Decreased Serum Antibody Responses to Recombinant *Pneumocystis* Antigens in HIV-Infected and Uninfected Current Smokers

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Serologic studies can provide important insights into the epidemiology and transmission of *Pneumocystis jirovecii*. Exposure to *P. jirovecii* can be assessed by serum antibody responses to recombinant antigens from the major surface glycoprotein (MsgC), although factors that influence the magnitude of the antibody response are incompletely understood. We determined the magnitudes of antibody responses to *P. jirovecii* in comparison to adenovirus and respiratory syncytial virus (RSV) in HIV-infected and uninfected patients and identified predictors associated with the magnitude of the response. We performed a cross-sectional analysis using serum samples and data from 153 HIV-positive and 92 HIV-negative subjects enrolled in a feasibility study of the Veterans Aging Cohort 5 Site Study (VACS 5). Antibodies were measured using an enzyme-linked immunosorbent assay (ELISA). Independent predictors of antibody responses were determined using multivariate Tobit regression models. The results showed that serum antibody responses to *P. jirovecii* MsgC fragments were significantly and independently decreased in current smokers. Antibodies to *P. jirovecii* also tended to be lower with chronic obstructive pulmonary disease (COPD), hazardous alcohol use, injection drug use, and HIV infection, although these results were not statistically significant. These results were specific to *P. jirovecii* and did not correlate with adenovirus. Antibody responses to RSV were in the inverse direction. Thus, current smoking was independently associated with decreased *P. jirovecii* antibody responses. Whether smoking exerts an immunosuppressive effect that affects the *P. jirovecii* antibody response, colonization, or subsequent risk for disease is unclear; prospective, longitudinal studies are needed to evaluate these findings further.

*Pneumocystis jirovecii* has long been recognized as a major cause of pneumonia in HIV-infected patients and other immunocompromised hosts. Emerging evidence suggests that chronic, low-level infection or “colonization” with *P. jirovecii* may play a role particularly in lung diseases such as chronic obstructive pulmonary disease (COPD). Prior or ongoing exposure to *P. jirovecii* without evidence of clinical *Pneumocystis* pneumonia (PCP) has been identified using sensitive molecular tools to detect *P. jirovecii* in respiratory tract samples in a number of patient groups, including smokers and those with chronic lung disease, HIV infection, or other forms of immunosuppression (3, 26, 29). The consequences of colonization are under investigation, but studies suggest that *P. jirovecii* colonization is associated with higher serum levels of proinflammatory markers (4) and may play a role in the pathogenesis of COPD (27, 30).

Another method to detect exposure to *P. jirovecii* is through measurement of serum antibody responses, which obviates the need for respiratory tract sampling and may be easier to obtain in larger-scale studies. Serologic studies of *P. jirovecii* can provide important insights into the epidemiology and transmission of disease. Serologic testing for *P. jirovecii* also offers future potential as a diagnostic tool for identification of active PCP or *P. jirovecii* colonization.

Although serologic studies of *P. jirovecii* infection have previously been hampered by the lack of suitable reagents, the use of recombinant *P. jirovecii* antigens such as the major surface glycoprotein (Msg) and Kexin, another *P. jirovecii* antigen, has shown promise in measuring *P. jirovecii* antibodies in humans (7, 8, 9, 10, 20, 28). Msg is encoded by a multigene family and is capable of antigenic variation. We developed three overlapping fragments (MsgA, MsgB, and MsgC1) of an Msg clone
and showed that MsgC1 was best at distinguishing HIV-infected patients who recovered from PCP from patients who never had PCP. We then developed three variants (MsgC3, MsgC8, and MsgC9) of MsgC1 and used them to better define the reactivity to this fragment. These clones exhibit 77% to 99% homology at the amino acid level (10) and are useful in studying antibody responses in different geographic locations. Although the factors associated with the magnitude of the antibody response remain incompletely understood, our studies thus far have demonstrated that prior PCP, age, geography, and failure to take PCP prophylaxis are associated with high serum P. jirovecii antibody levels in HIV-infected patients (6, 10, 39).

