis* pneumonia (PCP), the similarity between the inflammatory response in *Pneumocystis* colonization and COPD, and the development of airway obstruction and emphysema in a non-human primate model of *Pneumocystis* colonization (Table 1). We will first review the epidemiology of *Pneumocystis* colonization and then outline the specific data implicating *Pneumocystis* in COPD.

Pneumocystis colonization

Pneumocystis is a eukaryotic pathogen that causes pneumonia in immunocompromised hosts. Despite the fact that PCP is responsible for a large degree of morbidity and mortality in immunosuppressed populations, little is understood about the organism's epidemiology. The historic inability to culture *Pneumocystis* reliably has severely limited study of its life cycle and transmission. Originally classified as a protozoan, genetic analysis has found greater homology between *Pneumocystis* and certain fungi (31–33). The infectious form of the organism has not been identified, and routes of transmission are not well understood. *Pneumocystis* appears to be ubiquitous in the environment as nearly all children develop anti-*Pneumocystis* antibodies early in life (34–36). Traditionally, PCP has been thought to result from reactivation of latent infection acquired during childhood. This theory has never been definitively proven, and active or de novo infection from person-to-person exposure or from exposure to environmental sources of *Pneumocystis* may also be responsible for infections. In addition, studies in laboratory animals have shown direct transmission among animals (37–40).

The development of molecular techniques to identify and genotype *Pneumocystis* has provided an important tool with which to explore its epidemiology. PCR detects low numbers of organisms, particularly in cases where routine histochemical staining methods are negative (41,42). Use of PCR, particularly nested PCR, for detection of *Pneumocystis* has led to discovery of the organism among asymptomatic subjects. Presence of *Pneumocystis* in respiratory specimens from subjects without signs or symptoms of clinical infection and who do not progress to infection has been defined as colonization. In healthy subjects, *Pneumocystis* colonization is uncommon. Several studies have failed to detect any evidence of *Pneumocystis* colonization in autopsy studies of lungs from non-immunocompromised subjects without lung disease (42–45).

Pneumocystis colonization may occur in patients who are immunocompromised for reasons other than HIV infection. Nevez et al. examined bronchoscopic alveolar lavage (BAL) fluid from 82 patients whose immunosuppression was caused either by use of corticosteroids or by an underlying malignancy (46). Thirteen (16%) of these samples were positive for *Pneumocystis* by non-nested PCR. The prevalence of colonization in asymptomatic HIV-infected subjects appears to be higher than that seen in the general population with estimates ranging from 12 to 46% depending on the subject population and respiratory samples studied (42,44,47–50). Colonization appears to be more frequent in symptomatic HIV-infected persons. A recent study found that 69% of HIV-infected subjects who presented with respiratory symptoms, but were found not to have PCP by traditional staining methods, actually had BAL or induced sputum that contained *Pneumocystis* DNA by nested PCR (51).

***Pneumocystis* colonization is increased in COPD and correlates with disease severity**

The first link of *Pneumocystis* to COPD came from epidemiologic studies demonstrating that *Pneumocystis* colonization is higher in patients with COPD than healthy subjects or those with other lung disorders (Table 2). Calderon and colleagues initially reported that 10% of patients with chronic bronchial disease were colonized with *Pneumocystis* (52). They later studied 37 patients with chronic bronchitis and found that 41% of them were colonized (53). The increased colonization seen in COPD is not just a function of lung disease, but seems specific to COPD. For example, two studies have reported about 7% colonization in cystic fibrosis patients, whereas patients with COPD had a colonization frequency around 40% (54,55). A recent study by Helweg-Larsen and colleagues examined HIV-negative patients admitted to the hospital with suspected bacterial pneumonia (56). Of these subjects, 16 of 367 (4%) were colonized with *Pneumocystis*, and 63% of these patients had COPD compared to 20% of non-colonized patients. Morris and colleagues examined the prevalence of *Pneumocystis* colonization in patients undergoing lung transplantation for severe COPD or other end-stage lung diseases (57). They performed PCR on the native lung tissue removed at time of transplant and found that 37% of those with severe COPD were *Pneumocystis*-colonized compared to only 9% of those with other severe lung diseases. In multivariate analysis, a diagnosis of COPD was the only important predictor of colonization (odds ratio (OR) = 7.3, 95% confidence interval (CI) = 2.4–22.4, $p < 0.001$). Other clinical characteristics such as age, use of oral corticosteroids, and use of trimethoprim-sulfamethoxazole were not related to colonization risk. Not all studies have found the same association with colonization and COPD, however. A study by Maskell and colleagues found that colonization was associated with use of oral corticosteroids and not with COPD, but this study examined subjects with very mild airway obstruction and these subjects may have been less likely to be colonized (58).

