Epidemiology and Clinical Significance of *Pneumocystis* Colonization

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***Pneumocystis* pneumonia has long been recognized as a cause of morbidity and mortality in immunocompromised populations, particularly those with HIV infection. *Pneumocystis* colonization—that is, detection of the organism or its DNA, without signs or symptoms of pneumonia—has recently been described, and accumulating evidence suggests that it may be an important clinical phenomenon. Sensitive molecular techniques such as polymerase chain reaction are frequently used to identify *Pneumocystis* colonization. Low levels of *Pneumocystis* in the lungs may stimulate pulmonary inflammation and may play a role in the development of lung diseases such as chronic obstructive pulmonary disease. In this review, we discuss evidence for the occurrence of *Pneumocystis* colonization in animals as well as the epidemiology and risk factors for *Pneumocystis* colonization in various human populations. We also evaluate the clinical significance of *Pneumocystis* colonization and its relationship to lung disease.**

Pneumocystis jirovecii, previously known as *Pneumocystis carinii* f. sp. *hominis,* has long been recognized as a cause of opportunistic infections in the lower respiratory tract. Individuals with weakened immune systems, especially those with AIDS, are most commonly affected. *Pneumocystis* may also be present in the respiratory tract when clinical pneumonia is absent. The detection of *Pneumocystis* in individuals without signs and symptoms of *Pneumocystis* pneumonia (PCP) has been defined as colonization. Other terms that have been used include "carriage" and "subclinical infection." Although *Pneumocystis* can be visualized in respiratory specimens by microscopic examination using traditional staining methods, its detection in individuals without pneumonia often requires use of polymerase chain reaction (PCR). The clinical significance of *Pneumocystis* colonization is not yet fully understood, but it may be important for several reasons. Individuals colonized with *Pneumocystis* may be at risk of development of PCP or may transmit *Pneumocystis* to others. In individuals receiving long-term anti-*Pneumocystis* prophylaxis, colonization may lead to the selection of mutations that have been associated with drug resistance. Furthermore, the presence of *Pneumocystis* in the lungs, even at low levels, may stimulate a host inflammatory response that leads to lung damage and may play a role in the progression of lung diseases such as chronic obstructive pulmonary disease (COPD).

In the present review, we will describe techniques to detect *Pneumocystis* colonization and will discuss its effects in animals. The epidemiology of *Pneumocystis* colonization in humans will be summarized, along with the risk factors associated with it. Finally, its clinical significance will be discussed.

DETECTION METHODS

Immunohistochemical stains. Because *Pneumocystis* cannot be grown in culture, its detection relies on identification of its microscopic morphology. Staining methods such as methenamine silver, Wright, or Giemsa and/or the use of immunofluorescence-targeted antibodies are traditional methods for diagnosis of PCP in infected hosts when the organism burden is relatively high. Although some investigators have detected *Pneumocystis* colonization by using traditional staining methods [1], these methods are generally not adequate for detection of *Pneumocystis* colonization, and researchers have turned to more sensitive molecular techniques.

PCR. The development of PCR has been instrumental in advancing the study of *Pneumocystis* colonization. In 1990, detection of *P. jirovecii* by PCR was first described by Wakefield et al. [2]. The DNA sequence of the *Pneumocystis* large subunit of mito-

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Table 1. Summary of studies of *Pneumocystis* colonization in children.

Study	Subjects, no.	Diagnostic sample	Diagnostic method	Population	Colonized with *Pneumocystis*, %
Vargas et al. 1999 [11]	695	Autopsy lung	IHC stain	IC infants dying of SIDS and other causes	9.4
Vargas et al. 2001 [9]	74	NPA	Nested PCR	IC with respiratory infection	32.0
Morgan et al. 2001 [1]	79	Autopsy lung	IHC stain	Infants dying of SIDS	13.9
Nevez et al. 2001 [12]	178	NPA	Nested PCR	IC with bronchiolitis	24.3
Kasolo et al. 2002 [13]	75	Autopsy lung	PCR	Children with and without HIV and dying of non-PCP respiratory illness	17.3
Totet et al. 2003 [14]	240	NPA	Nested and real-time PCR	IC with bronchiolitis	24.6
Beard et al. 2005 [15]	58	Autopsy lung (4 samples)	Nested PCR	IC infants dying of various causes	100.0
Vargas et al. 2005 [16]	112	Autopsy lung (1 sample), tracheal aspirate	Nested PCR	IC infants dying in community/hospital	44.6
Vargas et al. 2007 [17]	130	Autopsy lung	GMS stain	IC infants dying in the community	32.3
Larsen et al. 2007 [18]	422	NPA	Real-time PCR	IC infants hospitalized with acute respiratory infection	15.9

