Lung Cytokines and Systemic Inflammation in Patients with COPD

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Lung Cytokines and Systemic Inflammation in Patients with COPD

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Abstract

Rationale: Chronic obstructive pulmonary disease (COPD) is characterized by lung and systemic inflammation. The role of cytokines in local and systemic inflammation in COPD is not well understood. This study aimed to compare plasma and bronchoalveolar lavage (BAL) fluid cytokine levels in COPD and non-COPD subjects with the intent of better understand their potential roles in driving local and systemic inflammation.

Methods: This cross-sectional study analyzed data from 65 subjects: 31 with COPD confirmed by spirometry and 34 non-COPD controls. All subjects underwent spirometry, plasma sample collection, and bronchoscopy/BAL. Levels of 21 inflammatory cytokines were measured in the plasma (systemic inflammation) and BAL (lung inflammation) using a multiplex assay.

Results: COPD subjects were overall older (median age 59 vs 36; p = <0.001) and had higher incidence of history of pneumonia and hypertension; otherwise, groups were similar in respect to gender, smoking history, history of asthma and other comorbidities. COPD subjects had higher plasma levels of 12 measured cytokines (in pg/ml) as compared to non-COPD controls: tumor necrosis factor (TNF)-α, 6.5 vs 3.7 (p = 0.005), interleukin (IL)-8, 5.1 vs 2.9 (p = 0.008), IL-21, 1 vs 0.4 (p = 0.006), IL-7, 5.9 vs 4.3 (p = 0.022), IL-10, 8.5 vs 5.1 (p = 0.036), interferon (IFN)-γ, 12.6 vs 9 (p = 0.021), IL-12p70, 1.8 vs 0.7 (p = 0.025), IL-2, 1.4 vs 0.9 (p = 0.014), IL-17A, 4.4 vs 1.7 (p = 0.026), IL-23, 34.7 vs 11.3 (p = 0.026), macrophage inflammatory protein (MIP)-3 α (CCL20), 24.4 vs 19.8 (p = 0.035), and fractalkine (CX3CL1), 68.9 vs 34.3 (p = 0.011). In contrast, cytokine levels in the BAL fluid of COPD subjects were not elevated compared with controls.

Conclusion: Elevated levels of cytokines were identified in the plasma of COPD subjects when compared to controls, supporting the role of these mediators as one of the mechanisms of systemic inflammation in COPD. In contrast, lung cytokines were not elevated suggesting that inflammation in the setting of COPD may not originate and/or perpetuate in the lungs, or that the BAL fluid is not an optimal source of information when evaluating inflammation in COPD. Although the role of these cytokines remains uncertain, anti-cytokine therapy might modulate inflammation in COPD and perhaps improve outcomes.

Introduction

Chronic obstructive pulmonary disease (COPD) is a growing global epidemic condition with raising morbidity and mortality as population age. It has been projected that COPD, which ranked sixth as the cause of death in 1990, will become the third leading cause of death worldwide by 2020 [1]. Smoking is the most well-known risk factor for COPD; however, non-smokers may develop chronic airflow obstruction through occupational exposure to dust and chemicals and indoor pollution from biomass. Other recognized risk factors are genetic predisposition and low socioeconomic status [1].

COPD is characterized by progressive airflow limitation from remodeling of small airways and destruction of lung parenchyma. In addition to local pulmonary injury, it has been increasingly recognized that COPD carries significant extra pulmonary effects that may contribute to the severity of disease in individual patients [2]. For example, COPD is associated with cardiovascular diseases, osteoporosis, diabetes, and metabolic syndrome more frequently than expected from common etiological factors such as smoking, suggesting a causal association between these comorbidities and the systemic effects of COPD. This is a topic of increasing interest given the large burden of hospitalizations and mortality associated with this condition [3]. The mechanisms linking COPD to systemic manifestations and comorbidities remains unelucidated, but a potential mechanism is systemic inflammation [2].

