Published in final edited form as:
Immunol Res. 2011 August ; 50(2-3): 175–180. doi:10.1007/s12026-011-8218-x.

Pneumocystis infection and the pathogenesis of chronic obstructive pulmonary disease

Karen A. Norris and
Department of Immunology, University of Pittsburgh School of Medicine, E 1057 Biomedical Science Tower, Pittsburgh, PA 15261, USA

Alison Morris
Department of Immunology, University of Pittsburgh School of Medicine, E 1057 Biomedical Science Tower, Pittsburgh, PA 15261, USA. Department of Medicine, Division of Pulmonary, Allergy and Critical Care Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Karen A. Norris: kan1@pitt.edu

Abstract

With increases in the immunocompromised patient population and aging of the HIV+ population, the risk of serious fungal infections and their complications will continue to rise. In these populations, infection with the fungal opportunistic pathogen *Pneumocystis jirovecii* remains a leading cause of morbidity and mortality. Infection with *Pneumocystis* (Pc) has been shown to be associated with the development of chronic obstructive pulmonary disease (COPD) in human subjects with and without HIV infection and in non-human primate models of HIV infection. In human studies and in a primate model of HIV/Pc co-infection, we have shown that antibody response to the Pc protein, kexin (KEX1), correlates with protection from colonization, Pc pneumonia, and COPD. These findings support the hypothesis that immunity to KEX1 may be critical to controlling Pc colonization and preventing or slowing development of COPD.

Keywords

Pneumocystis; HIV; SIV; Pulmonary disease; COPD

Introduction

Pulmonary disease remains a leading cause of morbidity and mortality in HIV infection despite the availability of combination antiretroviral therapy (ART). Chronic obstructive pulmonary disease (COPD) (which encompasses airway obstruction and/or emphysema) is of particular importance in the current era of HIV infection because it is accelerated in those with HIV and is likely to increase as this population lives longer with chronic HIV. Recent studies suggest that ART correlates with decreased pulmonary function in HIV-infected individuals, although the nature of the association is unknown. Pathogenesis of HIV-related COPD is not completely understood, but it is hypothesized that accelerated disease could result from co-infections that may up-regulate pulmonary HIV replication or amplify pulmonary inflammatory responses leading to tissue damage. Our laboratories have focused

Correspondence to: Karen A. Norris, kan1@pitt.edu.

on the role of the fungal opportunistic pathogen, *Pneumocystis jirovecii*, and its role as a potential co-factor in the development of COPD.

COPD

Chronic obstructive pulmonary disease is a general term for a group of conditions, including emphysema, which are characterized by chronic or recurrent airflow obstruction. Emphysema is defined pathologically by abnormal, permanent enlargement of the air spaces distal to the terminal bronchioles with associated destruction of the alveolar walls [1, 2]. While smoking has long been recognized as the primary risk factor for the development of COPD, only 15–20% of smokers develop COPD. Factors that determine which smokers will develop significant disease are largely unknown, but disease progression is likely influenced by environmental and genetic factors [3].

The pathophysiology of COPD includes a persistent inflammatory response, and it has been proposed that the mechanism of tissue damage involves the recruitment and activation of neutrophils, macrophages and T cells with concomitant up-regulation of several cellular proteases and inflammatory cytokines [3–5]. Cytokines and chemokines, particularly the neutrophil chemoattractants, interleukin (IL)-8 and leukotriene B_4, as well as tumor necrosis factor (TNF)-α and interferon (IFN)-γ, have been reported to contribute to emphysematous changes both in humans and in animal models of COPD [3, 5]. In addition, several studies have suggested a primary role for macrophages in the development of COPD [3, 5–8].

