

Separating sensitization and regulatory T-cell functions in patients with food allergy

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Food allergies have become a common health issue in westernized countries over the past several years, and symptoms can range from development of a rash to life-threatening anaphylaxis. It is well established that type 2 immune effector cells, including T_H2 cells, mast cells, and basophils, play a critical role in allergic reactions to foods. However, the mechanisms driving the early immune events that result in loss of tolerance to common food allergens and development of food allergy remain unclear.

In this issue of the *Journal*, Noval Rivas et al¹ address this question and demonstrate the role of type 2 innate lymphoid cells (ILC2s) in this process using a previously reported mouse model of food allergy. Because of an activating mutation (I14raF709) in the IL-4R α chain that results in enhanced signaling through IL-4 and IL-13, the mice used in this study are prone to the development of food allergy.² Importantly, the authors were able to use this enhanced phenotype to examine mechanisms thought to be key in the sensitization events of food allergy, particularly the IL-33/IL-33 receptor ST2 axis, through generating I14raF709 mice that lacked expression of the IL-33 receptor ST2 (*Il1rl1*^{-/-}).

Interestingly, the authors report that ILC2s, and in particular IL-4 expressed by ILC2s, are necessary for the development of food allergy in this model. Moreover, they find that IL-33 is important for driving IL-4 expression by ILC2s and that this IL-4 is critical for the activation of mast cells and inhibition of the induction of regulatory T (Treg) cell responses.

Taken together, the authors conclude that IL-33 induction in the small intestine primes ILC2s to produce IL-4, which inhibits the induction of allergen-specific Treg cells and potentiates production of peanut-specific IgE and mast cell degranulation. This process then leads to the development of food allergy. Conversely, in IL-33 receptor-deficient mice, this ILC2 IL-4 response is blunted, and the induction of Treg cells is not inhibited, which leads to the development of oral tolerance and protection from food allergy.

This study provides interesting new insights into how excessive signaling through IL-4R α , especially in the context of IL-33, might play a critical role in the early stages of establishing a food-induced allergic response. Although it has previously been established that ILC2s play important roles in the early stages of allergic lung inflammation,³ this study demonstrates that they also play a role in the establishment of food-induced allergic responses. This work also highlights the potential role for ILC2-derived IL-4 during allergic inflammation. Traditionally, ILC2s have not been thought to be major producers of IL-4, but recent work has shown that ILC2s can produce meaningful levels of IL-4, especially in response to thymic stromal lymphopoietin.⁴ The authors of the current study did not assess whether thymic stromal lymphopoietin levels were increased in this model, but based on the reduced frequency of IL-4⁺ ILC2s in the *Il1rl1*^{-/-} mice, it is clear that IL-33 also plays a role in the production of IL-4 by ILC2s.

The authors also demonstrate that transfer of wild-type ILC2s, but not IL-4-deficient ILC2s, can restore allergic sensitization in I14raF709 *Il1rl1*^{-/-} mice, raising the question of whether ILC2-derived IL-4 could play a role in the activation of B-cell class-switch recombination to IgE. Although recent studies have shown interactions between innate lymphoid cells and conventional T cells,⁵ there is little known about the cross-talk between innate lymphoid cell populations and B cells. Along these lines, it would be interesting to determine whether the specific antibody response is altered in I14raF709 *Il1rl1*^{-/-} mice beyond their lack of specific IgE. One possibility is that the loss of ST2 signaling and subsequent reduction in ILC2-derived IL-4 levels could favor the induction of protective antibody responses, such as peanut-specific IgG₄ or IgA, which could play a role in protection from the development of food allergy.

Although this work advances our understanding of the processes that might function to drive initiation of food allergies, other questions remain unanswered. The issue of discriminating the absence of sensitization versus the induction of tolerance is particularly complex. It has been demonstrated previously that IL-33 actually promotes Treg cell function in nonallergic mouse models,^{6,7} and the work presented here clearly demonstrates that IL-33 signaling to Treg cells is necessary for their suppressive function, at least *in vitro*. The authors propose that the ability of IL-33 to induce Treg cell function *in vivo* might be counterbalanced by the negative effects of ILC2-derived IL-4 on Treg cell function, but they did not directly assess whether IL-33 could induce a protective Treg cell response in this model.