NOTE. GMS, Gomori methenamine silver; IC, immunocompetent; IHC, immunohistochemical; NPA, nasopharyngeal aspirate; PCR, polymerase chain reaction; SIDS, sudden infant death syndrome.

chondrial (mtLSU) rRNA was cloned and used as a primer for amplification. The advantage of the mtLSU locus as a target for PCR is that it is a multicopy gene and thus enhances sensitivity. Other modifications to PCR-based detection improve sensitivity further. For example, nested PCR, in which a second round of PCR using primers internal to the originals is performed, has been implemented to increase detection of *Pneumocystis* colonization. PCR results should be interpreted with caution, because false-positive and false-negative results can occur. Scrupulous laboratory technique must be observed to prevent contamination of samples, and proper controls must be performed to determine evidence of PCR inhibitors.

EPIDEMIOLOGY OF *PNEUMOCYSTIS* COLONIZATION IN ANIMALS

Pneumocystis colonization is well documented in many species of mammals, including shrews, rats, voles, squirrels, and brown hares. In commercial rat colonies, nested PCR used to analyze oral swab samples has shown that 98% of adult rats become colonized with *Pneumocystis* shortly after they are received from the vendor [3, 4]. In newborn rats, Icenhour et al. demonstrated that 80% of neonatal rats carried *Pneumocystis* in the oral cavity within 2 h after birth [3]. The prevalence reached 97% after 24 h. Within 48 h, the entire population were carriers.

Pneumocystis colonization has been reported in wild and laboratory nonhuman primates. In a group of free-living healthy macaques, *Pneumocystis* colonization was assessed by nested PCR analysis of deep-nasal swab samples [5]. The prevalence of *Pneumocystis* colonization ranged from 11% to 100%, depending on the month of testing, and its duration ranged from <1 month to 6 months. Laboratory primates with simian immunodeficiency virus (SIV) can also develop *Pneumocystis* colonization, both naturally and after intrabronchial inoculation [6, 7]. This colonization state may persist for >40 weeks without development of acute PCP.

EPIDEMIOLOGY OF *PNEUMOCYSTIS* COLONIZATION IN HUMANS

Children. Primary exposure to *P. jirovecii* is common in young children, as is demonstrated by the increase in anti-*Pneumocystis* antibody titers during the first few years of life [8, 9]. For example, in a study of 233 Spanish children, the overall seroprevalence was 73% [10]. Furthermore, there was evidence of an age-related increase in seroprevalence, from 52% at age 6 years to 66% at 10 years and to 80% at age 13 years [10]. Among healthy, immunocompetent infants in Chile, the seroconversion rate reached 85% by 20 months of age [9].

Pneumocystis colonization may be detected directly in infants and young children and may be associated with conditions such as bronchiolitis and sudden infant death syndrome (SIDS) (table 1). Vargas et al. obtained nasopharyngeal aspirates (NPAs) during mild respiratory infections in immunocompetent infants [9]. Of 74 infants studied, 24 (32%) were found to carry *Pneumocystis* DNA. Although *Pneumocystis* colonization was detected in the setting of mild respiratory infection, the fact that 14 (21%) of 67 children seroconverted without antecedent respiratory symptoms suggests that primary infection can also be asymptomatic in children. Interestingly, 3 (13%) of the 24 infants who tested positive for

Table 2. Summary of studies of *Pneumocystis* colonization in immunocompetent adults.

Study	Subjects, no.	Diagnostic sample	Diagnostic method	Population	Colonized with *Pneumocystis*, %
Wakefield et al. 1990 [2]	10	BAL	PCR	IC	0
Peters et al. 1992 [19]	15	Autopsy lung	PCR	Death from trauma or cardiovascular disease	0
Leigh et al. 1993 [20]	20	IS	PCR	IC homosexual/heterosexual males	0
Nevez et al. 1997 [21]	169	BAL	Heminested PCR	HIV negative	19.5
Vargas et al. 2003 [22]	28	Nasal swab	Nested PCR	Nonpregnant women	0
Medrano et al. 2005 [23]	50	OW	Nested PCR	IC	20
Nevez et al. 2006 [24]	30	Sputum	Nested PCR	IC	0

NOTE. BAL, bronchoalveolar lavage; IC, immunocompetent; IS, induced sputum; OW, oropharyngeal wash; PCR, polymerase chain reaction.

