The Effects of Smoking on the Lipopolysaccharide Response and Glucocorticoid Sensitivity of Alveolar Macrophages of Patients With Asthma

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Background: **Cigarette smoking in asthma patients causes insensitivity to inhaled glucocorticoids (GCs). We tested the hypothesis that smoking causes GC insensitivity in alveolar macrophages (AMs) obtained from patients with asthma.**

Methods: **Nineteen asthmatic nonsmokers (ANSs) and 13 asthmatic smokers (ASMs) underwent BAL. AMs were cultured with or without dexamethasone, 0.1 to 1,000 nmol/L, for 2 h before lipopolysaccharide (LPS) [1 μg/mL] stimulation. After 6 h, supernatants were harvested for enzyme-linked immunosorbent assay, and messenger RNA was collected for real-time (RT)-polymerase chain reaction (PCR).**

Results: **ASMs had higher numbers of AMs per milliliter of BAL fluid than ANSs (1.98 vs 0.75×10^6 cells/mL, respectively; $p = 0.007$). Cigarette smoking significantly attenuated the LPS response for all three cytokines tested among ANSs vs ASMs (tumor necrosis factor [TNF]-α, 31.6 vs 10.6 ng/mL, respectively ($p = 0.01$); interleukin [IL]-6, 25.8 vs 10.8 ng/mL, respectively ($p = 0.002$); IL-8, 62.5 vs 36.1 ng/mL, respectively ($p = 0.001$)). There was no difference in dexamethasone dose-response curves between ANSs and ASMs ($p > 0.05$ for all comparisons). The inhibitory concentration of 50% (IC_{50}) for IL-6 was 120.6 vs 83.3, respectively, and for TNF-α it was 4.9 vs 8.6, respectively; an IC_{50} was not achieved for IL-8. RT-PCR also showed no difference in the suppression of cytokine messenger RNA levels between groups, with IL-8 being the most GC-insensitive cytokine.**

Conclusion: **Cigarette smoking in patients with asthma increases the number of airway AMs and attenuates their response to LPS, which may have implications in host immune function. Cigarette smoking does not alter the GC sensitivity of AMs in patients with asthma. There was differential cytokine sensitivity, with IL-8 being the least GC-sensitive cytokine. GC-insensitive IL-8 production from AMs may be a mechanism by which neutrophils are attracted into the airways.** ***(CHEST 2009; 136:163–170)***

Abbreviations: AM = alveolar macrophage; ANS = asthmatic nonsmoker; ASM = asthmatic smoker; CO = carbon monoxide; ELISA = enzyme-linked immunosorbent assay; GC = glucocorticoid; IC_{50} = inhibitory concentration of 50%; ICS = inhaled corticosteroid; IL = interleukin; LPS = lipopolysaccharide; PCR = real time polymerase chain reaction; RPMI = Roswell Park Memorial Institute; RT = real time; TNF = tumor necrosis factor

Cigarette smoking increases the number of inflammatory cells in the airways of healthy subjects, including neutrophils and alveolar macrophages (AMs).[1–3] Similarly, smoking increases neutrophil numbers in the airways of patients with asthma,[4,5] although to our knowledge, the effect of smoking on AMs in patients with asthma has not been studied. These cells are of importance in asthma because they are key players in the innate immune response and are capable of releasing a broad range of cytokines and chemokines.[6]

Acute cigarette smoke exposure up-regulates the production of some inflammatory mediators from macrophages, most notably interleukin (IL)-8,[7,8] but also down-regulates other cytokines involved in the macrophage innate immune response, including tumor necrosis factor (TNF)-α, IL-1, and IL-6.[8,9] Chen et al[10] reported that AMs from healthy long-term smokers secrete lower levels of proinflammatory cytokines in response to stimulation with the Toll-like receptor 4 agonist lipopolysaccharide (LPS

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[also known as *bacterial endotoxin*]), compared to nonsmoking control subjects. This was associated with impaired activation of the transcription factor nuclear factor-κB and reduced p38 mitogen-activated protein kinase activation. The reduced AM innate immune response in smokers may have implications for host defense against microbial pathogens.

