Elevated serum interleukin-5 levels in severe chronic obstructive pulmonary disease

ABBS – Lab Note

Elevated serum interleukin-5 levels in severe chronic obstructive pulmonary disease

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Running Head: IL-5 and COPD

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Chronic obstructive pulmonary disease (COPD) is a progressive inflammatory lung disorder, which is now ranked equal third as a leading cause of death [1]. Fixed airflow obstruction that is measured by spirometry following inhaled bronchodilator is essential to its diagnosis, and in its advanced stages, systemic manifestations such as inflammation and cachexia are also characteristic. In the lung, pathological features include increased numbers of airway neutrophils, a cytotoxic (Tc) predominant lymphocytic response associated with lymphoid follicles containing B and T cells, and proinflammatory cytokines including interleukin (IL)-6 and IL-8. Lung fibrosis and parenchymal destruction, otherwise known as emphysema can also feature in addition to small airways obstruction, and some evidence suggest these processes are linked to the proteolytic enzymes, matrix metalloproteinases (MMP).

While the predominant immune response in allergic asthma resembles the T_H2 pattern, a T_H1 lymphocytic response appears to predominate in COPD. Interferon gamma (IFN-γ) attracts T_H1 and Tc1 cells into the lung and this results in persistent inflammatory activation, with possible Tc1-related lung damage via type 1 pneumocyte apoptosis [2]. However, the distinction between asthma and COPD in terms of inflammatory and immune responses is often not clear [2]. In a study that examined bronchoalveolar lavage (BAL) specimens from COPD patients, IL-4 and IL-13 expression were increased largely by pulmonary Tc2 lymphocytes, and levels were inversely correlated with the degree of fixed airflow obstruction or COPD severity [3]. Up to 40% of patients with stable COPD have been shown to exhibit sputum eosinophilia [4, 5] that is most apparent during acute COPD exacerbations, thus resembling the immunological profile which is typical of acute asthma exacerbations. This may be primarily mediated by the cytokines IL-5, eotaxin and RANTES [6], via increased eosinophil production and release from bone marrow [5].

Systemic inflammation in COPD has been best demonstrated by the presence of raised high-sensitive C-reactive protein levels, which have been shown to correlate with disease severity, but not with prognosis or accelerated lung function decline. Increasing COPD severity has been

associated with increasing plasma concentrations of IL-13, CCL2 (MCP-1), CCL4 (MIP-1β), CCL11 (eotaxin) but not with IL-1β in stable COPD subjects. In a study that measured multiple serum cytokines in 21 stable COPD patients, serum CCL11 (eotaxin) and IL-6 were elevated compared with controls. This contrasted cytokines from the T_H2 and T_H17 lineage which did not show significant differences.

A distinct CD4+ T-cell subset, T_H17 cells, produce IL-17A that regulates neutrophil emigration and systemic granulopoietic responses to both pulmonary bacterial and allergen challenges. While this T_H17 pathway is involved in host defence against extracellular bacteria and autoimmune disease and has been linked to allergic asthma, bacterial pneumonia and acute rejection in lung transplantation, its involvement in COPD is unclear.

COPD-related inflammation continues to progress, even despite the cessation of smoking. It is unclear if this is due to persistent microbial colonisation, to persistent toxic stimuli in a susceptible individual, or possibly due to autoantigens that develop in repetitively damaged tissue. In this context, we aimed to measure serum cytokine levels representative of the T_H1, T_H2 and T_H17 inflammatory pathways by multiplex immunobead-based assay in those with severe COPD, mild-moderate COPD and those without COPD.

Here, we studied participants with and without physician-diagnosed COPD who were either ex-smokers or current smokers with at least a 10 pack-year history of smoking. Non-smokers with fixed airflow obstruction and emphysema documented by high-resolution computerised tomography chest scanning were also included in the study as comparison. Fixed airflow obstruction was defined as a ratio of post-bronchodilator forced expiratory volume in one second/ forced vital capacity (FEV_1/FVC) below the lower limit of the predicted normal range. As per the Global Initiative for Chronic Obstructive Lung Disease (GOLD) Strategy Document, severe COPD was defined as a FEV_1 less than 50% predicted, and mild-moderate COPD was defined by a FEV_1 greater than or equal to 50% predicted in this study. Control participants included those that were

