Pneumocystis jirovecii colonization in chronic pulmonary disease


Summary:
Pneumocystis jirovecii causes pneumonia in immunosuppressed individuals. However, it has been reported the detection of low levels of Pneumocystis DNA in patients without signs and symptoms of pneumonia, which likely represents colonization. Several studies performed in animals models and in humans have demonstrated that Pneumocystis induces a local and a systemic response in the host. Since P. jirovecii colonization has been found in patients with chronic pulmonary diseases it has been suggested that P. jirovecii may play a role in the physiopathology and progression of those diseases. In this report we revise P. jirovecii colonization in different chronic pulmonary diseases such as chronic obstructive pulmonary disease, interstitial lung diseases, cystic fibrosis and lung cancer.

KEY WORDS: Pneumocystis jirovecii, colonization, chronic pulmonary disease.

INTRODUCTION

Pneumocystis jirovecii (human-derived Pneumocystis) is an atypical opportunistic fungus with lung tropism and worldwide distribution that causes pneumonia in immunosuppressed individuals (Calderón et al., 2010). Although non-immunosuppressed patients rarely develop Pneumocystis pneumonia (PCP) (Cano et al., 1993), it has been demonstrated that in respiratory samples of some subjects without signs and symptoms of pneumonia, can be detected low levels of Pneumocystis DNA that likely represent colonization (Wissmann et al., 2010). Other terms that have been used include “carriage”, “subclinical infection” and “asymptomatic infection” (Peterson et al., 2005).

The first evidence reported of P. jirovecii colonization in patients with chronic bronchial diseases without immunosuppression was observed by using immunofluorescence with monoclonal antibodies and standard stains. It was found that 10 % of patients was colonized (Calderón et al., 1996). Current detection of Pneumocystis colonization has been greatly facilitated by the development of sensitive molecular techniques, such as polymerase chain reaction (PCR) (Calderón et al., 2010). The rate of P. jirovecii colonization reported in chronic pulmonary diseases is diverse as a result of the used different sampling or detection procedures and the area where the study was performed (see Morris et al., 2008b for review), since P. jirovecii infection is apparently linked with environmental factors and probably there are low and high-risk geographical areas (Varela et al., 2004).

It has been reported that Pneumocystis induce in animals models alveolar macrophage activation, elevation of proinflammatory interleukins, changes in lung surfactant, type I pneumocyte degenerative changes, type II pneumocyte reparatory hypertrophy, alterations in alveolus-capillary permeability, diffuse alveolar damage and lung fibrosis (Prévost et al., 1998; Rice et al., 1993; Thomas et al., 2007). In addition, a study using a culture system revealed that Pneumocystis...
major surface glycoprotein induces interleukin (IL)-8 and monocyte chemoattractant protein-1 release from a human alveolar epithelial cell line (Benfield et al., 1999). Besides, it has been recently demonstrated that colonized patients with chronic obstructive pulmonary disease (COPD) have higher systemic proinflammatory cytokine levels than do noncolonized patients (Calderón et al., 2007). These data suggest that Pneumocystis could play a role in the progression of chronic pulmonary diseases.

On the other hand, colonized patients can act as a reservoir and source of infection for susceptible subjects. In fact, the first molecular evidence that transmission of P. jirovecii from colonized immunocompetent carriers to susceptible subjects may occur has been recently provided (Rivero et al., 2008). What’s more, colonized individuals could represent a reservoir to P. jirovecii strains with dihydropteroate synthase (DHPS) gene mutations, potentially related with sulfadiazines resistance (Stein et al., 2004). In this sense, the finding of Pneumocystis DHPS gene mutations in two colonized patients with chronic bronchitis, who were not previously exposed to sulfa drugs (Calderón et al., 2004), suggests either possible occurrence of spontaneous mutations or, most probably, transmission of mutant strains from HIV-infected patients (frequently submitted to sulfa prophylaxis).

In this report we revise our current knowledge about P. jirovecii colonization in four major chronic lung diseases: COPD, interstitial lung diseases (ILDs), cystic fibrosis (CF) and lung cancer.

