Antibiotic-induced *Malassezia* spp. expansion in infants elicits intestinal immune dysregulation and increased airway inflammation in mice

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Background: The neonatal immune system undergoes important developmental changes that are dependent on microbial colonization post-birth. Early-life antibiotic use impacts the gut microbiome, leading to immune dysregulation and increased risk of childhood asthma. However, it is currently unknown if the fungal microbiome (mycobiome) contributes to antibiotic-induced immune dysregulation conducive to allergic asthma. We aimed to evaluate the effect of antibiotic-induced mycobiome changes on neonatal immune development and experimental airway inflammation model.

Methods: We ran an observational, prospective clinical study of 47 young infants (<6 months of age) receiving antibiotics. We compared the bacterial and fungal microbiome in fecal samples collected before and after antibiotics via shallow shotgun (bacteria) and ITS2 (fingi) sequencing, as well as qPCR for fungal and bacterial DNA. We then compared immune development and susceptibility to airway inflammation in gnotobiotic mice colonized with consortia of 12 mouse-derived bacteria (Oligo-MM12), or bacteria with *Candida albicans*, *Saccharomyces cerevisiae* and/or *Malassezia restricta*, to evaluate the effect of *Malassezia* colonization on host immunity, differentiating it from other common fungal colonizers.

Results: Antibiotic use decreased bacterial and increased fecal fungal DNA and induced expansion of *Malassezia* spp. in infants. *M. restricta* colonization increased Th2 cells, eosinophils, and delayed macrophage maturation in the colonic lamina propria. *M. restricta* also increased migratory dendritic cells, eosinophils, Th2, and Th17 in mesenteric lymph nodes, suggesting elevated immune responses deemed critical in atopy development. *M. restricta* also increased house dust mite-induced airway inflammation, with elevated cellularity and marked eosinophilia in the bronchoalveolar lavage fluid obtained from challenged mice.

Discussion: This translational work shows that fungal overgrowth and expansion of *Malassezia* spp. are previously overlooked collateral effects of infant antibiotic use, which causally contribute to immune dysregulation and increased susceptibility to allergic airway inflammation in mice.