

Letter to the Editor

Airway microbiome and responses to corticosteroids in corticosteroid-resistant asthma patients treated with acid suppression medications

To the Editor:

Patients with refractory, difficult to treat asthma often have gastroesophageal reflux disease (GERD).¹ Studies to date emphasize the complexity of interactions between the respiratory and gastrointestinal system. This makes it challenging to discern the mechanisms that link reflux and lung disease.¹ Acid suppression medications—proton pump inhibitors (PPIs) and histamine receptor 2 antagonists (H2 blockers)—are widely prescribed in patients with asthma and chronic cough. Studies also report that esophageal microbiota are subject to change in health and disease, including in patients with esophagitis and GERD.² In large population studies, the use of acid suppression drugs has been associated with the development of community-acquired pneumonia, often of *Streptococcal* etiology.^{3,4} Importantly, *Streptococcus* spp had been reported to be able to tolerate an acidic environment better than other microorganisms.⁵ To date there are a lack of studies about the contribution of GERD and acid suppressor medications on airway microbiome composition in asthma, disease severity, and response to corticosteroid treatment.

Our group recently evaluated airway microbiome in bronchoalveolar lavage samples (BAL) of asthmatic patients identified clinically as corticosteroid resistant (CR) or corticosteroid sensitive based on responses to oral prednisone treatment.⁶ Fourteen of 29 CR asthmatics examined demonstrated airway expansion of specific gram-negative bacteria. We demonstrated that pathogenic (*Haemophilus parainfluenzae*), but not commensal (*Prevotella melaninogenica*), bacteria can inhibit corticosteroid responses of asthmatic BAL macrophages and peripheral blood monocytes.⁶ The relationship between the airway microbiome and corticosteroid resistance in the remaining CR asthmatics was not defined. To determine other factors that contribute to the development of corticosteroid resistance in the remaining patients, we performed *post hoc* assessments of these patients' medication history. We found that 6 of 15 CR asthmatics from this group had been using PPIs (n = 3) or H2 blockers (n = 3). These patients were categorized *post hoc* as patients treated with acid suppression medications. GERD was not formally evaluated in this study. The study was approved by the Institutional Review Board at National Jewish Health, Denver, Colorado. Detailed patient characteristics were provided in the original study.⁶ All study patients were not smokers. Microbiome comparisons between the 2 groups of CR asthma patients treated or not treated with acid suppression medications were performed individually for each taxa using the 2-part test in Explicet 2.8.4 software (www.explicet.org; Boulder, Colo).

By subgrouping CR asthmatics into patients treated with acid suppression medications or not on acid suppression medications, we found that CR asthmatics treated with acid suppression medications had unique alterations in their airway microbiome, with an increase in percentage of sequences for phylum

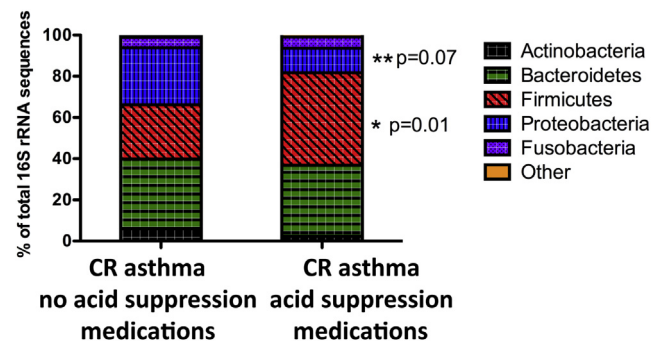


FIG 1. Changes in the airway microbiome composition of CR asthmatics treated with acid suppression medications. Distribution of major bacterial phyla based on 16S ribosomal RNA (rRNA) sequencing data of BAL samples of CR asthmatics on and not on acid suppression medications is provided.