Pneumocystis had apnea episodes, compared with none of 50 infants in whom *Pneumocystis* DNA was not detectable. Nevez et al. reported a 24% prevalence of *Pneumocystis* colonization when nested PCR was used to analyze NPAs from 178 infants with bronchiolitis [12]. Similar levels of *Pneumocystis* colonization (i.e., 14%–25%) have been noted by others who have investigated the immunocompetent pediatric population with acute respiratory syndromes or chronic lung diseases [13, 14, 18].

Autopsy studies have played an important role in our understanding of *Pneumocystis* colonization in infants and suggest a possible relationship between such colonization and SIDS. Vargas et al. examined autopsy lung tissue from 534 infants dying either at home or in the hospital [11]. Using immunohistochemical analysis, they found that 3% of all specimens were positive for *Pneumocystis.* Of the infants who died at home because of SIDS, 25% had detectable *Pneumocystis* DNA, compared with only 2.9% of infants who died in the hospital because of other causes ($P = .002$). In an additional analysis of 161 SIDS cases, 32% of infants from Chile and the United Kingdom had *Pneumocystis* as determined by microscopy of lung tissue. Morgan et al. stained lung tissue from infants dying of SIDS and found that 14% of them had *Pneumocystis* cysts as determined by microscopy [1]. This detection rate might have been somewhat higher if PCR had been used, because Vargas et al., using nested PCR to analyze autopsy lung specimens, recently found that 52% of infants dying in the community were colonized with *Pneumocystis,* compared with 20% of infants dying in the hospital ($P = .006$) [16]. Beard et al. reported an even higher prevalence: 100% of 58 infants who died from either SIDS or other causes tested positive for *Pneumocystis* colonization, based on nested PCR analysis of autopsy lung specimens [15]. The high level of detection in that study might be the result of differences in PCR techniques, geographic variation in *Pneumocystis* colonization, or differences in the subjects studied. A recent study suggests that the association between *Pneumocystis* colonization and SIDS is not one of direct causality, because the proportion of infants with *Pneumocystis* as determined by microscopy was similar whether they died of SIDS or other causes [17].

Healthy, immunocompetent adults. The prevalence of *Pneumocystis* colonization among healthy adults (those without immunosuppressive conditions or lung disease) in published studies has differed (table 2). Using nonnested PCR, Peters et al. examined autopsy lung specimens from 15 nonimmunosuppressed subjects but did not identify *Pneumocystis* DNA in any of them [19]. Also, Wakefield et al. were unable to detect *Pneumocystis* in bronchoalveolar lavage (BAL) fluid from 10 healthy subjects [2]. Several other studies, which used nested PCR to analyze oral wash samples, induced sputum samples, or nasal-swab samples from healthy volunteers, also failed to find evidence of *Pneumocystis* colonization [20, 22, 24]. There are some studies that have detected *Pneumocystis* colonization in healthy subjects, however. The largest study of nonimmunosuppressed subjects used nested PCR to analyze BAL specimens and found that 33 (20%) of 169 subjects were colonized with *Pneumocystis* [21]. In a more recent study of healthy subjects, the prevalence of *Pneumocystis* colonization as determined by nested PCR analysis of oropharyngeal wash samples was 20% [23]. That study examined hospital administrative workers, who, although they did not have known direct contact with patients with PCP, might have greater exposure than most healthy subjects in the community and, therefore, a higher level of *Pneumocystis* colonization. The differences in studies could be explained by varying occupational or geographic exposures in the populations, differences in techniques, or differing characteristics of the subjects.

HIV-infected adults. In contrast to what has been reported for the healthy population, *Pneumocystis* colonization clearly occurs in the HIV-infected population. The prevalence of *Pneumocystis* colonization varies depending on the specific population studied (table 3). In a study of HIV-infected patients hospitalized with pneumonia and demonstrated not to have PCP, nested PCR analysis of induced sputum samples or BAL samples showed that 69% of subjects were colonized with *Pneumocystis* [28]. A study of autopsy lung tissue from HIV-infected

Table 3. Summary of studies of *Pneumocystis* colonization in adults infected with HIV.

