Systemic Inflammation in Patients with Chronic Obstructive Pulmonary Disease Who Are Colonized with *Pneumocystis jiroveci*

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In chronic obstructive pulmonary disease, high levels of airway and systemic inflammatory markers are associated with a faster decrease in lung function. Our study shows that patients colonized by *Pneumocystis jiroveci* have higher proinflammatory cytokine levels than do noncolonized patients. This suggests that *Pneumocystis* may play a role in disease progression.

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality worldwide. COPD is a slowly progressive condition characterized by airflow limitation that is not fully reversible [1]. The airflow limitation is associated with a chronic inflammatory response in both airways and lung parenchyma. In addition to the presence of chronic local inflammation in the respiratory organs, there is increasing evidence of the important role of systemic inflammation in patients with COPD [2].

Smoking is considered to be the major cause of COPD, but only a small portion (15%–20%) of all smokers develop the disease. Factors that determine which smokers will develop significant disease are largely unknown [1]. Interest has focused on the potential role of infectious agents as cofactors in accelerating the progression of airway obstruction by increasing inflammatory response [3].

Pneumocystis jiroveci (human-derived *Pneumocystis*) is an atypical opportunistic fungus with lung tropism and worldwide distribution that causes pneumonia in immunosuppressed individuals. Basic research on *Pneumocystis* infection has been hampered by the lack of a reliable in vitro culture system; nevertheless, through the use of molecular techniques and experimental models, progress has been made over the past decades in our understanding of the epidemiological and clinical features of the infection [4]. Thus, in recent studies performed in Europe, *P. jiroveci* carriage was found in 6%–40% of patients with chronic pulmonary diseases [5–8], and it was suggested that *Pneumocystis* could be involved in the progression of COPD by means of the capacity of *Pneumocystis* during very early stages of the infection to induce, in animal models, alveolar macrophage activation, proinflammatory interleukin elevation, and changes in pulmonary surfactant [8, 9]. Moreover, in a study involving a human model, an association between *Pneumocystis* colonization and severity of airflow obstruction in smokers was revealed [10]. However, it has not yet been established whether *P. jiroveci* infection could have a role in worsening the natural course of COPD. We addressed the question of whether *Pneumocystis* colonization could increase systemic inflammatory response in patients with COPD.

Materials and methods. Fifty-one nonselected patients with COPD without pneumonia and without risk factors for HIV infection who were consecutively treated in our Department of Internal Medicine (Virgen del Rocio University Hospital, Seville, Spain) were included. Every patient underwent a clinical and biological examination using a standardized protocol, and sputum and serum samples were collected for analysis. The study protocol was approved by our hospital's ethics committee. Informed consent was obtained from all patients included in the study.

Identification of *P. jiroveci* colonization was performed by analyzing sputum samples using a 2-step protocol for a nested-PCR assay that amplifies a portion of the gene encoding the mitochondrial large-subunit (mt LSU) ribosomal RNA (rRNA) [9]. Briefly, DNA from *P. jiroveci* was extracted using a commercial kit (Qiagen). During the first round of amplification, the external primers pAZ102-E and pAZ102-H were used. This yielded a 346–base pair fragment. The second round of amplification used the internal primers pAZ102-X and pAZ102-Y and yielded a 260–base pair product. Both rounds of PCR comprised 35 amplification cycles. Amplicons were analyzed by electrophoresis on a 1.5% agarose gel containing ethidium bromide, and the bands were visualized by UV. To prevent contamination, pipettes with filters were used in all manipu-

lations. DNA extraction and preparation of the reaction mixture were performed in 2 different rooms, using separate laminar-flow hoods. The PCR procedure and analysis of PCR products were performed in another room. Control samples were run simultaneously with sputum samples. Positive control samples were bronchoalveolar lavage specimens from patients with *Pneumocystis* pneumonia; negative control samples were autoclaved water in the PCR mixture in the absence of the DNA template controls.

Proinflammatory cytokine (IL-8, TNF-α, and IL-6) levels were measured in serum samples, using commercially available specific EIA kits (R&D System), according to the manufacturer's instructions. The limits of detection for IL-8, TNF-α, and IL-6 were <3 pg/mL, 0.06 pg/mL, and 0.70 pg/mL, respectively. The reproducibility of these assays was confirmed by repeated measurements on successive days in the same serum specimens.

Statistical analysis was performed using SPSS software, version 13.0 (SPSS). The Mann-Whitney U test was used for qualitative variables; Student's t test was applied for quantitative variables, and the variance test was used to confirm normal distribution. A P value <.05 was considered to be statistically significant.