Firmicutes ($P = .0134$) (Fig 1). This was mainly due to expansion of the genus *Streptococcus* ($P = .0028$) and reduced percentage of sequences for phylum *Proteobacteria* ($P = .07$). No expansions of other *Firmicutes*, including genus *Staphylococcus* was observed. Analysis of *Streptococcus* sequences revealed that the predominant species of genus *Streptococcus* in CR patients on acid suppression medications were *S mitis* (present in 6 of 6 patients) and *S pseudopneumoniae* (present in 2 of 6 CR patients). Both of these strains belong to the opportunistic pathogens in the *S mitis* group and are related to *S pneumoniae*.^{7,8} In addition, a variety of commensal *Streptococcus* spp were also observed (*S oralis*, *S perioris*, *S infantis* – all *S mitis* group commensals; *S sanguinis*, *S parasanguinis*—*S sanguinis* group commensals closely related to *S mitis* group commensals; *S salivarius*—a *S thermophilus* group commensal).

In the current study, we used primary human bronchial epithelial cells to examine cellular responses to various *Streptococcus* spp. The details of the cell culture conditions are provided in this article's Online Repository at www.jacionline.org.

Incubation of primary bronchial epithelial cells with *S mitis* and *S pseudopneumoniae* (*S pseudo*) resulted in dose-dependent p38 mitogen activated protein kinase (MAPK) activation (Fig 2, A and B) in the cells as detected by Western blot. The highest p38 MAPK activation was observed with the cells cultured in the presence of 100:1 *S mitis* or *S pseudo*. In contrast, cells cultured with the airway commensal organism *S oralis* poorly activated p38 MAPK. Incubation of primary bronchial epithelial cells with *S mitis* or *S pseudo* reduced cellular responsiveness to corticosteroids *in vitro* (Fig 2, C and D) as detected by reduced ability of dexamethasone (DEX) to induce mitogen-induced kinase phosphatase 1 and reduced the ability of DEX to suppress IL-8 as shown by real-time PCR. Cells cultured with *S oralis* remained sensitive to corticosteroid treatment.

Our data suggest that opportunistic pathogens of the *S mitis* group, *S mitis* and *S pseudo*, in comparison to commensal *S oralis* reduce bronchial epithelial cell responses to corticosteroids and, therefore, may support persistent inflammation in the lung. The bacterial factors responsible for differential activation of

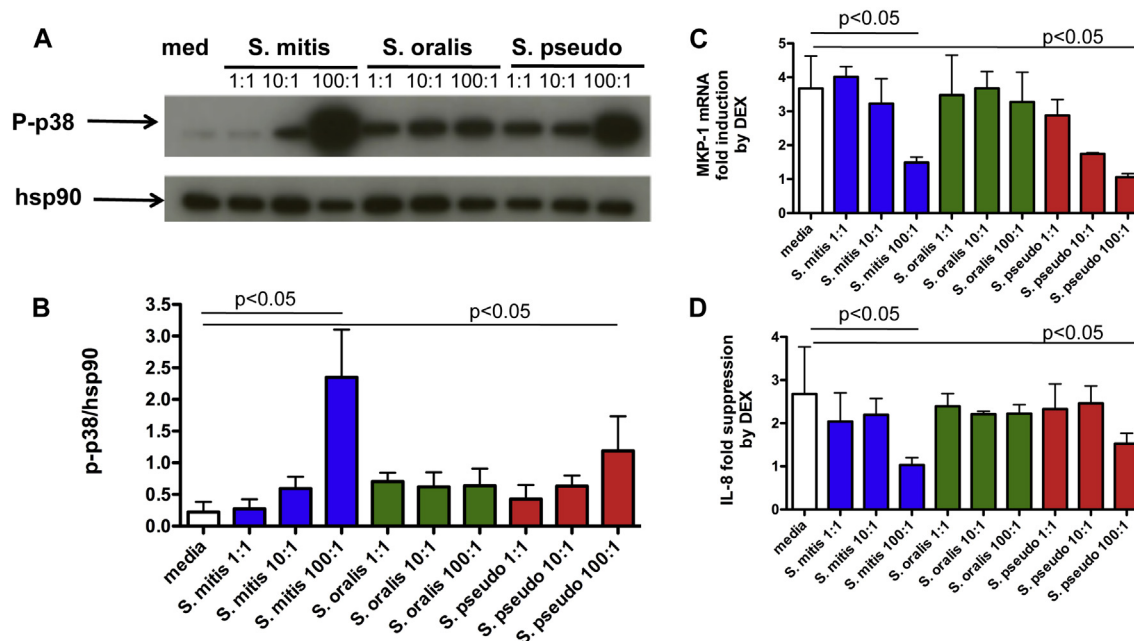


FIG 2. Effects of different *Streptococci* on primary bronchial epithelial cells activation and response to corticosteroids *in vitro*. **A** and **B**, p38 MAPK activation in the cells as detected by Western blot. **C** and **D**, Cellular production of mitogen-induced kinase phosphatase 1 (MKP-1) and IL-8 mRNA in response to corticosteroids *in vitro* as shown by real-time PCR. The experiments were done in 3 different primary bronchial epithelial cell lines.