Study	Subjects, no.	Diagnostic sample	Diagnostic method	Population	Colonized with *Pneumocystis*, %
Leigh et al. 1993 [20]	70	IS/BAL	PCR	HIV infected	10.0 (CD4 >400), 20.0 (CD4 <400), 40.0 (CD4 <60, on PCP prophylaxis), 10.0 (non-PCP illness)
Nevez et al. 1997 [23]	34	BAL	Heminested PCR	HIV infected	14.3
Rabodonirina et al. 1997 [25]	80	BAL	Nested PCR	HIV infected with respiratory symptoms	31.3
Nevez et al. 1999 [26]	5	BAL	Heminested PCR	HIV-infected inpatients	20.0
Matos et al. 2001 [27]	52	IS, OW	Nested PCR	HIV infected with respiratory symptoms	28.8
Huang et al. 2003 [28]	32	IS, BAL	Nested PCR	HIV infected with respiratory symptoms	68.8
Wakefield et al. 2003 [29]	16	BAL	Nested PCR	HIV infected	43.8
Morris et al. 2004 [30]	91	Autopsy lung	Nested PCR	HIV-infected men dying of non-PCP causes	46.2

NOTE. BAL, bronchoalveolar lavage; IS, induced sputum; OW, oropharyngeal wash; PCP, *Pneumocystis* pneumonia; PCR, polymerase chain reaction.

men dying from causes other than PCP reported that 46% of these subjects were colonized with *Pneumocystis* [30]. *Pneumocystis* colonization prevalences of 20%–43% have been reported by studies using PCR analysis of BAL specimens from HIV-positive patients [2, 29]. Another study has found *Pneumocystis* colonization in approximately one-third of asymptomatic patients with HIV who were receiving anti-PCP prophylaxis and whose sputum, on the basis of cytological analysis, was found to be negative for *Pneumocystis* [25]. Factors that may contribute to the wide variations in the prevalence of *Pneumocystis* colonization in HIV-infected individuals include the different populations tested, the method used to collect the respiratory specimen, and the different sensitivities of the various diagnostic techniques.

Risk factors for *Pneumocystis* colonization have been examined in the HIV-infected population to determine the clinical characteristics that might predispose to colonization. Factors such as a history of PCP, use of PCP prophylaxis, and use of antiretroviral medications have not been found to be associated with the risk of *Pneumocystis* colonization [28, 30]. Published studies differ as to whether CD4 cell count is associated with *Pneumocystis* colonization. Leigh et al. reported that *Pneumocystis* colonization increased as the CD4 cell count decreased [20]: they found that the prevalence of *Pneumocystis* colonization was 10% in subjects with CD4 cell counts >400 cells/μL, 20% in those with CD4 cell counts <400 cells/μL but >60 cells/μL, and 40% in those with CD4 cell counts <60 cells/μL ($P = .03$). In contrast, both Huang et al. and Morris et al. reported that there was no association between CD4 cell count and the risk of *Pneumocystis* colonization [28, 30]. Other factors that have been reported to increase the risk of *Pneumocystis* colonization in HIV-infected individuals are a history of smoking and the place of residence: in a study of the Multicenter AIDS Cohort, smokers had a higher risk of *Pneumocystis* colonization (odds ratio [OR], 2.9; $P = .02$) than did nonsmokers, and residents of Los Angeles had a lower risk of *Pneumocystis* colonization (OR, 0.14; $P = .002$) than did subjects from other study sites [30].

Adults with non-HIV immunosuppression. *Pneumocystis* colonization also occurs in non–HIV-infected individuals with various medical conditions (table 4). In a group of non–HIV-infected immunosuppressed patients, 13 (16%) of 82 were colonized with *Pneumocystis* [26]. Diagnoses of these subjects included conditions such as multiple myeloma, sarcoidosis, chronic lymphoid leukemia, and diabetes mellitus. Interestingly, CD4 cell count played a significant role in the HIV-negative subjects

Table 4. Summary of studies of *Pneumocystis* colonization in immunosuppressed adults not infected with HIV.

