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EDITORIAL COMMENTARY

# TLRs toll for Tregs

See corresponding article on Page 1201

Food allergy (FA) is an immune mediated pathologic condition in response to normally innocuous food antigens with the prevalence increasing in both Western and fast-developing countries. Food allergens are derived from common food proteins of plant and animal origin. The adverse immune responses in FA consist of IgE-mediated immediate hypersensitivity reactions, non-IgE-mediated reactions, and conditions involving mixed IgE-mediated and cell-mediated immune reactions.[1–3] The allergic responses are mediated by the participation of a range of innate and adaptive immune cells, such as mast cells, basophils, and eosinophils, type 2 innate lymphoid cells (ILC2s) as well as activated Th2 cells and B cells switched to the production of IgE.

Although the processes by which the immune regulatory mechanisms maintain tolerance to allergens remain enigmatic, the pathogenesis of allergic diseases is generally accepted to involve an ineffective immune tolerance to allergens. Regulatory T cells (Tregs) are a subset of CD4+ T lymphocytes, characterized by the expression of transcription factor FOXP3.[4] They play a key role in maintaining immune tolerance to food antigens and commensal microbiota in the gut. The professional antigen presenting cells, such as dendritic cells (DCs), process and present peptides from food antigens on their MHC class II molecules, and subsequently orchestrate antigen-specific T cell responses. The induction of protective T cell responses requires naïve T cells to receive signals via the TCR, costimulatory molecules, and cytokine receptors. These signals are generally provided by tolerogenic DCs in the gut-associated lymphoid tissue (GALT),[5] the largest immune organ in the body. Currently, mechanisms regulating oral tolerance to food antigens are not well understood, but may involve gut microorganisms as sources of natural antigens that continuously stimulate the GALT and induce mucosal immune tolerance to food antigens together with the molecular components derived from commensal bacteria.[5] DCs are stimulated when they encounter pathogen-associated molecular patterns (PAMPs) from microorganisms that are recognized by pathogen recognition receptors, of which TLRs represent one of the most important and evolutionarily conserved families. TLRs recognize conserved molecular structures expressed by microorganisms, including bacteria, viruses, fungi, and protozoa. TLR engagement by their cognate ligands in DCs alerts the immune system to pathogenic threat and leads to the activation of innate immune response, resulting in the production of proinflammatory cytokines, chemokines, and increased phagocytic capacity and antigen presentation along other innate effector mechanisms. Pathogen recognition via TLRs activates the innate immunity, which then results in the orchestration of ensuing adaptive immune response. Although a number of TLRs, including the LPS receptor TLR4, are found on the plasma membrane, TLR3, TLR7, TLR8, and TLR9 reside on the endomembranes, in intracellular locations, where they sense the presence of intracellular pathogens such as virus and bacteria or their microbial derived ligands. For example, TLR3 recognizes double-stranded RNA structures whereas TLR7/8 sense single-stranded RNA and TLR9 can detect nonmethylated CpG DNA from virus or bacteria.

TLR recognition of PAMPs are generally accepted to occur through innate immune cells such as DCs, which may prime Tregs; however, accumulating evidence suggests that TLR signaling may directly control Tregs suppressive functions. For example, stimulation of human Tregs with TLR5 ligand, flagellin increases their suppressive function.[6] Bacterial polysaccharides as TLR2 ligands can also induce Tregs through DCs[7] and TLR2-deficient mice display reduced numbers of CD4+ CD25+ Treg cells.[8] In vitro, TLR2 ligand $Pam_3Cys$, but not LPS (TLR4) or CpG (TLR9), was shown to act directly on Tregs in a MyD88-dependent fashion, augmenting Treg proliferation in vitro and in vivo.[9] Interestingly budesonide treatment of asthmatic patients results in increased TLR2 and TLR4 expression on their Tregs and ligation of TLR4 by LPS was found to increase FOXP3 expression in Tregs from both asthmatic patients and healthy donors.[10] Overall accumulating evidence suggests that steady state or de novo expression of a number of TLRs expressed in diverse locations in Tregs have functional roles that remain to be determined.

The study by Hong et al., published in this issue, adds a new twist to the emerging role of TLRs as positive regulators of Treg functions in the intestine. Far from being an inert intracellular innate immune

Abbreviations: DC, Dendritic cell; FA, Food allergy; GALT, gut-associated lymphoid tissue; GATA3, GATA binding protein 3; IFNAR, type I IFN receptor; ILC2, Type 2 innate lymphoid cell; iTreg, Induced regulatory T; polyI:C, polyinosinic polycytidylic acid; T-bet, T-box transcription factor; Treg, Regulatory T.



