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A “LEAP” forward in understanding immune mechanisms of oral tolerance to peanut.

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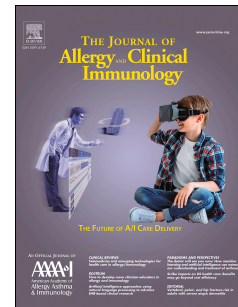
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- 1 **Title: A “LEAP” forward in understanding immune mechanisms of oral tolerance to peanut.**
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Peanut allergy affects about 2% of children in Western countries and is likely increasing in incidence¹. Peanut allergy typically develops during infancy and usually persists throughout life, and therefore early-life interventions aimed at peanut allergy prevention are essential for reducing disease burden. There is increasing evidence that non-oral exposure to environmental peanut, such as through the skin or respiratory tract, is a risk factor for peanut allergy development, whereas ingestion of peanut is associated with tolerance². The Learning Early About Peanut Allergy (LEAP) study was the first randomized controlled trial to demonstrate the importance of early introduction of dietary peanut to prevent peanut allergy in at-risk infants³. In this landmark study, 640 children ages 4-11 months with severe eczema and/or egg allergy were randomized to either peanut consumption or avoidance. The primary outcome of the study was prevalence of peanut allergy at 60 months of age as determined by oral food challenge. Among the 530 subjects evaluated for the primary outcome, the prevalence of peanut allergy was 13.7% in the avoidance group and 1.9% in the consumption group, indicating an 86% relative risk reduction with peanut consumption. To determine if unresponsiveness to peanut was sustained, a follow-on study (LEAP-on) asked 550 participants who completed the LEAP trial to avoid peanut for 12 months⁴. Peanut avoidance was not associated with an increase in peanut allergy, suggesting that tolerance to peanut had been established in the early consumption group. The striking results from these randomized trials, along with observational data that peanut allergy rates were low in societies where early-life peanut consumption was common, prompted an addendum to the guidelines for the management of food allergy in the United States⁵. An expert panel sponsored by the National Institute of Allergy and Infectious Diseases recommended that infants with severe eczema, egg allergy or both have peanut-containing foods introduced to their diet at 4-6 months of age to reduce the risk of peanut allergy.

While the LEAP and LEAP-on studies provided strong evidence that early dietary introduction of peanut was protective against peanut allergy, the immune mechanisms responsible for the induction of

tolerance to ingested peanut remain unclear. It has long been known that ingestion of food antigens can result in a systemic state of immunological unresponsiveness to the antigen, a phenomenon known as oral tolerance. Several immune mechanisms for oral tolerance have been proposed, including clonal deletion of antigen-specific T cells, induction of T cell anergy, and the generation of antigen-specific regulatory T cells (Tregs)⁶. Due to the inability to perform in-depth mechanistic studies in human infants, the relative contribution of these proposed mechanisms to tolerance induction during early dietary introduction of peanut is unknown. Elucidating the key cellular and molecular pathways responsible for tolerance induction is not only important for improving interventions aimed at peanut allergy prevention but will also likely improve the efficacy of peanut-specific immunotherapies.

In this issue of *The Journal of Allergy and Clinical Immunology*, Krempski et al.⁷ have developed a mouse model simulating the clinical findings of the LEAP study as a tool for investigating the immune mechanisms by which early peanut introduction protects against peanut allergy. In their model, 7–12-week-old mice were fed a total of 1 g of a commercial peanut butter product (equivalent to 220 mg of peanut protein) by oral gavage 3-5 times per week for a total of 6 weeks. The authors estimated that this feeding regimen would be similar to a human infant consuming 6-7 grams of peanut protein per week, which is the recommended dose for infants at high-risk for peanut allergy⁵. To determine if feeding peanut protected mice from developing peanut allergy, peanut flour was administered to the respiratory tract or skin weekly for 4 weeks to mimic environmental exposure to peanut. As expected, mice fed control buffer developed peanut-specific IgE and IgG1 following inhalational or epicutaneous exposure to peanut flour and developed anaphylaxis upon peanut challenge. In contrast, peanut-fed mice had low levels of peanut-specific immunoglobulins following airway or skin exposure to peanut and were protected from peanut-induced anaphylaxis. Mice allowed to consume peanut butter *ad lib* prior to airway exposure to peanut also did not develop peanut allergy, indicating that natural feeding of peanut butter was also protective. Moreover, peanut-fed mice switched to a peanut-free diet for 4

59 weeks after inhaled peanut exposure were still protected from peanut allergy, suggesting that feeding
60 peanut appeared to induce long-term tolerance rather than temporary desensitization in mice.