Study	Subjects, no.	Diagnostic sample	Diagnostic method	Population	Colonized with *Pneumocystis*, %
Nevez et al. 1999 [26]	82	BAL	PCR	Various causes of immunosuppression	15.9
Maskell et al. 2003 [31]	18	BAL	Nested PCR	Receiving prednisolone (>20 mg/day)	44.0
Vargas et al. 2003 [22]	33	Nasal swabs	Nested PCR	Pregnant women	15.5
Helweg-Larsen et al. 2002 [32]	17	BAL, tracheal aspirate, sputum	Nested PCR	Patients with suspected pneumonia and receiving corticosteroids	58.8

NOTE. BAL, bronchoalveolar lavage; PCR, polymerase chain reaction.

Table 5. Summary of studies of *Pneumocystis* colonization in adults with pulmonary diseases.

Study	Subjects, no.	Diagnostic sample	Diagnostic method	Population	Colonized with *Pneumocystis*, %
Calderon et al. 1996 [33]	50	Sputum	IHC stains, IF stains	Chronic bronchitis	10.0
Sing et al. 1999 [34]	63	BAL	Nested PCR	IC with primary pulmonary disease	19.0
Varela et al. 1998 [35]	45	Sputum	IF stains	Cystic fibrosis	0.0
Probst et al. 2000 [36]	141	IS, bronchial aspirate, BAL	Nested PCR	CLD	21.3
Visconti et al. 2000 [37]	78	BAL	Nested PCR	IC with primary pulmonary disease	2.6
Sing et al. 2001 [38]	95	Sputum	Nested PCR	Cystic fibrosis	7.4
Helweg-Larsen et al. 2002 [32]	367	BAL, IS, tracheal aspirate	Nested PCR	Bacterial pneumonia	4.4
Matos et al. 2003 [39]	34	BAL	IHC stains, IF stains, nested PCR	IC with primary pulmonary disease	35.3
Calderon et al. 2004 [40]	37	Sputum	Nested PCR	Chronic bronchitis	40.5
Morris et al. 2004 [41]	68	Lung resection	Nested PCR	COPD and other lung diseases	19.1 (COPD)
de la Horra et al. 2004 [42]	20	Autopsy lung	Touchdown PCR	Death from lung cancer	100.0 (SCLC), 20.0 (non-SCLC)
Respaldiza et al. 2005 [43]	88	Sputum, OW	Nested PCR	Cystic fibrosis	21.6
Nevez et al. 2006 [24]	50	Sputum	Nested PCR	COPD	16.0
Vidal et al. 2006 [44]	80	BAL	Nested PCR	Interstitial lung disease	33.8
Calderon et al. 2007 [45]	51	Sputum	Nested PCR	COPD	54.9

NOTE. BAL, bronchoalveolar lavage; CLD, chronic lung disease; COPD, chronic obstructive pulmonary disease; IC, immunocompetent; IF, immunofluorescence; IHC, immunohistochemical; IS, induced sputum; OW, oropharyngeal wash; PCR, polymerase chain reaction; SCLC, small-cell lung cancer.

colonized with *Pneumocystis*. When cellular immunity was evaluated, ~30% were found to be colonized with *Pneumocystis* when either the CD4 cell count was <400 cells/μL or the $CD4^+$:$CD8^+$ T cell ratio was <1 [26]. Iatrogenically induced immunosuppression by corticosteroids has also been linked to a higher risk of *Pneumocystis* colonization. In a study of 93 subjects undergoing diagnostic bronchoscopy with BAL, Maskell et al. detected *Pneumocystis* DNA in 8 (44%) of the 18 who were being treated with prednisolone dosages of >20 mg/day, compared with 9 (12%) of the 75 who were not being treated with glucocorticoids (OR, 5.9; $P = .004$) [31]. This linkage also was noted by Helweg-Larsen et al. in their evaluation of 80 patients with bacterial pneumonia: 75% of *Pneumocystis*-positive subjects had received corticosteroids, compared with 13% of the noncarriers ($P = .001$) [32]. The altered immunity associated with pregnancy also may increase the risk of *Pneumocystis* colonization. In a prospective study of 33 asymptomatic women during their third trimester of pregnancy, 15% had detectable *Pneumocystis* DNA in their nares, compared with none of 28 nonpregnant women ($P = .04$) [22].

