Effects of Early House Dust Mite Exposure on the Immunologic Response in Nasal Mucosal Cells of Rhesus Macaque Monkeys

Jonathan Liang, MD 1; Justin Fontaine, BS 2; Rebecca Fishman, BS 2; Joan Gerriets, PhD 2; Lisa Miller, PhD 2,3
1 Department of Otolaryngology, University of California Davis Medical Center, Sacramento, CA
2 Department of Anatomy, Physiology, and Cell Biology, School of Veterinary Medicine, University of California, Davis, CA
3 California National Primate Research Center, Davis, CA

Introduction
Nasal epithelial cells are the first cells exposed to environmental stimuli that are associated with airway inflammation. These cells defend against allergens and microorganisms through both physical and chemical barriers. They play an active role in the immunologic response through the production of a variety of cytokines, chemokines, and other mediators. Interleukin-8 (IL-8) and interleukin-6 (IL-6) are pro-inflammatory cytokines, and IL-6 also plays a key role in the Th2-type immune response. House dust mites (HDM) are complex organisms that produce a plethora of different proteins and other macromolecules. Dermatophagoides pteronyssinus (Der p) and Dermatophagoides farinae are the major causes of allergic disease. HDM present to the airway epithelium. We aim to study whether early allergen exposure with house dust mites affects nasal epithelium in a similar fashion. Changes in the immune response and inflammatory profile of nasal epithelial cells can have major implications in the approach and treatment of allergy-mediated diseases, such as rhinitis, sinusitis, laryngitis, and asthma.

Methods & Materials

Animals
We studied male rhesus macaque (Macaca mulatta) monkeys housed at the California National Primate Research Center. The animals were sensitized to Der p1 and Der p2 antigens via subcutaneous injection at 2 and 4 weeks of age. Between 1 month of age and 6 months of age the animals were exposed to 11 cycles of filtered air (FA) or house dust mite allergen (HDM). Each cycle consisted of 3 days (2 hours per day) of HDM followed by 11 days of FA. HDM exposure was performed in a chamber with a 2% aliphylized extract of Der p. All animals were raised in filtered air conditions until 3 years of age. All animals were necropsied at 3 years of age, and nasal mucosal tissues were removed. This concept of persistent airway remodeling after early exposure (HDM) and control (FA) animals. Although inflammation plays a key role in allergic response, other pro-inflammatory mediators may be involved. In this study, the production of a variety of cytokines, chemokines, and other mediators was measured.

Cell Culture
All animals were necropsied at 3 years of age, and nasal epithelial cells from the septum and turbinates were isolated by protease treatment and cryopreserved. Cells were plated on 12-mm transwells with Bronchial Epithelial Growth Media (BEGM) supplemented with 2% serum. The media was changed every 2 days. Cells were cultured in BEGM until 80% confluent under microscopy. Cells were then cultured via air-liquid interface (ALI) method for 7 days. The media was changed every 2 days.

IL-6 Results
FA animals showed significant dose-dependent differences in relative IL-6 expression both apically (ANOVA, p = 0.019) and basolaterally (ANOVA, p = 0.002). HDM animals showed significant dose-dependent differences in relative IL-6 expression only apically (ANOVA, p = 0.029). HDM animals showed significant increased relative IL-6 apical expression (t-test, p = 0.021) and decreased relative IL-6 basolateral expression compared with controls (p = 0.033).

IL-8 Results
FA animals showed a significant dose-dependent difference in relative IL-8 expression basolaterally (ANOVA, p = 0.006), and a similar trend apically (ANOVA, p = 0.047). HDM animals did not show any significant dose-dependent differences in relative IL-8 apically or basolaterally. There were no significant differences in relative IL-8 expression between HDM animals and FA animals at any HDM exposure concentration.

Conclusions
House dust mite exposure demonstrates a dose-dependent response in relative IL-6 expression for both early exposure (HDM) and control (FA) animals. No difference in relative IL-8 expression was seen between early exposure (HDM) and control (FA) animals. Although inflammation plays a key role in allergic response, other pro-inflammatory mediators may be involved. At high concentrations of HDM allergen, relative apical IL-6 expression is heightened and relative basolateral IL-6 expression is dampened in early HDM exposure animals. This suggests that early life allergen exposure may lead to alcaline remodeling in nasal mucosal cells with a persistent or enhanced Th2-type immune response.

References