The importance of inflammation in the pathogenesis of COPD is suggested by the observations that while smoking can induce an inflammatory response in the lungs, smokers with COPD have an increased inflammatory response compared to smokers without COPD, and inflammatory cellular infiltration correlates with airflow obstruction [9–11]. The infiltration of inflammatory cells and presence of bronchus-associated lymphoid tissue (BALT) are suggestive of an adaptive immune response, and there is evidence of persistence of the inflammatory response despite smoking cessation [3, 12]. Together, these findings suggest an ongoing adaptive immune response, but the stimuli for this response are not known. Several lines of evidence have suggested that persistent or repeated colonization with microbial pathogens may lead to the chronic inflammatory response associated with COPD, and microbial pathogens may be particularly important in HIV-associated COPD given the increased susceptibility to infection in HIV-infected individuals [12–18].

HIV and COPD

Several studies have demonstrated an increased risk of development of COPD in HIV-infected individuals [19–21]. Diaz reported, both by pulmonary function testing and by chest computed tomography (CT) scanning, that 37 percent of HIV-infected smokers without a history of pulmonary infections had emphysema [22]. No HIV-negative controls matched by smoking history had emphysema by either pulmonary function measures or chest CT. Gelman et al. [23] reported that significantly higher numbers of HIV-infected persons had evidence of focal air-trapping on CT scan compared to HIV negative subjects. The participants with air-trapping had worse pulmonary function including lower one-second forced expiratory volume (FEV_1) and diffusing capacity for carbon monoxide (Dlco). HIV-infected individuals, even those who do not smoke, have an increased risk of respiratory symptoms and airflow obstruction [19, 24, 25]. Crothers et al. [26] showed that HIV-infected veterans are more likely to have a diagnosis of COPD than non-HIV-infected controls and that HIV infection was an independent predictor of COPD. Recent studies from our group have confirmed an increased frequency of respiratory symptoms and pulmonary function abnormalities in HIV-infected persons, and in addition to smoking, these studies showed that ART was a risk factor for irreversible airway obstruction [27, 28]. While there

may be a direct relationship between ART and pulmonary damage, it is also possible that restoration of the immune response to subclinical infections following successful ART may promote a pathogenic inflammatory response leading to chronic obstructive disease [27, 28].

Pneumocystis and COPD

Our group and others have accumulated evidence in both humans and primate models of HIV infection that *Pneumocystis* is an important pathogen linked to the development of COPD [27–35]. Epidemiologic studies have shown a higher prevalence of Pc colonization in patients with COPD compared to other pulmonary diseases, using highly sensitive nested PCR for detection of Pc in respiratory samples [32, 36–39]. In these studies, colonization is generally defined as detection of Pc in respiratory samples from individuals without symptoms of clinical infection, and there is increasing evidence that colonization with Pc may be of clinical significance [40]. We have studied the frequency of Pc colonization in persons with varying degrees of COPD, but similar smoking histories [32]. Patients with more advanced COPD (as defined by the Global Health Initiative on Obstructive Lung Disease (GOLD) classification) [1] had a higher frequency of Pc colonization (OR = 2.4 for each increase in GOLD class, $P = 0.002$). Pc colonization prevalence was also evaluated in patients with other end-stage lung disease to determine if the high level of colonization was associated specifically with COPD, or was associated with end-stage lung disease in general. We found that of Pc-colonized subjects, 73% carried a diagnosis of COPD compared to only 32.2% of those not colonized (odds ratio [OR] = 5.8, 95% CI = 1.6–20.6, $P = 0.007$). This difference in colonization was not due to other clinical variables such as use of immunosuppressive therapy or severity of pulmonary disease [32]. These results are consistent with other epidemiologic studies of Pc colonization among persons with various pulmonary diseases [40]; however, a direct causal effect has not been demonstrated.

In HIV-infected individuals, we have shown that the prevalence of Pc colonization is high and occurs even in individuals with high CD4+ T cells counts on ART [41]. Recently, we demonstrated a link between Pc colonization and airway obstruction in HIV+ outpatients, and those who were colonized with Pc had significantly lower spirometric values compared to non-colonized subjects [31]. In addition, those who were Pc-colonized had a greater longitudinal decrease in pulmonary function [31]. These results are the first to demonstrate a link between Pc colonization and airway obstruction in HIV and to demonstrate greater decline in airway function prospectively.

