

488 Blocking the inhibitory receptor PD-1 prevents allergic immune response and anaphylaxis in mice



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RATIONALE: Food allergy and acute anaphylaxis can be life-threatening. While T follicular helper (Tfh) cells are indispensable in the allergic immune responses, the immunologic mechanisms that regulate the production of antibodies that mediate anaphylaxis are not fully understood. The aim of this study was to investigate the role of the inhibitory receptor programmed cell death protein 1 (PD-1), which is highly expressed on Tfh cells, in allergic immune responses using an animal model of peanut allergy and anaphylaxis.

METHODS: Naïve wild-type mice were exposed to peanut flour intranasally and then challenged with peanut extract to induce systemic anaphylaxis. The roles of PD-1 were examined by blocking antibodies and using gene-deficient animals. A hapten model and passive cutaneous anaphylaxis were used to characterize allergen-specific antibodies.

RESULTS: Treatment with anti-PD-1 enhanced development of Tfh cells and germinal center B cells in mice exposed to peanut flour. Nonetheless, anti-PD-1 or anti-PD-L1 fully protected mice from developing anaphylaxis. Anti-PD-1 treatment or genetic deficiency of PD-1 in CD4⁺ T cells inhibited production of peanut-specific IgE and increased the levels of IgG. The passive cutaneous anaphylaxis showed that peanut-specific IgG generated in anti-PD-1-treated animals and transferred to naïve animals prevented, rather than provoked, anaphylaxis. Anti-PD-1 promoted production of antibodies with low affinity for an antigen in the hapten model.

CONCLUSIONS: Blockade of the PD-1/PD-L1 pathway is protective against allergic immune responses. The direct interaction between Tfh cells and B cells may play a pivotal role in controlling antibody quality and clinical manifestation of allergic diseases.

489 Intestinal Barrier Dysfunction Accompanies Peanut Allergy In A Genetically-Susceptible Mouse Model And Identifies Angiopoietin-like 4 As A Biomarker



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RATIONALE: Peanut is the #1 cause of fatal food-associated anaphylaxis. Increased intestinal epithelial cell (IEC) barrier permeability is a feature of peanut allergy and correlates with anaphylaxis severity; its mechanism is unknown. We investigated IEC barrier dysfunction in the CC027/GeniUnc mouse.

METHODS: CC027/GeniUnc and control C3H/HeJ female mice were sensitized to peanut without adjuvant by oral gavage. Mice were orally challenged with peanut and FITC-dextran 4 kDa (FD4) for IEC barrier permeability analysis, and rectal temperature was recorded. Murine small intestinal epithelial cells (IECs) were isolated for RNA-seq and *ex vivo* monolayer cultures. Sera was collected from mice and peanut allergic pediatric patients for ELISA.

RESULTS: Peanut-sensitized CC027 mice during challenge had elevated plasma FD4 levels. IEC only from CC027 peanut-sensitized mice in *ex vivo* culture demonstrated peanut-associated decreased transepithelial electrical resistance (TEER) and increased passage of FD4. RNA-seq analysis identified differentially expressed CC027 IEC pathways enriched in cell

cycle and lipid metabolism pathways. IEC angiopoietin-like 4 (*Angptl4*) RNA was highly upregulated during challenge. Serum Angptl4 was elevated in peanut-sensitized CC027 mice. Serum ANGPTL4 was elevated in peanut-allergic pediatric patients.

CONCLUSIONS: Intestinal barrier dysfunction is associated with peanut challenge of sensitized CC027/GeniUnc mice. *Ex vivo* IEC monolayer cultures recapitulate *in vivo* barrier dysfunction and allow for mechanistic studies of barrier integrity. RNA-seq analysis of IEC after peanut challenge identified differentially expressed Angptl4, which is significantly higher in sera of peanut-sensitized CC027 mice and peanut-allergic pediatric patients. The CC027 mouse is a unique animal model for studying mechanisms of intestinal barrier dysfunction in peanut allergy.