match as much as possible, being either current smokers, ex-smokers or non-smokers with a ratio of FEV_1/FVC within the normal predicted range. Exclusion criteria included a self-reported or physician-diagnosis of asthma, a respiratory infection within the preceding 4 weeks, current use of oral corticosteroids, known bronchiectasis or alternative systemic inflammatory disorder, and significant co-morbidity. The study protocol was approved by Austin Health Human Research Ethics Committee, and all participants provided written, informed consent. Venous blood was collected, serum isolated and tested using bead-based multiplex immunoassay for cytokines (MIA, 9 Bio-Plex Panel B, BioRad Laboratories Inc.) - IL-12p70, IL-5, IL-13, IL-17A, IL-7, G-CSF, IL-1β, CCL2 and CCL4. Thawed serum samples were diluted in a ratio of 1:3 with the human serum specific diluent. High PMT standard settings were prepared with "blank" negative controls in duplicate. Ninety-six-well plates were coated with beads, followed by the addition of samples and standards and detection antibodies, then streptavidin-phytoerythrin as per manufacturer's instructions. The beads were resuspended and then the fluorescence output was read and calculated on the Bio-Plex array reader; all relevant controls were included to validate the cytokine analyis. Non-parametric analysis of variance (Kruskal-Wallis test) was used to compare the means between the three groups. Spearman rank correlations were used to compare values between individual cytokines. A conventional cut-off of $p < 0.05$ was used to determine statistical significance.

The demographic and lung function data of the 35 participants are summarised in Table 1. There was a male predominance in the combined COPD group (n = 13, 68%), and a female predominance in the control group (n = 11, 69%). Those with COPD were on average between 11 and 12 years older than the control participants ($p = 0.01$). Significant differences in lung function were seen between participant groups, where the mean FEV_1 was 37% of predicted for those with severe COPD, 70% of predicted for those with mild-to-moderate COPD and 109% of predicted for control subjects. Gas transfer factor measurements were significantly lower for COPD patients with severe airflow obstruction, when compared with those who had mild-to-moderate disease

[40.4% of predicted (95% confidence interval (CI) 26-54) versus 55.8% of predicted (95%CI 43-69)].

Individual levels of each cytokine are illustrated in Figure 1a, where there were no differences between control and COPD groups, and between COPD severity categories, for most of the cytokines tested. A significant difference in serum IL-5 levels was noted between those who had severe COPD to those who had mild-to-moderate COPD or controls (Figure 1a, p = 0.005). Although most participants had undetectable levels of IL-5, half the individuals in the severe COPD group (n = 4) had detectable levels with a mean (SEM) level of 6.4 (3.2) pg/ml. Similarly, half with severe COPD had detectable IL-13 levels, although the trend in levels between groups did not quite reach statistical significance (p = 0.079) (Figure 1a). A modest correlation was noted between the T_H2 type cytokines, IL-5 and IL-13 (Spearman r = 0.429, p = 0.0102). However, moderate correlations were noted between lineages, namely IL-17A and IL-12p70 (Spearman r = 0.52, p = 0.0015), as well as between IL-17A and IL-13 (Spearman r = 0.55, p = 0.0006) (Figure 1b, 1c).

This study has documented levels of serum cytokine that are representative of the T_H1, T_H2 and T_H17 pathways in COPD patients with mild-to-moderate and severe airflow obstruction. Although a T-lymphocyte type 1 response that primarily involves IFN-γ predominates in COPD [2], our detection of serum IL-5 levels in those with severe COPD and a lower gas transfer factor supports the body of evidence that suggests type 2 responses co-exist, especially during exacerbations. Oral corticosteroid therapy has been shown to reduce sputum eosinophilic cationic protein and blood eosinophil count in patients with stable COPD in whom asthma was excluded, and an anti-IL-5 receptor-α monoclonal antibody might be useful in the subgroup of COPD patients with sputum eosinophilia.

The overlap between asthma and COPD has now been formally recognized to be a separate COPD phenotype [7, 8], as sputum eosinophilia can occur in patients with stable COPD despite

this immunological response being typically associated with asthma [5]. Mean systemic IL-5 levels up to 80 pg/mL have been measured by ELISA in individuals with childhood persistent asthma, although much lower levels are generally reported [9]. In a study of stable COPD patients with bronchial asthma excluded by a pulmonologist, the eosinophilic chemotactic protein, serum eotaxin, was found to be higher when compared with controls, but this study did not report corresponding serum IL-5 values. When compared with smokers with no or minimal lung disease as defined by $FEV_1/FVC > 0.7$, $FEV_1 \geq 80\%$ and low attenuation areas (LAA) ≤ 2.4, plasma IL-5 levels were statistically higher for those with radiological evidence of emphysema (LAA ≥ 8), airflow obstruction ($FEV_1/FVC > 0.7$, $FEV_1 \leq 60\%$) or both. While a history of childhood asthma is often inaccurate when it is recalled in adulthood, this prospective history is generally unavailable in the clinical setting. As our study participants did not self-report a history of lifetime asthma, it is therefore still possible that those with severe COPD may have had end-stage, insidious, allergic asthma that was otherwise not detectable.

