Pulmonary cytokine composition differs in the setting of alcohol use disorders and cigarette smoking

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Burnham EL, Kovacs EJ, Davis CS. Pulmonary cytokine composition differs in the setting of alcohol use disorders and cigarette smoking. Am J Physiol Lung Cell Mol Physiol 304: L873–L882, 2013. First published April 19, 2013; doi:10.1152/ajplung.00385.2012.—Alcohol use disorders (AUDs), including alcohol abuse and dependence, and cigarette smoking are widely acknowledged and common risk factors for pneumococcal pneumonia. Reasons for these associations are likely complex but may involve an imbalance in pro- and anti-inflammatory cytokines within the lung. Delineating the specific effects of alcohol, smoking, and their combination on pulmonary cytokines may help unravel mechanisms that predispose these individuals to pneumococcal pneumonia. We hypothesized that the combination of AUD and cigarette smoking would be associated with increased bronchoalveolar lavage (BAL) proinflammatory cytokines and diminished anti-inflammatory cytokines, compared with either AUDs or cigarette smoking alone. Acellular BAL fluid was obtained from 20 subjects with AUDs, who were identified using a validated questionnaire, and 19 control subjects, matched on the basis of age, sex, and smoking history. Half were current cigarette smokers; baseline pulmonary function tests and chest radiographs were normal. A positive relationship between regulated and normal T cell expressed and secreted (RANTES) with increasing severity of alcohol dependence was observed, independent of cigarette smoking (P = 0.0001). Cigarette smoking duration was associated with higher IL-1β (P = 0.0009) but lower VEGF (P = 0.0007); cigarette smoking intensity was characterized by higher IL-1β and lower VEGF and diminished IL-12 (P = 0.0004). No synergistic effects of AUDs and cigarette smoking were observed. Collectively, our work suggests that AUDs and cigarette smoking each contribute to a proinflammatory pulmonary milieu in human subjects through independent effects on BAL cytokine activation in the lung has been reported, including enhanced induction of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 (13, 33, 39, 57). Similarly, cigarette smoke exposure has also been reported to alter alveolar macrophage release and bronchoalveolar lavage (BAL)
Delineating the combined effects of alcohol and cigarette smoke exposure on TNF-α and IL-1β may be particularly useful because these cytokines are known to be integral to pneumococcal growth and dissemination (46, 63). Furthermore, a broader knowledge regarding combined alcohol and smoking effects on additional pulmonary mediators could also provide insight to the clinical relevance of more recently proposed pathogenic mechanisms underlying pneumococcal invasion, including the role for adaptive immunity (28, 36).

Our group has previously explored the effects of AUDs, with and without concomitant cigarette smoking, on components of innate immunity that may influence predisposition for pneumococcal infection (7, 10, 12). We have established a cohort of subjects with AUDs with a carefully characterized cigarette-smoking history and lung disease assessment, along with a cohort of control subjects matched on age, smoking, and sex. In these investigations, we sought to characterize the cytokine, chemokine, and growth factor milieu within the lung of those with AUDs who are either current smokers or non-smokers. Our goals were to determine whether additive or synergistic alterations related to AUDs and cigarette smoking were present that could influence the development of pneumococcal pneumonia. We also sought to establish whether dose-response relationships between the severity of alcohol misuse and cigarette smoke exposure could be detected. We hypothesized that the combination of AUDs and cigarette smoking would be associated with an increased quantity of proinflammatory mediators in BAL, including IL-1β, IL-6, and TNF-α, compared with either factor alone. Conversely, we postulated that the levels of anti-inflammatory mediators, including IL-10 and IL-1 receptor antagonist (IL-1RA), would be diminished.

MATERIALS AND METHODS

Subject screening, recruitment, and enrollment. Subjects with AUDs were recruited between 2008 and 2010 at the Denver Comprehensive Addictions Rehabilitation and Evaluation Services (Denver CARES) center, an inpatient detoxification facility affiliated with Denver Health and Hospital System in Denver, CO. Control subjects without AUDs were recruited from the Denver VA Medical Center’s smoking cessation clinic and via approved flyers posted on the University of Colorado’s Anschutz Medical Campus in Aurora, CO. The institutional review boards at all participating sites approved this study, and all subjects provided written informed consent before their participation in this protocol.