One of the strongest pieces of evidence linking *Pneumocystis* colonization and progression of COPD is the finding that colonization correlates with degree of airway obstruction independent of smoking history. Morris and colleagues examined *Pneumocystis* colonization in lung samples of patients with varying degrees of airway obstruction as characterized by Global Health Initiative on Obstructive Lung Disease (GOLD) stage, but who had similar smoking histories (57). Colonized subjects had significantly lower FEV₁ and FEV₁/forced vital capacity (FVC) percent predicted (Figure 1) and colonization was more common as GOLD stage increased (Figure 2). Over a third (36.7%) of those with severe COPD (GOLD stage IV) were colonized compared to 5.3% of those with less severe COPD (stages 0 to III) (57). Although this study does not prove causality, it strongly suggests that *Pneumocystis* colonization affects COPD progression.

Increased emphysema and *Pneumocystis* colonization in HIV-infected smokers

Another line of evidence linking *Pneumocystis* colonization and COPD is the tendency for HIV-infected subjects to develop both emphysema and colonization. HIV-infected subjects are at increased risk for development of emphysema, particularly if they smoke (59,60). Diaz reported that almost 40% of HIV-infected smokers without a history of pulmonary infections had emphysema by pulmonary function and/or chest computed tomography (CT)-scanning (60). No HIV-negative controls matched by smoking history had emphysema by either pulmonary function measures or chest CT. CD8⁺ T lymphocyte levels in BAL were also significantly increased in those HIV-infected smokers with emphysema compared to HIV-positive and HIV-negative smokers without emphysema.

Another study found that a significantly higher number of HIV-infected subjects than HIV-negative controls had evidence of focal air-trapping on CT scan (59). Subjects with air-trapping also had worse obstructive changes in pulmonary function including lower FEV₁ and diffusing capacity for carbon monoxide (Dlco). Even HIV-infected subjects who do not smoke appear

to have an increased risk for developing emphysema. Recent data from a multicenter study of autopsy lungs in HIV infection has shown that 16% of patients with HIV infection who were non-smokers had pathological emphysema (Morris, unpublished data). Although there was no HIV-negative control group, this prevalence of emphysema in a non-smoking population is much higher than would be expected. It has been postulated that latent or subclinical infections might play a role in the development of HIV-associated emphysema, but there have been no studies to date that have explored this hypothesis.

It is possible that *Pneumocystis* is one such subclinical infection that might play a role in the accelerated emphysema seen in HIV infection. Morris and colleagues have investigated the epidemiology of *Pneumocystis* colonization in HIV-infected subjects (50). They examined lung specimens from subjects who died from causes other than PCP and found that 46% of HIV-infected subjects had *Pneumocystis* detectable in their lungs. Interestingly, clinical factors such as degree of immunosuppression based on CD4 cell count, history of previous PCP, or use of PCP prophylaxis did not influence colonization risk. One of the strongest independent risk factor for *Pneumocystis* colonization was having a history of cigarette smoking (OR = 4.5, 95% CI = 1.27–15.6, $p = 0.02$). Although lung function data were not available in this population, this observation raises the possibility that the accelerated emphysema seen in HIV-infected smokers may in fact be related to *Pneumocystis* colonization.

