

T cells (Nishimura et al., 2009), and Reisner and colleagues corroborate this. Through deconvolution of variance between groups, the authors identified TCR- $\beta$  sequences which were enriched in AT of mice lacking Perf-DCs. Several of the identified TCR- $\beta$  sequences varied at the nucleotide level while encoding synonymous codons. Enticingly, this implies Perf-DCs constrain T cell clones, which recognize specific antigen(s) present in AT.

The authors conclude that deletion of populations of T cells in adipose tissue helps limit fat mass expansion. This is a provocative hypothesis and wholly consistent with their data. However, it raises several further lines of questions for which their model does not provide answers. From an immunological perspective, what are the postulated antigens? From where are they derived? Are they adipocyte or even foreign derived? Answering these questions would undoubtedly reveal a fundamental aspect of the relationship between adipose tissue and the immune system and likely shed light on why there is such a robust immune response in adipose tissue.

These findings also do not address the nature of the signal that causes an increase in fat mass in Perf-DC-deficient mice. Though incompletely understood, the regulation of fat mass is under neuroendocrine control, with hormones, most notably leptin being released from adipose tissue, signaling to the feeding and energy regulatory centers of the central nervous system. How does deletion or expansion of T cells alter this homeostatic loop? It is conceivable that the weight regulatory effects of Perf-DCs might not be limited or even found in the adipose tissue.

Although the immune response to metabolic perturbations is most robust in adipose tissue, metabolically induced and specifically obesity induced changes in immune populations also occur in other important tissues, including the liver, intestine, pancreas, and hypothalamus. Indeed, perhaps the most parsimonious explanation for the current observations is that Perf-DCs limit T cell populations in the hypothalamus or brainstem in ways that locally modulate neurons critical for feeding behavior, satiety and energy expenditure. Answering these ques-

tions will provide key, fundamental insights into both metabolism and immunology.

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# Licensed to Ill: IL-9 Generation in Immature Mast Cells Permits Food-Elicited Anaphylaxis

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**Food-specific IgE is central to the pathobiology of food allergy, but not sufficient to induce disease. Chen et al. (2015) demonstrate that food-elicited reactions require an immature mast cell that generates IL-9 to induce its own maturation.**

Acute allergic reactions to foods can range in severity from mild mouth itching or abdominal discomfort to severe life-threatening anaphylaxis. Because there is no cure or disease-modifying therapy, the management of food allergic patients is limited to the use of epinephrine for accidental ingestions and the strict avoid-

ance of diagnosed food allergens. This prescription is complicated by the finding of elevated food-specific immunoglobulin E (IgE), indicative of sensitization, in individuals with no clinical reaction to the implicated food. Indeed, although approximately 8% of U.S. children are sensitized to peanut, the prevalence of

clinical allergy is approximately 1%–2% (Liu et al., 2010). The factors that regulate clinical reactions to food antigens have been sought for some time but are poorly understood.

The natural history of repeated oral antigen administration in mice and humans is the generation of antigen-specific

tolerance and the acquisition of regulatory T cells. Murine models of food allergy circumvent this by administering oral antigens in the presence of strong adjuvants (cholera toxin, staphylococcal enterotoxin), by using mutant mice with disrupted regulatory T cell function, or by sensitizing mice through either disrupted skin or peritoneal injection with alum. Genetic susceptibility to experimental food allergy in mice is widely acknowledged to be strain-specific, despite the significant induction of IgE in most strains. A constant feature among these models is the involvement of activated mast cells (MCs) (reviewed in [Oyoshi et al., 2014](#)).

MCs are well known effectors of allergic inflammation. Activated MCs release secretory granule-associated mediators such as histamine, generate lipid mediators such as cysteinyl leukotrienes (cysLTs) and platelet activating factor (PAF), and produce pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF- $\alpha$ ) and interleukin-13 (IL-13). There are two major subclasses of MCs, innate and induced. Innate connective tissue MCs (CTMCs), derived independently of thymic function from fetal liver progenitors, reside in stromal tissues, while induced mucosal MCs (MMC9s), derived from bone marrow (BM) progenitors, are found in the epithelium of the gut and lung in the setting of type-2-cell-mediated inflammation.

The study by [Chen et al. \(2015\)](#) induced experimental food allergy in BALB/c mice with ovalbumin (OVA)+alum intraperitoneal sensitization and repeated oral OVA challenges. This model has been previously demonstrated to induce expansion of MMCs in the small intestine ([Brandt et al., 2003](#)). Moreover, depletion of MMCs by an antibody directed to c-Kit or inhibition of MC activation by an antibody to IgE or the use of Fc $\epsilon$ R1 null mice significantly reduced the development of diarrhea and gut edema elicited by oral antigen challenge, indicating the importance of MC activation for the elicitation of clinical reactions. Importantly, in the same OVA+alum model, mice with a targeted deletion of either the IL-9 receptor (IL9R) or IL-9, a cytokine which promotes intestinal mastocytosis, were protected from MMC expansion in the intestine and MC degranulation and diarrhea induced by oral antigen, despite intact generation of OVA-specific IgE and IgG ([Osterfeld](#)

[et al., 2010](#)). Thus IL-9 appears to be a second signal required for clinical reactivity to oral food antigens.