Proposed mechanisms of systemic inflammation in COPD include systemic oxidative stress, activation of circulating inflammatory cells and increased levels of circulating inflammatory cytokines [4, 5]. Inflammation in COPD involves both innate immunity (neutrophils, macrophages, eosinophils, mast cells, natural killer cells, gamma delta T cells, and dendritic cells) and adaptive immunity (T and B lymphocytes),...
but also involves the activation of airway and alveolar epithelial cells, endothelial cells, and fibroblasts. Cigarette smoke and other irritants activate the release of multiple chemotactic mediators, which attract circulating neutrophils, monocytes, and lymphocytes into the lungs [4, 5]. Irritant-activated epithelial cells produce inflammatory mediators, including tumor necrosis factor (TNF) alpha, interleukin (IL)-1 beta, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-8. This inflammation persists even when smoking is stopped, suggesting that there are self-perpetuating mechanisms, although these have yet to be identified [6, 7]. It is possible that memory T cells, bacterial colonization, and/or autoimmunity may drive persistent inflammation in COPD [7].

Several studies have explored the systemic inflammatory state in COPD by demonstrating elevated levels of inflammatory markers, inflammatory cells, and a variety of inflammatory mediators such as cytokines in the serum and sputum of patients with COPD [4, 8–16]. Based on our literature review, most studies measuring local inflammatory responses in COPD have been performed in induced sputum and not in bronchoalveolar lavage (BAL) samples such as in our study. Cytokines are extracellular signaling proteins that play important roles in inflammatory and immune responses. Depending on their effects promoting or inhibiting inflammatory mechanisms, cytokines are normally classified as pro-inflammatoty-inflammatory, although it is not uncommon for many cytokines to exert both types of effects depending on a variety of factors or conditions. Chemokines are a family of structurally-related cytokines with chemo-attractant properties that play a central role in the recruitment of leukocytes to sites of inflammation. Elevated levels of several pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6; the chemokine IL-8, the anti-inflammatory cytokine IL-10 and vascular endothelial growth factor (VEGF) have been found to be elevated in the induced sputum of individuals with COPD [17]. A pattern of elevated serum levels of IL-6, IL-8, TNF-α, and VEGF has also been described in different studies. The combined elevation of these cytokines in the serum was shown to be significantly associated with severity of disease [14]. The exact role of each cytokine is not completely elucidated but some studied examples are the skeletal muscle weakness associated with IL-6, cachexia associated with TNF-α and IL-1β and hypoxemia also associated with TNF-α [18].

A more profound and detailed understanding of local and systemic inflammatory responses in COPD is necessary in view of the growing interest in studying it as a systemic condition and in the search and development of new therapies [19]. The purpose of this study was to compare inflammatory cytokine profiles in the plasma and BAL of COPD and non-COPD subjects.

**Methods**

**Study design and subjects**

This was a pilot cross-sectional study analyzing the data of a subgroup of subjects included in the study “The Impact of Oxidative Stress on HIV-Induced Lung Disease” conducted at the University of Louisville in Louisville, Kentucky, USA. The study enrolled 132 subjects from February 2014 to October 2016 and included HIV, non-HIV, COPD and non-COPD subjects. All participants provided written informed consent and the study was approved by the Institutional Review Board (IRB), approval # 13.0442. HIV subjects were excluded for the purposes of our study and 65 non-HIV subjects were selected; 31 with a diagnosis of COPD and 34 non-COPD controls. The diagnosis of COPD was made based on the current GOLD guidelines, defined as FEV₁/FVC ratio of less than 0.70 [1]. All subjects were seen in an outpatient basis and were stable from a respiratory standpoint. They underwent spirometry, blood sample collection and outpatient bronchoscopy with BAL.

**Spirometry**

Spirometry was conducted following the guidelines of the American Thoracic Society. A post-bronchodilator forced expiratory volume in 1 second (FEV₁)/forced vital capacity (FVC) < 0.7 confirmed airflow limitation and the diagnosis of COPD.

**Samples**

**Serum:** Venous blood (4cc) was collected using Vacutainer tubes and allowed to clot at room temperature. Following centrifugation at 300 x g for 10 min, the serum was separated by aspiration, aliquoted and stored frozen at -80°C until assayed.