Non-human primate models of HIV-associated COPD

Our laboratory has used non-human primate models to investigate Pc colonization, PCP, and HIV-related COPD [29, 34, 35, 42–46]. Simian immunodeficiency virus (SIV) and chimeric HIV-SIV viruses (SHIVs), generated by insertion of HIV genes into the SIV backbone, have been used extensively to study viral pathogenesis and in pre-clinical testing of antiretroviral drugs and vaccine candidates [47]. Although neither SIV nor SHIV infection of non-human primates completely mimics human infection with HIV, infection of susceptible macaques with these viruses are the most useful models to study disease progression, immune dysfunction, and opportunistic infections because of the similarities to human infection with HIV. Additionally, these models have been useful in studies of long-term consequences of HIV infection [47]. We have found that SIV- and SHIV-infected macaques are excellent models of pulmonary disease because of the natural susceptibility to Pc, the relatively rapid development of pulmonary function deficits similar to HIV-related COPD, and the similarity between HIV and SHIV-induced changes in T and B lymphocyte subpopulations [29, 34, 35, 42, 44, 46, 48–51].

We have used both SIV (deltaB 670) and $SHIV_{89.6P}$ infection of rhesus and cynomolgus macaques to investigate natural transmission and experimental infection with *Pneumocystis* [29, 42, 44, 46]. Although both infection models induce a persistent decline in peripheral blood CD4+ T cells, a key difference is the rate of CD4+ T cell decline, which occurs by 2–3 weeks post-inoculation with SHIV, compared to 6–12 months to achieve sufficient T cell depletion for natural Pc colonization in SIV-infected monkeys (~500 cells/μl) [45, 48].

An important feature of this SIV/SHIV model is the protracted course of Pc colonization, followed by acute PCP [35, 42, 44]. Of particular interest is the observation that Pc-induced inflammatory response in the lungs of SIV-and SHIV-infected macaques is evident very early after Pc colonization and persists for weeks to months before respiratory symptoms are observed [35, 42, 44]. In a longitudinal study of SHIV-infected macaques using serial pulmonary function testing, quantitative chest CT scanning, analyses of bronchoalveolar lavage (BAL) and blood, immunologic studies, and lung pathology, we found that SHIV-infected monkeys that became colonized with Pc developed progressive obstructive pulmonary disease characterized by anatomic emphysema and irreversible airflow obstruction, while animals infected with virus alone did not develop these changes [35]. These experiments showed that changes in pulmonary function were not a result of SHIV infection alone, but were instead associated with Pc colonization, lending further support to a growing body of evidence that suggests an association between Pc colonization and the development of COPD.

Because COPD and Pc infection are associated with vigorous inflammatory responses, we evaluated inflammation indicators in serial BAL samples following SHIV infection and Pc colonization [35]. Interestingly, while there were no significant changes in absolute number or percentage of T cells, macrophages, neutrophils, or CD4+/CD8+ T cell ratios in BAL of infected monkeys regardless of Pc status during the study course (up to 12 months post-SHIV infection), serial cytokine and chemokine analysis of BALF revealed significant changes from baseline in SHIV/Pc+, but not in SHIV/Pc-colonized monkeys. Increases in inflammatory mediators in BAL samples were only detected after Pc colonization was evident, with significant increases in IL-4, IL-5, IL-6, granulocyte macrophage colony-stimulating factor (GM-CSF), and lymphotoxin-α, and transient increases in IL-8, IL-13, interferon IFN-γ, macrophage inflammatory protein (MIP)-1α, and TNF-α were observed in SHIV following Pc colonization ($P < 0.05$ compared to baseline). Conversely, SHIV-infected macaques that remained Pc-negative did not exhibit increases in BAL cytokine levels, except for TNF-α at later time points [35]. These results demonstrate that SHIV infection alone had little effect on induction of inflammatory mediators in alveolar spaces, while Pc colonization induced a pro-inflammatory and Th2-skewed cytokine response, which was coincident with declining pulmonary function.