Adults with primary lung disease. *Pneumocystis* colonization has also been detected in subjects with various lung diseases (table 5). Probst et al. investigated 141 patients with respiratory disorders, such as COPD, cystic fibrosis, and lung cancer [36], and found that >21% were colonized with *Pneumocystis*. Other studies that have examined patients with acute and chronic pulmonary disorders have found *Pneumocystis*-colonization prevalences that varied from 2.6% to 35% [34, 37, 39]. More recently, researchers in Spain evaluated patients with suspected interstitial lung diseases and found that 34% were colonized with *Pneumocystis* [44]. In that study, cigarette smokers had a higher risk of *Pneumocystis* colonization than did nonsmokers (OR, 3.2; $P = .02$). Published studies differ on whether subjects with cystic fibrosis (CF) are colonized with *Pneumocystis*. One study reported that no patients with CF were colonized with *Pneumocystis*, but it used staining methods rather than PCR [35]. Other reports have demonstrated that the prevalence of *Pneumocystis* colonization in patients with CF ranges from 7.4% to 22% [38, 43].

A potentially important association between *Pneumocystis* colonization and either chronic bronchitis or COPD has been found. Calderon et al. reported that 10% of patients with chronic bronchial disease were colonized with *Pneumocystis* by staining [33]. They later studied 37 patients with chronic bronchitis and found that 41% of them were colonized with *Pneumocystis* using nested PCR [40]. This prevalence of *Pneumocystis* colonization has also been reported in patients with COPD. In a study of patients with various respiratory disorders, the highest prevalence of *Pneumocystis* colonization, 41%, was seen in subjects with COPD [36].

Among smokers with COPD, 37% of those with severe COPD (Global Health Initiative on Obstructive Lung Disease stage IV) were colonized with *Pneumocystis,* compared with 5.3% of those with less-severe COPD (stages 0–III) ($P = .004$) [41]; this percentage was much higher than the 9.1% in subjects with other end-stage lung diseases (i.e., those not due to COPD) ($P = .007$). Interestingly, the association between *Pneumocystis* colonization and the severity of COPD was independent of smoking history, suggesting that *Pneumocystis* colonization might result in progression of COPD. Another recent study found that 55% of subjects with COPD had *Pneumocystis* colonization as determined by nested PCR analysis of sputum samples [45]. One study did not report an association between *Pneumocystis* colonization and COPD; however, that study included only subjects with very mild disease [31].

Another interesting association with *Pneumocystis* colonization has been detected in lung cancer. de la Horra et al. found that all of 10 patients with small-cell lung cancer had *Pneumocystis* DNA in their lung tissue, compared with only 2 of 10 patients who had non–small cell lung cancer and no patients without underlying pulmonary pathology ($P < .0001$) [42]. Larger studies are necessary to verify this association and to determine its clinical relevance.

CLINICAL CONSEQUENCES OF *PNEUMOCYSTIS* COLONIZATION

Pneumocystis colonization likely represents more than an interesting epiphenomenon and likely has several potentially important clinical effects. First, *Pneumocystis* colonization might lead to acute PCP in susceptible hosts and/or result in transmission of the organism to others. *Pneumocystis* colonization might stimulate pulmonary inflammation and lead to lung damage. As suggested by the associations between *Pneumocystis* colonization and SIDS, COPD, and lung cancer, *Pneumocystis* colonization might be an important cofactor involved in the progression of certain lung diseases.

Acute PCP and transmission of PCP. It is unknown whether *Pneumocystis* colonization leads to development of PCP. There have been few long-term studies that have followed individuals colonized with *Pneumocystis* in order to determine the magnitude of the risk, if any, for development of PCP. If individuals colonized with *Pneumocystis* are at risk for development of PCP, they should likely be offered *Pneumocystis* prophylaxis to prevent disease; however, current data are insufficient to justify such a recommendation.

Even if individuals colonized with *Pneumocystis* do not themselves develop PCP, they might transmit the organism to others. Exposure to animals colonized with *Pneumocystis* leads to colonization of healthy animals and to the development of clinical disease in immunosuppressed animals. Several studies have demonstrated that immunocompetent mice can develop *Pneumocystis* colonization after being housed with mice with severe combined immunodeficiency (SCID) that have PCP [46]. If the immunocompetent mice that are colonized with *Pneumocystis* are then housed with SCID mice, the latter mice will develop PCP [47]. Also, healthy, immunocompetent mice colonized with *Pneumocystis* can transmit the organism to other immunocompetent mice that can subsequently transmit the organism to SCID mice.