In the present study, we sought to compare serum antibody responses to MsgC in HIV-positive and HIV-negative patients and to characterize factors associated with the magnitude of the antibody response. In particular, we were interested in the relationship of smoking history and COPD to serum P. jirovecii antibody responses given prior studies suggesting an immunosuppressive effect of cigarette smoking (12, 22) and a greater likelihood of P. jirovecii colonization among smokers and in patients with more severe COPD (26, 27). We also asked whether factors associated with antibody responses to P. jirovecii were unique to P. jirovecii or whether the serum antibody response to other respiratory pathogens would be similar by comparing the results to those for two other common respiratory pathogens, namely, adenovirus and respiratory syncytial virus (RSV).

MATERIALS AND METHODS

Subjects. We performed a cross-sectional analysis using serum samples and paired clinical data from a feasibility study of 153 HIV-positive and 92 HIV-negative subjects enrolled in the prospective, observational Veterans Aging Cohort 5 Site Study (VACS 5). These patients represent a subset of the total of 1,031 HIV-positive and 740 HIV-negative veterans who were participating in VACS 5. Although in the parent cohort the HIV-positive and HIV-negative participants were block matched by age, race, gender, and site of care, this matching was not retained in the feasibility cohort, as this was a convenience sample of subjects who were interested in participating in blood sampling. Subjects were enrolled between 2001 and 2002 from the outpatient infectious disease and general medicine clinics at the Veterans Affairs (VA) Medical Centers in Atlanta, Bronx, Houston, Los Angeles, and Manhattan. Institutional review boards approved the study at all locations, and patients provided informed consent.

Data collection. At study enrollment, all subjects completed a self-administered questionnaire (available at the VACS website, www.vaco.org). From this questionnaire, we obtained data on smoking and substance use. As in other studies, smoking status was based on self-reporting (34). Current smokers were defined as those who reported current or any smoking within the last 4 weeks, and former smokers were defined as those who quit >4 weeks ago. Subjects were classified as injection drug users (IDU) if they reported that they had ever used injection drugs or if they reported their HIV risk factor as injection drug use. We classified as injection drug users (IDU) if they reported that they had ever used injection drugs or if they reported their HIV risk factor as injection drug use.

Because levels of antibodies to MsgC antigens were left censored (lower limit of detection of 1 U) and levels of antibodies to RSV and adenovirus were right censored (levels of >300 U), we conducted Tobit analyses of antibody data with lower and upper bounds as appropriate. This allowed us to retain all subjects, including those with results at the lower or upper limits of these assays, in our analyses. In Tobit multivariate linear regression models, we evaluated predictors that have been associated with P. jirovecii antibody responses in previous studies, such as geographic site, and variables that were significantly associated with antibody responses at a P value of <0.1 in unadjusted linear regression models. Predictors that were significant at a P value of <0.1 for at least one of the antibody responses were retained in all final adjusted models. Although current smoking, IDU, and hazardous alcohol use are behaviors that tend to covary with each other, these variables were not significantly correlated and were thus retained as separate variables in the models. To approximate a normal distribution, we used a natural logarithmic transformation of MsgC samples were obtained for research purposes and were drawn at a median of 18 days (interquartile range, 0 to 69 days) after the baseline enrollment date in VACS 5.

Pneumocystis IgG antibody responses. Quantitative P. jirovecii IgG antibody responses were measured using an enzyme-linked immunosorbent assay (ELISA) as previously described (6, 10, 39). Serum specimens to be analyzed and the standard reference serum were tested against four recombinant MsgC fragments (MsgC1, MsgC3, MsgC8, and MsgC9); phosphate-buffered saline (PBS) without one antigen was used as a negative control. The reactivity of each serum specimen to MsgC was corrected by subtraction of the reactivity of that serum to PBS (mean optical density [OD] with MsgC − mean OD with PBS), and the results were quantified using the method of Bishop and Kovacs (2). A standard serum with specificity for each construct in turn was developed by mixing a pool of four to six sera with high reactivity to that construct. A standard curve was generated for each construct on each day of the assay and was used to determine the units of reactivity to the MsgC constructs. Each standard serum pool was assigned a value of 100 units of reactivity to its target MsgC construct in 100 µl of a 1/100 dilution of serum. Test sera were assayed at 1/100 to 1/200 dilutions to fit the linear portions of the standard curves, and units of reactivity were calculated taking the dilution into account. Using these quantitative methods, results were not considered undetectable, but those with values below the limit of detection of the standard curve were assigned a value of 1 U. This methodology allowed us to retain all subjects in analyses and evaluate the serologic data in terms of the magnitude of the antibody response, which may be influenced by a number of factors, including burden of last exposure (such as recent recovery from active PCP), time from last exposure (such as recent versus distant past), and host immune responses.

