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Eosinophils in Fat: Pink Is the New Brown

Stephen D. Lee[1] and Peter Tontonoz[1,*]
[1]Howard Hughes Medical Institute, Department of Pathology and Laboratory Medicine, University of California, Los Angeles, Los Angeles, CA 90095, USA
*Correspondence: ptontonoz@mednet.ucla.edu
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Subcutaneous white adipose tissue can be induced to undergo "browning" and acquire thermogenic capacity in response to physiological stimuli such as cold exposure or exercise. In this issue of *Cell*, Qiu et al. and Rao et al. demonstrate that pink-staining eosinophils and alternatively activated macrophages play key roles in an immune cascade mediating this metabolic switch.

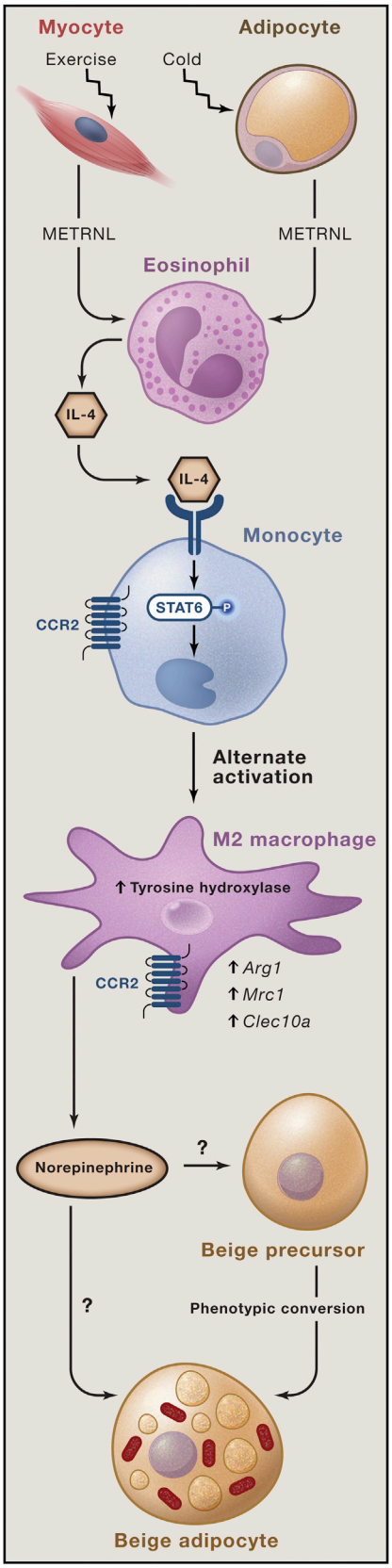
Two papers in this issue of *Cell* (Qiu et al., 2014; Rao et al., 2014) define molecular pathways that regulate the ability of white adipose tissue (WAT) to acquire thermogenic capacity through browning. Maintenance of body temperature in mammals is achieved through a combination of shivering and nonshivering thermogenesis. For many years, nonshivering thermogenesis in adipose tissue was thought to occur exclusively in brown adipose depots. In contrast to white adipocytes that store energy as triglycerides, brown adipocytes generate heat from the metabolism of fatty acids by virtue of their ability to express the mitochondrial uncoupling protein UCP1. More recently, it has been appreciated that a subpopulation of cells within subcutaneous white adipose depots, known as "beige" or "brite" adipocytes, can also engage in thermogenesis. Moreover, the activity and abundance of these beige cells can be increased in response to enhanced thermogenic demand, leading to the so-called "browning" of WAT. Coupled with the observation that cells in adult human brown adipose depots resemble beige adipocytes (Wu et al., 2012), the discovery of browning has fueled interest in manipulating beige adipocyte abundance or activity for the treatment of metabolic diseases (Bartelt and Heeren, 2014).

UCP1 is crucial to thermogenesis in both brown and beige adipocytes, but the factors that regulate its expression differ in the two cell types. Brown adipose tissue is densely innervated, and consequently, thermogenesis in this tissue is regulated primarily by the sympathetic nervous system. In contrast, white adipose depots are poorly innervated, suggesting that alternate pathways must control UCP1 expression. Factors influencing UCP1 expression in white depots have been described previously, including starvation hormones, atrial and brain natriuretic peptides, myokines, and bone morphogenic proteins (Rosen and Spiegelman, 2014). Rao et al. (2014) and Qiu et al. (2014) now identify a role for eosinophils in the alternative activation of adipose tissue macrophages in response to exercise and chronic cold exposure, respectively. Eosinophils are circulating immune cells that derive their name from the fact that they are avidly stained by the pink histochemical dye eosin. Once activated by eosinophil-derived IL-4, adipose tissue macrophages release catecholamines, activate β-adrenergic signaling, and drive thermogenesis (Qiu et al., 2014; Rao et al., 2014).