Contrary to reports evaluating inflammatory cellular infiltration associated with human and animal COPD studies, no significant changes in absolute numbers or percentages of T cells or neutrophils in BAL fluid of either monkey group was observed, even after significant pulmonary function decline was evident. We did, however, observe an increased frequency of BALT in SHIV- and Pc-co-infected macaques compared to those infected with SHIV alone, similar to the findings of increase BALT in COPD subjects [12]. The lack of significant cellular infiltration in the BAL in the Pc+ monkeys with COPD may be due to the fact that the primate model is capturing very early events in disease progression, whereas most human studies of COPD are in persons with advanced disease. We previously showed infiltration of CD8+ T cells and neutrophils in SIV/Pc-infected macaques [42]; however, this occurred in response to intrabronchial inoculation with a higher Pc dose than the natural colonization of SHIV-infected macaques [35]. It is likely that as infection progresses or Pc

burden increases, a CD8+ T cell- and neutrophil-dominant response may develop and amplify inflammation-mediated pulmonary damage, as observed in human COPD.

Susceptibility and resistance to Pc colonization and PCP

There is strong evidence that Pc colonization is associated with the development of COPD; however, less is known regarding the mechanisms of Pc transmission and the factors that contribute to host susceptibility to Pc carriage [40, 52, 53]. Pc cannot be reliably cultured in vitro; thus, epidemiologic studies and studies of the mechanisms of transmission are limited. Immunologic control of Pc infection and prevention of PCP are strongly correlated with CD4+ T cells, although B cells also play an important role [54–56]. High seroprevalence rates (80–100%) in children by 8 years of age support the concepts that Pc is ubiquitous and that exposure to Pc occurs early in life [52, 53]. Consistent with these studies, our laboratory and others have shown high Pc seroprevalence in adult non-human primates [44, 57], suggesting the persistence of serological memory or Pc-specific long-lived plasma cells in response to natural Pc exposure. Antibodies to a Pc kexin-like protease may be particularly important because immune responses to Pc kexin have been associated with control of Pc infection in immunosuppressed murine models [58, 59].

In experimental studies to evaluate the dynamics of the humoral response to Pc colonization and infection in macaques, our group has developed an enzyme-linked immunosorbent assay (ELISA) using a recombinant Pc-derived protein fragment, KEX1, which covers a 110 amino acid internal conserved region of the Pc kexin protein [42]. We found that the KEX1 ELISA was effective in tracking natural transmission of Pc among immunocompetent macaques co-housed with Pc-colonized primates and that serologic evidence of Pc colonization in these animals was consistent with the detection of Pc DNA in BAL fluid by nested PCR [42, 44]. In the SHIV-Pc co-infection model, anti-KEX1 plasma IgG antibody titers were also shown to be effective in evaluating changes in Pc colonization status and predicting susceptibility to Pc colonization following SHIV-induced immunosuppression [45]. In these studies, higher baseline plasma anti-KEX1 IgG titers and higher numbers of KEX1-specific antibody-secreting cells prior to SHIV-induced immunosuppression correlated with prevention or delay of Pc colonization after SHIV immunosuppression and Pc exposure [45]. In addition, higher baseline plasma anti-KEX IgG titers correlated with improved kinetics and increased magnitude of a KEX1-specific IgA levels in the lung upon Pc exposure [45]. Macaques with high baseline anti-KEX1 IgG titers maintained this response throughout SHIV infection, remained free from detectable Pc colonization for up to 1 year post-SHIV infection and maintained normal lung function [45]. In contrast, macaques with low or undetectable anti-KEX titers at baseline were more likely to become colonized with Pc following SHIV infection and develop obstructive pulmonary diseases, although they did develop anti-KEX1 antibody response following colonization [45].

