Microbial Regulation of IgE Production in Early Life

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Background

- The prevalence of food allergies is estimated at 5% of adults and 8% of children *(Sicherer and Sampson, JACI. 2014),* and studies support an increasing prevalence of food allergy.
- Rapid increases in prevalence cannot be explained by genetic variation, suggesting a role of the environment in shaping allergic sensitization to food.
- Reduced microbial diversity in early life has been noted in children developing food sensitization or eczema. *(Azad, Clin Exp Allergy, 2014; Abrahamsson, JACI, 2012; Nylund, BMC Microbiol, 2015)*
- Mice with food allergy exhibit a specific gut microbiota signature capable of transmitting disease susceptibility *(Noval-Rivas, JACI. 2013)*.
- Increased susceptibility to experimental food allergy has been found in antibiotic treated mice and germ-free (GF) mice *(Stefka, PNAS. 2014)*. Selective colonization of germ-free mice demonstrated a clostridia-containing microbiota protects against sensitization to food allergens.
- IgE and basophils are regulated by the intestinal microbiota *(Cahenzil, Cell Host Microbe, 2013; Hill, Nat Med. 2012).*

Aim

To determine the impact of intestinal microbiota on susceptibility to peanut allergy using novel models of adjuvant-free sensitization and human intestinal microbial transplant.

Methods

ANTIBIOTIC TREATMENT:

- C57BL/6 mice were gavage-fed daily with a cocktail of antibiotics (ABX), including ampicillin, metronidazole, neomycin, and vancomycin.

GERM-FREE MICE AND HUMAN MICROBIOTA TRANSPLANT:

- C57BL/6 mice were maintained under germ-free conditions in the gnotobiotic facility at ISMMS. Germ-free mice were colonized with fecal microbiota from a healthy pediatric donor. A frozen stool specimen was prepared for administration by gavage (described in Faith JJ, Sci Transl Med, 2014) and administered in the isolator.

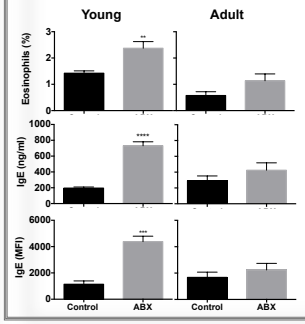
ADJUVANT-FREE ORAL SENSITIZATION MODEL:

- Mice received an intragastric administration of 100 mg of ground peanut, once a week for 6 weeks. Peanut administered to germ-free mice was autoclaved prior to administration.

OUTCOME MEASURES:

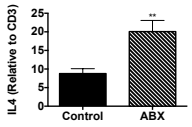
- Anaphylaxis in response to intraperitoneal injection of 1 mg of defatted crude peanut extract was measured by body temperature 30 min after challenge.
- Total IgE and peanut-specific IgG1 in serum were measured by ELISA.
- Circulating eosinophils and surface IgE on basophils were quantified by flow cytometry.
- Cytokine expression in the Peyer's patches (PP) was measured by real time RT-PCR by using SYBR green detection.
- Basophil activation tests were performed using blood incubated with media alone or with crude peanut extract for 90 minutes. Basophils were identified as CD3-CD19-CD49+IgE+, and activation measured by CD200R.

Early life treatment with antibiotics increases eosinophils, IgE, and gastrointestinal IL-4 expression



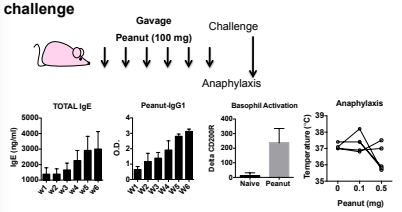
Left: Young (3 weeks) and adult mice (5 weeks) were treated with ABX for 4 weeks. Blood eosinophils were quantified by flow cytometry (CD45+/CD3-CD19-/Ly6G-/SiglecF+/CD11b+). Surface IgE expression on blood basophils was also quantified by flow cytometry, and serum IgE was measured by ELISA.. ** p<0.01, *** p<0.001, **** p<0.0001.