**Bronchoalveolar lavage:** A bronchoscope was wedged into a distal segment of a bronchus. After the administration of local anesthesia (lidocaine 1% solution), a total of 180 ml of saline solution in three 60 ml aliquots were instilled into a lobe of the lung, with gentle aspiration after each aliquot. The lavage fluid was filtered through sterile gauze and then centrifuged at 800 x g for 10 minutes. The cell pellet was resuspended in Hanks’ balanced salt solution and the supernatants aliquoted and stored at -80°C.

**Cytokine measurements**

Serum and BAL samples were thawed and centrifuged at 10,000 x g for 5 minutes prior to use in the assays. The concentrations of twenty-one different cytokines and chemokines in plasma and BAL samples were measured using Milliplex MAP High Sensitivity Human T cell panel kits (HSCTYTMAG-28SK, EMD Millipore, Billerica, MA) according to the manufacturer’s instructions. The measured cytokines/chemokines were: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IL-17A, IL-21, IL-23, IFN-γ, TNF-α, CCL3 (MIP-1α), CCL4 (MIP-1β), CCL20 (MIP-3α), CXCL11 (I-TAC) and CX3CL1 (Fractalkine). Cytokine/chemokine levels were reported in picogram per milliliter (pg/mL). Cytokine levels in the BAL fluid were adjusted for total protein to account for potential dilution.

**Statistical analysis**

Categorical variables were summarized with frequencies and percentages and differences between groups were tested using Chisquared or Fisher’s Exact tests. Continuous variables were summarized with medians and interquartile ranges and the Mann-Whitney U-test was used to test for differences between groups. We considered a two-sided p value < 0.05 as statistically significant. We used the software R version 3.3.2 for statistical analysis.
Results

Subject Characteristics
The demographics and characteristics of the subjects are shown in Table 1. Sixty-five subjects were included in the study; 31 with the diagnosis of COPD and 34 non-COPD controls. COPD subjects were overall older than non-COPD (median age 59 vs 36; p = <0.001). History of bacterial pneumonia was more common in the COPD group (45% vs 15%; p = 0.013) as was the history of hypertension (55% vs 26%; p = 0.025). Groups were otherwise similar in respect to gender, smoking history and comorbidities such as asthma, diabetes and chronic kidney disease.

Cytokine Levels
Plasma cytokine levels are shown in Table 2. COPD subjects had significantly higher mean plasma levels of twelve of the twenty measured cytokines: TNF-α, IL-8, IL-21, IL-7, IL-10, IFN-γ, IL-12p70, IL-2, IL-17A, IL-23, MIP-3α, and fractalkine. Levels of the remaining measured plasma cytokines in comparison to non-COPD subjects were not significantly different.

Table 3. Bronchoalveolar Lavage Cytokine Levels

Table 4. Correlation between cytokine level and pulmonary function

As shown in Table 4, BAL levels of GMSCF and TNF-α were positively correlated with FEV1/FVC. In the plasma, levels of fractalkine, IL-12p70, IL-17A, IL-2, IL-21, IL-6, IL-7, IL-8 and TNF-α were negatively correlated.
Table 4. Correlation between cytokine level and FEV1/FVC.

<table>
<thead>
<tr>
<th>Cytokine (BAL)</th>
<th>Correlation</th>
<th>P-value</th>
<th>Cytokine (Plasma)</th>
<th>Correlation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GMSCF</td>
<td>0.319</td>
<td>0.048</td>
<td>0.286</td>
<td>0.239</td>
<td></td>
</tr>
<tr>
<td>FRACKTALINE</td>
<td>0.094</td>
<td>0.121</td>
<td>0.181</td>
<td>0.099</td>
<td></td>
</tr>
<tr>
<td>IL-12/20</td>
<td>0.061</td>
<td>0.712</td>
<td>0.415</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>IL-17A</td>
<td>0.002</td>
<td>0.992</td>
<td>0.325</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>0.053</td>
<td>0.056</td>
<td>0.347</td>
<td>0.055</td>
<td></td>
</tr>
<tr>
<td>IL-17I</td>
<td>0.108</td>
<td>0.514</td>
<td>0.365</td>
<td>0.225</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>0.007</td>
<td>0.966</td>
<td>0.330</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>IL-7</td>
<td>0.073</td>
<td>0.538</td>
<td>0.442</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>IL-8</td>
<td>0.124</td>
<td>0.411</td>
<td>0.518</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

BAL: bronchoalveolar lavage; GMSCF: granulocyte macrophage colony-stimulating factor; IFN: interferon; IL: interleukin; TNF: tumor necrosis factor

Table 5. Correlation between cytokine level and FEV1.