Elevated serum cytokine levels might be due to a “spill-over” effect of the pathological process in the lung into the systemic circulation [10], and a linear and predictable nature cannot be presumed. In a key study of eleven subjects with COPD of moderate severity of whom four underwent a peripheral blood study, increased numbers of Tc2-like cytokine-expressing cells were isolated from BAL fluid, and this was consistent with other data supporting increased IL-4 and IL-13 expression by pulmonary T_c2 lymphocytes [3]. In comparison with the increased numbers of (CD8+) Tc1-like cells detectable in blood, only around one-sixth of (CD8+) Tc2-like, (CD4+) T_H1-like, and (CD4+) T_H2-like cells were detectable in the blood compared with BAL fluid. This suggests that expression of IL-5 by pulmonary T_c2 lymphocytes is likely to have been increased, especially for some of our participants who had severe COPD.

Our correlation data suggests moderate interrelationships between serum IL-17A levels and the cytokine IL-12p70, a promoter of T_H1 differentiation, and the cytokine IL-13 that is inhibitory to T_H1 cells. These represent cytokines from the three different subsets of effector CD4+ T cells, in

addition to T-regulatory cells. The interrelationships are consistent with naïve precursor T_H17 cells being inhibited by T_H1 and T_H2 cytokines, although the interpretation of our finding was limited without IFN-γ and IL-4 levels.

Over-expression of IL-13 in the lungs of adult mice has been shown to induce many features associated with COPD. These include emphysema, mucus goblet cell hyperplasia, and airway inflammation associated with macrophages, lymphocytes and eosinophils and increased matrix metalloproteinases. Low or undetectable levels of tissue IL-13 have been reported in lung specimens of human subjects with severe emphysema, whereas plasma levels of IL-13 have been shown to be modestly elevated in phenotypes associated with emphysema in another clinical study, with and without airflow obstruction.

In terms of limitations, we acknowledge our study had modest numbers of participants (n=35), although numbers were comparable to the similar study by Bade and colleagues (n=30). As the measurement of cytokines in the systemic compartment lacks sensitivity when compared with either BAL fluid or surgical lung biopsy, this might have attenuated or effectively eliminated associations between serum cytokines and the severity categories of COPD. Other tests that would have been useful to be included, including the measurement of IFN-γ, IL-8 and IL-4 for the interpretation of serum IL-17A and its relationship to T_H1 and T_H2 lymphocytic pathways; and objective measures of allergic and asthma such as blood eosinophil count, bronchial provocation and/or skin prick testing. Further studies are needed to clarify whether serum IL-5 might be a biomarker for severe COPD.

This study aimed to document the systemic immune responses in participants with COPD of varying severity, and appears to show an enhanced systemic T_H2 expression of IL-5 levels for participants who had severe airflow obstruction and a lower mean gas transfer factor. Although the cohort of COPD patients is very small these findings support the concept that although the immune

mechanisms of COPD and asthma are classically different, increasing disease severity and clinical overlap of these two obstructive lung diseases can blur the distinction.

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Table 1 Baseline spirometry and smoking characteristics

Subjects, n = 35	Severe COPD	Mild-moderate COPD	Controls
Subjects, n	8	11	16
Age (years) *	68.6 (13)	72.5 (9.8)	59.2 (12)
Male: female	4 : 4	9 : 2	5 : 11
FEV_1 post-BD (% predicted) §	36.6 (8.0)	69.8 (13)	109 (15)
FVC post-BD (% predicted)	80.9 (9.9)	104 (10)	111 (14)
FEV_1/FVC post-BD (ratio) §	30.9 (4.9)	50.7(11)	78.3 (5.9)
Smoking history (pack-years) †	36.6 (28)	50.3 (24)	17.9 (0-65) [a]
Ex/ Current/ Non-smoker, n	7 / 0 / 1	8 / 3 / 0	7 / 3 / 6
T_LCO (% predicted)	40.4 (17)	55.8 (20)	--

Abbreviations: BD, bronchodilator; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; T_LCO, transfer factor of the lung for carbon monoxide

Results are expressed as mean (SD) except where otherwise indicated

[a] range § $p < 0.0001$, † $p = 0.0041$, * $p = 0.01$

Figure 1 (A) Serum cytokine levels between control and COPD groups. Only serum IL-5 levels showed statistical significance between severe COPD and controls (p = 0.005) and between severe COPD to mild-moderate COPD (p = 0.005). There was no statistical significance for all other serum cytokines, IL-13 (p = 0.079), G-CSF (p = 0.47), IL-12p70 ((p = 0.41), IL-17A (p = 0.815), CCL2 (p = 0.18), IL-1β (p = 0.345), IL-7 (p = 0.063) and CCL4 (p = 0.89). **(B, C)** Relationships between serum IL-17A, IL-13 and IL-12p70 levels using log scale graphs.