To further assess the B cell responses in SHIV-infected macaques with and without Pc colonization, peripheral blood mononuclear cells (PBMC) were evaluated by memory B cell ELISPOT assay in order to assess the Pc KEX1-specific memory response [45]. PBMC were evaluated at approximately 1 year post-SHIV infection [45]. SHIV-infected monkeys that remained Pc-negative throughout the study had significantly higher percentages of KEX1-specific memory B cells than monkeys that became Pc-colonized [45]. There was no significant difference in plasma viral loads or CD4+ T cell levels between monkeys that became Pc-colonized and those that remained Pc-negative, suggesting that susceptibility to Pc infection was not a result of a more severe viral infection or defective T cell response [45]. Likewise, plasma antibody titers to SHIV antigens were comparable between Pc+ and Pc− groups, suggesting that the failure to prevent Pc colonization was not due to more severe suppression of humoral immunity [45].

These experimental studies, which suggest a role for KEX1 antibody in predicting susceptibility to Pc infection, are supported by a recent longitudinal serologic survey of HIV-infected individuals We evaluated plasma anti-KEX1 antibody levels in HIV-infected persons with PCP and those with other AIDS-defining illnesses, both before and after their AIDS-defining illness [60]. Antibody titers to both KEX1 and another Pc antigen (major surface glyco-protein) increased after acute PCP, but low baseline KEX1 levels were associated with subsequent development of PCP, even in persons not yet at risk for PCP by CD4+ T cell count criteria.

The relationship between anti-Pc KEX1 plasma antibody titers and the degree of COPD was also examined in a study of HIV negative smokers [30]. While over 60% (96/153) of the study participants had detectable anti-KEX1 titers, multivariate analyses revealed that a low or undetectable anti-KEX1 antibody titer was an independent predictor of more severe airway obstruction. No similar relationship was found with anti-influenza antibody titers, which suggests that low Pc antibody association is not merely a marker of a poor humoral immune response.

Together, these findings suggest that low plasma anti-KEX1 IgG levels may be a novel, early marker Pc colonization associated with the development of COPD or of future PCP risk. These findings lend additional support to the hypothesis that Pc is involved in the pathogenesis or progression of COPD and suggest that decreased antibody response to Pc antigens might be an important mechanism by which individuals become colonized. Nevertheless, several issues remain unresolved, including a clearer understanding of the nature of the protective immune response to *Pneumocystis*, the mechanism of B cell help in the context of diminished CD4+ T cell levels of HIV infection, the role anti-Pc antibodies in control of infection in HIV-infected individuals, and whether the immune response to KEX1 is a correlate of protection or is directly involved in the protective response.

Acknowledgments

This work was supported by NIH, National Heart Lung Blood Institute grants HL077095-05 and HL083432-05 (KAN); HL083461 and HL 090339 (AM).

References

1. Global Initiative for Chronic Obstructive Lung Disease. NHLBI/WHO workshop report. Bethesda: National Heart, Lung, and Blood Institute; April. 2001 Global strategy for the diagnosis, management and prevention of chronic obstructive pulmonary disease. Update of the management sections. GOLD website (www.goldcopd.com)
2. Snider GL. Chronic obstructive pulmonary disease: a definition and implications of structural determinants of airflow obstruction for epidemiology. Am Rev Respir Dis. 1989; 140(3 Pt 2):S3–8. [PubMed: 2675711]
3. Barnes PJ. Mediators of chronic obstructive pulmonary disease. Pharmacol Rev. 2004; 56(4):515–48. [PubMed: 15602009]
4. Shapiro SD. Proteinases in chronic obstructive pulmonary disease. Biochem Soc Trans. 2002; 30(2): 98–102. [PubMed: 12023833]
5. Curtis JL, Freeman CM, Hogg JC. The immunopathogenesis of chronic obstructive pulmonary disease: insights from recent research. Proc Am Thorac Soc. 2007; 4(7):512–21. [PubMed: 17878463]
6. Churg A, et al. Macrophage metalloelastase mediates acute cigarette smoke-induced inflammation via tumor necrosis factor-alpha release. Am J Respir Crit Care Med. 2003; 167(8):1083–9. [PubMed: 12522030]
7. Shapiro SD. The macrophage in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 1999; 160(5 Pt 2):S29–32. [PubMed: 10556166]

