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Early View

Research letter

Current smoking status is associated with reduced sputum immunoglobulin M and G expression in chronic obstructive pulmonary disease

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TITLE: Current smoking status is associated with reduced sputum immunoglobulin M and G expression in chronic obstructive pulmonary disease.

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TAKE HOME MESSAGE: Current smoking status in subjects with mild to moderate chronic obstructive pulmonary disease was associated with reduced total induced sputum IgM and IgG. This reduction in immunoglobulins was independent of inhaled corticosteroid use.

To the Editor:

The relationship between smoking status and total immunoglobulin (Ig) levels in patients with chronic obstructive pulmonary disease (COPD) is not fully understood. Decreased Ig levels have been observed in patients with COPD[1–4], a disease largely attributed to current or former smoking, and cigarette smokers[5,6]. These studies focused predominately on serum (systemic) or salivary (upper respiratory tract) Igs. Levels of Igs in induced sputum, a more direct measure of airway Igs, are less well studied. Given the vital role Igs perform in microbial defence and tissue homeostasis, we assessed airway Igs in induced sputum of smokers and patients with COPD.

To begin, we obtained 99 de-identified, banked, frozen induced sputum supernatant and matched serum samples from the Airways Disease Endotyping for Personalised Therapeutics (ADEPT) study (six sites, European and North American locations; *Clinical Trial #*NCT01274507; Janssen Pharmaceutical). All subjects provided written informed consent to participate (*Hamilton Integrated Research Ethics Board* Project #2031). Control subjects had no clinically significant medical condition, airflow limitation, or a history of any chronic pulmonary disease (n=45; 44.4% male; mean predicted %FEV_1: 106.5% ± 11.0%). Control subjects classified as non- or ex-smoker (NES) had quit smoking for greater than one year prior to sample collection and had ≤ 10 smoking pack-year history. Current smoking status was self-reported and confirmed by urinary cotinine. Patients with COPD were diagnosed by a physician and identified as having an FEV_1/FVC < 0.7, FEV_1 bronchodilator reversibility < 12%, and postbronchodilator FEV_1 value < 80%. Subjects were enrolled during stable disease, defined by a period of no exacerbations within three months prior to sample collection. COPD stage was classified by severity of lung function, ≥ 50% (n=45; 64.4% male; mean predicted %FEV_1: 57.7% ± 11.6%) and < 50% predicted FEV_1 (n=9; 66.7% male; mean predicted %FEV_1: 45.1% ± 5.5%)**.**

Total IgM, IgG, and IgA in induced sputum supernatant and serum was assessed by ELISA as per manufacturer's instructions (BMS2091/ BMS2096/ BMS2098; ThermoFisher Scientific, ON, Canada). Blood draw and sputum induction[7] (as per Pizzichini *et al.* with a plug weight of ≥ 50mg and squamous cell content ≤ 20%) were performed during the same visit. All sputum samples were processed using dithiothreitol (DTT) for optimal mediator release from mucus plugs. We observed no change in total sputum IgM, IgG, and IgA based on COPD severity, sex, body mass index, nor age (data not shown). Bacterial colonisation has been associated with altered Ig levels but was not assessed in this study[8]. ADEPT control subjects included current (46.7%), ex- (4.4%) and never smokers (48.9%). All patients with COPD had a smoking history of at least 10 pack years; 51.1% of mild to moderate COPD (≥ 50% predicted FEV_1) and 55.6% of severe COPD (< 50% predicted FEV_1) subjects were current smokers. Notably, current smoking status was associated with decreased total sputum IgM. Moreover, sputum IgG showed a trend towards decline in current smokers (p value = 0.07) (**Figure 1A**). In a previous study, decreased induced saliva IgM and IgG were observed in cigarette smokers[9], suggesting that cigarette smoke suppresses Igs throughout the respiratory tract. Moreover, serum total Ig levels were not changed based on any available clinical parameters, including current smoking status (data not shown). Given the decreased sputum Ig trends in current smokers, we next performed a power calculation to determine sufficient sample size for a sputum Ig and smoking status study. We calculated that 56 subjects per group would be required to accurately assess IgG trends based on an alpha of 0.05 and beta of 0.2. To this end, a second independent cohort was recruited from the Guangzhou Institute of Respiratory Health, China (GIRH).