Subjects with AUDs were eligible to participate if they met all of the following criteria at study entry: 1) an Alcohol Use Disorders Identification Test (AUDIT) score of ≥8 for men or ≥5 for women, 2) alcohol use within the 7 days before enrollment, and 3) age of ≥21 yr. The AUDIT questionnaire is a standardized set of 10 questions that detect current and previous alcohol abuse, which has been validated in a variety of clinical settings (45). Furthermore, to meet eligibility as a control, control subjects’ AUDIT values were required to be <8 for men or <5 for women. Screening of potential control subjects centered on matching these control subjects to AUD subjects in terms of age, sex, and smoking history. With the use of AUDIT data, both subjects and controls were stratified into zones 1–4, classifying the severity of their alcohol consumption (37, 50). Zone stratification was performed to enable comparisons between AUDs and abstinence, as well as between AUDs and low-risk drinking. AUDIT scoring zones clinically correlated to abstinence (zone 1), low-risk drinking (zone 2), mild to moderate alcohol misuse with a lower risk of alcohol dependence (zone 3), and severe alcohol misuse (zone 4).

Cigarette-smoking history was assessed by self-report. In subjects who were either actively smoking cigarettes, or who had previously smoked, the number of packs per day smoked was collected, as well as total number of years smoking, to calculate the pack-year history. In previous (but not current) smokers, the number of years that had elapsed since stopping smoking was also recorded. Current smokers were also analyzed according to packs per day smoked. Nonsmokers were characterized as subjects who did not smoke at all in the past year, moderate smokers currently consumed <1 pack of cigarettes per day, whereas heavy smokers consumed 1 or more packs per day (31). Analyzing subjects in larger groups according to smoking intensity was performed to optimize the clinical applicability of the results, given the variability of cigarette smoking reported in our cohort.

In an effort to minimize the effects of comorbidities on outcome variables, AUD subjects and controls were ineligible to participate in the study if they met any of the following criteria: 1) prior medical history of liver disease (documented history of cirrhosis, total bilirubin ≥2.0 mg/dl, or serum albumin <3.0), 2) prior medical history of gastrointestinal bleeding (due to the concern for varices), 3) prior medical history of heart disease (documentation of ejection fraction <50%, myocardial infarction, or severe valvular dysfunction), 4) prior medical history of renal disease (end-stage renal disease requiring dialysis, or a serum creatinine ≥2 mg/dl), 5) prior medical history of lung disease defined as an abnormal chest radiograph or spirometry (forced vital capacity or forced expiratory volume in 1 s <75%), 6) concurrent illicit drug use defined as a toxicology screen for cocaine, opiates, or methamphetamines, 7) prior history of diabetes mellitus, 8) prior history of human immunodeficiency virus, 9) failure of the patient to provide informed consent, 10) pregnancy, or 11) abnormal nutritional status. The nutritional status was assessed using the nutritional risk index (NRI) with the subject’s albumin, height, and usual weight values in the following equation (17): NRI = 1.519 (albumin in g/l) + (current weight/usual weight) * 100 + 0.417. Subjects were considered to have a normal nutritional status if the NRI was ≥90 and an abnormal NRI if it was <90. Potential subjects >55 yr of age were also excluded to minimize the presence of concomitant but asymptomatic comorbidities.

Study protocol. Eligible subjects with AUDs were admitted to the University of Colorado Hospital Clinical and Translational Research Center (CTRC) for bronchoscopy. All bronchoscopy procedures were performed utilizing telemetry monitoring and standard conscious sedation protocols as previously described (25). The bronchoscope was wedged into a subsegment of either the right middle lobe or the lingula. Three to four 50-ml aliquots of sterile, room temperature 0.9% saline were sequentially instilled and recovered with gentle aspiration. The first aspirated aliquot was not utilized in experiments. The first aspirated aliquot was not utilized in experiments for this investigation. The second and subsequent aliquots were combined and used in experiments as representative of the distal airspaces. AUD subjects were discharged ~24 h after completion of bronchoscopy. Control subjects had bronchoscopy performed in a similar fashion but were discharged the same day.