Adenovirus and respiratory syncytial virus IgG ELISA. IgG antibodies against adenovirus and respiratory syncytial virus were quantitated using commercially available kits (IBL, Minneapolis, MN) according to the manufacturer's instructions. 100 µl of serum responses were run in triplicate in 96-well plates (Corning). 0.1% Bovine serum albumin (BSA) buffer was added to each microwell well, and the wells were incubated at room temperature for 60 min in the dark. The wells were washed three times with wash buffer (PBS-Tween 20), and 100 µl of conjugate was added. The wells were then incubated at room temperature for 30 to 60 min in the dark and washed three times, and 100 µl of substrate was added to each well. Color was developed for 20 min in the dark, and the reactions were stopped by the addition of 100 µl of stop solution (0.5 M M4). The optical density was read at 405 nm and IgG antibodies were quantitated using the provided positive and negative standards.

Statistical analysis. Complete antibody responses and survey, administrative, laboratory, and pharmacy data were available for all subjects in these analyses with the exception of three subjects with missing AUDIT data from the baseline survey. These subjects were assumed to have nonhazardous alcohol use and were retained in multivariate models. Statistical analysis was performed with Stata statistical software (v. 10.0). P values of ≤0.05 was considered significant.

Descriptive statistics were used to compare the demographic and clinical characteristics of patients. Median values of nonnormally distributed continuous data were compared using the Wilcoxon rank sum test, and categorical values were compared using the chi-square test. Antibody responses in former smokers, however, were significantly different from those in current smokers (P < 0.05 for P. jirovecii constructs). Therefore, we combined former smokers and those who had never smoked into one category of nonsmokers in linear regression models, given the limited number of those who had never smoked in this cohort.

Because levels of antibodies to MsgC antigens were left censored (lower limit of detection of 1 U) and levels of antibodies to RSV and adenovirus were right censored (at levels of >300 U), we conducted Tobit analyses of antibody data with lower and upper bounds as appropriate. This allowed us to retain all subjects, including those with results at the lower or upper limits of these assays, in our analyses. In Tobit multivariate linear regression models, we evaluated predictors that have been associated with P. jirovecii antibody responses in previous studies, such as geographic site, and variables that were significantly associated with antibody responses at a P value of <0.1 in unadjusted linear regression models. Predictors that were significant at a P value of <0.1 for at least one of the antibody responses were retained in all final adjusted models. Although current smoking, IDU, and hazardous alcohol use are behaviors that tend to covary with each other, these variables were not significantly correlated and were thus retained as separate variables in the models. To approximate a normal distribution, we used a natural logarithmic transformation of MsgC samples.
TABLE 1. Baseline characteristics of subjects according to HIV status

| Characteristic          | HIV+ veterans (n = 153)* | HIV- veterans (n = 92)* | P value |
|-------------------------|--------------------------|-------------------------|---------|
| Age, yr                 | 48 (42–54)               | 52 (46–57)              | <0.001  |
| Male sex, %             | 97                       | 100                     | 0.2     |
| Race/ethnicity, %       |                          |                         | 0.02    |
| Black                   | 57                       | 39                      |         |
| White and other         | 22                       | 34                      |         |
| Hispanic                | 22                       | 27                      |         |
| Smoking status, %       |                          |                         | 0.5     |
| Never smoked            | 19                       | 20                      |         |
| Former smoker           | 29                       | 36                      |         |
| Current smoker          | 52                       | 45                      |         |
| Pack yr of smoking      | 17 (5–30)                | 15 (6–38)               | 0.4     |
| Hazardous current alcohol use, % | 11                       | 15                      | 0.4     |
| IDU ever, %             | 41                       | 15                      | <0.001  |
| COPD, %                 | 7                        | 0                       | 0.008   |
| Bacterial pneumonia, %  | 7                        | 0                       | 0.001   |
| PCP, %                  | 2                        | 0                       | 0.2     |
| CD4 cells, no.          | 370 (240–540)            | 370 (240–540)           |         |
| CD4 <200 cells, %       | 16                       | 16                      |         |
| HIV RNA, copies/ml      | 758 (50–17,199)          | 758 (50–17,199)         |         |
| HIV RNA <400 copies, % | 46                       | 46                      |         |
| On cART, %              | 78                       | 78                      |         |
| Antibody level, U       |                          |                         |         |
| MsgC1                   | 1.4 (0.7–2.6)            | 1.9 (1.1–2.7)           | 0.06    |
| MsgC8                   | 3.1 (2.1–4.1)            | 3.5 (2.6–4.1)           | 0.09    |
| RSV                     | 183 (129–236)            | 192 (157–251)           | 0.06    |
| Adenovirus              | 243 (187–281)            | 198 (144–248)           | <0.001  |

* Continuous variables are given as median (interquartile range).

