Airway exposure initiates peanut allergy by involving the IL-1 pathway and T follicular helper cells in mice

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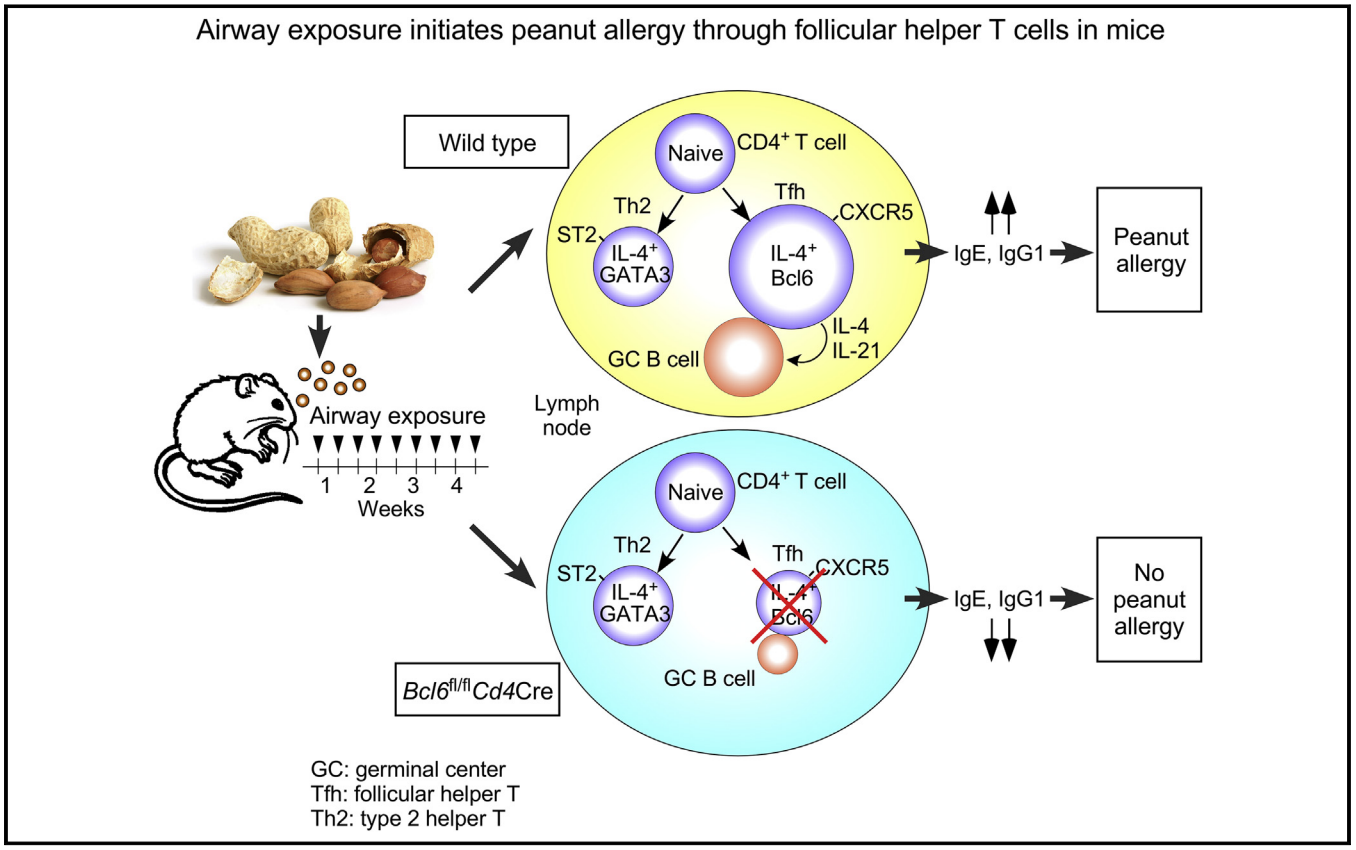
GRAPHICAL ABSTRACT



Background: Little is currently known regarding the immunologic mechanism(s) that initiate peanut allergy. Notably, peanut proteins have been detected in house dust, and their levels correlate with peanut allergy prevalence.