8. Taylor AE, et al. Defective macrophage phagocytosis of bacteria in COPD. Eur Respir J. 2010; 35(5):1039–47. [PubMed: 19897561]
9. Keatings VM, Barnes PJ. Granulocyte activation markers in induced sputum: comparison between chronic obstructive pulmonary disease, asthma, and normal subjects. Am J Respir Crit Care Med. 1997; 155(2):449–53. [PubMed: 9032177]
10. Lacoste JY, et al. Eosinophilic and neutrophilic inflammation in asthma, chronic bronchitis, and chronic obstructive pulmonary disease. J Allergy Clin Immunol. 1993; 92(4):537–48. [PubMed: 8409114]
11. O'Shaughnessy TC, et al. Inflammation in bronchial biopsies of subjects with chronic bronchitis: inverse relationship of CD8+ T lymphocytes with FEV1. Am J Respir Crit Care Med. 1997; 155(3):852–7. [PubMed: 9117016]
12. Hogg JC, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. N Engl J Med. 2004; 350(26):2645–53. [PubMed: 15215480]
13. Hogg JC. Role of latent viral infections in chronic obstructive pulmonary disease and asthma. Am J Respir Crit Care Med. 2001; 164(10 Pt 2):S71–5. [PubMed: 11734471]
14. Hogg JC. Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. Lancet. 2004; 364(9435):709–21. [PubMed: 15325838]
15. Morris A, Sciurba FC, Norris KA. *Pneumocystis*: a novel pathogen in chronic obstructive pulmonary disease? Copd. 2008; 5(1):43–51. [PubMed: 18259974]
16. Sethi S. Infectious etiology of acute exacerbations of chronic bronchitis. Chest. 2000; 117(5 Suppl 2):380S–5S. [PubMed: 10843981]
17. Sethi S. Bacterial infection and the pathogenesis of COPD. Chest. 2000; 117(5 Suppl 1):286S–91S. [PubMed: 10843957]
18. Sethi S, Murphy TF. Infection in the pathogenesis and course of chronic obstructive pulmonary disease. N Engl J Med. 2008; 359(22):2355–65. [PubMed: 19038881]
19. Diaz PT, Clanton TL, Pacht ER. Emphysema-like pulmonary disease associated with human immunodeficiency virus infection. Ann Intern Med. 1992; 116(2):124–8. [PubMed: 1727615]
20. Diaz O, et al. Role of inspiratory capacity on exercise tolerance in COPD patients with and without tidal expiratory flow limitation at rest. Eur Respir J. 2000; 16(2):269–75. [PubMed: 10968502]
21. Kuhlman JE, et al. Premature bullous pulmonary damage in AIDS: CT diagnosis. Radiology. 1989; 173(1):23–6. [PubMed: 2781013]
22. Diaz PT, et al. HIV infection increases susceptibility to smoking-induced emphysema. Chest. 2000; 117(5 Suppl 1):285S. [PubMed: 10843956]
23. Gelman M, et al. Focal air trapping in patients with HIV infection: CT evaluation and correlation with pulmonary function test results. AJR Am J Roentgenol. 1999; 172(4):1033–8. [PubMed: 10587143]
24. Diaz PT, et al. Respiratory symptoms among HIV-seropositive individuals. Chest. 2003; 123(6): 1977–82. [PubMed: 12796177]
25. O'Donnell CR, et al. Abnormal airway function in individuals with the acquired immunodeficiency syndrome. Chest. 1988; 94(5):945–8. [PubMed: 3263260]
26. Crothers K, et al. Increased COPD among HIV-positive compared to HIV-negative veterans. Chest. 2006; 130(5):1326–33. [PubMed: 17099007]
27. George MP, et al. Respiratory symptoms and airway obstruction in HIV-infected subjects in the HAART era. PLoS One. 2009; 4(7):e6328. [PubMed: 19621086]
28. Gingo MR, et al. Pulmonary function abnormalities in HIV-infected patients during the current antiretroviral therapy era. Am J Respir Crit Care Med. 2010
29. Fernandes EF, et al. Colonization with *Pneumocystis* in a simian model of AIDS results in chronic inflammation and airflow obstruction. Am J Resp Crit Care Med. 2005; 2:A867.
30. Morris A, et al. Relationship of *Pneumocystis* antibody response to severity of chronic obstructive pulmonary disease. Clin Infect Dis. 2008; 47(7):e64–8. [PubMed: 18724825]
31. Morris A, et al. Airway obstruction is increased in *Pneumocystis*-colonized human immunodeficiency virus-infected outpatients. J Clin Microbiol. 2009; 47(11):3773–6. [PubMed: 19759224]