In 2011, Nguyen et al. (2011) reported that macrophages contribute to brown adipocyte thermogenesis in response to cold exposure. In the current study, Qiu et al. (2014) show that type II immune signaling pathways also contribute to the browning of subcutaneous white fat. They employ loss-of-function mouse models to define the immune cascade responsible for cold adaptation, beginning with IL-4/13 release and terminating with tyrosine hydroxylase-mediated synthesis of catecholamines in alternatively-activated macrophages (Figure 1). Mice with a global deletion of IL-4/13, IL-4 receptor α (IL-4Rα), STAT6, or chemokine receptor 2 (CCR2) or mice lacking eosinophils altogether show reduced energy expenditure under thermal stress and an inability to maintain body temperature when housed at 4°C. Furthermore, in each case, the ability to defend body temperature correlates with subcutaneous WAT browning.

The loss of browning in CCR2 knockout mice suggests that monocytes are important to the process. Qiu et al. (2014) confirm this by showing that conditional disruption of IL-4Rα or tyrosine hydroxylase (which catalyzes the rate-limiting step in catecholamine synthesis) in monocytes prevents browning. To address whether this pathway might be harnessed for potential therapy, they administer recombinant IL-4 to mice in an effort to drive the beige adipocyte thermogenic program. Indeed, IL-4 treatment increases UCP1 expression and promotes energy expenditure in the setting of thermal stress, and this effect is abrogated by deletion of IL-4Rα. Finally, administration of IL-4 also improves the metabolic phenotype of mice with diet-induced obesity, as evidenced by decreased body mass and improved insulin responsiveness.

Rao et al. (2014) uncovered the importance of eosinophils to browning using a completely independent approach. They were pursuing their recent discovery of an exercise-induced splice isoform of PPARγ coactivator 1a, Pgc1α4 (Ruas et al., 2012). Muscle-specific Pgc1α4-transgenic mice are lean and show increased adipose tissue thermogenesis. Combining microarray and proteomics approaches, Rao et al. (2014) identify



Meteorin-like (METRNL) as a myokine that contributes to the phenotype of Pgc1α4-transgenic mice. The *Metrnl* gene is highly expressed in both muscle and fat and can be induced by either resistance exercise (downhill running) in skeletal muscle or by cold exposure in WAT. Overexpression of *Metrnl* in mice increases energy expenditure, promotes thermogenic gene expression, and increases the abundance of beige adipocytes. Similarly, administration of recombinant METRNL to mice also stimulates thermogenic gene expression, and this is accompanied by weight loss in a diet-induced model of obesity.

In the course of pursuing the mechanism of METRNL action, Rao et al. (2014) noted that *Metrnl* overexpression induces genes involved in type II immune activation. Rao et al. (2014) follow up this observation by testing whether the METRNL-induced browning of adipose tissue is secondary to immune cell activation. Studies in pure cell populations suggest that recombinant METRNL does not act directly on adipocytes or macrophages; however, its effects in vivo are dependent on the IL-4/13 signaling cascade of alternately activated macrophages. Fluorescence-activated cell sorting (FACS) analysis of adipose tissue reveals increased numbers of eosinophils in mice overexpressing METRNL. The authors subsequently demonstrated that eosinophils are indeed required for METRNL-induced browning. Finally, a neutralizing antibody raised against METRNL prevents the accumulation of eosinophils in adipose tissue and reduces the expression of genes associated with alternative macrophage activation and thermogenesis, thereby implicating METRNL in the physiological browning response.

Figure 1. A Type II Immune Cascade Activates Thermogenesis in White Adipose Tissue

Cold exposure or exercise induces the secretion of Meteorin-like (MTRNL), a peptide that triggers the production of the type II cytokines IL-4 and IL-13 by eosinophils in adipose tissue. These cytokines cause the alternative (M2) activation of adipose tissue macrophages through activation of STAT-6 signaling and induce their production of catecholamines. The local release of catecholamines from alternatively activated macrophages increases the abundance of thermogenic, UCP1-expressing "beige" adipocytes in a process known as browning.

Together, the complementary approaches of Rao et al. (2014) and Qiu et al. (2014) provide compelling evidence for the involvement of an eosinophil-derived signal in the physiological adaptation of WAT depots to thermogenic challenges. At the same time, these papers also raise a number of important questions for future investigation, including: what is the cellular target for METRNL? How does cold exposure elicit eosinophil recruitment to and release of anti-inflammatory cytokines in adipose tissue? And do catecholamines released by alternately activated macrophages act on beige progenitors or on mature white adipocytes? The identification of the "pink-brown" cascade, combined with recent reports suggesting that beige adipose tissue activity can contribute meaningfully to whole-body thermogenesis (Cohen et al., 2014; Shabalina et al., 2013), seems certain to fuel interest in beige adipocytes as potential targets for metabolic therapeutics.

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