<table>
<thead>
<tr>
<th>Cytokine (BAL)</th>
<th>Correlation</th>
<th>P-value</th>
<th>Cytokine (Plasma)</th>
<th>Correlation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GMSCF</td>
<td>0.014</td>
<td>0.004</td>
<td>0.001</td>
<td>0.101</td>
<td></td>
</tr>
<tr>
<td>FRACKTALINE</td>
<td>0.119</td>
<td>0.036</td>
<td>0.360</td>
<td>0.024</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.332</td>
<td>0.237</td>
<td>0.144</td>
<td>0.199</td>
<td></td>
</tr>
<tr>
<td>IL-17A</td>
<td>0.345</td>
<td>0.011</td>
<td>0.147</td>
<td>0.972</td>
<td></td>
</tr>
<tr>
<td>IL-8</td>
<td>0.026</td>
<td>0.877</td>
<td>0.576</td>
<td>-0.081</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.367</td>
<td>0.021</td>
<td>0.609</td>
<td>0.003</td>
<td></td>
</tr>
</tbody>
</table>

BAL: bronchoalveolar lavage; GMSCF: granulocyte macrophage colony-stimulating factor; IFN: interferon; IL: interleukin; TNF: tumor necrosis factor

Discussion

Our study found significantly elevated levels of twelve cytokines and chemokines in the systemic circulation of subjects with COPD in comparison with non-COPD controls. In the BAL, cytokine levels were not different in COPD and non-COPD, except for TNF-α, which levels were elevated in the non-COPD group. COPD is associated not only with an abnormal inflammatory response of the lung parenchyma but also with systemic inflammation implicated in the pathogenesis of many of the comorbidities linked to this condition including weight loss, skeletal muscle dysfunction, cardiovascular disease, depression, and osteoporosis, making this a popular study subject. Known mechanisms of local inflammation in COPD include oxidative stress, activation of resident inflammatory cells and increased production of proinflammatory cytokines. In local immune (e.g. macrophages) and non-immune cells (e.g. epithelial cells), it is known that cigarette smoke and air pollutants are triggers for these local mechanisms. On the other hand, the origin of systemic inflammation in COPD is not yet completely elucidated. The hypothesis that inflammatory cells “spill-over” into the systemic circulation has been explored, however, it has not been proven [20–22]. Smoking, per se, has been explored as a mechanism for systemic inflammation in COPD. Some cytokines appear to be elevated as a consequence of smoking, particularly, IFN-γ, TGF-β, IL-8 and MCP-1 [14]. The inflammatory process, however, seems to continue despite smoking cessation. This is supported by evidence of persistent presence of inflammatory cells and mediators and continued - despite slower - deterioration of lung function in ex-smokers [6, 7, 23, 24].

Similarly to our findings, elevated levels of several cytokines in the systemic circulation of COPD subjects have been previously demonstrated in multiple studies. Some of the cytokines commonly found to be elevated were IL-1β, IL-6, IL-16, IL-8, TNF-α and VEGF [11, 14–16, 23, 25]. Selvarajah et al. measured fourteen cytokines in serum from never-smokers, ex-smokers, current smokers, and COPD subjects (GOLD stages 1-3) and found that the combined - not individual - increase of certain cytokines in COPD subjects was associated with disease severity, indicating that patterns of cytokine expression might be more relevant [14]. Aaron et al. measured sputum and serum cytokine levels in past and current smokers with COPD, smokers with no COPD, and non-smokers with COPD in three different occasions. Systemic and sputum biomarkers were associated with clinical variables that predict severity in COPD, but also showed a high degree of intra-subject variability by using repeated biomarker measurements over time, suggesting that one-time measurements may not be diagnostically reliable. As previously mentioned, the association between severity of disease and cytokine levels has been explored [14–16, 23]. According to GOLD guidelines FEV1/FVC is used for diagnosis of COPD and FEV1 is used to classify severity of disease [1]. In our study, we found significant negative correlation between levels of nine plasma cytokines and FEV1/FVC and three cytokines and FEV1.