61 Next, Krempski et al. used their model to investigate the immune mechanisms by which feeding
62 peanut protected mice from sensitization to environmental peanut exposure. They found that feeding
63 peanut butter inhibited development of IL-4-producing T follicular helper (Tfh) cells in lung-draining
64 lymph nodes, which are essential for inducing peanut-specific IgE⁸. Because inducible Tregs have been
65 shown to mediate oral tolerance to food antigens⁹, they next evaluated how feeding peanut affected
66 Treg development. Interestingly, they did not observe an increase in the number or frequency of Tregs
67 in lung-draining or mesenteric lymph nodes from peanut-fed mice compared to mice fed control buffer.
68 To determine if feeding peanut affected Treg phenotype, they performed single cell RNA sequencing on
69 CD4+ T cells from lung-draining lymph nodes of peanut-fed mice after airway sensitization to peanut.
70 They identified a cluster of CD4+ T cells with a typical Treg transcriptional profile that expressed high
71 levels of the inhibitory molecule cytotoxic T-lymphocyte associated protein 4 (CTLA-4), but not the
72 immunosuppressive cytokines IL-10 or TGF- β . To investigate the role of CTLA-4 in mediating protection
73 against peanut allergy, they administered anti-CTLA-4 blocking antibody to peanut-fed or control mice
74 during airway sensitization to peanut. Treatment with anti-CTLA-4 antibody resulted in increased
75 numbers of Tfh and germinal center B cells in lung-draining lymph nodes, which corresponded with
76 elevated levels of peanut-specific antibodies. Furthermore, blocking CTLA-4 reversed the protective
77 effects of feeding peanut on anaphylaxis, indicating that the CTLA-4 pathway plays an important role in
78 preventing sensitization to environmental peanut.

79 The investigations by Krempski et al. introduce a novel mouse model for simulating early peanut
80 introduction and provide new insights into how feeding peanut protects against peanut allergy
81 development. While any preclinical animal model of human disease has its limitations, the current
82 studies are strengthened by a rigorous attempt to use relevant sources and quantities of peanut protein

for feeding, demonstrating protection from peanut sensitization using multiple exposure routes, and confirming experimental results in different mouse genotypes and sexes. Still, the use of adult mice is a limitation regarding the translation of their findings to human infants. While the authors observed that the mice used for their experiments have an immature immune system based on CD8+ T cell immunophenotyping, it is likely that other aspects of the adaptive immune response significantly differ between adult mice and human infants. Future studies using neonatal mice would help address this concern and increase the translational impact of their animal model. This study also highlights an important regulatory role for CTLA-4 in mediating tolerance to food allergens and provides pre-clinical evidence that augmenting CTLA-4 signaling may increase the efficacy of allergen-specific immunotherapy. Indeed, abatacept, a soluble CTLA-4-Ig fusion protein, is being investigated as an adjuvant to peanut oral immunotherapy (NCT04872218). Still, several questions remain, including the mechanism by which CTLA-4 protects mice from airway sensitization to peanut and where CTLA-4-expressing Tregs are induced in response to peanut feeding. Interestingly, the authors found that adoptive transfer of Tregs from mesenteric lymph nodes of peanut-fed mice did not confer protection against airway sensitization to peanut, raising the possibility that peanut-specific Tregs cells might expand and become activated in the gut mucosa as described in other models of oral tolerance¹⁰. Another limitation is that the protective role of CTLA-4 was only demonstrated in an airway sensitization model, so it is unclear if a similar mechanism also mediates protection against sensitization through the skin or gut. The mouse model presented by Krempski et al. will provide a valuable tool to address these questions and others regarding the regulatory mechanisms that mediate tolerance to food allergens.

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Figure Legends:

Figure 1. Ingestion of peanut protects mice from peanut allergy caused by environmental peanut exposure. During peanut avoidance, exposure to environmental peanut through the airway or skin results in increased numbers of IL-4-producing T follicular helper (Tfh) cells and germinal center (GC) B cells in tissue-draining lymph nodes, leading to increased levels of peanut-specific immunoglobulins and development of peanut allergy. In contrast, peanut consumption induces a T regulatory (Treg) cell population that expresses high-levels of CTLA-4, which suppress Tfh and GC B cell expansion following environmental peanut exposure. This results in decreased production of peanut-specific immunoglobulins and development of tolerance to peanut. Created with BioRender.com.