Laboratory processing. BAL samples were transported to the laboratory in sterile 50-ml conical tubes. BAL fluid was immediately centrifuged (900 g, 5 min) after collection to separate cellular and acellular components. Specimens that were not assayed immediately were aliquoted and stored at −80°C.

Cytokine/growth factor assays. Acellular BAL fluid was used in assays to assess differences in cytokine/chemokine/growth factors within the alveolar space using a multiplex bead array (Bio-Rad Laboratories, Hercules, CA) according to manufacturer instructions and as previously described by our own laboratories (2, 16). Multiplex bead array cytokines, chemokines, and growth factors included: IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, eotaxin, fibroblast growth factor (FGF), granulocyte colony stimulating factor, granulocyte-macrophage colony stimulating factor, interferon (IFN)-γ, IFN-γ-induced protein 10 (IP-10, also known as CXCL10), monocyte chemotactic protein-1 (also

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Table 1. Demographics of subjects with AUDs and controls

<table>
<thead>
<tr>
<th></th>
<th>AUD Subjects, n = 20</th>
<th>Control Subjects, n = 19</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>42.7 ± 7.0</td>
<td>41.4 ± 6.7</td>
<td>0.58</td>
</tr>
<tr>
<td>Current smokers, %</td>
<td>50%</td>
<td>47%</td>
<td>1.0</td>
</tr>
<tr>
<td>Pack-year smoking, mean</td>
<td>13 ± 12</td>
<td>9 ± 14</td>
<td>0.41</td>
</tr>
<tr>
<td>Pack-year smoking, median</td>
<td>10 [0-24]</td>
<td>0 [0-20]</td>
<td>0.18</td>
</tr>
<tr>
<td>Packs per day, mean</td>
<td>0.5 ± 0.4</td>
<td>0.4 ± 0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>Packs per day, median</td>
<td>0.5 [0-0.5]</td>
<td>0 [0-0.6]</td>
<td>0.27</td>
</tr>
<tr>
<td>Sex, % men</td>
<td>65%</td>
<td>47%</td>
<td>0.34</td>
</tr>
<tr>
<td>Race, %</td>
<td>0.05</td>
<td></td>
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|                          |                       |                          |         |
| White                    | 40% (8/20)            | 74% (14/19)              |         |
| African-American         | 10% (2/20)            | 16% (3/19)               |         |
| Latino                   | 30% (6/20)            | 11% (2/19)               |         |
| Native American          | 20% (4/20)            | 0% (0/19)                |         |
| AUDIT Score              | 25.4 ± 9.1            | 2.3 ± 1.8                | <0.0001 |
| Zone 1 (abstinence)      | 0/20                  | 5/19                     | <0.0001 |
| Zone 2 (low-risk drinking)| 0/20                  | 14/19                    |         |
| Zone 3 (moderate alcohol misuse) | 4/20 | 0/19 |         |
| Zone 4 (severe alcohol misuse) | 16/20 | 0/19 |         |
| Days per week drinking  | 5.1 ± 1.7             | 0.7 ± 0.9                | <0.0001 |

AUD, alcohol use disorder; AUDIT, alcohol use disorders identification test; WBC, white blood cell. *Comparison of AUDIT zones between alcohol use disorder subjects and control subjects using Pearson’s test.

RESULTS

Baseline demographics of enrolled subjects. Twenty subjects with AUDs were enrolled in these investigations, along with 19 healthy controls (Table 1). Ages between the two groups were similar as were the percentage of current smokers. There was a nonsignificant difference in the number of men between groups. Racial differences were present between the AUDs and control groups; more Caucasian subjects were enrolled in the control group, whereas more Native Americans were enrolled in the AUD group. Cigarette-smoking habits did not vary significantly by age (P = 0.87), by sex (P = 1.0), or by race (P = 0.08). The AUD group had significantly higher AUDIT scores than did controls, with AUD subjects falling exclusively in consumption zones 3 and 4, whereas control subjects fell in zones 1 and 2.

Cell counts and differentials from BAL were similar between the AUD and non-AUD subjects although smokers in each group had higher total cell counts (P = 0.002 for comparison across all AUD and non-AUD subjects, stratified by smoking, Table 2). Similarly, smokers both with and without AUDs had significantly higher total monocyte counts (P = 0.002). The percentage of each cell type did not vary by alcohol or smoking history.