We also measured cytokine levels in BAL fluid and surprisingly found none of the cytokines or chemokines to be significantly elevated in COPD subjects in comparison with non-COPD. In fact, level of TNF-α was found to be elevated in the non-COPD group. Before we explore these findings, it is important to highlight that most studies exploring local inflammation in COPD have utilized induced sputum and not BAL samples as we were able to perform in our study. In addition, many of the studies using BAL measurements were conducted with the purpose of comparing local cytokine profiles in COPD versus asthma and not necessarily with the aim of contrasting BAL with serum levels such as we aimed with our analysis. Therefore, we consider that our study took a distinct approach when seeking a better understanding of inflammation in COPD, enabling one to compare BAL (local) versus plasma (systemic) in COPD versus non-COPD subjects.

Multiple studies have previously explored local inflammation in COPD by analysis of induced sputum with measurement of different cytokine levels. The findings have been overall inconsistent when sputum or BAL levels were compared to serum levels, as well as when levels were compared between COPD and non-COPD groups [9, 12, 15, 23, 26, 27]. Considering that we found low levels of locally present cytokines in COPD - which differs from most studies in our literature search - we bring the following points to discussion. First, inflammation in the setting of COPD may not originate and/or perpetuate in the lungs. As mentioned above, previous studies have shown that some COPD subjects continue to have decline in lung function despite smoking cessation [6, 7, 23, 24]. This suggests that inflammation in COPD could be generated systemically and not locally, which could potentially, or at least partially, explain the fact that to this day we have not found effective local therapies that prevent decline in lung function and significantly decrease morbidity and mortality in COPD. Second, BAL fluid may not be the optimal source of information when evaluating inflammation in COPD and selecting the correct biological fluid.
Our study has several limitations including a small sample size of sixty-five subjects and measurements coming from a one-time isolated sample. It has been previously demonstrated that many biomarkers that are apparently associated with COPD exhibit a high degree of intra-subject variability [15]. Therefore, measuring cytokine levels at different time points and analyzing patterns of concomitantly elevated cytokines in certain groups would be an optimal approach. As subjects in the COPD group were overall older, another limitation of our study is the fact that our data analysis was not adjusted for age. It is known that aging is accompanied by immune changes leading to a chronic inflammatory state with potential increase in levels of inflammatory cytokines [28]. This was a pilot study with a small sample size, consequently with not enough power to conduct an analysis adjusted for age. Another potential confounder to be mentioned is the increased incidence of pneumonia in the COPD group, which could alter the inflammatory profile of these subjects leading to increased levels of inflammatory cytokines [29].

Strengths of our study include the ability of obtaining plasma and BAL fluid samples in an outpatient basis, when subjects were not currently experiencing or being treated for an exacerbation. In addition, the control and study populations were for the most part similar. Finally, our analysis differs from most studies in this field by the fact that we were able to collect standardized bronchoscopic BAL fluid samples and not only induced sputum samples for measurement of cytokine levels and comparison with plasma. Understanding in detail the local and systemic inflammatory processes of COPD is essential to elucidate the mechanisms that could potentially be altered. Our study identified elevated levels of several inflammatory cytokines in the plasma of COPD subjects when compared to controls, implying and supporting the role for systemic inflammation in this condition. Locally measured cytokine levels in the BAL were not elevated, suggesting that inflammation in COPD may not originate or not perpetuate in the lungs. In addition, the BAL may not be an optimal source of information when evaluating inflammation in COPD.

It is well known that COPD is a global epidemic and there is a major need for therapies that stop the decline in lung function and substantially decrease morbidity and mortality. Apart from the obvious large need for smoking cessation, ideal therapy would be the one targeting the underlying processes that progressively and continuously drive damage. Systemic anti-cytokine therapy might be part of a future solution in modulating inflammation and perhaps improving outcomes in this condition of great health burden. COPD is a very complex heterogeneous condition and so is the research needed to explore it. We suggest future large population studies that take into consideration patterns of plasma cytokine level elevation with measurements at different time points. Such approach could lead us to potential answers regarding which cytokines or their receptors to target and in which populations.

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