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Alveolar macrophage inflammatory mediator expression is elevated in the setting of alcohol use disorders

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ABSTRACT

Alcohol use disorders (AUDs) are associated with increased susceptibility to pulmonary diseases, including bacterial pneumonia and acute respiratory distress syndrome (ARDS). Alveolar macrophages (AMs) play a vital role in the clearance of pathogens and regulation of inflammation, but these functions may be impaired in the setting of alcohol exposure. We examined the effect of AUDs on profiles of cytokines, chemokines, and growth factors in human AMs isolated from bronchoalveolar lavage (BAL) samples from 19 AUD subjects and 20 age-, sex-, and smoking-matched control subjects. By multiplex bead array, the lysates of AMs from subjects with AUDs had significant elevation in the cytokine tumor necrosis factor α (TNF-α), as well as chemokine (C-X-C motif) ligand 8 (CXCL8), CXCL10, and chemokine (C–C motif) ligand 5 (CCL5) ($p < 0.05$). Additionally, a 1.8-fold increase in IL-1β, 2.0-fold increase in IL-6, 2.3-fold increase in interferon gamma (IFN-γ), 1.4-fold increase in CCL3, and a 2.3-fold increase in CCL4 was observed in the AUD group as compared to the control group. We also observed compensatory increases in the anti-inflammatory cytokine IL-1RA ($p < 0.05$). AUD subjects had 5-fold higher levels of CXCL11 mRNA expression ($p < 0.05$) and a 2.4-fold increase in IL-6 mRNA expression by RT-PCR as well. In these investigations, alcohol use disorders were associated with functional changes in human AMs, suggesting that chronic alcohol exposure portends a chronically pro-inflammatory profile in these cells.

Introduction

Alcohol use disorders (AUDs), including alcohol abuse and alcohol dependence, affect approximately 17 million adults in the United States each year (SAMHSA, 2012). The lifetime prevalence of AUDs is over 30% in the United States, with a higher prevalence in males than females (Hasin, Stinson, Ogburn, & Grant, 2007). AUDs are characterized by recidivism and are potentially lethal, shortening lifespans of affected individuals by more than a decade (SAMHSA, 2012; Schuckit, 2009). In addition, alcohol use leads to significant functional derangements in nearly every organ system and is responsible for a substantial portion of the health-care burden in almost all populations (Schuckit, 2009).

AUDs have been associated with an enhanced susceptibility to a number of pulmonary diseases, including bacterial pneumonia and acute respiratory distress syndrome (ARDS) (Moss, Bucher, Moore, Moore, & Parsons, 1996; Moss et al., 1999; SAMHSA, 2012; van der Poll & Opal, 2009). In addition to an increased risk for contracting community-acquired bacterial pneumonia, AUD patients who succumb to community-acquired pneumonia tend to have greater symptom severity, increased length of hospitalization, more frequent intensive care unit admission, increased need for mechanical ventilation, and poorer survival (de Roux et al., 2006; Fernández-Solá et al., 1995). Individuals with AUDs also display an increased risk for the development of ARDS, and in this setting, have a more treacherous clinical course, with greater chances of developing non-pulmonary organ dysfunction and increased mortality (Moss et al., 1996, 1999).

Alveolar macrophages (AMs) are the primary effector of pulmonary innate immunity, equipped with a variety of processes implicating them in the pathophysiology of both pneumonia and ARDS. They are characterized by the ability to avidly phagocytose pathogens in the terminal airways, a function vital to the defense against bacterial pneumonia (Nelson & Summer, 1998). Another

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function, perhaps just as important in defending against pneumonia, is the ability to recruit granulocytes from the systemic circulation by generating chemotactic mediators. Additionally, AMs are the primary cells that ingest apoptotic granulocytes in ARDS to facilitate resolution of pulmonary inflammation, a process known as efferocytosis (Boé et al., 2010; Haslett, 1999).

The relationship of AUDs to impaired AM function may therefore contribute to the observed associations between bacterial pneumonia and ARDS that have been consistently described. Several studies conducted in animal models have reported that AM functions are significantly altered in the setting of alcohol consumption, including a diminished ability to phagocytose bacteria (Boé et al., 2010; Rimland & Hand, 1980; Romero et al., 2014). Human studies have shown increased intrapulmonary oxidative stress in the setting of AUDs, but knowledge of the effects of AUDs on human AM functions remains limited (Antony, Godbey, Hott, & Queener, 1993; Brown, Harris, Bechara, & Guidot, 2001; D'Souza, Nelson, Summer, & Deaciuc, 1996; Guidot & Brown, 2000).