A total of 152 induced sputum supernatant samples were collected from subjects recruited by the GIRH *(Clinical Trial #NCT03240315;* First Affiliated *Hospital* of *Guangzhou Medical University)*. Of those patients, 54 were current smokers and 98 non/ex-

smokers. This study followed inclusion criteria from the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) study. While these inclusion criteria were less stringent than the ADEPT study, subject characteristics between the cohorts were comparable, with the exception of sex since the GIRH cohort was entirely male, which is a reflection of the high male smoking prevalence in China. Subject stratification into lung function groups was identical to that of the ADEPT study, with COPD patients being defined as having an $FEV_1/FVC < 0.7$ and subsequent predicted FEV_1 value either $\geq 50\%$, (mild to moderate) or $< 50\%$ (severe). Included in this cohort were 39 control subjects (mean predicted $\%FEV_1$: 99.1% ± 9.2%), 52 patients with mild to moderate COPD (mean predicted $\%FEV_1$: 75.3% ± 15.7%), and 61 patients with severe COPD (mean predicted FEV_1: 36.0% ± 8.9%). Induced sputum supernatant was collected as per Bafadhel *et al.* and processed using phosphate buffered saline (PBS)[10]. Total IgM, IgG, and IgA were measured by ELISA in the GIRH cohort as for the ADEPT study. Mirroring observations in the ADEPT cohort, current smoking status, but not COPD severity or other clinical parameters, was associated with decreased induced sputum IgG and IgM in the GIRH cohort (**Figure 1A**). While current smoking status was not associated with decreased IgA (**Figure 1A**), total sputum IgA was decreased in severe COPD as compared to subjects with milder disease and controls with normal lung function (**Figure 1B**), mirroring previous observation in COPD[1,2]. ¶

Of note, the range of total sputum IgM and IgA were variable between the cohorts. Procedural differences in sputum processing, likely contributed to observed differences in total Ig recovery between cohorts; ADEPT samples were processed using DTT while GIRH samples were processed in PBS. To address DTT interference, we processed Ig standard with or without 0.1% DTT for 15mins, at which timepoint the sample was diluted to 0.025% DTT with PBS, and added to the ELISA plate, mimicking the sputum processing protocol by Pizzichini *et al.*[11]. We observed DTT interference in Ig signal for IgM (54.7% reduction;

PBS: 130 ng/mL ± 18, DTT: 59 ng/mL ± 5, p value = <0.0001) and IgA (7.7% reduction; PBS: 8.0 ng/mL ± 0.3, DTT: 7.3 ng/mL ± 0.5, p value = 0.02). IgG standard binding signal was increased 15.5% (PBS: 115 ng/mL ± 11, DTT: 133 ng/mL ± 10, p value = 0.01) with the inclusion of a DTT processing step. Therefore, the DTT processing step in the ADEPT cohort may contribute to reduced recoverable total IgM compared to the GIRH cohort. Notably, the increased IgA yield in the ADEPT cohort is likely attributable to improved IgA release from the mucus plug[12]. Given, similar cigarette smoking trends were conserved between cohorts, we propose the GIRH cohort validated the ADEPT cohort current smoking status observation. Overall,-despite the difference in sample processing, current smoking status was associated with reduced sputum IgM and IgG in both cohorts.

Next, we assessed the relationship between current smoking status and COPD severity with regard to sputum Ig levels. In subjects with normal lung function, current smoking status was not associated with any change in sputum IgM, IgG, nor IgA (**Figure 1C**). GIRH control subjects included current (38.5%), ex- (38.5%) and never smokers (23.1%). In contrast, current smoking status was associated with decreased sputum IgM and IgG in mild to moderate COPD (38.5% current smokers). Of note, subjects with the most severe disease did not have decreased IgM nor IgG based on current smoking status (31.1% current smokers). While the mechanisms are currently not understood, it is possible that less abundant T cell help in patients with early disease that smoke contribute to lower IgM and IgG levels[13]. We did not observe changes in total sputum IgA in either mild to moderate or severe disease based on current smoking status. It is plausible that the development of lymphoid follicles and follicle-associated IgA may compensate for mechanisms leading to decreased sputum IgA[14]. Alternatively, increased expression of transforming growth factor beta in patients with COPD[15] may lead to preferential IgA class switching[16]. Further research is warranted to investigate how cigarette smoke reduces sputum IgM and IgG levels. Overall, current

smoking status was associated with reduced sputum IgM and IgG in subjects with mild to moderate COPD, but not in subjects with normal lung function or subjects with severe disease.

Inhaled corticosteroids (ICS) form a major part of the treatment strategy for COPD patients. ICS have previously been observed to be immunosuppressive and, consequently, may reduce Ig levels in patients. We sought to understand the impact of ICS upon induced sputum Ig levels. In the GIRH COPD cohort, 42.3% of mild to moderate and 78.7% of severe COPD subjects were receiving ICS treatment at the time of sample collection. However, ICS usage was not associated with decreased total Ig isotype in either COPD severity (**Figure 1D**). This highlights the impact of current smoking status on reduced levels of sputum IgM and IgG in patients with COPD.

