Non-canonical alternatives: What a macrophage is 4

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Macrophages play pleiotropic, niche-specific roles in all tissues and organs. As immune sentinels, tissue macrophages regulate immune activation and inflammation; in turn, their function is modulated by inflammatory mediators deriving from such activation. Recent papers have established unanticipated roles for interleukin 4 and the alternative activation of tissue macrophages in the organismal response to diverse environmental stressors.
metabolic syndrome, type 2 diabetes, atherosclerosis, and nonalcoholic fatty liver disease. The immune system provides a critical pathogenic link between obesity and its pernicious sequelae (Shoelson et al., 2006; Schenk et al., 2008). Adipocytes appear to be important in activating the proinflammatory cascades that drive insulin resistance with increasing adiposity (Schenk et al., 2008). Macrophages represent the numerically dominant immune cell population in white adipose tissue (WAT) at baseline and are specifically recruited to the WAT of obese mice and humans (Weisberg et al., 2003; Xu et al., 2003), where they promote both the disposal of cellular debris resulting from adipocyte death and adipose tissue remodeling. Adipose-associated macrophages also play a central role in sustaining insulin sensitivity by adipocytes. Execution of the AAM program depends on signaling through PPAR-γ, and mice with a macrophage-specific deletion in PPAR-γ expression exhibit worsened insulin resistance and glucose intolerance when challenged with high fat diets (Bouhlel et al., 2007; Odegaard et al., 2007). PPAR-γ expression is induced by IL-4 and IL-13, but the biologically relevant source of these cytokines in fat has been unclear. Although originally identified as adaptive cytokines produced by Th2-polarized CD4+ T cells, an expanding number of innate cells—including granulocytes and diverse populations of innate lymphocytes—have now been identified as robust producers of these cytokines (Oliphant et al., 2011; Wills-Karp and Finkelman, 2011). Wu et al. (2011)

Broadly speaking, classically activated macrophages (CAM; M1 macrophages) play important roles in defense against bacteria, protozoa, and viruses and drive proinflammatory tissue damage; alternatively activated macrophages (AAM; M2 macrophages) contribute to defense against helminthes, drive allergy pathogenesis, suppress inflammation (in part by antagonizing CAM responses), regulate wound healing, and drive fibrosis (Murray and Wynn, 2011a,b). It should be noted that the CAM/AAM paradigm in no way exhausts the polarization capacity of macrophages (the exquisite tissue specificity of resident macrophages aside), but despite its many caveats and weaknesses, the paradigm has provided theoretical and experimental guidance to the deconvolution of macrophage phenotypes and biology over the last decade (Murray and Wynn, 2011a,b). Indeed, the utility of the CAM/AAM paradigm has recently spilled beyond classical immunobiology. Following on the heels of a considerable body of work defining a role for AAM in restraining deleterious proinflammatory responses in obesity, recent studies have implicated AAM in defense against both cold stress and cognitive stress.

Eosinophils, AAMs, and obesity

The obesity pandemic continues unabated (Finucane et al., 2011), bringing in its wake dramatic increases in the incidence of common metabolic and end-organ sequelae such as metabolic syndrome, type 2 diabetes, atherosclerosis, and nonalcoholic fatty liver disease. The immune system provides a critical pathogenic link between obesity and its pernicious sequelae (Shoelson et al., 2006; Schenk et al., 2008). Adipocytes appear to be important in activating the proinflammatory cascades that drive insulin resistance with increasing adiposity (Schenk et al., 2008). Macrophages represent the numerically dominant immune cell population in white adipose tissue (WAT) at baseline and are specifically recruited to the WAT of obese mice and humans (Weisberg et al., 2003; Xu et al., 2003), where they promote both the disposal of cellular debris resulting from adipocyte death and adipose tissue remodeling. Adipose-associated macrophages also play a central role in promulgating obesity-associated inflammation (Schenk et al., 2008). Notably, the tissue macrophages normally resident in the WAT of lean animals have an AAM-like phenotype, and progression of obesity is associated with a switch to a CAM-like phenotype (Fig. 1 A; Lumeng et al., 2007; Odegaard et al., 2007). The NALP3 inflammasome may have a role in this phenotypic switch (Vandanmagsar et al., 2011), perhaps via the sensing of cholesterol crystals (Duewell et al., 2010) released by dying adipocytes. In addition, multiple lines of evidence support a pathogenic role for WAT CAM in obesity-induced insulin resistance (Chawla et al., 2011).