PCP produces permanent COPD-like changes

Pulmonary function changes have not been studied in the setting of *Pneumocystis* colonization, but acute PCP has been associated with the development of obstructive pulmonary changes. A case series of 10 patients with PCP reported a high incidence of small airways dysfunction as demonstrated by a reduced forced expiratory flow (FEF) 25–75% (61). Another study of 169 patients with HIV found that DLco, FEV₁, and peak flow were decreased in those with acute PCP (62). These obstructive changes appear to be permanent and not just a function of acute infection. The Pulmonary Complications of HIV Infection Study, a prospective cohort of over 1,100 HIV-infected subjects followed for a median of four years, performed serial pulmonary function studies in subjects at pre-defined intervals and after an episode of pneumonia. In a study of pulmonary function after PCP in this cohort, HIV-infected subjects with PCP had accelerated declines in FEV₁, FEV₁/FVC, and DLco beyond that expected from age and smoking history (63). These changes in pulmonary function were indistinguishable from those seen clinically with COPD and persisted for years after the acute infection resolved.

Similarity of the inflammatory response in COPD and *Pneumocystis* colonization

Airflow limitation in COPD is progressive and associated with an abnormal inflammatory response in the lungs. While the inflammatory response in COPD is complex and heterogeneous, it most often includes neutrophil, CD8+ lymphocyte and macrophage infiltration (7,9,64,65). It has long been proposed that the mechanism of tissue damage involves the recruitment and activation of these inflammatory cells with concomitant up-regulation of several cellular proteases and inflammatory cytokines. These cells seem to play an important role in development of disease as the numbers of CD8+ lymphocytes and neutrophils are correlated with disease severity (7–10,64,66). Macrophages produce chemokines that lead to an influx of neutrophils and CD8+ T-lymphocytes. The neutrophils are potent producers of proteases that may contribute to tissue destruction. The CD8+ T-lymphocytes in turn produce cytokines such as IFN- γ and IL-8 which perpetuate recruitment and activation of macrophage and neutrophils (67–69). In fact, the lymphocyte in the context of lymphoid follicles may be responsible for the primary events initiating the interaction with microbial antigens, thus precipitating the inflammatory cascade (70). TNF- α production by macrophages and CD8+ T-lymphocytes is also elevated and contributes to the inflammatory process by activating nuclear

factor (NF)- κ B NF- κ B subsequently upregulates a number of inflammatory molecules, including IL-8 and proteases.

One hypothesis of COPD pathogenesis is the protease/antiprotease theory (71). Several proteases, particularly neutrophil elastase and matrix metalloproteases likely play a role in the degradation of extracellular matrix proteins, particularly elastin and collagen, leading to the tissue damage observed. In addition, it has been proposed, based on the increased incidence of emphysema in patients deficient in alpha-one antitrypsin, that an imbalance between proteases and their inhibitors results in tissue injury. Increased levels of cytokines and chemokines, particularly the neutrophil chemoattractants, IL-8 and leukotriene (LT) B₄, as well as TNF- α and IFN- γ , have been reported to contribute to emphysematous changes both in humans and in animal models of COPD (72).

Acute PCP elicits a pronounced pulmonary inflammatory response characterized by increases in neutrophils and lymphocytes, primarily CD8+ T cells. There is an enhanced production of pro-inflammatory cytokines such as IFN- γ , TNF- α and IL-8 (73). The immune response in colonized humans has not been studied, but preliminary studies indicate that IFN- γ and TNF- α are increased in BAL samples of non-HIV-infected subjects colonized with *Pneumocystis* (Morris, unpublished data). In a model of *Pneumocystis*-colonized rhesus macaques, a cascade of cellular infiltrates and mediator release that is similar to that seen in acute PCP occurs. Board and coworkers examined the immune response to *Pneumocystis* colonization in a simian model of AIDS in which simian immunodeficiency (SIV)-infected monkeys were inoculated with macaque-derived *Pneumocystis* (74). Some animals developed PCP while others remained in a protracted state of asymptomatic colonization.