32. Morris A, et al. Association of chronic obstructive pulmonary disease severity and *Pneumocystis* colonization. Am J Respir Crit Care Med. 2004; 170(4):408–13. [PubMed: 15117741]
33. Morris AM, et al. Permanent declines in pulmonary function following pneumonia in human immunodeficiency virus-infected persons. The pulmonary complications of HIV infection study group. Am J Respir Crit Care Med. 2000; 162(2 Pt 1):612–6. [PubMed: 10934095]
34. Norris KA, et al. *Pneumocystis* colonization, airway inflammation, and pulmonary function decline in acquired immunodeficiency syndrome. Immunol Res. 2006; 36(1–3):175–87. [PubMed: 17337778]
35. Shipley TW, et al. Persistent *Pneumocystis* colonization leads to the development of chronic obstructive pulmonary disease in a nonhuman primate model of AIDS. J Infect Dis. 2010; 202(2): 302–12. [PubMed: 20533880]
36. Sing A, et al. *Pneumocystis carinii* carriage in immunocompetent patients with primary pulmonary disorders as detected by single or nested PCR. J Clin Microbiol. 1999; 37(10):3409–10. [PubMed: 10488221]
37. Probst M, et al. Detection of *Pneumocystis carinii* DNA in patients with chronic lung diseases. Eur J Clin Microbiol Infect Dis. 2000; 19(8):644–5. [PubMed: 11014633]
38. Helweg-Larsen J, et al. Detection of *Pneumocystis* DNA in samples from patients suspected of bacterial pneumonia–a case-control study. BMC Infect Dis. 2002; 2(1):28. [PubMed: 12445330]
39. Calderon E, et al. *Pneumocystis jiroveci* isolates with dihydropteroate synthase mutations in patients with chronic bronchitis. Eur J Clin Microbiol Infect Dis. 2004; 23(7):545–9. [PubMed: 15175932]
40. Morris A, et al. Epidemiology and clinical significance of *Pneumocystis* colonization. J Infect Dis. 2008; 197(1):10–7. [PubMed: 18171279]
41. Morris A, et al. Prevalence and clinical predictors of *Pneumocystis* colonization among HIV-infected men. AIDS. 2004; 18(5):793–8. [PubMed: 15075515]
42. Board KF, et al. Experimental *Pneumocystis carinii* pneumonia in simian immunodeficiency virus-infected rhesus macaques. J Infect Dis. 2003; 187(4):576–88. [PubMed: 12599074]
43. Patil SP, et al. Immune responses to *Pneumocystis* colonization and infection in a simian model of AIDS. J Eukaryot Microbiol. 2003; 50 (Suppl):661–2. [PubMed: 14736208]
44. Kling HM, et al. *Pneumocystis* colonization in immunocompetent and simian immunodeficiency virus-infected cynomolgus macaques. J Infect Dis. 2009; 199(1):89–96. [PubMed: 19014344]
45. Kling HM, et al. Relationship of *Pneumocystis jiroveci* humoral immunity to prevention of colonization and chronic obstructive pulmonary disease in a primate model of HIV infection. Infect Immun. 2010; 78(10):4320–30. [PubMed: 20660609]
46. Croix DA, et al. Alterations in T lymphocyte profiles of bronchoalveolar lavage fluid from SIV- and *Pneumocystis carinii*-coinfected rhesus macaques. AIDS Res Hum Retroviruses. 2002; 18(5): 391–401. [PubMed: 11897041]
47. Joag SV. Primate models of AIDS. Microbes Infect. 2000; 2(2):223–9. [PubMed: 10742694]
48. George MP, et al. Pulmonary vascular lesions are common in SIV- and SHIV-env-infected macaques. AIDS Res Hum Retro-viruses. 2010
49. Guillot J, et al. Phylogenetic relationships among *Pneumocystis* from Asian macaques inferred from mitochondrial rRNA sequences. Mol Phylogenet Evol. 2004; 31(3):988–96. [PubMed: 15120396]
50. Kling H, Shipley T, Norris K. Abnormalities in peripheral blood B lymphocyte populations in SHIV89.6P-infected macaques. Comp Med. 2011 (In press).
51. Kuhrt D, et al. Evidence of early B-cell dysregulation in simian immunodeficiency virus infection: rapid depletion of naive and memory B-cell subsets with delayed reconstitution of the naive B-cell population. J Virol. 2010; 84(5):2466–76. [PubMed: 20032183]
52. Morris A. Is there anything new in *Pneumocystis jirovecii* pneumonia? Changes in P. *jirovecii* pneumonia over the course of the AIDS epidemic. Clin Infect Dis. 2008; 46(4):634–6. [PubMed: 18190280]
53. Morris A, et al. Current epidemiology of *Pneumocystis* pneumonia. Emerg Infect Dis. 2004; 10(10):1713–20. [PubMed: 15504255]