RESULTS

Characteristics of the cohort. The HIV-positive patients in this feasibility cohort from VACS 5 were significantly younger and more likely to be black than the HIV-negative patients (Table 1). Nearly all patients were male. There was no significant difference between smoking status and HIV-negative alcohol use between HIV-positive and HIV-negative patients. Significantly more HIV-positive patients were IDU. Of HIV-positive patients, 16% had a CD4+ cell count of below 200, and 78% were on combination ART (cART). Overall, 9% of HIV-positive patients had a diagnosis of COPD, compared to only 1% of HIV-negative patients (P = 0.01), and 7% of HIV-positive patients had prior bacterial pneumonia, compared to none of the HIV-negative patients (P = 0.008). Only 2% of the HIV-positive and none of the HIV-negative patients had PCP in the baseline study period, limiting our power to examine associations between P. jirovecii antibody responses and prior PCP in this cohort. When comparing antibody responses by HIV status, adenovirus responses were significantly higher among the HIV-positive patients than among HIV-negative patients (P < 0.001), while P. jirovecii antibody responses to the MsgC1 construct and RSV antibody responses tended to be lower in the HIV-positive patients but this difference was not statistically significant (P = 0.06 for both).

Correlation between P. jirovecii, RSV, and adenovirus antibody responses. P. jirovecii antibody responses to MsgC8 were highly correlated with MsgC3 and also with MsgC9 (Pearson’s rank correlation coefficient of ≥0.8 for both). Therefore, we present the results of analyses for MsgC1 and MsgC8. However, antibody responses to RSV and adenovirus had only very weak correlations with each other or with P. jirovecii antibody responses. There was no association between having a low (<25th percentile) or high (>75th percentile) antibody response to RSV or adenovirus with low or high P. jirovecii antibody titers (data not otherwise shown).

Predictors of antibody responses in unadjusted analyses. Current smoking and a greater number of pack years of exposure to cigarettes were strongly and consistently associated with decreased serum P. jirovecii antibody responses to the MsgC1 and MsgC8 constructs (Table 2). When stratified by smoking status, P. jirovecii antibody responses were significantly different in current smokers, former smokers, and those who had never smoked (P value for overall comparisons < 0.05), with significantly lower levels in current smokers than in nonsmokers. In addition, we found that serum antibody levels decreased for both P. jirovecii antigens according to greater pack years of exposure to cigarettes and were lowest among patients with 40 pack years or more of smoking. Similarly, as the average number of cigarettes smoked per day increased among current smokers, levels of antibody to MsgC8 decreased significantly, levels for MsgC1 also decreased, but this was not statistically significant (data not otherwise shown). In contrast to P. jirovecii responses, RSV antibody responses were higher for current smokers and for those patients with heavier smoking histories than for nonsmokers. Antibody responses to adenovirus displayed no associations with smoking status or pack years of smoking.

HIV infection was associated with significant differences in antibody responses in unadjusted analyses (Table 2). HIV-positive patients tended to have lower antibody responses to MsgC1 and had significantly lower antibody responses to MsgC8 and RSV in unadjusted Tobit regression models. In contrast, HIV-positive patients had significantly higher levels of antibody to adenovirus. There were no significant differences in P. jirovecii antibody responses according to baseline CD4 cell count and HIV viral load.

In terms of other predictors, hazardous alcohol use tended to be associated with decreased antibody responses to MsgC1 and MsgC8 and with increased antibody responses to RSV (Table 2). IDU was associated with significantly decreased P. jirovecii antibody responses to MsgC8 and tended to be associated with decreased responses to MsgC1. P. jirovecii antibody responses did not vary significantly by age, although RSV antibody responses were significantly lower and adenovirus responses were substantially higher in older subjects. MsgC8 and adenovirus responses also varied by race/ethnicity. There was no difference in antibody responses according to prior PCP or recent exposure to PCP preventive therapy.