bronchial epithelial cells by *S mitis* and *S pseudo* in comparison to *S oralis* need to be defined, but likely they are related to the virulence factors produced by these bacteria.^{7,8} The reason for the expansion of *Streptococci* in patients on acid suppression medications requires further investigation. *Streptococci* are the predominant organisms in the esophagus, representing approximately 40% of the bacterial communities.² It is possible that reflux in severe asthmatics makes them prone to exchanges of bacterial microbiota between esophagus and airways (aerodigestive exchanges). It has also been suggested that PPIs may directly target proton pumps of certain bacteria that contain P-type ATPase enzymes and/or indirectly affect the environment of bacteria by increasing the pH.⁹ Therefore the expansions of *Streptococci* may occur due to various factors, that is, aerodigestive exchanges due to GERD, the direct response to acid suppression medications and/or due to the changes in bacterial environment that provide favorable conditions for *Streptococci* growth in the airways.

In conclusion, our study demonstrates expansion of *Streptococci* in the airways of CR asthmatics taking acid suppression medications. We also provide evidence that certain strains of *Streptococci* can influence the response of bronchial epithelial cells to corticosteroids. We therefore suggest that comprehensive evaluation of aerodigestive interactions in severe asthma should include the potential role of acid suppression medications (PPIs and H2 blockers) and their effects on the esophageal and lower airway microbiome as it relates to corticosteroid response.

Elena Goleva, PhD^a

J. Kirk Harris, PhD^b

Charles E. Robertson, PhD^c

Leisa P. Jackson, BS^a

Richard J. Martin, MD^{c,d}

Donald Y. M. Leung, MD, PhD^{a,b}

From ^athe Department of Pediatrics and ^dthe Department of Medicine, National Jewish Health, Denver, Colo; and ^bthe Department of Pediatrics and ^cthe Department of Medicine, University of Colorado at Denver and Health Sciences Center, Aurora, Colo. E-mail: golevae@njhealth.org.

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METHODS

S. pseudopneumoniae (BAA-960), *S. mitis* (ATCC 49456) and *S. oralis* (ATCC 9811) were obtained from ATCC (Manassas, Va) and cultured in brain heart infusion broth according to ATCC's instructions. On the day of the experiment, bacterial concentrations were calculated based on OD at 620 nm and standard curves generated for each strain to determine the relationship between colony forming units and OD. Bacteria were pelleted and resuspended in bronchial epithelium basal medium (BEBM) (Lonza, Walkersville, Md) without supplements and antibiotics and added to the cells. Primary bronchial epithelial cell lines from asthma patients obtained from National Jewish Health BioBank (Denver, Colo) were expanded on collagen-coated dishes in BEBM with supplements (Lonza). Following the expansion, the cells were cultured overnight in BEBM without supplements and antibiotics, pretreated with bacteria for 15 minutes followed by 3 hours of treatment with 10^{-6} mol/L DEX or medium and analyzed by Western blot and real-time PCR. We used 5×10^4 cells per condition (bacterium-cell ratio 1:1, 10:1, and 100:1). It was estimated that asthmatic

airways contain a median of 2,000 bacterial genomes (maximum up to 200,000 bacterial genomes) per cm^2 of airway surface.^{E1} Lung morphometry measurements estimated about 30,000 epithelial cells per cm^2 in human bronchioli.^{E2} Based on these data, we estimate the bacterial load in the asthmatics airways is about 10 bacteria per airway epithelial cell. This load can be greater during the infection. Therefore the ratio of 100 bacteria per cell is likely to occur *in vivo*.

Cellular protein extraction, RNA preparation, Western blot, and real-time PCR were performed as previously described by us.^{E3}

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