The early period after inoculation was marked by an influx of CD8+ T cells and neutrophils regardless of whether fulminant PCP or asymptomatic colonization resulted. Interestingly, CD8+ cell and neutrophil infiltration persisted throughout the course of infection in those animals with prolonged colonization and those who progressed to acute PCP. IFN- γ TNF- α and IL-8 also increased during colonization (75). The intensity and persistence of the inflammatory response seen in this model raises the possibility that *Pneumocystis*-induced lung damage results from colonization. Such inflammation may play either a causative or adjunctive role in the evolution of lung diseases in which *Pneumocystis* colonization is common.

In humans, the systemic inflammatory response to *Pneumocystis* colonization has been examined. A study of non-immunosuppressed individuals with chronic bronchial disease found that those patients colonized with *Pneumocystis* had a significantly higher peripheral lymphocyte count than those not colonized. In addition, the mean CD4+ count also appeared higher in the colonized individuals. While the exact clinical significance of this finding was unclear, it was postulated that the increase in the CD4+ cells and overall lymphocyte counts seen in those colonized by *Pneumocystis* might be related to exacerbations of the underlying chronic bronchitis.

Pneumocystis colonization may also contribute to the development of emphysema through release of endogenous proteases and stimulation of protease release in the host lung. Increased protease activity occurs in the lungs during acute PCP and may be responsible for some of the parenchymal destruction seen in the disease. Host-derived matrix metalloproteases (MMP)-2 and 9 are increased during acute PCP (77,78). *Pneumocystis*-derived proteases may also contribute to lung destruction. Rat *Pneumocystis* has protease, elastase, and chymase activity that can degrade the alveolar-capillary interface (77,79). A novel collagenase has also been identified in the lungs of rats with PCP, and it is thought that the enzyme is *Pneumocystis*-derived (77). *Pneumocystis* also has kexins, a family of serine proteases, on its cell surface

(80–82). Although the function of kexin is not completely understood, it may also contribute to lung damage from *Pneumocystis*.

***Pneumocystis* colonization and airway obstruction in a simian model of AIDS**

Few studies have addressed the clinical significance of prolonged *Pneumocystis* colonization and whether colonization results in pulmonary damage. The SIV/*Pneumocystis* primate model offers the opportunity to investigate the consequences of persistent colonization. As discussed above, *Pneumocystis*-induced inflammatory response in the lungs of SIV-infected macaques is evident very early after *Pneumocystis* inoculation, persists for weeks to months before any respiratory symptoms associated with acute PCP are observed and might result in airflow obstruction (74).

To assess airflow obstruction as a consequence of SIV infection or SIV infection with *Pneumocystis* colonization, pulmonary physiologic maneuvers using whole body plethysmography and a forced deflation technique were performed longitudinally in SIV-infected and SIV-infected/*Pneumocystis*-colonized animals (85). SIV-infected, *Pneumocystis*-colonized monkeys had significant airflow obstruction compared to normal or SIV-infected monkeys, supporting the hypothesis that colonization with *Pneumocystis* in the context of SIV-induced immunosuppression leads to pulmonary impairment.

This model was modified using simian immunodeficiency/human immunodeficiency chimeric virus-infected cynomolgus macaques and spontaneous development of *Pneumocystis* colonization produced by exposure to infected monkeys (85). To further test the link between *Pneumocystis* colonization and lung disease, longitudinal studies of pulmonary function in these animals were performed comparing changes in SHIV-infected alone to SHIV-infected/*Pneumocystis*-colonized animals (85). *Pneumocystis*-colonized animals developed airway obstruction as determined by decreases in most pulmonary function parameters while SHIV-infected animals did not change significantly (Figure 3). These changes demonstrated that *Pneumocystis* colonization leads to the development of airflow obstruction.