Predictors of antibody responses in adjusted analyses. Current smoking remained significantly associated with decreased antibody responses to MsgC1 and MsgC8 in models adjusting for other potential factors, including HIV status (Table 3).
Hispanic race/ethnicity is black, and that for current smoking is nonsmoking (former smokers and those who had never smoked).

While current smoking was the only significant predictor of antibody responses to MsgC1, older age, white race, and a history of IDU were also significantly associated with decreased responses to MsgC8. Patients with hazardous alcohol use tended to have decreased responses to MsgC1 and MsgC8.

Whereas independent predictors of antibody responses to RSV and adenovirus differed from those identified with P. jirovecii. Current smoking was significantly associated with increased antibody responses to RSV. Hazardous alcohol use also tended to be associated with higher RSV antibody responses. In contrast,
antibody responses to adenovirus displayed no relationships with current smoking, hazardous alcohol use, or age. However, adenovirus antibody responses were significantly higher in HIV-infected patients and tended to be lower in whites when controlling for HIV status.

**DISCUSSION**

In this study, we sought to determine factors associated with the magnitude of serum antibody responses to *P. jirovecii* and to contrast these with factors associated with RSV and adenovirus responses. Our data reveal a previously unrecognized low serum antibody response to *P. jirovecii* MsgC fragments in current smokers. We also found a dose-response effect, with lower *P. jirovecii* antibody levels in heavier smokers. The association of decreased antibody responses was significant for both *P. jirovecii* MsgC1 and MsgC8 constructs, although responses to *P. jirovecii* MsgC fragments can vary by geographic region (10). Our results can inform future studies that incorporate *P. jirovecii* serology to assess transmission, exposure, or disease activity.

Our findings suggest that smoking may decrease host humoral immune responses to *P. jirovecii*. This may be a potential mechanism accounting in part for the increased risk for PCP among current smokers described in other studies (24, 25). Indeed, smoking has broad immunosuppressive effects on the host cellular and humoral immune responses (12, 22). In rodent models, chronic exposure to cigarette smoke or nicotine inhibits T cell responsiveness, which may account for the decreased antibody response to T cell-dependent antigens that has been demonstrated in these animals (38). Chronic nicotine exposure also correlates with increased influenza virus titers in the lung, along with decreased migration of leukocytes to an area of infection or inflammation (36). While smoking stimulates an inflammatory response with an increase in the number of alveolar macrophages and systemic leukocytosis, the antimicrobial properties of these cells are significantly impaired (37).

The discovery of low levels of antibodies to *P. jirovecii* in current smokers raises the possibility that low serum antibody levels are a risk factor for *P. jirovecii* colonization. This is consistent with prior studies demonstrating a greater likelihood of respiratory tract colonization with *P. jirovecii* in association with current smoking and COPD (26, 27, 30). Decreased serum antibody responses may reflect altered immune responses to *P. jirovecii* in the setting of smoking that allows colonization with *P. jirovecii* to occur. The relationship between serum antibody responses and respiratory tract colonization with *P. jirovecii* has not been directly compared in published reports.

How antibody responses to *P. jirovecii* change with smoking cessation is unclear. Although we used a relatively short window of smoking cessation to define former smokers, the physiologic effects of smoking cessation and the time course of changes following smoking cessation vary widely. Hematologic and immunologic responses have been demonstrated to occur within days to weeks of smoking cessation (1, 14, 23). Antibody responses to *P. jirovecii* in those who quit smoking may return to levels observed in those who have never smoked, as we found that *P. jirovecii* antibody levels in former smokers were more similar to those in persons who had never smoked than to those in current smokers. Repeated measures of serum antibody responses to *P. jirovecii* among patients before and after smoking cessation would be required to address this question further.