**CHRONIC OBSTRUCTIVE PULMONARY DISEASE**

COPD is a major cause of illness, death, and the use of health care resources globally. The disease causes approximately 2.75 million deaths annually, and the number is projected to increase. At present, it is the 4th most common cause of death among adults, and it is estimated to rank 3rd by 2020 (Chapman et al., 2006; Lopez et al., 2006). COPD is a slowly progressive condition characterized by airflow limitation that is not fully reversible. The airflow limitation is associated with a chronic inflammatory response in both airways and lung parenchyma (MacNee, 2005). Smoking is considered to be the major cause of COPD, but only a small portion of all smokers develops the disease. It has been hypothesized that cofactors, as P. jirovecii colonization, may play a role in COPD development (Morris et al., 2008a). Additionally, smoking would be a risk factor for P. jirovecii colonization in both HIV-infected individuals (Morris et al., 2004a) and subjects with chronic pulmonary diseases (Vidal et al., 2006).

Actually, cigarette smoking results in impaired mucociliary clearance, which could facilitate P. jirovecii colonization. In short, Pneumocystis lower respiratory tract colonization, together with smoking, could be a cofactor that increase or maintain inflammatory response, accelerating COPD progression. The rate of P. jirovecii colonization reported among patients with COPD is usually high. It shows however significant differences (16 to 55 %) that suggest major geographical disparities in P. jirovecii epidemiology (Calderón et al., 2007; Nevez et al., 2006). Interestingly, among the COPD patients from a same geographical area P. jirovecii colonization is more prevalent in those with severe COPD (Morris et al., 2004b).

β-glucans are major structural components of cyst wall of Pneumocystis (Lebrón et al., 2003). β-glucans potentely activate alveolar macrophages to release pro-inflammatory cytokines and chemokines through nuclear factor-κB (NF-κB) utilizing cellular receptors and signalling pathways distinct from bacterial lipopolysaccharide. β-glucans induce both cytokine generation and associated neutrophilic lung inflammation. In addition to Pneumocystis clearance, macrophages also strongly initiate inflammatory responses in the lung following interactions with β-glucan components of the organism, including secretion of tumor necrosis factor-α (TNF-α), IL-6, IL-8, macrophage inflammatory protein-2, eicosanoid metabolites, and reactive oxidant species (Lebrón et al., 2003). These exuberant host inflammatory responses, though necessary for clearance of infection, further promote lung injury resulting in airspace inflammation and excessive morbidity. The pulmonary inflammatory response against Pneumocystis resembles that associated with COPD and could therefore contribute to the pathogenesis of airway and parenchyma damage in subjects with this disease (Morris et al., 2008a).

An interesting study showed that Pneumocystis colonization correlates with degree of airway obstruction independent of smoking history (Morris et al., 2004b). This study did not prove causality, but it strongly suggests that Pneumocystis colonization affects COPD progression. In this sense, patients with COPD colonized by P. jirovecii were found to develop a higher systemic inflammatory response (increased levels of several circulating cytokines and acute-phase reactants including IL-8, IL-6 and TNF-α) than do noncolonized patients (Calderón et al., 2007). In COPD patients, systemic inflammatory response has been linked to weight loss, muscle wasting and accelerate decrease in lung function (Gan et al., 2004). As a cause of systemic inflammatory response, Pneumocystis colonization could therefore play a role in the pathophysiology of COPD.
INTERSTITIAL LUNG DISEASES

ILDs are a heterogeneous group of disorders of the lower respiratory tract in which injury to the lung parenchyma leads to a cascade of inflammatory and immune processes that cause lung fibrosis. The exact mechanism by which connective tissue proliferation occurs in some cases is unknown, although infectious agents have been implicated in the pathogenesis of ILDs (Egan et al., 1997, du Bois et al., 2005). In this way, it has been suggested that pulmonary inflammation, together with the adaptive immune response triggered by alveolar injury caused by Pneumocystis, could play a role in the pathogenesis of ILDs and in the progression of the disease to fibrosis. The rate of P. jirovecii colonization in patients with ILDs ranged from 30 to 34 % (Vidal et al., 2006; Martín-Juan et al., 2008; Shimizu et al., 2009) and it is similar among different kind of ILDs (Martín-Juan et al., 2008) (Table I).

