A humanized mouse model of anaphylactic peanut allergy

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Background: Food allergy is a growing health problem with very limited treatment options. Investigation of the immunologic pathways underlying allergic sensitization to foods in humans has been greatly constrained by the limited availability of intestinal tissue and gut-resident immune cells. Although mouse models have offered insights into pathways of food sensitization, differences between rodent and human immune physiology limit the extension of these findings to our understanding of human disease.

Objective: We sought to develop a strategy for the generation of mice with humanized adaptive immune systems, complete with tissue engraftment by human mast cells that are competent to mount specific IgE-mediated responses and drive systemic anaphylaxis on ingestion challenge.

Methods: Nonobese diabetic severe combined immunodeficient mice lacking the cytokine receptor common gamma chain ($\gamma_c^{-/-}$) and carrying a human stem cell factor transgene were engrafted with human hematopoietic stem cells. The impact of peanut (PN) feeding and IgE neutralization on the development of immune responses, mast cell homeostasis, and anaphylactic food allergy was assessed in these animals.

Results: Humanized nonobese diabetic severe combined immunodeficient common gamma chain–deficient stem cell factor (huNSG) mice exhibited robust engraftment with functional human T and B lymphocytes and human mast cells were found in significant numbers in their tissues, including the intestinal mucosa. Following gavage feeding with PN, they mounted specific antibody responses, including PN-specific IgE. When enterally challenged with PN, they exhibited mast-cell–mediated systemic anaphylaxis, as indicated by hypothermia and increases in plasma tryptase levels. Anti-IgE (omalizumab) treatment ablated this anaphylactic response.

Conclusions: huNSG mice provide a novel tool for studying food allergy and IgE-mediated anaphylaxis. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *IgE, mast cells, anaphylaxis, humanized mice, tryptase, peanut allergy*

Food allergy is a rising health challenge with a lack of effective treatments. As such, new tools are required to probe the earliest cellular and molecular signals driving allergic food sensitization and those responsible for maintaining the response. Furthermore, innovative approaches are needed for the preclinical evaluation of treatment strategies emerging from basic research. Although substantial advances in delineating pathways of allergic sensitization to foods and effector mechanisms of hypersensitivity have recently been made in human subjects, progress has been constrained by the limited availability of the key tissues required to analyze local intestinal responses (eg, intestinal mucosa, mast cells, and gut-associated lymphoid tissue).

Mouse models have been applied with significant success but, for the most part, have required non- or less physiologic methods of sensitization (intraperitoneal priming or using adjuvant) or genetic manipulations to enhance sensitivity. In addition, inherent differences in the immune physiology of rodents and humans limit the interpretation of findings from such models. Notably, the high-affinity IgE receptor, FcεRI, displays a wider distribution on human leukocytes, including antigen-presenting cells (APCs), and soluble CD23 (FcεRII) complexed to IgE can stimulate IgE synthesis in human but not mouse B cells via CD21.[1,2] The differences in antibody Fc biology extend to the interaction between IgG and FcγRIIb, which exerts a critical brake on degranulation and anaphylaxis and is generally stronger in mice than in humans,[3,4] with human skin mast cells expressing primarily activating FcγRIIa.[5]

We reasoned that a model in which rodents harbor a fully humanized adaptive immune system capable of generating food-specific IgE following allergen ingestion, as well as fostering the development of the human innate effector cells of anaphylaxis, would provide such a tool. Here, we describe the conditions for the generation of such mice, their physiological response to peanut (PN) allergen, and their utility as a source of human mast cells. Furthermore, we demonstrate the impact of omalizumab-mediated IgE neutralization on PN-induced anaphylaxis.

METHODS

Mice

Nonobese diabetic (NOD) severe combined immunodeficiency (SCID) common gamma chain–deficient (NSG) stem cell factor (SCF) (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl} Tg(PGK1-KITLG*220)441Daw/SzJ) mice were

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Abbreviations used
APC: Antigen-presenting cell
HSC: Hematopoietic stem cell
huNSG: Humanized nonobese diabetic severe combined immunodeficient common gamma chain–deficient stem cell factor
NOD: Nonobese diabetic
NSG: Nonobese diabetic severe combined immunodeficiency common gamma chain–deficient
PN: Peanut
SCF: Stem cell factor
SCID: Severe combined immunodeficiency

purchased from the Jackson Laboratory, and bred in a biosafety level 2 facility at Boston Children's Hospital. NSG mice were maintained in autoclaved cages with Sulfatrim oral suspension (Sulfamethoxazole/Trimethoprim, HiTech Pharmacal, Amityville, NY) in the sterilized drinking water. All procedures were carried out under protocols approved by the local institutional animal care and use committee.

Stem cells

Cord blood–derived flow-sorted human $CD34^+$ hematopoietic stem cells (HSCs) were purchased from AllCells (Alameda, Calif). Such cells are obtained under Institutional Review Board– or Human Subject Committee–approved protocols. Cells (5×10^4-10^5) were injected intravenously via the retro-orbital sinus into 3- to 6-week-old mice. Engraftment was monitored in samples of peripheral blood using flow cytometry monthly for the 4-month engraftment period preceding allergen sensitization.