Objective: This study aimed to develop a new mouse model for peanut allergy and to investigate the immunologic mechanisms involved in peanut allergen sensitization.

Methods: To mimic environmental exposure, naive mice were exposed to peanut flour by inhalation for up to 4 weeks. We then analyzed serum levels of IgE antibody and challenged mice with peanut proteins. Immunological mechanisms involved in sensitization were analyzed using cytokine reporter mice, an adoptive cell transfer model, and gene knockout mice.

Results: When exposed to peanut flour by inhalation, both BALB/c and C57BL/6 mice developed peanut allergy, as demonstrated by the presence of peanut-specific IgE antibodies and manifestation of acute anaphylaxis on challenge. A large number of follicular helper T (Tfh) cells were also detected in

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This work was supported by grants from the National Institutes of Health (NIH) (R01 AI71106) and the Mayo Foundation.

Disclosure of potential conflict of interest: H. Kita's institution received grant NIH R01 AI71106 from National Institute of Allergy and Infectious Diseases for this work and grants from National Institute of Allergy and Infectious Diseases and National Heart, Lung, and Blood Institute for other works. J. Dolence's institution received NIH T32 Training Grant in Allergic Diseases and support for travel from NIH T32 Training Grant in Allergic Diseases for this work. The rest of the authors declare they have no relevant conflicts of interest.

Received for publication May 31, 2017; revised November 2, 2017; accepted for publication November 8, 2017.

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0091-6749/$36.00

https://doi.org/10.1016/j.jaci.2017.11.020

draining lymph nodes of allergic mice. These cells produced IL-4 and IL-21, and they more robustly promoted peanut-specific IgE production than T_H2 cells did. Genetic depletion of Tfh cells decreased IgE antibody levels and protected mice from anaphylaxis, without affecting T_H2 cells. Furthermore, peanut flour exposure increased lung levels of IL-1α and IL-1β, and mice deficient in the receptor for these cytokines showed a significant decrease in Tfh cells compared with in wild-type mice.

Conclusions: Tfh cells play a key role in peanut allergy, and the IL-1 pathway is involved in the Tfh response to peanut allergen exposure. (J Allergy Clin Immunol 2017;■■■:■■■-■■■.)

Key words: *Follicular T cells, IL-4, IgE, allergy, peanut, IL-1, IL-33*

Abbreviations used

BAL:	Bronchoalveolar lavage
CTX:	Cholera toxin
eGFP:	Enhanced green fluorescent protein
FACS:	Fluorescence-activated cell sorting
GC:	Germinal center
IL-1RAcP:	IL-1R-associated protein
IN:	Intranasal
IP:	Intraperitoneal
IV:	Intravenous
MCPT-1:	Mast cell protease-1
mLN:	Mediastinal lymph node
LN:	Lymph node
OVA:	Ovalbumin
OX40L:	OX40 ligand
PD-1:	Programmed cell death protein 1
Tfh:	Follicular helper T
TLR4:	Toll-like receptor 4
TSLP:	Thymic stromal lymphopoietin
WT:	Wild-type

Food allergy is a growing public health concern that impacts approximately 4% of adults and 8% of children in the United States,[1] and frequencies of food allergies are on the rise worldwide.[2] In particular, the incidence of peanut allergy has more than tripled in US children, increasing from 0.4% in 1997 to 1.4% in 2008.[3] In contrast to milk or egg allergies that are commonly outgrown in childhood, peanut allergy is often life-long.[2] The majority of fatal food-induced anaphylaxis is associated with peanut allergy,[4] and avoidance of peanut exposure is difficult to achieve due to the popularity of peanut in our society.[5] As a result, peanut allergy can negatively impact quality of life, as well as the psychosocial status of patients and their families.[5] Therefore, it is critical to better understand the immunologic mechanisms involved in development and persistence of peanut allergy and to identify novel strategies to prevent and/or to effectively treat this disease.