In the current study, Chen et al. used this model to identify the cellular source of IL-9. The investigators first identified lineage-negative (Lin<sup>-</sup>) IL-9-producing cells in the lamina propria of several sensitized and challenged murine strains. As expected, they found a correlation between the number of IL-9<sup>+</sup> cells and the degree of intestinal mastocytosis, MC activation (as determined by the detection of mouse MC protease-1, mMCP-1, in the serum), and antigen-induced diarrhea and hypothermia across strains. IL-9-producing cells expressed c-Kit and the receptor for IL-33 (ST2), contained transcripts for mMCP-1 and the transcription factor GATA-2 but not GATA-3, and released mMCP-1 on ex-vivo IgE cross-linking, identifying these as MCs and not type 2 innate lymphoid cells (ILC2s). Notably these cells generated very high amounts of IL-9 (0.4 pg/ml/cell) and generated higher amounts (1.2 pg/ml/cell) with IL-33 stimulation.

MMC9s develop exclusively in peripheral tissues from BM-derived agranular MC progenitors (MCps) which seed the intestine and lung of naive mice and expand in the setting of type 2 cell-mediated inflammation. MCps are defined by their expression of the integrin  $\beta$ 7, which is required for MCp homing to the small intestine ([Gurish et al., 2001](#)) and the inflamed lung ([Abonia et al., 2006](#)), and which is progressively lost with maturation of MCps into hypogranular early MMCs (eMMC) and then fully granulated MMCs ([Bankova et al., 2015](#)). Chen et al. found that while Lin<sup>-</sup>cKit<sup>+</sup>ST2<sup>+</sup> $\beta$ 7<sup>hi</sup> MCps did not produce IL-9, a hypogranular Lin<sup>-</sup>cKit<sup>+</sup>ST2<sup>+</sup> $\beta$ 7<sup>lo</sup> cell did. They went on to identify this cell as an intermediate in MC lineage development by several methods. Using the adoptive transfer of purified BM MCps (Lin<sup>-</sup>Ly6C<sup>-</sup>Fc $\epsilon$ R1<sup>-</sup>CD41<sup>-</sup>CD71<sup>-</sup>FLK2<sup>-</sup>CD150<sup>-</sup>cKit<sup>+</sup> $\beta$ 7<sup>hi</sup> cells) into irradiated recipients to reconstitute intestinal MCps, they showed that antibody directed to  $\alpha$ 4 $\beta$ 7 integrin prevented MCp reconstitution of the intestinal compartment, as expected, and also prevented the development of IL-9-producing hypogranular MMCs (MMC9s), mature MMCs, MC degranulation, and diarrhea in response to OVA+alum sensitization and oral OVA challenge. Furthermore, isolated intestinal MCps acquired

IL-9 generating capacity when cultured ex vivo with a combination of IL-4, TGF- $\beta$ , and IL-33, and isolated IL-9-producing MMC9s cultured with the conventional MC growth factors stem cell factor (SCF) and IL-3 generated granulated MCs, which lost the capacity to generate IL-9.

Next, the investigators assessed the requirements for MMC lineage development in vivo ([Figure 1](#)). Murine strains that were resistant to experimental food allergy (IL-4<sup>-</sup>, IL4R<sup>-</sup>, STAT6<sup>-</sup>, or T cell-deficient) had reduced intestinal MCps, MMC9s, MMCs, and diarrhea in response to oral antigen, suggesting regulation at the level of MCps. By contrast, Ab-mediated depletion of CD4<sup>+</sup> cells demonstrated no effect on intestinal MCps, but reduced MMC9s, MMCs, and clinical response to oral antigen, suggesting CD4<sup>+</sup> T cell regulation of the MC transition from MCps to MMC9s, likely by T helper 2 (Th2) cells. Finally, both IL-9-deficient and IL9R-deficient strains had normal numbers of MCps and MMC9s, but reduced mature MMCs, MC activation, and response to oral antigen, indicating that the generation of IL-9 is a critical checkpoint to generate a fully mature MMC. As the transfer of WT, but not IL-9-deficient, MMC9s to irradiated IL-9-deficient mice restored the induction of intestinal mastocytosis, MC reactivity, and response to oral antigen, MMC9s generated the critical cytokine for their own maturation. Finally, the authors demonstrated elevated amounts of transcripts for IL-9 and MC selective proteases such as trypsin, chymase, and carboxypeptidase in duodenal biopsies from patients with food allergy, as compared to control subjects.