BAL measurements for IL-2, IL-4, IL-5, IL-9, IL-17, eotaxin, FGF, and MIP-1α were below the detection limit of the assay for the majority of subjects (>50%) assessed; therefore, results for these factors were not analyzed further.

Dose-dependent association between BAL RANTES and alcohol use. Correlations in BAL cytokines were examined in relation to the AUDIT scores from the 39 total subjects collected at the time of bronchoscopy. A positive correlation was observed between the quantity of BAL RANTES and subjects’ AUDIT scores (P = 0.58, P = 0.0001, Fig. 1A). Comparing RANTES across AUDIT zones confirmed that, with increasing severity of alcohol dependence, RANTES was increased (P = 0.001, Fig. 1B). Moreover, values for both zone 3 (P = 0.005) and zone 4 (P = 0.0004) were significantly greater than those from subjects in zone 2 (low-risk drinkers), accounting for multiple comparisons. No significant correlations were observed between AUDIT scores and any other cytokine, chemokine, or growth factor in BAL, including IL-1β (P = 0.83), IL-6 (P = 0.94), TNF-α (P = 0.25), IL-15 (P = 0.11), IP-10 (P = 0.31), IL-8 (P = 0.91), or IFN-γ (P = 0.35). Among all subjects with AUDs, BAL RANTES was positively associated with IP-10 (ρ = 0.53, P = 0.02).

Dose-dependent association of cytokines in BAL and cigarette smoking. Potential associations between cigarette-smoking history and BAL cytokines were examined. Pack-year data were missing from 4 of the 19 currently smoking AUD and control subjects. Five current nonsmokers had stopped smoking more than 1 yr before bronchoscopy. In the cohort of current smokers, there was a significant difference in total monocyte counts (P = 0.002) between AUD and non-AUD subjects. The percentage of each cell type did not vary by alcohol or smoking history.
subjects were stratified into four groups based on a history of AUDs and current cigarette smoking. Ten subjects did not have an AUD and did not smoke; nine did not have an AUD but did smoke; ten had an AUD but did not smoke; and ten had an AUD and smoked. Significant differences were noted to be present across the four groups for BAL IL-1β (P = 0.001), RANTES (P = 0.001), IL-12 (P = 0.0006), and VEGF (P = 0.0003) (Fig. 4). After post hoc within-group comparisons were performed, cigarette smoking, but not alcohol, appeared to be driving both the higher values of BAL IL-1β, as well as the lower values of BAL IL-12 and VEGF. Examining the data among AUD subjects only revealed that subjects with AUDs who smoked cigarettes had significantly elevated BAL IL-1β compared with AUD subjects who did not smoke. However, values for the AUD group who smoked were not significantly different from non-AUD smoking controls. Similarly, for BAL IL-12 and VEGF, among AUD subjects only, lower values were observed in AUD subjects who smoked. However, as with IL-1β, values for the AUD group who smoked were not significantly different from non-AUD subjects who smoked. In contrast, BAL RANTES values among subjects with AUDs were significantly higher compared with non-AUD subjects, regardless of cigarette smoking. In the AUD subjects only, RANTES values were not significantly more elevated among cigarette smokers. Therefore, our data suggest that smoking influences BAL IL-1β, IL-12, and VEGF, independent of AUDs, whereas an AUD history influences RANTES, independent of smoking.

Correlations between demographic factors and cytokine values.

To determine whether specific, unalterable demographic characteristics might have influenced our BAL data, we examined the relationship of age, race, and sex with different cytokine values. We observed that age was associated with decreased IL-7 (P = 0.01). Although our cohort consisted primarily of white subjects, we did examine a number of African-American, Hispanic, and Native Americans as well. However, of the 19 evaluable cytokines/growth factors within BAL, we did not observe any significant differences referable to race to persist after correcting for multiple comparisons.

Correlations between demographic factors and cytokine values.
Similarly, there were no remarkable cytokine differences between women in the cohort compared with men.

