

# Flora-Induced Immunoregulation of Allergic and Inflammatory Disease

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Increasing evidence suggests that immunomodulatory molecules on the commensal microbiota play an important role in regulating immune responsiveness. Recent work has begun to reveal the broad role of microbiota-derived signals in the regulation of a variety of disorders, including diabetes and obesity. We have shown that mice lacking Toll-like receptor 4 (TLR4), the receptor for bacterial lipopolysaccharides, display aberrant Th2-biased hyperreactivity to food antigens. Intragastric administration of a peanut allergen with the mucosal adjuvant cholera toxin induces allergen-specific IgE, elevated plasma histamine levels, and anaphylactic symptoms in TLR4 mutant mice, but not in MHC matched controls. When the composition of the microbiota is reduced and altered by antibiotic administration (beginning at 2 weeks of age), TLR4 wild-type mice become as susceptible to the induction of allergy as their TLR4 mutant counterparts. Both allergen-specific IgE and Th2 cytokine responses are reduced in antibiotic-treated mice in which the microbiota is allowed to repopulate.<sup>1</sup>

More recent unpublished work has shown that in TLR4 wild-type mice, a putative regulatory population of plasmacytoid DC (pDC), with constitutive expression of IL-10, is detectable in the mesenteric lymph nodes (MLNs), which drain the gut-associated lymphoid tissue, but not in the spleen. The constitutive expression of IL-10 mRNA by this pDC population is impaired in TLR4 mutant mice. Antibiotic administration eliminates the constitutive expression of IL-10 by MLN pDC, further supporting a role for the microbiota in stimulating the expression of these cytokines. CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> regulatory T cells (Tregs) isolated from the spleen and MLN of

TLR4 mutant mice have normal in vitro regulatory function but are impaired in their ability to secrete IL-10 in response to T-cell receptor triggering in vitro. Our findings suggest that TLR4 mutant mice are highly susceptible to allergic responses to food antigens because they lack populations of microbiota-induced regulatory DCs and T cells, which are present in wild-type mice.

The incidence of allergic sensitization to food is increasing dramatically.<sup>2</sup> A number of studies already have associated polymorphisms in TLR4, and/or its co-receptor CD14, with an atopic phenotype<sup>3-7</sup>; TLR4 agonists are under development as immunotherapeutics for the treatment of allergic disease.<sup>8</sup> Our animal model data suggest that microbiota-derived TLR4 signals are important developmentally during the transition to weaning. A relatively short course of broad spectrum antibiotics led to alterations in immune system function by altering the composition of the commensal microbiota; after antibiotic treatment TLR4 wild-type mice developed allergic responses to food antigens similar to those seen in TLR4 mutant mice.<sup>1</sup> Analogously, repeated courses of oral antibiotics during infancy (as are commonly given for the treatment of ear infections) might contribute to the increasing incidence of allergic responses to food. A better understanding of how microbiota-induced TLR4 signals modulate the function of antigen-presenting cells and T cells will inform strategies for the development of TLR4 agonists that mimic these immunoregulatory signals. For example, prophylactic co-administration of this type of TLR4 agonist during antibiotic treatment regimens in infancy would maintain stable TLR4-induced immunoregulatory signals under conditions that alter the composition of the microbiota, and might provide an effective and novel strategy for preventing sensitization to food antigens.

TLR4 signals from the commensal microbiota also influence the Th1/Th17-mediated inflammation associated with the development of colitis in IL-10<sup>-/-</sup> mice. Spontaneous *Helicobacter*-dependent colitis is exacerbated in IL-10<sup>-/-</sup> mice that bear a mutation in TLR4. We found that TLR4-mediated signals play two interrelated roles in the exacerbation of disease in TLR4<sup>-/-</sup> x IL-10<sup>-/-</sup> mice.<sup>9</sup> FoxP3<sup>+</sup> Tregs accumulate in the colonic lamina propria of TLR4<sup>-/-</sup> x IL-10<sup>-/-</sup> mice, acquire the ability to produce interferon g (IFN-g), and fail to protect against disease. In addition, dysregulated control of epithelial cell turnover in TLR4<sup>-/-</sup> mice results in the persistence of antigen-presenting cells (APCs) bearing apoptotic epithelial fragments in the colonic lamina propria of *Helicobacter*-infected mice. Our data suggest that, in TLR4<sup>-/-</sup> mice, IL-10 secretion by these APCs holds an inflammatory response in check. In mice that lack both IL-10 and TLR4 mediated signals, aberrant regulatory cell function and dysregulated control of epithelial homeostasis combine to exacerbate inflammation. Taken together with new genome-wide association studies identifying a genetic locus that modifies TLR4 signaling as a novel risk factor for inflammatory bowel disease,<sup>10</sup> our results suggest the possibility of exploring TLR4-based therapies for the treatment of intestinal inflammation.