In terms of other factors associated with *P. jirovecii* antibody responses, we also found that antibody responses to *P. jirovecii* tended to be lower in patients with hazardous alcohol use, IDU, and HIV infection, although these results did not all reach statistical significance. While point estimates differed in magnitude and level of significance in multivariate models for MsgC1 and MsgC8, the direction of association was nonetheless consistent for most predictors. As with smoking, alcohol use has also been associated with immunosuppressive effects in the lung (13). It is unclear if the association of decreased MsgC8 responses with IDU reflects residual confounding related to smoking. Our findings suggest that hazardous alcohol use and IDU may be important cofactors to consider in assessing *P. jirovecii* antibody responses and colonization. In addition, prior studies have found that episodes of PCP are associated with greater *P. jirovecii* antibody responses (10). More severely impaired lung function in COPD patients has been associated with undetectable serum antibodies to Kexin, another *P. jirovecii* antigen (28). We did not find significant associations between prior PCP or diagnoses of COPD with serum *P. jirovecii* antibody responses in our cohort, potentially due to limited power given the relatively small number of patients with either of these conditions in our study. The association of *P. jirovecii* with COPD is nonetheless particularly intriguing and may have relevance for HIV-infected patients, who have an increased risk for COPD (5, 11).

In order to determine whether the antibody responses to *P. jirovecii* were due to a more general deficiency in the humoral response in patients or whether the results were specific for *P. jirovecii*, we also compared antibody responses to RSV and adenovirus. Both of these viruses are frequent causes of infection. In contrast to the case for *P. jirovecii*, our study shows that current smokers have high levels of serum antibodies to RSV. There was also a trend toward a dose effect, with higher levels of antibody to RSV found in heavier smokers. Although comparison is somewhat difficult, these RSV results are generally consistent with data from two other studies. In the first of two seroepidemiological studies, the frequency of serum antibodies to a panel of respiratory viruses was generally higher among smokers, but this did not reach statistical significance with RSV (32). However, in the second study, multiple linear regression analysis showed that antibodies to RSV were associated with decreased lung function (33).

The levels of serum antibody to adenovirus in this study displayed a pattern of reactivity different from the reactivity to *P. jirovecii* or to RSV. Antibody results for adenovirus were not associated with smoking, alcohol, or IDU but were significantly higher in patients with HIV infection and tended to be lower in white participants. Serum antibodies to adenovirus are frequent in the general populations throughout the world (31, 32). With over 50 adenovirus serotypes, cross-reactivity is common and can make interpretation of serologic data difficult. While our results may be explained by recent exposure to or infection with these organisms, we did not have data on recent viral infections to determine whether recent exposure to ade-
novirus was more common among HIV-infected patients or whether current smokers were more likely to have had recent RSV infection as potential explanations for our measured serum antibody responses to these two common viruses.

Nonetheless, including measurements of antibody responses to two common respiratory tract organisms enhances our findings. First, it increases the likelihood that the responses we observed are specific to *P. jirovecii*. Second, the lack of correlation between low antibody responses for the three organisms makes it unlikely that low responses to *P. jirovecii* within an individual are due to a generalized deficiency in humoral immune responses.

Our study has a number of limitations. This is a cross-sectional analysis, with a relatively small number of patients with COPD or prior PCP. These analyses do not take into account possible seasonal variation in antibody responses. Although we obtained information on smoking status, hazardous alcohol use, and IDU from patients’ self-reporting, we relied on ICD-9 diagnoses of COPD and PCP. We may therefore have underestimated the prevalence of these conditions, as administrative data tend to have a high specificity but moderate sensitivity (18, 19, 35, 40). We may also underestimate COPD in the absence of pulmonary function testing (21). In addition, we do not have data to correlate airway tract colonization with serum antibody results. Future studies should include analysis of repeated samples over time and should pair serum samples with respiratory tract specimens and pulmonary function testing.

In conclusion, we found a previously unrecognized decreased serum antibody response to *P. jirovecii* MsgC1 and MsgC8 fragments in current smokers. The antibody response to *P. jirovecii* also tended to be lower in patients with COPD, hazardous alcohol use, IDU, and HIV infection. These results were specific to *P. jirovecii*, as antibody responses to RSV tended to be in the inverse direction, and did not correlate with adenovirus. Our results can inform future serologic studies that use *P. jirovecii* antibody levels to assess transmission, exposure, or disease activity, as these data suggest that host factors such as current smoking influence magnitude of the immune response. The implications of a low serum antibody response to *P. jirovecii* are not fully understood, although low levels of serum antibody to *P. jirovecii* have been associated with more severe COPD (28). It is also not clear if low serum antibody responses reflect a greater risk for developing PCP, although smoking has been found to be a risk factor for the development of PCP. Prospective, longitudinal studies are needed to evaluate these findings further.

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