Interestingly, in a large study carried out in 240 patients with ILDs (Martín-Juan et al., 2008), patients with ILDs who were carriers of P. jirovecii showed a significant higher peripheral blood total leukocyte and eosinophil levels compare to non-carriers. Also, the differential cell counts in the bronchoalveolar lavage fluid revealed a low CD4+/CD8 ratio in carrier patients, mainly because of an increased CD8 T-cell count, although the differences did not reach statistical significance. This finding suggests that Pneumocystis colonization may be related to an exacerbation of these diseases. Further studies are needed in order to confirm the clinical significance of these findings.

CYSTIC FIBROSIS

CF is the most common serious hereditary disorder in the Caucasian populations. It is caused by several mutations in the CF transmembrane conductance regulator gene that map on the large arm of chromosome 7 (7q31.2) and encodes a chloride channel involved in electrolytic exchanges through the plasma membrane, leading to a defect in the mucociliary clearance and a thickness of the bronchial mucus (Rowe et al., 2005). Several organs are affected in this disease and pulmonary damage is the most serious clinical problem. An important factor that contributes to the development of pulmonary disease in patients with CF is persistent infection by opportunistic pathogens, mainly Pseudomonas aeruginosa, Staphylococcus aureus, Haemophilus influenza and Burkholderia cepacia (Rowe et al., 2005).

Recent researches indicate that a wide range of fungi can colonize the respiratory tract of patients with CF. Aspergillus fumigatus is the main fungus involved in fungal-related CF complication, however it is not the fungal infection itself which is mainly responsible for respiratory failure, instead the fungus grows in the upper respiratory way of CF patients, where it provokes repeated immune stimulation, such mechanism leads to Allergic bronchopulmonary aspergillosis (ABPA), a chronic hypersensitivity condition that can lead to morbidity and ultimately to respiratory failure (Antunes et al., 2010).

Only a few published studies carried out in Europe have evaluated the prevalence of Pneumocystis colonization in patients with CF, reporting ranges from 1.3 %

<table>
<thead>
<tr>
<th>Interstitial lung diseases</th>
<th>Patients studied (n)</th>
<th>Patients with P. jirovecii colonization detected by molecular techniques (%)</th>
<th>Patients with P. jirovecii colonization detected by conventional and immunofluorescence staining (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idiopathic pulmonary fibrosis</td>
<td>69</td>
<td>22 (31.8 %)</td>
<td>4 (5.7 %)</td>
</tr>
<tr>
<td>Fibrosing lung disease associated with connective tissue disease</td>
<td>38</td>
<td>13 (34.2 %)</td>
<td>2 (5.2 %)</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>35</td>
<td>10 (28.5 %)</td>
<td>2 (5.7 %)</td>
</tr>
<tr>
<td>Cryptogenic organizing pneumonia</td>
<td>31</td>
<td>9 (29 %)</td>
<td>1 (3.2 %)</td>
</tr>
<tr>
<td>Diffuse alveolar haemorrhage</td>
<td>20</td>
<td>8 (40 %)</td>
<td>2 (10 %)</td>
</tr>
</tbody>
</table>

Modified from Martín-Juan et al., 2008.

Table I. – Prevalence of Pneumocystis colonization in individuals with interstitial lung diseases from Andalucía (Spain).

<table>
<thead>
<tr>
<th>Country</th>
<th>Number of patients</th>
<th>Prevalence of Pneumocystis colonization (%)</th>
<th>Median age (years)</th>
<th>Chemoprophylaxis with co-trimoxazole (%)</th>
<th>Authors, year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germany</td>
<td>95</td>
<td>7.4 %</td>
<td>23.2</td>
<td>No</td>
<td>Sing et al., 2001</td>
</tr>
<tr>
<td>Spain</td>
<td>88</td>
<td>21.6 %</td>
<td>15.8</td>
<td>2.3 %</td>
<td>Respaldiza et al., 2005</td>
</tr>
<tr>
<td>France</td>
<td>76</td>
<td>1.3 %</td>
<td>18.5</td>
<td>39.5 %</td>
<td>Gal et al., 2010</td>
</tr>
</tbody>
</table>

Table II. – Prevalence of Pneumocystis jirovecii colonization detected by using molecular methods among patients with cystic fibrosis.