In closing, we investigated total induced sputum supernatant IgM, IgG, and IgA in two independent cohorts. Current smoking status in subjects with mild to moderate COPD was associated with reduced total induced sputum IgM and IgG. This reduction was not associated with current ICS usage. Our findings highlight the need for studies investigating mechanisms of cigarette smoke-mediated Ig immunosuppression, specifically during early disease progression. Of note, in two independent retrospective studies, COPD-related hospitalisations were reduced in a subset of COPD frequent exacerbators observed to have primary antibody deficiency syndromes following treatment with Ig replacement therapy (intravenous (IV) and subcutaneous Ig)[3,4]. While the impact of IVIg therapy on respiratory Ig is not known, targeted treatment of respiratory Ig may improve COPD-related hospitalisations in subjects with low Igs. Notably, we observed no difference in total serum Ig measured, emphasising the need for sampling induced sputum supernatant or bronchoalveolar lavage for assessment of immune processes in COPD. Ultimately, this study highlights the

need for further investigation into the impact of current cigarette smoking upon respiratory tract Igs in COPD patients.

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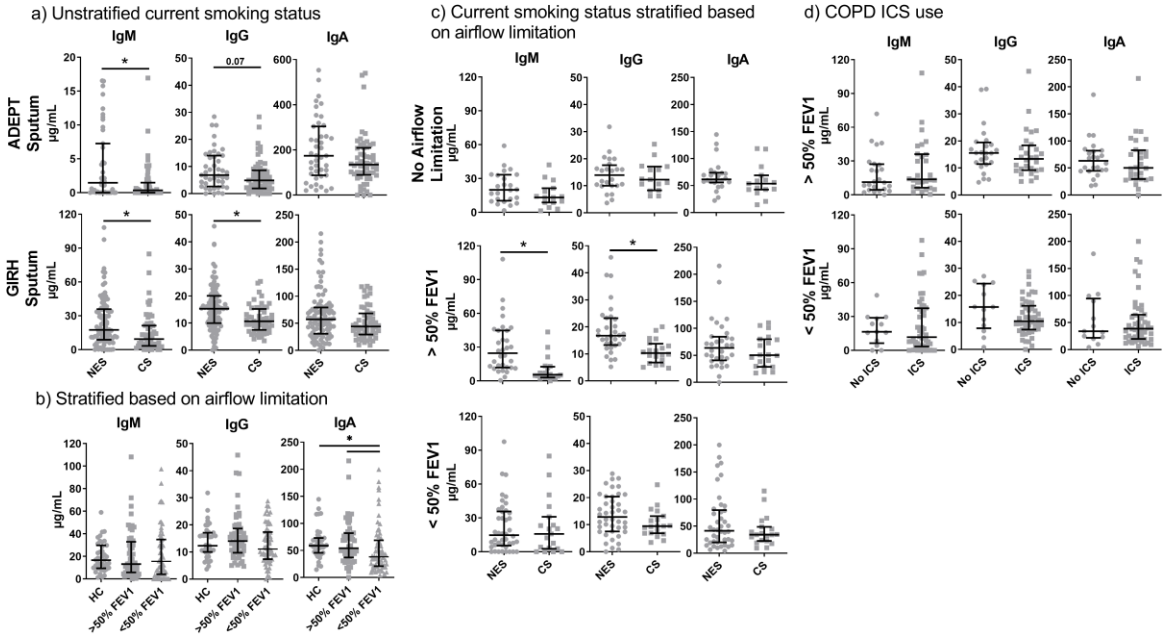
Figure

FIGURE 1. Current smoking status is associated with reduced IgM and IgG levels in the sputum of patients with COPD. a) Total sputum IgM, IgG, and IgA in the Airways Disease Endotyping for Personalized Therapeutics (ADEPT) study and the Guangzhou Institute of Respiratory Health (GIRH) cohorts stratified based on current smoking status. b) Total sputum IgM, IgG, and IgA in the GIRH cohort classified based on airflow limitation. c) Current cigarette smoking status impact on total sputum IgM, IgG, and IgA in the GIRH cohort stratified by airflow limitation. d) Sputum immunoglobulin expression based on COPD severity and inhaled corticosteroid (ICS) use in the GIRH cohort. Data in (a-d) are represented as median and interquartile range. Statistical differences were determined using two-tailed Mann-Whitney test (a, c-d). In (b), statistical differences were determined using Kruskal-Wallis test with Dunn's multiple comparison test. * $p = <0.05$. FEV1 - forced expiratory volume in one second. HC – healthy control/no airflow obstruction. NES - non and ex-smoker. CS - current smoker.


a) Unstratified current smoking status
IgM
IgG
IgA
ADEPT Sputum µg/mL
GIRH Sputum µg/mL
0.07
*
NES
CS
b) Stratified based on airflow limitation
µg/mL
HC
>50% FEV1
<50% FEV1
c) Current smoking status stratified based on airflow limitation
No Airflow Limitation µg/mL
> 50% FEV1 µg/mL
< 50% FEV1 µg/mL
d) COPD ICS use
No ICS
ICS