trigger the DC activation response? For one thing, white blood cells injected alone were apparently unable to evoke $CD4^+$ DC activation, as shown, but more investigation is needed to further substantiate that RBCs are both essential (platelets? white cell contribution?) and sufficient (highly purified RBCs?) for this. The current work certainly deserves credit for providing the inspiration to perform such studies.

Another question relates to the issue of "missing self." Clearly, this concept is well known for MHC class I molecules that can be downregulated under various pathological conditions, such as infection with large DNA viruses. However, is this also the case for CD47 on RBCs? And if so, could this then be a mechanism to engage erythrocytes in promoting immunity by delivering danger signals to DCs? Actually, we do not really know at this point whether missing CD47 occurs at all, for instance, during infections of the erythrocyte, and, if this does occur, whether it plays a critical and meaningful role. Clearly, one condition that springs to mind when considering erythrocytes as a target for infection is malaria. However, evidence so far has not revealed any effects of RBC *Plasmodium* infection on CD47 overall expression (Hempel et al., 2014). What is interesting nevertheless is that CD47 deficiency confers resistance to malaria infection (Banerjee et al., 2015), and the current work by Yi et al. might also provide a potential explanation for that.

Finally, there is the situation in humans. It is known that the human spleen has quite a different architecture than the rodent spleen, and this is particularly evident in the marginal-zone area. In fact, a marginal zone is not clearly identifiable in the human spleen (Steiniger, 2015). Nevertheless, the perifollicular zone of the human spleen, which also contains a subset of DCs, has been suggested to resemble, at least in some aspects, the rodent marginal zone (Pack et al., 2008). It would obviously be of interest to find out whether this novel and exciting concept of RBC-mediated control over DC activation also occurs in humans. Be that as it may, Yi and colleagues have certainly demonstrated that DCs see red when encountering erythrocytes that are denuded of CD47.

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Keeping Off the Weight with DCs

Ethan A. Edwin[1] and Anthony W. Ferrante, Jr.[1,*]
[1]Department of Medicine, Naomi Berrie Diabetes Center, Columbia University, 1150 St Nicholas Ave, New York, NY 10032, USA
*Correspondence: awf7@cumc.columbia.edu
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Long studied as modulators of insulin sensitivity, adipose tissue immune cells have recently been implicated in regulating fat mass and weight gain. In this issue of *Immunity*, Reisner and colleagues (2015) report that ablation of perforin-expressing dendritic cells induces T cell expansion, worsening autoimmunity and surprisingly increasing adiposity.

The immune and metabolic systems are tightly coupled so that substantial changes in one often alter the function and state of the other. In adipose tissue (AT), where these two systems interact intimately, increases or decreases in fat mass elicit cellular immune responses that can increase or decrease immune cell populations by as much as an order of magnitude, so that in the most obese individuals immune cells constitute the majority of cells in a fat depot. Conversely, perturbations in immune cell number and function can increase or reduce the efficiency of adipocyte lipid storage, and thereby alter systemic glucose and lipid metabolism. Sub-populations of adipose tissue macrophages (ATMs) and T cells have been the most intensively studied but eosinophils, mast cells, neutrophils, and iNKT cells also respond to changes in the metabolic state of adipose tissue and have been implicated in modulating local inflammation and systemic metabolism. A simple paradigm has emerged from early studies suggesting that changes in immune function and profiles that skew toward a type 1 cellular immune response impair adipocyte function and induce systemic insulin resistance.

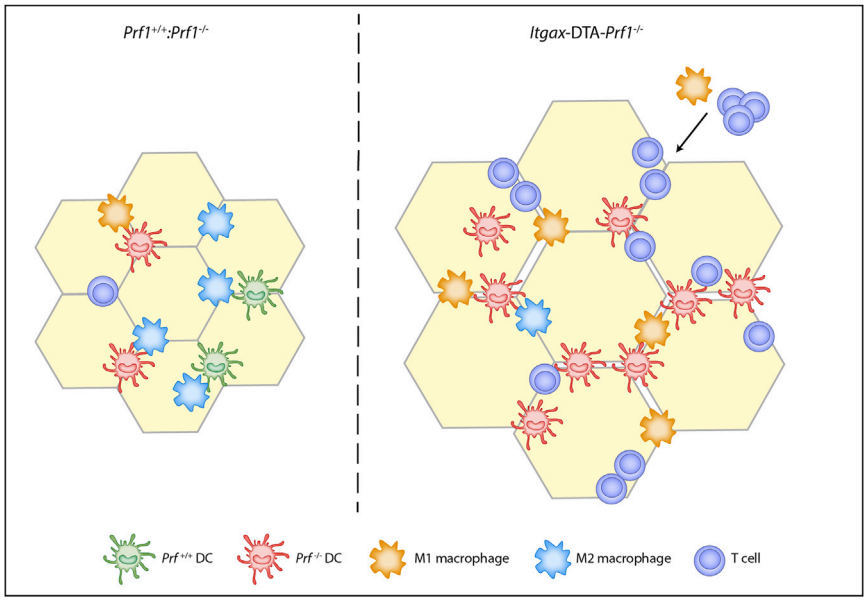

Figure 1. Deficiency of Perforin$^+$ DCs Leads to Expansion of T Cell Populations and Adipose Tissue Mass

The mixed marrow transplant design employed by Reisner and colleagues allows comparison of WT-*Prf1*$^{-/-}$ mice in which 50% of hematopoietic cells lack perforin (left panel) and *Itgax*-DTA- *Prf1*$^{-/-}$ mice in which all CD11c$^+$ (Itgax$^+$) cells lack perforin (right panel). In the *Itgax*-DTA-*Prf1*$^{-/-}$ mouse (right panel), their adipose tissue has an increase in T lymphocytes and M1 macrophages and is enlarged compared to adipose tissue (left panel) where half of all hematopoietic cells in WT-*Prf1*$^{-/-}$ mouse lack perforin.