We and others have previously investigated the cytokine composition of BAL fluid from control and AUD subjects (Burnham, Kovacs, & Davis, 2013). While examination of this fluid offers insight into the global pulmonary environment, it does not clearly identify which lung cells are responsible for the secretion of these cytokines. Additionally, cytokine measurements in lavage may be influenced by variable between-subject yield of the procedure, making measurements of these mediators difficult to normalize and interpret. We sought to characterize the AM population in a cohort of patients with AUDs by delineating the cytokine, chemokine, and growth factor profiles of these cells, and to determine whether a pro- or anti-inflammatory state exists in these immune effectors in the absence of overt pulmonary symptoms. We hypothesized that the AM population of AUD subjects would demonstrate an altered inflammatory mediator profile in comparison to control subjects.

Materials and methods

Subject screening, recruitment, and enrollment

Subjects with AUDs were recruited between May 2012 and January 2014 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, Colorado. Control patients without AUDs were recruited via approved print and electronic flyer advertisements in the Denver, Colorado community. This study was approved by the Colorado Multiple Institutional Review Board (COMIRB). All subjects provided written informed consent before their participation in the study.

The Alcohol Use Disorders Identification Test (AUDIT) questionnaire was used for identification of AUD subjects and appropriate controls. The AUDIT questionnaire is a well verified and widely used 10-question screening tool used to identify hazardous and harmful patterns of alcohol consumption in the clinical setting. A score of 8 and above in men, or 5 and above in women, identifies both heavy drinkers and those with AUDs, with a sensitivity of 50–90% and a specificity of about 80% (Reinert & Allen, 2002; Schuckit, 2009).

Subjects with AUDs were eligible to participate if they met the following criteria for study entry: i) an Alcohol Use Disorders Identification Test (AUDIT) score of greater than or equal to 8 for men or greater than or equal to 5 for women; ii) alcohol use within 7 days of enrollment; and iii) age greater than or equal to 21 years. AUD subjects were not approached for enrollment until a sober time extrapolated from their presenting breathalyzer value had been reached. To be eligible for the control arm of the study, subjects were required to have an AUDIT score of less than these values based on gender, corresponding to abstinence or a low risk of alcohol-related problems. Cigarette smoking history was assessed by self-report.

In an effort to minimize potential confounding by comorbidities, subjects were ineligible to participate in the study if they met any of the following criteria: i) prior medical history of liver disease (documented history of cirrhosis, total bilirubin $\geq$ 2 mg/dL, or albumin $<$ 3.0 g/dL), ii) prior medical history of gastrointestinal bleeding (because of the concern of varices), iii) prior medical history of heart disease (documentation of ejection fraction $<$50%, myocardial infarction, or severe valvular dysfunction), iv) prior medical history of renal disease (end-stage renal disease requiring dialysis, or a serum creatinine $\geq$2.0 mg/dL), v) prior medical history of lung disease defined as an abnormal chest radiograph or spirometry (percent predicted forced vital capacity [FEV_1] $<$75%), vi) prior history of diabetes mellitus, vii) prior history of HIV infection, viii) pregnancy, ix) abnormal nutritional risk index (Detsky et al., 1984), or x) age $>$55 years due to the potentially high prevalence of asymptomatic comorbidities in these individuals. A chest radiograph was taken of each subject before enrollment; subjects were excluded if there was any evidence of an acute process or a lesion that might necessitate clinical bronchoscopy. Subjects who failed to provide informed consent were also excluded.

Whole blood samples were obtained from all subjects upon enrollment for complete blood count (CBC), complete metabolic panel (CMP), and prothrombin time with international normalized ratio (INR). Urine samples were also obtained upon enrollment to screen for cocaine, opioids, and methamphetamines. Pulmonary function tests (PFTs) were completed prior to performance of the bronchoalveolar lavage (BAL).

Subject matching

Control subjects were group-matched to AUD subjects on the basis of age, gender, smoking status, and self-reported race.

Bronchoscopy BAL

Bronchoscopy procedures were performed in the inpatient Clinical and Translational Research Center (CTRC) at the University of Colorado Hospital. All procedures were performed utilizing telemetry monitoring and standard conscious sedation protocols as previously described (Burnham et al., 2011; Hunninghake, Gadek, Kawanami, Ferrans, & Crystal, 1979). The bronchoscope was wedged into a sub-segment of either the right middle lobe or the lingula. Three to four 50-mL aliquots of sterile, room-temperature, 0.9% NaCl solution were sequentially instilled and recovered with gentle aspiration. BAL fluid was then transported to the laboratory immediately in sterile 50-mL conical tubes for processing. The first aliquot was not utilized for the performance of assays in this investigation.

Cell count, differential, and viability from BAL samples

Using a small aliquot of the collected BAL fluid, cell count per milliliter was determined by microscopy as previously described (Burnham et al., 2011). Diff-quick™ (Andwin Scientific, Addison, IL) staining was performed. A minimum of 200 cells was evaluated to determine the differential cell types in all samples in a blinded manner. Viability of the cells was determined by Trypan blue exclusion, and was $>$97% for all samples analyzed.