The findings by Chen et al. add considerably to our understanding of experimental food allergy and MC biology. First, they provide substantial evidence for the role of MMCs, not basophils or CTMCs, in the IgE-dependent reactions elicited by oral food challenge. Second, using careful flow cytometric characterization of intestinal MMC populations across time, strains, adoptive transfers, and depletions, they provide clear evidence that MMC9s are hypogranular MC lineage intermediates developing in the setting of type-2-cell-mediated intestinal inflammation. Third, and perhaps most interesting for further study, they find that

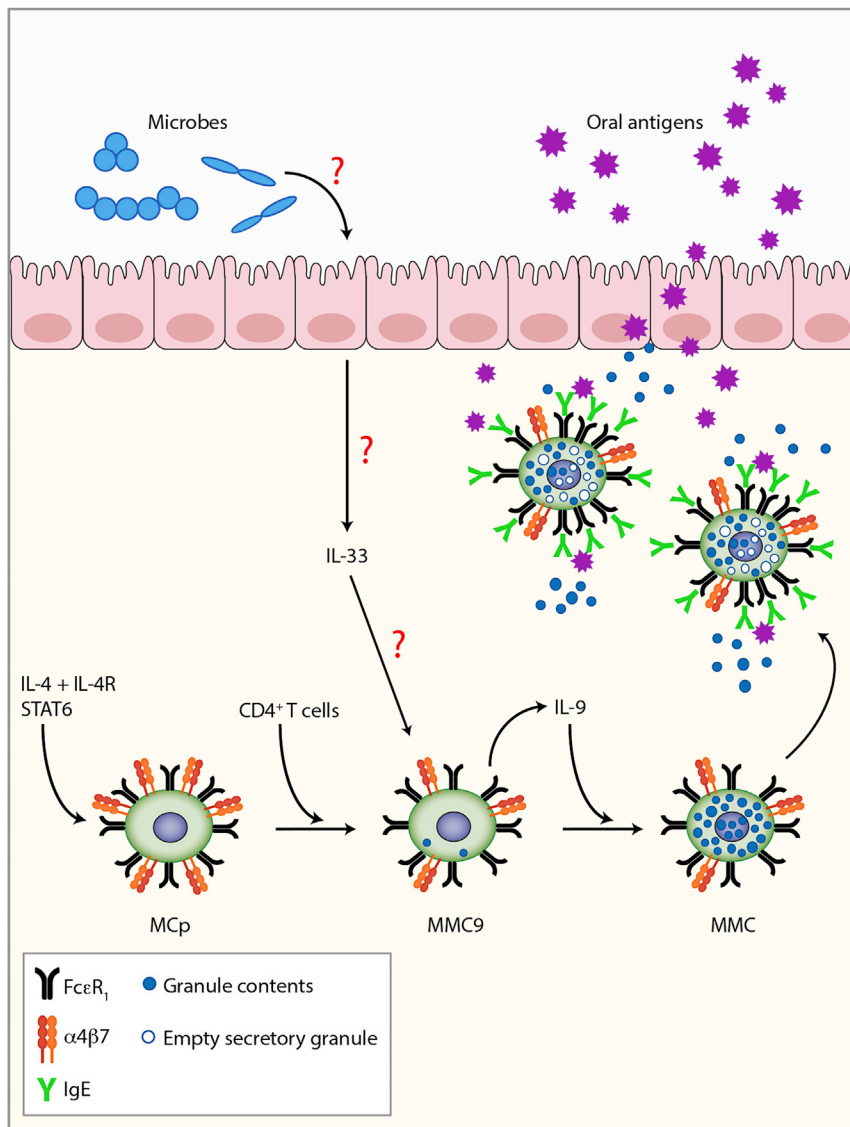


**Figure 1. Intestinal Mucosal Mast Cell Lineage Development in Experimental Food Allergy**

Mast cell progenitors (MCps) are recruited and expanded in the intestinal mucosa in the setting of T cell-dependent type-2-cell-mediated inflammation requiring IL-4 + IL4R and STAT6 signaling. MCp development into IL-9-producing MMCs (MMC9s) requires CD4<sup>+</sup> T cells and might be driven by stromal-derived signals such as IL-33 elicited by the intestinal microbiome. IL-9 production by MMC9s and possibly alternative sources drives MMC9 maturation into MMCs with characteristic granules, poor proliferative capacity, and potent reactivity to oral antigen challenge.

MMC9s participate in driving their own maturation into fully competent MMCs, but require as yet undetermined strain-related local tissue signals to activate this program.

The findings from this study have several potential implications. First, it is tempting to speculate that local MMC

expansion might provide a second tissue-level signal required for food allergy, and the lack of this population may explain the absence of clinical reactions in many patients with elevated food-specific IgE. Second, the granulated MMCs derived from MMC9s could no longer generate IL-9, indicating the limits of inter-

preting MC pathobiology based on the study of granulated cells. Furthermore, as cytokine producing hypogranular MC lineage intermediates are also seen in models of allergic pulmonary inflammation (Bankova et al., 2015) and helminth infection (Liu et al., 2013), further studies of these cells are likely to reveal additional roles in immunopathology. Third, as transfer of the gut microbiome from food allergic mice elicits MC activation and clinical reactivity to oral antigen in recipients (Noval Rivas et al., 2013), it seems likely that the local microbiota could play a significant role in intestinal MMC development and further study of these interactions will help shed light on the programs that drive MMC9 maturation and license them for ill.

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