Parasite, 2011, 18, 121-126
to 21.6% (Table II) (Sing et al., 2001; Respaldiza et al., 2005; Nevez et al., 2010). These differences in colonization rates could be related with differences in use of chemoprophylaxis with sulfa-drugs to prevent bacterial infections among the CF patients (Sing et al., 2001; Respaldiza et al., 2005; Gal et al., 2010). However, they could also be due to differences in population density, and in HIV infection or PCP incidence (Gal et al., 2010). On the other hand, several studies have shown seasonal changes of PCP incidence that seem to be associated with climate factors (Lubis et al., 2003; Varela et al., 2004; Sing et al., 2009). In this sense, the higher P. jirovecii colonization rates observed in patients with CF from Southern Europe than in patients from Northern Europe could be related with climate factors (Sing et al., 2001; Respaldiza et al., 2005; Gal et al., 2010).

Pneumocystis colonization does not appear to be a risk factor for subsequent PCP in CF patients, probably because the immune status of these patients is usually enough to control the colonization to pneumonia shift (Sing et al., 2001; Respaldiza et al., 2005; Montes-Cano et al., 2007). However, even without deep immunosuppression, in chronic lung conditions Pneumocystis colonization could provoke mild or moderate respiratory illness, with the pathogen acting as a co-morbidity factor that may stimulate pulmonary inflammation and may play a pathogenic role (Calderón et al., 2007; Morris et al., 2008b). Thus, it would be hypothesized that P. jirovecii colonization provokes airflow obstruction and inflammatory response in CF patients. However, to our knowledge, no evidence supporting this hypothesis was provided till now. In other words, it has not yet been established whether P. jirovecii colonization could have a role in worsening the natural course of CF.

On the other hand, our knowledge of how P. jirovecii interact with pathogenic bacteria in the respiratory tract of patients with CF is completely unknown but this interaction could have some impact in the pathophysiology of CF. In this sense, a potential antagonistic relation among P. jirovecii colonization and bacterial microbiota has been recently reported in the respiratory tract of patients with idiopathic interstitial pneumonia (Friaza et al., 2010).

LUNG CANCER

Tobacco smoking is the most important but not the only risk factor in lung carcinoma. The role of infectious agents in the development of human tumours was not demonstrated until recently (Kuper et al., 2000). Actually, it is estimated that 15% of all human cancers worldwide have an infectious origin (Pisani et al., 1997). Recently, it has been reported the first evidence of an association between small cell lung carcinoma and the presence of P. jirovecii colonization. In this study, in all the examined samples of small cell lung carcinoma P. jirovecii was detected (it was not found in metastases) (de la Horra et al., 2004). While the nature of this association is unclear, it nevertheless constitutes an important finding and open a new field for research since it is unknown either the P. jirovecii colonization is specifically facilitated by this tumour or induces the development of this type of neoplasm in combination with other factors.

CONCLUSIONS

There is a high prevalence of P. jirovecii colonization in patients with chronic pulmonary diseases (Calderón, 2009). Data from different studies suggest a potential role of P. jirovecii in the pathophysiology of chronic pulmonary diseases through inducing inflammatory changes with chronic lung injury, but also interacting with other agents as tobacco or pathogenic bacteria (Calderón et al., 2007; Morris et al., 2008a; Christensen et al., 2008; Friaza et al., 2010). Understanding the role of P. jirovecii colonization in patients with chronic lung diseases may help us identify a novel, treatable pathogenic agent.

On the other hand, P. jirovecii colonization in patients with chronic pulmonary diseases could represent a problem for public health since colonized patients could act as a major reservoir and source of infection for susceptible subjects (Calderón, 2009; Wissmann et al., 2010). In the future, advances as real time PCR could enhanced the sensitivity of Pneumocystis DNA detection and to make research on Pneumocystis colonization epidemiology much easier. In this way, the role of non invasive marker serum as β-glucan levels in colonized patients should be explored (Krajicek et al., 2008). Further investigation is need to better define the epidemiology and the role of P. jirovecii colonization in the natural course of chronic pulmonary diseases and to determine the exact mechanism of its effects.

ACKNOWLEDGEMENTS

This work was developed in the framework of the project “Pneumocystis Pathogenomics: Unravelling the Colonization-to-Disease Shift” into a Coordination Action of the European Commission (ERA-NET PathoGenoMics) and was supported by the Consejería de Salud Junta de Andalucía (PI-0391/07) and the Ministerio de Ciencia e Innovación (PI-080983).
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Received on June 30th, 2010
Accepted on October 31st, 2010