Although mice and humans differ in many ways, mouse models provide robust tools to elucidate the immunologic mechanisms of human diseases. In particular, both the skin and oral sensitization models for peanut allergy implicate T_H2 cells in driving the allergic response to peanut.[6-8] An IL-1-family cytokine, IL-33, has further been shown to play an important role in the development of $T_H2$responses in these models,[6,7] and long-lived peanut-specific memory B cells that replenish IgE^+ plasma cells likely sustain clinical reactivity in mice.[9] Nevertheless, the molecules and cell types that drive the development of peanut allergy in humans are not fully understood. Furthermore, a majority of earlier mouse models used mucosal adjuvants, such as cholera toxin (CTX)[7] and staphylococcal enterotoxin B,[10,11] or required genetic alteration of Toll-like receptor 4 (TLR4)[12] or IL-4 receptor,[13] making it difficult to determine the precise immunologic mechanisms involved in the initiation of peanut allergy.

Peanut allergen sensitization has previously been thought to occur as a consequence of the ingestion of dietary peanut products. However, ingestion of innocuous antigens generally results in oral tolerance,[14] and the majority of children with peanut allergy experience their first allergic reaction on their first ingestion of peanut.[15] Additionally, the recent Learning Early About Peanut Allergy (LEAP) study found that early dietary introduction of peanut prevented development of peanut allergy, whereas a greater proportion of children who avoided dietary peanut developed peanut allergy.[16] These data suggest that patients have been sensitized to peanut proteins in their environment. Indeed, peanut proteins are readily detectable in house dust at levels comparable to those of inhaled allergens, such as house dust mite,[17,18] and a dose-response relationship between environmental peanut exposure and the risk of peanut allergy has been observed.[19,20] A number of clinical studies have further demonstrated an association between atopic dermatitis and peanut allergy in children,[20] suggesting that allergic sensitization to peanut is mediated through impaired skin. Consistent with this, epicutaneous exposure to crude peanut extract was found to promote T_H2-type sensitization to peanut proteins in mice.[6]

Despite these studies, and the well-established evidence for the presence of peanut proteins in house dust, it remains unclear whether and how nonoral exposure initiates peanut allergy. To address this question, we developed a mouse model for inhalation-based peanut allergen sensitization. To mimic natural environmental exposure, we exposed naive mice to peanut flour by inhalation and found that these animals develop anti-peanut IgE antibodies and clinical symptoms resembling peanut allergy in humans. We further determined that follicular helper T (Tfh) cells that produce elevated levels of IL-4 and IL-21 are generated in draining lymph nodes and that they promote production of peanut-specific IgE. Thus, Tfh cells are likely to be critical for the development of peanut allergy in our model.

METHODS

See the Methods section of this article's Online Repository (available at www.jacionline.org) for more details.

Mice

BALB/c, C57BL/6, C.C3-*Tlr4*$^{Lps-d}$/J (*Tlr4*$^{-d}$), C.129-Il4^{tm1Lky}/J (4get), Tg(Cd4-cre)1Cwi/BfluJ (*Cd4*-Cre), B6.129P2-*Tcrb*^{tm1Mom}/J (*Tcrb*$^{-/-}$), B6Cr.129S4-*Tnfsf4*^{tm1Sug}/Pgn (*Tnfsf4*$^{-/-}$), B6;129S1-*Il1rap*^{tm1Roml}/J (*Il1rap*$^{-/-}$), and B6.129S7-*Il1r1*^{tm1Imx}/J (*Il1r*$^{-/-}$) mice were obtained from The Jackson Laboratory (Bar Harbor, Me). *Il1rap*$^{-/-}$ mice were subsequently backcrossed onto the BALB/c background for 10 generations. *Il1rl1*$^{-/-}$ (*ST2*$^{-/-}$) mice and *Crlf2*$^{-/-}$ (*Tslpr*$^{-/-}$) mice (both BALB/c background) were kindly provided by Dr Andrew McKenzie (MRC Laboratory of Molecular Biology, Cambridge, UK) and Dr Steven F. Ziegler (Benaroya Research Institute, Seattle, Wash), respectively, and were bred