This issue becomes clearer when we examine the results from the experiments in the I14raF709 *Il1rl1*^{-/-} mice. I14raF709 mice, whose T cells can fully respond to IL-33, still have food allergy and anaphylaxis, but I14raF709 *Il1rl1*^{-/-} mice, whose T cells cannot respond to IL-33, are protected from food allergy. This suggests that the effect of IL-33 on Treg cells is not required to

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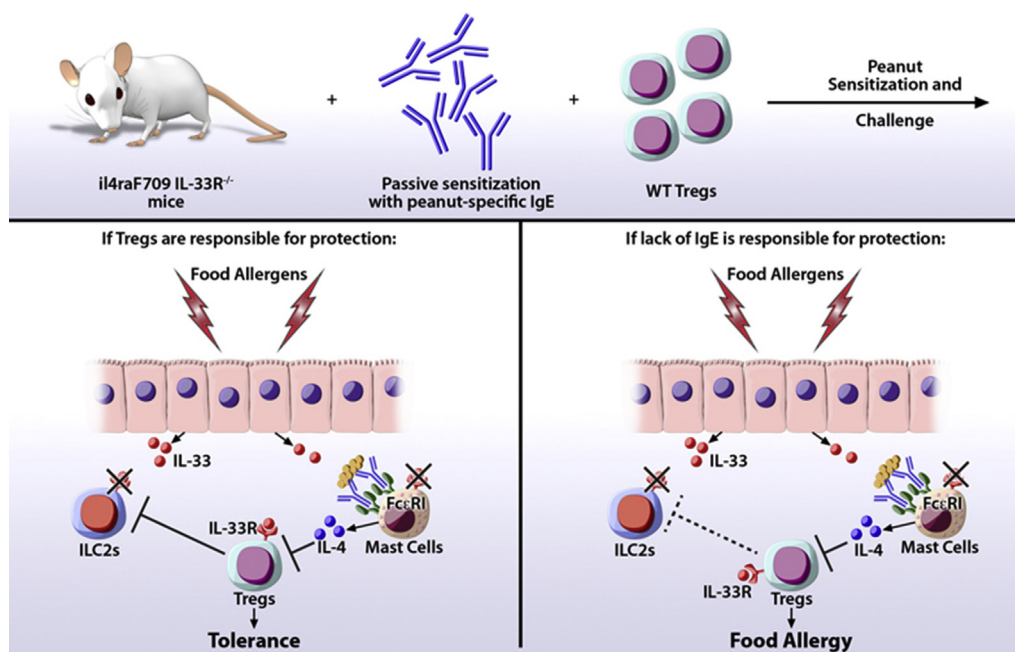


FIG 1. Discrimination between induction of tolerance and a lack of allergic sensitization. Noval Rivas and colleagues demonstrate that *il4raF709 IL-33R^{-/-}* mice are protected from food-induced anaphylaxis, but these mice also do not develop allergen-specific IgE. The depicted experiment could be used to determine whether induction of Tregs or lack of specific IgE is more important for this protection. *il4raF709 IL-33R^{-/-}* mice are passively sensitized with peanut-specific IgE and adoptively transferred with WT peanut-specific Tregs before peanut sensitization and challenge, as described by Noval Rivas and colleagues (top). If augmentation of Treg function by IL-33 is sufficient to inhibit activation of mast cells and ILC2s, even in the presence of allergen-specific IgE, then the result will be tolerance (bottom left). If the protection seen in *il4raF709 IL-33R^{-/-}* mice is due to a lack of sensitization, then addition of specific IgE will result in food allergy, even in the presence of Tregs that can respond to IL-33 (bottom right).

prevent induction of food allergy in this model. When interpreting the results from this study, it is also important to keep in mind that the *Il4raF709 Il1rl1^{-/-}* mice were not sensitized (ie, they did not produce peanut-specific IgE). Consequently, it is not possible to discriminate between the induction of tolerance and a lack of allergic sensitization as the process that protects the *Il4raF709 Il1rl1^{-/-}* mice from anaphylaxis. This question could potentially be tested by using passive sensitization of *Il4raF709 Il1rl1^{-/-}* mice and adoptive transfer of wild-type peanut-specific Treg cells (Fig 1, top). If protection is mainly mediated by the induction of tolerance through IL-33 (ie, through peanut-specific Treg cells), then the mice that receive wild-type Treg cells should be protected from anaphylaxis, even in the presence of allergen-specific IgE (Fig 1, bottom left), but if protection is dependent on the absence of sensitization, then transfer of wild-type Treg cells should not protect the animals from anaphylaxis (Fig 1, bottom right).

Finally, it will be important to determine how these results fit in with other studies using murine models of food-induced allergic responses. A recent study by Esterhazy et al⁸ found that specific subsets of intestinal dendritic cells were required for the induction of oral tolerance, but the study by Noval Rivas et al¹ did not implicate intestinal dendritic cells in their model. It is not clear whether these disparate results are due to differences in the model antigens used, the strain of mice, or other factors. Future studies are needed to address these questions to provide a more comprehensive understanding of the mechanisms involved in

the initiation of food allergies and to help in the development of effective therapeutic strategies for patients with food allergy.

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