Recent studies, however, have uncovered a more direct role for immune cells in adipocyte metabolism, a role independent of inflammation and in which lipid catabolism, especially by ATMs, regulate whole adipose tissue and body lipid metabolism. The catabolism of excess lipids by ATMs contributes directly to lipid fluxes through fat and into the circulation (Xu et al., 2013). Separately, genetic and pharmacologic studies that target immune cells function have uncovered another unexpected role for the immune system in adipose tissue biology. In immune cells, fat mass is modulated by targeted deletion or inhibition of nuclear receptors, surface receptors that regulate immune cell activation and recruitment, and inflammatory regulators. However, despite the identification of some immune molecules that can regulate fat mass, the cells responsible and the mechanisms remain completely obscure. Reisner and colleagues now identify a population of perforin$^+$ dendritic cells (Perf-DCs) (Zlotnikov-Klionsky et al., 2015) whose function contributes to adipose tissue expansion.

Previously, the Reisner laboratory defined a population of dendritic cells that in vitro kill CD8$^+$ T cells recognizing peptide-major histocompatibility complex (MHC) molecules in a process that employs perforin, a canonical cytotoxic polypeptide (Zangi et al., 2012). They had hypothesized that these cells would function in a tolerogenic fashion, limiting immune and inflammatory responses in vivo. Using a clever method to generate mice that lack perforin in CD11c$^+$ cells, the authors demonstrated a role for Perf-DCs in the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis and perhaps more surprisingly in regulating adipose tissue inflammation and mass.

The elimination of perforin-expressing dendritic cells was carried out by transplanting irradiated wild-type mice with mixed marrow from two donors: *Perforin*-deficient (*Prf1*$^{-/-}$) and *Itgax*-DTA mice. Immune cells arising from *Itgax*-DTA marrow expressed the lethal diphtheria toxin A under the *Itgax* (Cd11c) promoter. Thus, all CD11c$^+$ leukocytes in the *Itgax*-DTA;*Prf1*$^{-/-}$ chimeras derive from the *Prf1*$^{-/-}$ donor (Figure 1). One caveat of this approach is that approximately half of the non-CD11c$^+$ cells immune cells are also deficient in perforin. Given that CD8$^+$ T cell homeostasis is in part dependent on perforin (Badovinac et al., 2000), this partial deficiency of perforin in non-targeted populations might alter the homeostasis and clonality of some subpopulations, including cytotoxic T lymphocytes.

Consistent with in vitro observations and Perf-DCs playing a tolerogenic role, mice lacking Perf-DCs proved more susceptible to experimental autoimmune encephlalomyelitis (EAE); developing disease earlier and with greater severity. Unexpectedly, however, mice deficient in Perf-DCs also developed a metabolic phenotype while eating a low-fat diet in the absence of any experimental immune stimulus. Three months after transplant, mice fed a low-fat diet and lacking Perf-DCs gained significantly more weight than control mice with Perf-DCs intact. The weight gain was due to expansion of fat mass and adipocyte hypertrophy, and consistent with the increased adiposity, the Perf-DC-deficient mice were metabolically dysregulated with increased insulin resistance and impaired glucose tolerance. These findings echo the metabolic phenotype of mice lacking Perforin (*Prf1*$^{-/-}$) in all cells. Mice lacking *Prf1* weigh more than their littermate controls early in life, though by 26 weeks of age the weight of the *Prf1*$^{-/-}$ mice catches up to that of control animals (Revelo et al., 2015). The obesity phenotype of Perf-DC-deficient mice was further exacerbated when they were fed a high-fat diet; animals deficient in Perf-DCs became more obese when fed a high-fat diet than mice replete with Perf-DCs. This unexpected result led the authors to study the immune cell populations of adipose tissue.

On the basis of their previous in vitro data, the authors hypothesized Perf-DCs act to regulate the quantity of T cells in adipose tissue and limit their expansion. Indeed, deficiency of Perf-DCs increased the populations of CD4$^+$ and CD8$^+$ T cells in fat of *Itgax*-DTA; *Prf1*$^{-/-}$ mice. Remarkably, depletion of T cells in the Perf-DCs not only normalized the AT T cell population but also prevented the development of obesity. If their model is correct, then these data imply a restriction of the T cell receptor (TCR) repertoire in normal adipose tissue to which Perf-DCs contribute. A restricted adipose tissue TCR repertoire in lean mice has been reported by others, both in regulatory T cells (Feuerer et al., 2009) and CD8$^+$

T cells (Nishimura et al., 2009), and Reisner and colleagues corroborate this. Through deconvolution of variance between groups, the authors identified TCR-β sequences which were enriched in AT of mice lacking Perf-DCs. Several of the identified TCR-β 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 questions 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

Nora A. Barrett[1] **and K. Frank Austen**[1,*]
[1]Division of Rheumatology, Immunology and Allergy, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA
*Correspondence: fausten@research.bwh.harvard.edu
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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 avoidance 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