Smokers in whom COPD develops have a limited clinical response to glucocorticoid (GC) treatment.[11] AMs from COPD patients have been reported to be GC insensitive,[7] and thus may play a key role in the limited clinical response to GC therapy. A variety of mechanisms have been proposed to explain the GC resistance of AMs in patients with COPD, including decreased histone deacetylase 2 activity associated with chromatin remodeling.[12] GC resistance has been observed in clinical trials of asthmatic smokers (ASMs),[13–15] but we are unaware of any studies that have assessed changes in the sensitivity of AMs to GC caused by smoking in patients with asthma.

We hypothesized that the production of inflammatory cytokines would be altered by cigarette smoking in patients with asthma and smoking would change the sensitivity of these cells to GC. This article reports a comparison of the LPS-stimulated production of cytokines from AMs from smoking and nonsmoking patients with asthma, and the effects of GC on these responses.

Materials and Methods

Subjects

Nineteen asthmatic nonsmokers (ANSs) and 13 ASMs were recruited through media advertising. Inclusion criteria were as follows: physician diagnosis of asthma from childhood, current asthma symptoms as defined by guidelines,[16] reversibility of ≥ 200 mL to salbutamol or PC_{20} AMP (provocative concentration of adenosine monophosphate causing a 20% fall in FEV_1) of < 100 mg/mL, treatment with at least a short-acting inhaled β-agonist, and age < 50 years. Subjects receiving oral GCs were excluded. ASMs had a history of > 10 pack-years of cigarette smoking and were current smokers. Subjects with a clinical history of COPD or asthma exacerbation within 4 weeks, defined as worsening of asthma symptoms resulting in any change in asthma therapy implemented by a physician or a change in regular asthma therapy within 4 weeks, were excluded. Written informed consent was obtained, and the local ethics committee approved the study.

Study Design

A medical history, physical examination, allergen skin-prick test, exhaled carbon monoxide (CO), asthma control score,[17] spirometry, total lung capacity, and diffusing capacity were performed. Bronchoscopy was performed on a separate day.

Allergen Skin-Prick Test

House dust mite (*Dermatophagoides pteronyssinus*), cat hair, and grass pollen (Bayer; Elkhart, IN) allergens were used. Normal saline solution and histamine were used as negative and positive controls, respectively. Sensitization was defined as a wheal diameter 3 mm more than the negative control.

Pulmonary Function Measurements

Total lung capacity measurements were carried out as previously described.[18] Spirometry was performed using a dry wedge spirometer (Vitalograph; Buckinghamshire, UK) according to standard guidelines.[19]

Exhaled CO Measurement

Exhaled CO was measured (Micro CO meter; Micromedical Ltd; Basingstoke, UK). After breath-holding for 20 s, subjects exhaled slowly into the meter; reading results were reported in parts per million.

Bronchoscopy and BAL Processing

The bronchoscope was wedged in the right or left upper lobes, and a maximum of four 60-mL aliquots of prewarmed sterile 0.9% saline solution were instilled into each lung (maximum, 480 mL). The total volume instilled depended on how well the patient tolerated the procedure. The aspirated fluid was stored on ice until filtration (100-μm filter; BD Biosciences; Oxford, UK) and centrifugation at 400*g* for 10 min at 4°C. The cell pellet was washed in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 2 mmol/L L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin. Total viable cell count and total AM cell counts were determined by trypan blue exclusion (Neubauer hemocytometer). The cell suspension was adjusted to 1×10^6 AMs per milliliter in supplemented RPMI 1640 medium with 10% fetal calf serum. Cytospins were prepared for differential cell counts.

Cell Culture

Cells were plated at 1×10^5 cells per plate in 96-well plates and allowed to adhere for 2 h at 37°C in 5% carbon dioxide. The supernatant was removed and wells washed twice with RPMI medium. Cells were then preincubated for 2 h with dexametha-

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