**Correlations between resident lung cells and cytokine values.** To help establish the potential cells of origin responsible for cytokine production, correlations between the absolute number of different cell types obtained during BAL and cytokine values were examined. The total white blood cell number per milliliter of BAL fluid was positively associated with IL-1β (ρ = 0.61, P < 0.0001) but inversely associated with IL-12 (ρ = 0.49, P = 0.002) and VEGF (ρ = 0.48, P = 0.002). Results were similar for the absolute monocyte count that was positively associated with IL-1β (ρ = 0.58, P = 0.0001) and negatively associated with IL-12 (ρ = 0.52, P = 0.0006) and VEGF (ρ = 0.51, P = 0.0009). Finally, the absolute neutrophil count was most strongly associated with IL-1β (ρ = 0.48, P = 0.002) and IL-1RA (ρ = 0.48, P = 0.002) but inversely associated with IL-12 (ρ = 0.42, P = 0.007). No correlation between IL-10 or RANTES and the total white blood cell count or any specific cell type was observed.

**DISCUSSION**

In these investigations, AUDs and cigarette smoking were associated with alterations in pulmonary levels of specific cytokines, chemokines, and growth factors. The relationship of these two environmental mediators to measured BAL cytokines appeared related to different inflammatory pathways, and no evidence of synergism between AUDs and smoking was observed. Nevertheless, the elevation of BAL RANTES in association with AUDs, along with elevated IL-1β in association with cigarette smoking, suggests that an independent but potentially additive proinflammatory milieu may be present in individuals who are coexposed to both alcohol and cigarettes. To our knowledge, this is the first report in a sizable cohort of well-characterized human subjects of elevated BAL RANTES in association with AUD severity; alterations in BAL IL-12 and VEGF in the context of cigarette smoking and AUDs are also novel. We confirmed previously reported positive relationships between cigarette smoking with BAL IL-1β expression (31) and decreased VEGF expression (30, 60). Dose-response relationships between cigarette smoking with IL-1β, IL-12, and VEGF were also observed. Collectively, our work suggests that cigarette smoking contributes to a proinflammatory milieu characterized via increasing IL-1β, whereas increases in RANTES associated with AUDs may further accentuate this proinflammatory environment. Moreover, the association of tobacco with decreased IL-12 and VEGF suggests potential influences on cellular immunity and alveolar epithelial permeability related to these substances, independent of AUDs. Our observations provide new, clinically relevant insight regarding the relationship between AUDs, cigarette smoking, and pneumococcal pulmonary disease. These data may also help explain the severity of pneumococcal illness, as several of these cytokines, chemokines, and growth factors have been reported to have specific roles in colonization, infection, and clearance of pneumococcus.

Alveolar macrophages produce type I interferons in response to recognition of pneumococcal DNA. This recognition stimulates RANTES production in infected cells and neighboring alveolar epithelial cells (28). RANTES is a CC chemokine that serves as a chemoattractant for a variety of immune cell types (53). In our subjects with AUDs, RANTES levels correlated with levels of IP-10, a CXC chemokine secreted by cells in response to IFN-γ. In the setting of experimental pneumococcal pneumonia, pulmonary levels of RANTES and IP-10 may be increased (21, 40) and, along with other proinflammatory cytokines, appear to play a role in the pneumococcal immune response. In animal experiments using an EF3030 pneumococcal strain, RANTES also influenced the production of antibodies that limited the progression of nasopharyngeal pneumococcal carriage to frank pneumonia and influenced survival (40). Our observation that BAL RANTES was increased in a dose-dependent fashion among subjects with AUDs suggests a potential protective role of alcohol in pneumococcal infection. However, published investigations have alluded to a critical effect of alcohol metabolism on RANTES expression (70) and suggest that overexpression of RANTES may be pathological. Moreover, RANTES overproduction has recently been reported to decrease T cell progeny and increase myeloid progenitors in a murine model. This could influence immune responses to pathogens and may be important in aging-associ...
ated immunodeficiency (20). As a final consideration, down-regulation of RANTES receptor, CCR5, could also have affected our results; AUDs have previously been associated with a decrease in CD4+ T cell CCR5 density (41) that potentially influences the balance between cellular and humoral immunity. Understanding the effect of alcohol use on RANTES expression in the lung and nasopharynx, as well as its effect on CCR5, its receptor, will clarify the importance of this cytokine on pneumococcal susceptibility among those with AUDs.