Results. Of the 51 individuals with COPD included in the study, 28 (55%) were colonized by *P. jiroveci*. No other infections were detected by culture of sputum samples. No differences were detected with regard to age, sex, smoking habit, and functional respiratory parameters when comparing *P. jiroveci* carriers with noncarriers (table 1). However, patients with COPD who were colonized by *P. jiroveci* showed a higher level of proinflammatory cytokines than did noncolonized subjects (table 1).

Conclusions. The results of our study confirm the high prevalence of *P. jiroveci* colonization in patients with COPD and reveal, to our knowledge, for the first time, an association between *P. jiroveci* colonization and systemic inflammatory response in patients with this disease, suggesting a possible pathogenic link with COPD progression.

We now know that patients with COPD have increased levels of several circulating cytokines and acute-phase reactants, including TNF-α, IL-6, and IL-8 [11]. The presence of a systemic inflammatory response is linked to weight loss and muscle wasting. Moreover, some data suggest that subjects with increased systemic inflammatory markers experience an accelerated decrease in lung function and are at increased risk of hospitalizations for COPD in the future [11].

The origin of the systemic inflammation associated with COPD is unclear. Cigarette smoking is widely considered to be the starting point of the pathogenetic pathway in COPD. However, once COPD develops, cessation of smoking does not fully attenuate the inflammatory process associated with this condition. Several studies have revealed that smoking cessation resulted in a significant reduction of the age-related decrease in the forced expiratory volume in 1 s. Nevertheless, worsening airspace abnormality does not slow with cessation of smoking, and the presence of inflammatory processes remains in the airways and circulation of patients with COPD, despite smoking cessation [12]. This suggests that there are factors that increase or maintain the chronic inflammatory process once it has become established. In this sense, bacterial colonization of the lower respiratory tract or latent viral infection have been implicated as cofactors that increase or maintain inflammatory response, accelerating COPD progression [3, 13].

There is little evidence of a local inflammatory response to *Pneumocystis* in the immunocompetent host, and this subject has not been investigated in our study. However, some studies

Table 1. Demographic and clinical data for patients with chronic obstructive pulmonary disease (COPD) according to *Pneumocystis jiroveci* colonization.

Characteristics and laboratory data	COPD without *P. jiroveci* colonization ($n = 23$)	COPD with *P. jiroveci* colonization ($n = 28$)	P
Age, mean years $\pm$ SD	74 $\pm$ 8.6	70.1 $\pm$ 9.8	.14[a]
Male sex, %	91	75	.13[b]
Active smoker, %	39	33	.72[b]
Mean percentage from predicted FEV$_1$ $\pm$ SD	42.2 $\pm$ 15.3	47.7 $\pm$ 23.1	.52[a]
Circulating lymphocytes, mean cells/μL $\pm$ SD	1555.6 $\pm$ 901.1	1798.5 $\pm$ 731.5	.36[a]
Circulating leukocytes, mean cells/μL $\pm$ SD	11242.6 $\pm$ 4135.3	9954.5 $\pm$ 5344.4	.4[a]
IL-8 level, mean pg/mL $\pm$ SD	13.89 $\pm$ 13.87	21.26 $\pm$ 9.25	.028[a]
TNF-α level, mean pg/mL $\pm$ SD	3.57 $\pm$ 2.03	8.15 $\pm$ 10.6	.047[a]
IL-6 level, mean pg/mL $\pm$ SD	5.34 $\pm$ 5.45	16.95 $\pm$ 25.06	.038[a]

NOTE. FEV$_1$, forced expiratory volume in 1 s.
[a] Determined by Student's t test.
[b] Determined by Mann-Whitney U test.

involving immunocompetent animal models have revealed that *Pneumocystis* β-glucan acts as a potent inducer of alveolar macrophage activation, which initiates inflammatory response in the lungs, including secretion of TNF-α, IL-6, IL-8 macrophage inflammatory protein-2, eicosanoid metabolites, and reactive oxidant species [14]. Furthermore, another study using a culture system revealed that *Pneumocystis* major surface glycoprotein induces IL-8 and monocyte chemoattractant protein-1 release from a human alveolar epithelial cell line [15].

The pulmonary inflammatory response that occurs in response to *Pneumocystis* resembles that of COPD and could contribute to the pathogenesis of airway and parenchymal damage in subjects with this disease. Inflamed pulmonary parenchymal cells are a likely source of proinflammatory mediators that may reach the systemic circulation and/or contribute to the activation of the inflammatory cells during their transit through the pulmonary circulation, which could explain the ability of *Pneumocystis* colonization to induce a systemic inflammatory response.

Our results suggest that *P. jiroveci* is an infectious agent that may play a role in the pathophysiology of COPD. However, future studies are needed to further define the role of *Pneumocystis* infection in COPD and to determine whether anti-*Pneumocystis* treatment can attenuate the inflammatory process in colonized patients.

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