Flow cytometry

Cells were stained with the following antibodies: Brilliant Violet 421–conjugated anti-human CD45 (clone HI30), phycoerythrin (PE)-Cy7 anti-mouse CD45 (30-F11), Alexa Fluor 700 anti-CD3 (HIT3a), fluorescein isothiocyanate anti-CD4 (OKT4), PE-Dazzle594 anti–IL-4 (MP4-25D2), Alexa Fluor 647 anti-Foxp3 (259D), PerCP-Cy5.5 anti-CD127 (A019D5), PE-Cy7 anti-CD25 (BC96), PerCP-Cy5.5 anti–IFN-γ (4S.B3), PE anti–IL-10 (JES3-19F1), APC anti-CD19 (HIB19), PE-Cy7 anti–HLA-DR (L243), Brilliant Violet anti–c-Kit (104D2), and PE anti-FcεRI (AER-37 [CRA-1]) were purchased from Biolegend (San Diego, Calif). APC anti-IgE (Ige21) was obtained from Affymetrix eBioscience (San Diego, Calif). Anti-mouse CD16/32 (clone 93) and TruStain FcX Fc receptor blocking solution (both Biolegend) were used to prevent nonspecific binding. Dead cells were excluded using fixable viability dye eFluor 780 (Affymetrix eBioscience). Intracellular cytokine staining was performed after a 4-hour stimulation at 37°C with 500 ng/mL ionomycin, 500 ng/mL phorbol 12,13-dibutyrate, and 1 μg/mL brefeldin A (all Sigma-Aldrich, St Louis, Mo). Coordinate analysis of transcription factors and cytokine production was performed using BD Biosciences Cytofix and Cytoperm reagents as previously described.[6] Intestinal leukocyte isolation was performed according to established protocols.[7]

Food allergen sensitization and anaphylaxis

After 4 months of stem cell engraftment, mice were sensitized by intragastric feeding with 22.5 mg (5 mg protein) Skippy creamy peanut butter (Hormel Foods, Austin, Minn) in 250 μL 0.2 mol sodium bicarbonate, pH 8.0, weekly for 8 weeks. Control mice were sham-sensitized with sodium bicarbonate alone. Allergen challenge was performed by gavage feeding with 350 mg peanut butter suspended in 0.2 mol sodium bicarbonate. Temperature measurements were performed using microchip transponders implanted subcutaneously 48 hours before challenge, as we have previously described.[8] Omalizumab (αIgE) was administered weekly by intraperitoneal injection 48 hours before PN feedings at 120 mg/kg. Dosing was calculated to correspond to the 10 mg/kg used in humans, based on surface area conversion recommendations from the Food and Drug Administration and other sources,[9,10] and was designed to optimize the potential for IgE neutralization rather than an attempt to mimic therapeutic use in human patients.

ELISAs

PN-specific IgE was quantified by ELISA, capturing IgE onto plates coated with 3 μg/mL antihuman IgE (clone G7-18, BD Biosciences), and detecting with biotinylated PN extract (200 ng/mL; see Burton et al[11]). A standard curve was constructed using PN-allergic patient sera that had been previously quantified by Immunocap. Tryptase measurements were performed by using ELISA as previously described.[12]

Statistics

Data were plotted and analyzed in Prism 5.0f (GraphPad Software, Inc, La Jolla, Calif). Temperature curves were analyzed using repeated-measures 2-way ANOVA, with matching for each individual mouse across the time course. Tryptase and IgE values were log transformed before analysis by ANOVA with Bonferroni posttests. Data are represented as mean ± SEM, with an individual point for each mouse where applicable.

RESULTS

PN-fed humanized nonobese diabetic severe combined immunodeficient common gamma chain–deficient stem cell factor mice exhibit PN-specific IgE responses, PN-induced systemic anaphylaxis, and intestinal mast cell expansion

A number of murine models of food allergy have been developed by our group and others to probe mechanisms underlying both allergic sensitization to foods and the induction of tolerance.[8,13-18] One such model, in which an atopic tendency including susceptibility to food allergy is conferred by targeted insertion of a gene expressing an activated form of the IL-4 receptor α-chain, revealed the importance of IgE antibodies and mast cells in mediating food anaphylaxis.[8] Although there is a great deal of overlap in the pathways leading to immunologic sensitization and tolerance in humans and rodents, there are inherent differences that limit the application of findings from animal models to human disease. However, the direct study of human intestinal immune responses to food allergens has been hindered by the lack of available tissue. Food-allergic subjects are generally otherwise healthy and have no clinical indications for endoscopy and biopsy, which would be necessary to obtain primary tissue for investigational purposes. We reasoned that a compromise would be to reconstitute immunodeficient mice with human hematopoetic progenitor that would develop into the human adaptive immune cells capable of generating T_H2 and IgE responses as well as the effector innate immune cells (mast cells) of immediate hypersensitivity. Such humanized mice would permit us to test food allergy mechanisms and specifically the role of IgE antibodies and mast cells in a humanized setting.

A number of approaches have been described for the generation of humanized mice, generally using immunodeficient mice reconstituted with human PBMCs or stem cells.[19-23] We elected to use a stem cell repopulating model, in which $CD34^+$ cord blood HSCs (5×10^4) are injected into NOD SCID common gamma chain $(\gamma_c)^{-/-}$ (NSG) mice and develop into a human immune system. We predicted that, in addition to reconstituting full T- and B-cell adaptive immune function, it would be necessary in an allergy model for the recipient mice to be