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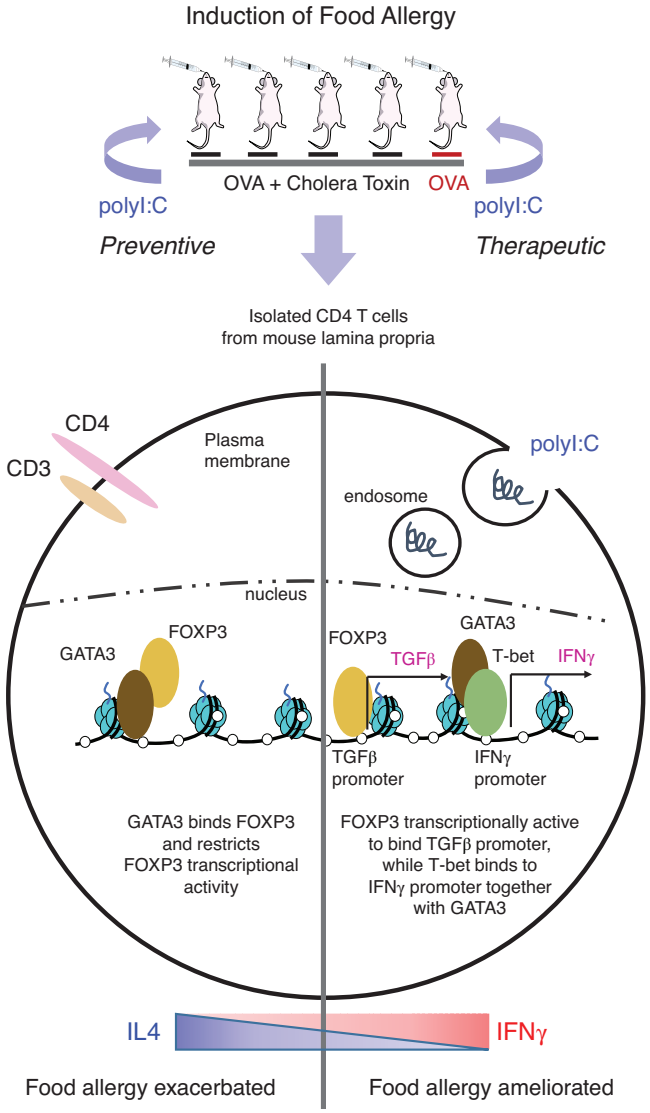


**FIGURE 1** The cartoon depicts transcriptional effects of polyI:C in T cells alleviating pathological immune response in FA

receptor, TLR3 is expressed by the lamina propria T cells, and following triggering by polyinosinic polycytidylic acid (polyI:C), an analogue of double-stranded RNA mirroring viral infection, results in the activation of induced Tregs (iTregs) (Fig. 1). The study shows that polyI:C exposed lamina propria CD4+ T cells in the colon express high levels of FOXP3 and IFN$\gamma$, with potent immunosuppressive functions, which can be mirrored by polyI:C exposure of splenic naïve CD4+ T cells in vitro since they exhibited higher suppressive capacity in inhibiting effector T cell proliferation in comparison to Tregs expressing solely FOXP3. The authors discovered a selective association between the transcription factors, T-bet (T-box transcription factor, driving Th1) and GATA binding protein 3 (GATA3, driving Th2) from the immunoprecipitates of the cell lysates, obtained from the lamina propria CD4+ T cells of mice orally administered polyI:C or alternatively from splenic CD4+ T cells, activated by polyI:C in vitro. The T-bet levels in iTregs were found markedly augmented following both in vivo or in vitro exposure to polyI:C. The authors used immunoprecipitation and later chromatin immunoprecipitation assays elegantly to demonstrate that T-bet competitively and selectively complexed with GATA3, thus liberating and rendering FOXP3 transcriptionally active. Upon release from GATA3, FOXP3 was found to translocate and associate with the promoter of TGF-$\beta$, a key cytokine involved in the differentiation of iTregs. Whether T-bet stoichiometry is a defining parameter for the reported competitive interaction with GATA3 to take place remains unclear, the study identifies T-bet levels positively correlate with increased Treg activity.

In an attempt to test preventive and therapeutic effects of administering polyI:C in a model of Th2-mediated FA, the authors gavage-fed mice with polyI:C for a week prior to chronic feeding of OVA and cholera toxin for 4 consecutive weeks. On the fifth week, mice were rechallenged with high dose of OVA alone or were administered polyI:C together with OVA after establishing a similar type of FA. Remarkably, administration with polyI:C prevented the development of FA as well as inhibited existing FA, in a Treg-dependent manner. The Treg dependence of polyI:C mediated beneficial effects were further substantiated by the in vivo depletion of Tregs using anti-CD25 antibody, which reversed polyI:C mediated protection after an established FA. In conclusion, activation of TLR3 by polyI:C administration induces IFN$\gamma$+ FOXP3+ Tregs, which prevented FA development and inhibited existing FA in mice.

The biologic effects of triggering TLRs in Tregs themselves are fascinating, but further research is required to reveal the versatile role and mechanism of function of TLRs found on the surface or in endocytic locations in Tregs. The study by Hong et al. opens a Pandora's box of questions, relating to which TLR isoforms are expressed at steady states and their dynamic expression upon inflammation or infection by local Treg subtypes. Additional questions remain unestablished with regards to the observed effects of polyI:C augmenting T-bet expression in CD4+ T cells. What are the processes leading to increased protein levels of T-bet? Does T-bet expression involve type I IFN receptor signaling, particularly through IFN beta ($\beta$), an antiviral molecule as the main target of polyI:C mediated transcriptional activity, which may then rev up the transcriptional activity on the T-bet promoter or simply via increasing T-bet mRNA stability in an autocrine/paracrine manner?

The study overall opens up a new window of opportunity for immunotherapy to treat patients with FA by shifting the balance between immunity and tolerance. Future studies are needed for comprehensive analyses of the molecular and biochemical processes associated with the TLRs pathways in T cells that support their immunosuppressive functions.

## DISCLOSURE

The author declares no conflicts of interest.

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