CONCLUSION

Despite the enormous public health burden of COPD, we still do not understand the factors that determine differential development and progression of disease in smokers. Various microbes may be involved in the pathogenesis of COPD by perpetuating the “vicious circle” of airway colonization, inflammation, and destruction. *Pneumocystis* is an agent recently proposed to play a role in COPD by inducing inflammatory changes that contribute to development and progression of disease. The presence of *Pneumocystis* in the lung may lead to chronic lung injury and worsening of pulmonary function. Additional studies are needed to better define the role of *Pneumocystis* in COPD and to determine the exact mechanism of its effects. Understanding the role of *Pneumocystis* colonization may help us identify a novel, treatable risk factor for progression of COPD.

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Figure 1.

Comparison of FEV₁ and FEV₁/FVC in *Pneumocystis*-colonized subjects and *Pneumocystis* negative. $p = 0.006$ for both comparisons. Abbreviations: FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 second, percent predicted.

Figure 2. Association of GOLD stage and prevalence of *Pneumocystis* colonization. Reproduced with permission from *American Journal of Respiratory and Critical Care Medicine*. Abbreviations: GOLD, Global Health Initiative on Obstructive Lung Disease.

Figure 3.

Change in forced expiratory volumes and forced expiratory flow values for non-colonized monkeys from baseline (uninfected) to follow-up (SHIV-infected, *Pneumocystis*-negative) and for *Pneumocystis*-colonized monkeys from baseline (uninfected) to follow-up (SHIV-infected, *Pneumocystis*-colonized). Average follow-up of 8 months. * $p = 0.02$ for comparison of change in FEV_{0.4} and FEF₂₅₋₇₅ in non-colonized versus *Pneumocystis*-colonized. Abbreviations: FEV_{0.4}, forced expiratory volume during first 0.4 seconds; FEF₂₅₋₇₅, forced expiratory flow over mid 50% of forced vital capacity; Pc, *Pneumocystis*.

Table 1
Evidence linking *Pneumocystis* colonization and COPD

Increased *Pneumocystis* colonization in COPD patients compared to other lung diseases
Accelerated emphysema in HIV-infected subjects
Increased *Pneumocystis* colonization in HIV-infected smokers
Development of permanent COPD-like change after PCP
Similarity of inflammatory response in *Pneumocystis* colonization and COPD
Development of airway obstruction and emphysema in primate model of *Pneumocystis* colonization

Abbreviations: COPD, chronic obstructive pulmonary disease, PHP, *Pneumocystis*, pneumonia.

Table 2Summary of studies reporting prevalence of *Pneumocystis* colonization in COPD

Study	n	Population	Technique/sample	% colonized
Calderon, 1996	50	Patients with chronic bronchial disease	IHC stains, IF stains/sputum	10.0%
Sing, 1999	8	Patients with COPD	Nested PCR/BAL	37.5%
Probst, 2000	37	Patients with COPD	Nested PCR/BAL and sputum	40.5%
Helweg-Larsen, 2002	23	In-patients with suspected bacterial pneumonia and COPD	Touch-down PCR/BAL, sputum, and tracheal aspirates	43.5%
Maskell, 2003	23	Patients with COPD undergoing bronchoscopy	Nested PCR/BAL	17.4%
Calderon, 2004	37	Patients with chronic bronchitis	Nested PCR/sputum	40.5%
Morris, 2004	68	Smokers undergoing lung surgery	Nested PCR/lung tissue	36.7% of GOLD IV
				5.3% of GOLD 0–III

Abbreviations: BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary disease; GOLD, Global Initiative on Obstructive Lung Disease; IF, immunofluorescence; IHC, immunohistochemical; PCR, polymerase chain reaction.