Although the association of serious pneumococcal infections and cigarette smoking have been reported consistently in the literature, reasons for this association are incompletely understood. A recent series examining BAL from a large cohort of smokers with no pulmonary symptomatology, normal pulmonary function tests, and chest radiographs provides convincing evidence that current smoking in and of itself leads to an increased total number of recovered cells in BAL (27), suggesting that smoking without frank pulmonary disease can contribute to a proinflammatory state within the lung. In our series of smokers, IL-1β values were most strongly associated with the absolute number of monocytes. The IL-1 family of cytokines is important in early innate immune responses (58); notably, a dose-dependent association between cigarette smoking with BAL IL-1β has previously been reported (31). In animal models exposed to cigarette smoke extract, IL-1β production and signaling along with pulmonary neutrophilia are believed to occur through Toll-like receptor (TLR)4 activation (18). TLRs are key initiators in recognizing pathogen-associated molecular peptides, including pneumolysin, produced by the pneumococcus (32). Pneumolysin binds directly to TLR4, where it results in production of proinflammatory mediators and induces epithelial cell apoptosis (56). In a recently published investigation using an experimental model of pneumococcal infection, chronic cigarette smoke exposure followed by challenge with live S. pneumoniae was associated with increased IL-1β in lung homogenates, BAL neutrophilia,
increased pulmonary bacterial burden, and worsened clinical signs of pneumococcal pneumonia (42). Our investigations suggest the possibility that cigarette smoke exposure results in a baseline increase in the proinflammatory state of the lung. Upon exposure to products of the pneumococcus (i.e., pneumolysin), additional inflammation may further overwhelm the host response, resulting in an inability to clear the pathogen with subsequent disease progression, perhaps due to slowed resolution of neutrophilia in conjunction with damaged alveolar epithelium.

IL-12 is a regulatory cytokine that contributes to both innate and acquired immunity, with a crucial role in host resistance to pneumococcal infection (59). IL-12 is derived from lymphocytes and has the ability to stimulate the proliferation of T cells, induce NK cell formation, and induce IFN-γ production. Chronic cigarette smoke exposure has been associated with a significant increase in BAL IL-12 in a mouse model (8), but no reports in the literature comment on the effect of either alcohol or smoking on this cytokine in lung. Understanding the role of IL-12 on pneumococcal immunity in humans may be important in improving the efficacy of vaccines to combat this pathogen, particularly in high-risk groups (26). IL-12 has been proposed as an adjuvant with recombinant pneumococcal protein antigens in experimental mucosal vaccines (67). Ex vivo human BAL cells that had been treated using this strategy were determined to have increased TNF-α (and to a lesser extent, IFN-γ) production compared with exposure of cells with pneumococcal antigen alone (68). Nevertheless, progress in the use of IL-12 as an adjuvant for human pneumococcal vaccines requires further investigation to limit its toxicity and to optimize efficacious delivery to the mucosa (24).

Cigarette smoking has been associated with decreased BAL VEGF and VEGF receptor-2 expression; levels become more decreased as smoking-related lung disease progresses (30, 60). These abnormalities may be related to the direct effect of cigarette smoke on airway epithelial cells (61), where it can induce cellular apoptosis and necrosis (29). In contrast, moderate doses of alcohol experimentally enhance VEGF expression, as well its interaction with the VEGFR-2 receptor (14–15, 23). Abnormal VEGF homeostasis in the lung elicited by

![Graph A](image1.png)
![Graph B](image2.png)
![Graph C](image3.png)
![Graph D](image4.png)
cigarette smoking could be a factor in the development of complicated pneumococcal infections, such as empyema (66). Through potential effects on alveolar epithelial permeability, alterations in pulmonary VEGF could also play a role in the development of the acute respiratory distress syndrome (ARDS) that may complicate pneumococcal pneumonia. Significantly lower BAL VEGF levels have been reported both in the early and late phases of ARDS, compared with either healthy subjects (1) or patients at risk for ARDS (62). Collectively, these investigations suggest that aberrant pulmonary VEGF homeostasis due to effects of cigarette smoking coupled with effects of AUDs on alveolar epithelial integrity (11) may place individuals who use both substances at risk for increased morbidity in the setting of pneumococcal infection.