Below: Peyer's patches were isolated from control and ABX-treated mice (young mice only) for RNA isolation and qRT-PCR for IL-4 (normalized to CD3 expression). ** p < 0.01.



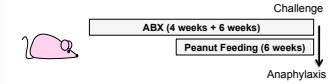
Microbiota is required for susceptibility to peanut allergy in an adjuvant-free oral sensitization model

1. Mice respond to high-dose peanut feeding with sensitization and mild anaphylaxis on challenge



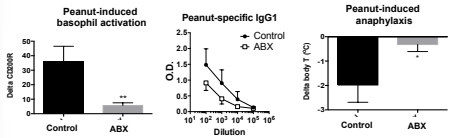
Mice (Balb/c) were gavaged with high-dose peanut once a week for 6 weeks. Total IgE and peanut-specific IgG1 was measured in serum weekly. At week 7, peanut-induced basophil activation was measured by flow cytometry, using the basophil activation marker CD200R. Mice were challenged by ip injection crude peanut extract, and body temperature monitored 30 min after each dose.

2. Antibiotic treatment suppresses sensitization to adjuvant-free high dose peanut feeding



Mice (C57BL/6) were treated with antibiotics (ABX) for 4 weeks. Total IgE was measured after 4 weeks and was elevated compared to control (not shown).

Mice were then fed with high dose peanut as above for 6 weeks. Peanut-induced basophil activation, peanut-specific IgG1, and peanut-induced anaphylaxis (1 mg CPE) were measured as above.



Acknowledgements

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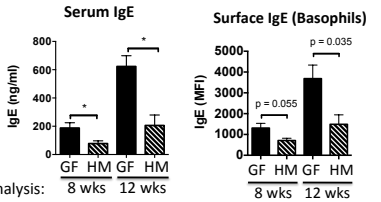
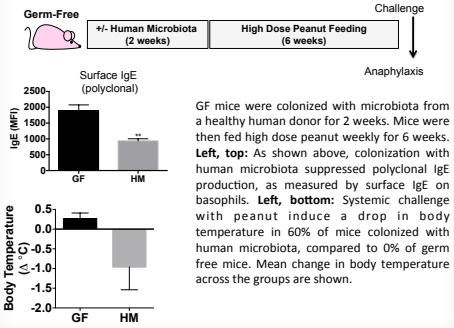
Human Intestinal Microbiota Transplant Suppresses IgE in Germ-Free Mice



Germ-free (GF) mice were colonized with human microbiota (HM) from a healthy pediatric donor at 4 or 8 weeks of age, and analyzed 4 weeks later. Germ-free mice had progressively increasing levels of total serum IgE and surface IgE on basophils with age. Colonization with human microbiota (HM) at either 4 or 8 weeks of age suppressed surface IgE and total serum IgE. * p<0.05.

Human Intestinal Microbiota Transplant Enhances Peanut Allergy in Germ-Free Mice



GF mice were colonized with microbiota from a healthy human donor for 2 weeks. Mice were then fed high dose peanut weekly for 6 weeks. **Left, top:** As shown above, colonization with human microbiota suppressed polyclonal IgE production, as measured by surface IgE on basophils. **Left, bottom:** Systemic challenge with peanut induce a drop in body temperature in 60% of mice colonized with human microbiota, compared to 0% of germ free mice. Mean change in body temperature across the groups are shown.

Summary

Antibiotic treatment in early life leads to a Th2 milieu in the Peyer's patch and elevated polyclonal IgE and circulating eosinophils. Despite evidence of immune deviation after antibiotic treatment, the microbiota is required for sensitization and anaphylaxis to peanut using an adjuvant-free model of sensitization.

The finding that the intestinal microbiota facilitates sensitization to peanut was replicated in germ-free mice with a "humanized" microbiota.

The "humanized microbiota" mouse model is a powerful tool for identifying functional properties of human microbiota- from comparing microbiota of healthy versus allergic subjects, to identifying strains with immunomodulatory activity.