## References

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## Q&A

Q: In the food-allergy model, have you tried to deplete plasmacytoid dendritic cells (pDCs)?

Also, where are the pDCs?

Dr Nagler: That is what we are going to do next. There is no evidence that pDCs are trafficking from the lamina propria or from the Peyer's patches to the mesenteric lymph nodes. We think there is some other dendritic cell population that initially samples the antigen and carries it to the pDC population, or carries some signal derived from that population to the mesenteric lymph node, which then regulates the response. It is possible that the initial Toll-like receptor 4 (TLR4)-dependent signal is generated by the epithelium, and that it is an epithelial-derived signal that educates the dendritic cells below the epithelium and regulates this pathway. Those questions are all approachable with the models we have now. We can make bone marrow chimeras, do epithelial-specific deletion of TLR4, and do pDC depletion to find out where this response is being generated.

Q: You mentioned that the food-allergy model is TLR4 signal independent. Does that mean Gram-negative bacteria are key in driving the food-allergy response in that model, as opposed to Gram-positive?

Dr Nagler: I do not want to imply a specific role for TLR4 in regulating human disease. TLR4 has been implicated in many different murine allergy models, although the genetics of food allergy are not known yet. It is not inconceivable, but I am trying to present this as a model of the ability of the microbiome to influence immune-mediated responses and the possibility of regulating those responses by manipulating the microbiome.

Q: But do you not think that, in the microbiome, species binding mainly to the TLR4 are Gram-negative?

Dr Nagler: I think that TLR4-mediated signals have both inflammatory and anti-inflammatory roles. So blocking one pathway or another suggests a potential for immunotherapeutics.

Q: Is atopic dermatitis or eczema induced in this food-allergy model?

Dr Nagler: Not in the model. However, that is associated with food allergy.

Q: So you see systemic changes and IgE?

Dr Nagler: Yes. One practical implication of this model that relates directly to your question is found in some soft data that suggest that the increase in food allergy is related to antibiotic use, especially in infancy. So if you were able to modulate at the same time you give antibiotics, or you reduced the use of antibiotics in viral infections, for which they are not needed, and you also provided a TLR4 agonist that could continue the TLR4 immunomodulatory signal at the same time you removed the bacterial pathogen population that is generating the inflammatory response, you perhaps could eliminate the influence of antibiotics on susceptibility to allergy, if that truly exists. This is a potential area of study.

Q: I am familiar with the cholera toxin model, but what happens if you do not use cholera toxin? What happens in C3H/HeJ mice by themselves in your model, and what happens if you treat the C3H/HeJ with antibiotics?

Dr Nagler: I did not show you results without cholera toxin because we did not study that. The allergic response in C3H/HeJ mice treated with antibiotics is unchanged.

Q: You described an  $MyD88^{-/-}$  x  $IL-10^{-/-}$  model in which TLR4 signal lines protect against inflammatory bowel disease [Rakoff-Nahoum S et al: *Cell* 2004;118:229-241]. We can interpret that as meaning that some constitutive group of epithelial cells required signaling between the flora and epithelial cells. Does that fit with your model?

Dr Nagler: No, completely the opposite.

Q: What do you think of that model now?

Dr Nagler: I suggest a unique role for TLR4. Eyal Raz at the University of California at San Diego has unpublished data on TLR9<sup>-/-</sup> x IL-10<sup>-/-</sup> mice, and they look like MyD88<sup>-/-</sup> x IL-10<sup>-/-</sup> mice. He does not have the big colony data we have, however, and his group did not look at the effect of *Helicobacter*—and the result is clearly changed by the presence or absence of *Helicobacter* infection.

Q: I think your data show nicely that there are a lot of models in which effects are modulated by the flora. In studies by Medzhitov and colleagues [Rakoff-Nahoum S et al: *Cell* 2004;118:229-241], the hygiene status in which these mice were held probably had a huge effect, and neurovirus infection of many mouse colonies has quite an effect on the epithelial layer.