Reciprocally, AAMs have a role in sustaining insulin sensitivity by adipocytes. Execution of the AAM program depends on signaling through PPAR-γ, and mice with a macrophage-specific deletion in PPAR-γ expression exhibit worsened insulin resistance and glucose intolerance when challenged with high fat diets (Bouhlel et al., 2007; Odegaard et al., 2007). PPAR-γ expression is induced by IL-4 and IL-13, but the biologically relevant source of these cytokines in fat has been unclear. Although originally identified as adaptive cytokines produced by Th2-polarized CD4+ T cells, an expanding number of innate cells—including granulocytes and diverse populations of innate lymphocytes—have now been identified as robust producers of these cytokines (Oliphant et al., 2011; Wills-Karp and Finkelman, 2011). Wu et al. (2011)
recently established that eosinophils are the main IL-4--producing cells in WAT and are required for the maintenance of AAM in nonobese mice (Fig. 1 A). Furthermore, a reciprocal relationship was found between obesity and WAT eosinophil frequency in both diet-induced and genetic models of obesity, as mice lacking eosinophils responded to caloric challenge with greater glucose dysmetabolism than controls. Helminth infection augmented adipose tissue eosinophilia, leading to sustained enhancement of glucose tolerance with caloric stress (Wu et al., 2011). The latter finding underscores the fact that responses to infection provide a useful theoretical framework for understanding part of the complex relationship between immunity and metabolism (Chawla et al., 2011). AAM-driven up-regulation of nutrient storage sequesters nutrients away from pathogens, and may thus provide an advantage to the host during the chronic, low intensity challenge of helminth infection. In contrast, CAM-driven insulin resistance, associated with acute, high intensity infection by bacteria, viruses, and protozoa, likely provides an advantage to the host by supporting the increased need for aerobic glycolysis to drive lymphocyte proliferation, macrophage functions, and dendritic cell activation (Krawczyk et al., 2010; Chawla et al., 2011). What benefit, if any, obesity-associated, CAM-driven inflammation might provide to the uninfected host remains unclear.

AAMs and adaptive thermogenesis

Obesity results from an imbalance between energy intake and expenditure. In addition to that expended in basal metabolic processes and physical activity, energy is expended through cold-induced nonshivering thermogenesis and diet-induced thermogenesis (Cannon and Nedergaard, 2004, 2011). These two processes occur largely via brown adipose tissue (BAT), an organ specialized for heat dissipation via uncoupling of mitochondrial respiration from ATP synthesis in a process mediated by uncoupling protein 1. Nonshivering thermogenesis and diet-induced thermogenesis are facultative (able to be turned on rapidly) and adaptive (able to undergo expansion of capacity over time through recruitment of increased BAT), and adrenergic stimulation is key to both characteristics (Cannon and Nedergaard, 2004, 2011). In the classical view of thermogenesis, the relevant adrenergic stimulation derives from norepinephrine released from sympathetic nerves that originate in the hypothalamus and innervate BAT. Nguyen et al. (2011) recently showed that severe cold stress up-regulates markers of alternative activation in adipose tissue macrophages and that \( \text{IL}-4^{-/-}/\text{IL}-13^{-/-} \) and \( \text{Stat}6^{-/-} \) mice are compromised in their ability to maintain their body temperature during such stress. In this model, AAM in adipose tissue directly produce norepinephrine, driving the adaptive thermogenic program in BAT and providing fuel for this by up-regulating lipolysis in BAT (Fig. 1 B; Nguyen et al., 2011). Using mice lacking IL-4 specifically on macrophages, the authors demonstrated that bolus injection of IL-4 promoted alternative activation of macrophages in BAT and WAT coincident with rapid increases in oxygen consumption. Collectively these data provide strong evidence for a role for IL-4 and AAM in the response to cold stress. However, many questions remain, including the relevant sources of IL-4 in BAT, whether such exposures lead to local tissue macrophage proliferation (Jenkins et al., 2011), and the relative role of macrophage- and neuron-derived norepinephrine in this response.

As an important sidebar, the great plasticity of macrophages, the fact that an in vivo macrophage is unlikely to encounter IL-4 or IL-13 in the absence of other stimuli, and the fact that many marker genes are not, in fact, specific for AAMs, have led to difficulties in defining AAMs (Murray and Wynn, 2011a,b). One solution is to return to the original observations of of Sionon Gordon, with the simplest practical definition being that AAMs are the result of IL-4/IL-13 stimulation of STAT6 via the IL-4Rα chain (Murray and Wynn, 2011b). This definition is effectively used by Nguyen et al. (2011), who employed a combination of mice lacking \( \text{Il4}^{-/-}/\text{Il13}^{-/-} \) and \( \text{Stat}6^{-/-} \), as well as those lacking IL-4Rα expression only by macrophages, to conclusively assign an AAM phenotype to the BAT and WAT macrophages that facilitate adaptive thermogenesis.

Mnemosyne and the AAM?