We have highlighted potential mechanisms whereby AUDs and cigarette smoking might influence the cytokine milieu in lung to increase the risk for infection with the pneumococcus, given that it is the most common etiological agent for community-acquired pneumonia worldwide. However, it should be noted that AUDs and cigarette smoking also have a significant impact on the predisposition for other common pulmonary infections. A major example would be the relationship between cigarette smoking and the development of influenza (19), where in murine models, cigarette smoke adversely affects the primary immune response to influenza (48). Notably, in the most recent H1N1 epidemic, cigarette smoking increased the risk for hospitalization in the context of this illness (65), further adding to the clinical relevance of this association. Chronic exposure to alcohol in animal models enhances the severity of influenza infection (35) and has also been associated with the development of seasonal influenza, but not specifically H1N1 (47). Improved understanding of the relationship between smoking, AUDs, and influenza could be important given pneumonia that commonly occurs after primary influenza infections. Tuberculosis is another important pulmonary disease frequently linked to AUDs and cigarette smoking. Worldwide, a very strong association between AUDs and tuberculosis has been reported, with ~10% of cases linked to alcoholism. Several countries have proposed measures to address both the pulmonary disease concomitantly with AUDs to improve the efficacy of directly observed therapies (44). The literature further supports cigarette smoking as a modifiable risk factor for tuberculosis infection as well as the development of symptomatic tuberculosis (3). Our work hopefully provides some insight for investigations related to these and other important alcohol- and smoking-related pulmonary diseases.

Our investigations are not without limitations. First, the total number of subjects enrolled in these investigations was not large, and therefore the power of the associations we observed is limited. Nevertheless, this is the largest cohort of closely matched human subjects with well-characterized alcohol use and cigarette smoking data examined via bronchoscopy that we have encountered in the literature. Given ethical issues with bronchoscopy in asymptomatic human subjects, we believe that our results provide a translational perspective to previously published animal and human work. Secondly, smoking and alcohol history were provided by self-report that might limit the accuracy of these data. Certainly, biological assessment of smoking (e.g., serum cotinine) could better clarify dose-response relationships between cigarette smoking and cytokines, but biological markers for severity of alcohol misuse are lacking. Nevertheless, subjects with AUDs were recruited from a detoxification center that had a long-standing treatment relationship with these individuals, enhancing the validity of their AUD characterization. Potential unmeasured confounders might have influenced our results, such as occult liver disease. However, screening laboratory work and pulmonary examination suggested no occult illness in our subjects or controls. Finally, we acknowledge that these investigations do not prove that alcohol or cigarettes are directly causal to the cytokine differences we observed. Our work does not explain the operative mechanisms underlying the reasons that AUDs, cigarette smoking, or both may contribute to pulmonary infections, including pneumococcal pneumonia. It does, however, provide a framework to understand the potential associations of AUDs and smoking on pulmonary cytokines, thereby directing future investigations to help unravel mechanisms of increased predisposition for pulmonary infections in this population.

In conclusion, we observed an association between the proinflammatory cytokine RANTES and AUDs that was independent of cigarette smoking. We confirmed that cigarette smoking was associated with an increase in the proinflammatory cytokine IL-1β, as well as decreases in IL-12 and VEGF. These abnormalities suggest an excessively proinflammatory state in the lung in the setting of AUDs and cigarette smoking and also potential influences on adaptive immunity (via IL-12) and alterations in epithelial permeability (via VEGF). Collectively, these observations can help delineate the relationship of AUDs and cigarette smoking on the predisposition for pneumococcal pulmonary infection and also provide insight regarding reasons for increased disease severity among these individuals.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: E.L.B., E.J.K., and C.S.D. conception and design of experiments; E.L.B., E.J.K., and C.S.D. performed experiments; E.L.B. prepared figures; E.L.B. drafted manuscript; E.L.B., E.J.K., and C.S.D. interpreted results of experiments; E.L.B., E.J.K., and C.S.D. approved final version of manuscript; C.S.D. performed experiments.

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BRONCHOALVEOLAR LAVAGE CYTOKINES IN ALCOHOLISM AND SMOKING