54. Empey KM, et al. Passive immunization of neonatal mice against *Pneumocystis carinii* f. sp. muris enhances control of infection without stimulating inflammation. Infect Immun. 2004; 72(11): 6211–20. [PubMed: 15501746]

55. Lund FE, et al. B cells are required for generation of protective effector and memory CD4 cells in response to *Pneumocystis* lung infection. J Immunol. 2006; 176(10):6147–54. [PubMed: 16670323]

56. Lund FE, et al. Clearance of *Pneumocystis carinii* in mice is dependent on B cells but not on P *carinii*-specific antibody. J Immunol. 2003; 171(3):1423–30. [PubMed: 12874234]

57. Demanche C, et al. Molecular and serological evidence of *Pneumocystis* circulation in a social organization of healthy macaques (Macaca fascicularis). Microbiology. 2005; 151(Pt 9):3117–25. [PubMed: 16151222]

58. Zheng M, et al. CD4+ T cell-independent DNA vaccination against opportunistic infections. J Clin Invest. 2005; 115(12):3536–44. [PubMed: 16308571]

59. Wells J, et al. Active immunization against *Pneumocystis carinii* with a recombinant P. *carinii* antigen. Infect Immun. 2006; 74(4):2446–8. [PubMed: 16552076]

60. Gingo MR, et al. Serologic responses to *Pneumocystis* proteins in human immunodeficiency virus patients with and without *Pneumocystis jirovecii* pneumonia. J Acquir Immune Defic Syndr. 2011s

Biography

Karen A. Norris