IL-4 has also been reported to play an important role in learning and memory. Extensive work has linked proinflammatory cytokine activity in the central nervous system (e.g., in infection, aging, and depression) with cognitive impairment, and studies have shown that T cell–deficient mice are impaired in learning and memory (Kipnis et al., 2004; Brynskikh et al., 2008). Following up on this, a recent study by Derecki et al. (2010) provided evidence for cognitive impairment in mice lacking IL-4, a finding correlated with increased proinflammatory cytokine production by meningeal macrophages. In the authors’ model, cognitive stress generated by training the mice to navigate a Morris water maze leads to increased stress up-regulates markers of alternative activation in adipose tissue macrophages and that \( \text{IL}-4^{-/-}/\text{IL}-13^{-/-} \) and \( \text{Stat}6^{-/-} \) mice are compromised in their ability to maintain their body temperature during such stress. In this model, AAM in adipose tissue directly produce norepinephrine, driving the adaptive thermogenic program in BAT and providing fuel for this by up-regulating lipolysis in BAT (Fig. 1 B; Nguyen et al., 2011). Using mice lacking IL-4 specifically on macrophages, the authors demonstrated that bolus injection of IL-4 promoted alternative activation of macrophages in BAT and WAT coincident with rapid increases in oxygen consumption. Collectively these data provide strong evidence for a role for IL-4 and AAM in the response to cold stress. However, many questions remain, including the relevant sources of IL-4 in BAT, whether such exposures lead to local tissue macrophage proliferation (Jenkins et al., 2011), and the relative role of macrophage- and neuron-derived norepinephrine in this response.

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proinflammatory cytokine production (TNF and IL-12) by meningeal myeloid cells (presumptively macrophages), with homeostasis being provided by the attendant accumulation of IL-4–producing T cells in meningeal spaces (Fig. 2). Provision of IL-4–sufficient T cells decreased inflammatory cytokine production by meningeal myeloid cells and partially corrected the defect in learning and memory in IL-4–deficient mice. It should be noted that although the IL-4–producing cells were stated to be Th2 cells, it remains unclear from the flow cytometric techniques used whether these are CD4+ T cells or an innate lymphocyte population. In addition, although it was suggested that IL-4 may drive alternative activation of meningeal macrophages, this was not demonstrated using the same criteria used by Nguyen et al. (2011). However, later studies showed that the cognitive defects in SCID mice uncovered by this model were partially ameliorated by provision of in vitro–generated AAM (Derecki et al., 2011). If cognitive drives the accumulation of functionally important IL-4–secreting lymphocytes to the meninges, this would provide a potential novel mechanism behind the “use it or lose it” admonition in terms of salvaging cognition from the ravages of aging, a state in which there are likely multiple causes for pernicious up-regulation of proinflammatory cytokine production in the central nervous system. The model also leads to a number of fascinating perplexities. What is the antigen receptor repertoire of such T cells? What drives their activation and meningeal accumulation? These studies were done within the theoretical framework of cognitive tasks driving lymphocyte accumulation and myeloid cell activation. A credible null hypothesis is that, in the absence of IL-4, the basal state of meningeal macrophages is marked by deleterious increased proinflammatory cytokine production. Far more problematic, however, is the use of the Morris water maze as an experimental model of learning and memory. It was stated that the water temperature was kept between 21 and 22°C (Derecki et al., 2010), which actually represents a severe cold stress for mice, whose thermoneural zone—in the much more forgiving air—is 29–32°C (Gordon, 1993). This provides a major confounder for all such studies. It appears more likely that the relevant stress derives from cold challenge rather than cognitive exertion. The study by Nguyen et al. (2011) provides interesting biological context here, although it would be premature to suggest morning ice baths as a potential prophylactic against the cognitive decline associated with aging.

Concluding remarks

These studies expand our understanding of the role of the AAM in homeostatic responses to diverse stresses (Figs. 1 and 2). They also raise the evolutionary question of what the ur-function of Th2 immunity was. A reasonable hypothesis deriving from these studies is that the selection pressures underlying the Th2/AAM response derived predominantly from a critical need to maintain tissue homeostasis, preserving organ function at baseline and in response to diverse environmental stressors, and that the antiinflammatory response casette was secondarily co-opted for its utility in anti-helminth responses and barrier immunity.

More broadly, these studies underscore the essential role of tissue macrophages in homeostasis and responses to stress. Macrophages constitute a significant fraction of the number of cells in every tissue, where they play critical, pleiotropic, niche-specific roles in development, homeostasis, repair, and regeneration in addition to host defense (Stefater et al., 2011). The current studies emphasize the critical need for optimal programming of tissue macrophages by the broader immunological environment. It should be noted that although the focus in these studies was on IL-4 and AAM, other cytokines (e.g., IL-10) and patterns of macrophage response and polarization are likely to be just as important (Fiorentino et al., 1991; Murray and Wynn, 2011b). These studies also strongly suggest that disease states associated with chronic production (or suppression) of such cytokines, as well as genetic polymorphisms and therapies that modulate their activity, are likely to drive biologically significant, as-yet unrecognized, off-target effects on diverse organs through effects on macrophage programming and function.

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