There is some evidence that exposure to humans with PCP can result in *Pneumocystis* colonization, but whether these colonized hosts can transmit disease is unknown. Studies of health-care workers (HCWs) have demonstrated that exposure to patients infected with PCP might result in *Pneumocystis* colonization. In a prospective study examining *Pneumocystis* colonization in immunocompetent HCWs, Miller et al. detected *Pneumocystis* DNA in 24% (4/17) of HCWs who were in contact with HIV-infected patients with PCP, compared with 11% (1/9) of those who were not in contact with such patients [48]. No HCWs had symptoms of respiratory infection, and none developed PCP. However, contact with patients with PCP might not have been responsible for the HCWs' *Pneumocystis* colonization, because genotype analysis demonstrated that 5 of 8 *Pneumocystis* strains identified in the HCWs were not observed in samples from the patients. Another study, by Vargas et al., found that deep-nasal swab samples from 3 hospital contacts of a patient with PCP had *Pneumocystis* but that those from 30 hospital workers without contact with the patient did not [49]. These results suggest that close occupational contact with patients with PCP can result in *Pneumocystis* colonization and might represent a potential source of disease transmission.

Inflammatory response to* Pneumocystis *colonization. Acute PCP elicits a pronounced pulmonary inflammatory response characterized by increases in pulmonary neutrophils and lymphocytes, primarily $CD8^+$ T cells. There is an enhanced production of proinflammatory cytokines such as interferon (INF)–γ, tumor-necrosis factor (TNF)–α, and interleukin (IL)–8. The immune response in humans colonized with *Pneumocystis* has not been studied, but, in animals colonized with *Pneumocystis,* a cascade of cellular infiltration and mediator release that is similar to that seen in acute PCP occurs. Board et al. examined the immune response to *Pneumocystis* colonization in a simian model of AIDS in which SIV-infected monkeys were inoculated with macaque-derived *Pneumocystis* [7]. Some of the monkeys developed PCP, whereas others remained in a protracted state of asymptomatic *Pneumocystis* colonization. The early period after inoculation was marked by an influx of pulmonary $CD8^+$ T cells and neutrophils, regardless of whether fulminant PCP or asymptomatic *Pneumocystis* colonization resulted. Interestingly, $CD8^+$ T-cell and neutrophil infiltration persisted throughout the course of infection, even in those monkeys that did not develop acute PCP.

INF-γ, TNF-α, and IL-8 in BAL samples also increased during *Pneumocystis* colonization [50]. The intensity and persistence of the inflammatory response seen in this model raises the possibility that lung damage results from *Pneumocystis* colonization. Such inflammation may play either a causative or an 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 nonimmunosuppressed individuals with chronic bronchial disease found that those patients colonized with *Pneumocystis* had a significantly higher peripheral-lymphocyte count than did those not colonized with the organism [51]. In addition, the mean $CD4^+$ lymphocyte count also appeared to be higher in the individuals colonized with *Pneumocystis.* Although the exact clinical significance of this finding is unclear, it was postulated that the increase in the $CD4^+$ T-cell and overall lymphocyte counts that was seen in individuals colonized with *Pneumocystis* might be related to an exacerbation of the underlying chronic bronchitis. A recent study found that subjects colonized with *Pneumocystis* who had COPD had higher circulating levels of TNF-α, IL-6, and IL-8 than did subjects with COPD who were not colonized with this organism [45].

CONCLUSION

There is growing evidence that *Pneumocystis* colonization is an important part of the organism's life cycle and has significant clinical consequences. Many host populations, including children, HIV-infected and non–HIV-infected immunosuppressed adults, individuals with chronic lung diseases, and HCWs, may be susceptible to *Pneumocystis* colonization. Certain risk factors, such as low CD4 cell count, smoking, and geographic location, appear to influence the risk of *Pneumocystis* colonization. Certain diseases, such as SIDS and COPD, are associated with a high prevalence of *Pneumocystis* colonization, but the association between *Pneumocystis* colonization and development of these diseases is unclear. There appears to be potential for individuals colonized with *Pneumocystis* to serve as a reservoir for maintenance and transmission of the organism in the host population. *Pneumocystis* colonization elicits a proinflammatory response in the host, similar to that which is seen in active PCP, and *Pneumocystis* might potentiate ongoing lung injury. However, the true clinical significance of *Pneumocystis* colonization is unknown. Future studies are necessary to define the epidemiology of *Pneumocystis* colonization, to determine its role in development and transmission of PCP, to detail the nature of the inflammatory response to *Pneumocystis* colonization, and to confirm the organism's role in the development of lung disease.

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