Leukocyte set points in metabolic disease

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Abstract

Vertebrate tissues comprise precise admixtures of parenchymal and hematopoietic cells, whose interactions are vital to proper tissue function. By regulating this interaction, vertebrates are able to mitigate environmental stress and coordinate dramatic physiologic adaptations. For instance, under conditions of chronic nutrient excess, leukocyte recruitment and activation increase in an effort to decrease excess nutrient storage and alleviate adipocyte stress. While basal equilibria may be reestablished upon normalization of nutrient intake, a new set point characterized by insulin resistance and chronic inflammation is established if the stress persists. Consequently, although this response is adaptive in settings of acute overfeeding and infection, it has catastrophic health consequences in the modern context of obesity. Understanding how leukocyte set points (numbers and activation status) are established, maintained, and regulated in tissues is, thus, critical to our understanding of, and intervention in, chronic metabolic diseases, such as obesity and diabetes.

Introduction

Obesity and associated health consequences comprise the greatest public health challenge of our time. Worldwide, ~ 1.5 billion people tip the scales as overweight, 300-500 million of whom are obese, placing nearly a quarter of humanity at dramatically increased risk for diabetes, cardiovascular disease, and many types of cancer [1,2]. While our considerable scientific investments have barely begun to slow the expansion of our waistlines, they have already yielded unexpected physiologic insights, perhaps the greatest of which is the discovery of the unprecedented level of cooperation between parenchymal cells and leukocytes necessary for proper function in metabolic tissues.

For almost two centuries, the stereotyped appearances of basic human tissues have been staples of medical text-books [3]. Despite this well-worn familiarity, it was not until recently that cellular inventory of these tissues revealed remarkable numbers of macrophages and other leukocytes tucked away among the more familiar parenchymal cells. However, rather than being randomly distributed, these leukocytes were arranged in stereotyped, reproducible patterns that were tissue-specific [4,5]. For example, liver samples consistently harbored similar numbers of macrophages (also known as Kupffer cells) arranged in the same peri-sinusoidal pattern, whereas brain tissues demonstrated similarly constant complements of microglia (resident macrophages of the central nervous system). Indeed, macrophage representation is significant and similar (~5-15%) across nearly every tissue and remarkably well conserved across vertebrate species [6,7]. Perhaps most interesting of all, however, is that depletion of macrophages from any given tissue results not in permanent loss, haphazard recolonization, or encroachment by other leukocyte lineages, but in rapid and precise restoration of the original macrophage complement in both spatial and numerical terms [8].

While the macrophage's infiltrative penchant has long been appreciated, the precision and rigidity of their arrangements in healthy tissues, the conservation of these patterns across vertebrate species, and their rapid and precise re-establishment following depletion suggest

that specific set points exist for ensuring an appropriate complement of leukocytes in each tissue. Furthermore, the temporal stability and aggressive re-establishment of macrophage-parenchyma relationships suggest that active mechanisms exist to maintain this interaction within specific parameters [7]. Indeed, recent work has begun to unearth the complex recruitment and retention networks dedicated to the maintenance and survival of resident leukocyte/macrophage populations, e.g. recruitment and survival of CX_3CR1^+ monocytes by tissue-derived CX_3CL1 and Csf1 [4,9]. Needless to say, such sophisticated arrangements — especially in tissues with little risk of infection — are not easily explained by traditional theories of host defense. Why then would parenchymal cells go to such lengths to accoutre themselves with macrophages? In answer, numerous studies have now demonstrated that resident macrophages shoulder critical, non-immunologic tissue functions: for example, microglia are required for proper synapse formation and function [10], resident intestinal macrophages are necessary to maintain gut epithelial integrity [11], and bone marrow resident macrophages act as critical components of the hemato-poietic stem cell niche [12]. Together, these observations suggest that, as a general principle, vertebrate tissues are critically dependent on resident tissue macrophages to perform their primary functions. In this review, we will explore these concepts as they operate within white and brown adipose tissue physiology. Specifically, we will review recent findings demonstrating novel and non-redundant roles for resident leukocytes in adipose tissue metabolism and discuss how alterations in leukocyte "set points" (numbers and activation status) contribute to the pathogenesis of metabolic disease.

Recent advances
Macrophage activation
Macrophages comprise a heterogenous and plastic leukocyte lineage [4,13]. While their physiologic roles are diverse and, in many instances, poorly-understood, their behavior may be broadly divided into two categories — classical (M1) and alternative (M2) activation — functionally defined by the phenotypes elicited during bacterial and parasitic infections, respectively [14-16]. These macro-phage activation programs can be modeled *in vitro* by stimulation of bone marrow-derived macrophages with interferon-γ (IFN-γ)/lipopolysaccharide (LPS) or interleu-kin-4 (IL-4), respectively [14]. Such a definition is likely to be an oversimplification of complex *in vivo* populations [13,17,18]; however, it serves as a useful rubric by which to begin to understand *in vivo* phenomena.

White adipose tissue
White adipose tissue is the primary site of long-term nutrient storage in vertebrates and the largest endocrine

organ in humans, secreting a variety of hormones termed "adipokines" that regulate systemic metabolism [19]. Healthy white adipose tissue is characterized histologi-cally by a predominance of adipocytes admixed with scattered adipose tissue macrophages, eosinophils, and Tregs (regulatory T cells) [20-23]. Under conditions of nutrient homeostasis, IL-4 secreted by eosinophils maintains adipose tissue macrophages in an alternatively activated (M2) phenotype necessary for proper adipocyte function [24]. Disruption of this circuit, either through eosinophil depletion [22] or through abrogation of macrophage alternative activation [24-28], impairs adipocyte storage and endocrine functions, resulting in obesity-induced insulin resistance. While the exact nature of the macrophage-adipocyte signaling mechan-ism and the stimulus for tonic eosinophil IL-4 produc-tion remain unclear, these data demonstrate the intimate interdependence between adipocytes and leukocytes for proper tissue function and, importantly, show that disruptions in the tissue set point result in significant functional consequences for the tissue and the organism as a whole.

Brown adipose tissue
In contrast to white adipose tissue, brown adipose tissue has little role in long-term storage of energy but is critically required for acclimation to cold temperatures via a process termed adaptive thermogenesis [29-31]. This stems from brown adipose tissue's ability to oxidize fatty acids via uncoupled respiration, which generates heat rather than ATP in the respiring mitochondria [32]. Indeed, active brown adipose tissue is capable of impressive metabolic feats: the 63g of brown adipose tissue present in an average adult human is estimated to catabolize 4.1kg of white adipose tissue each year [33]. Until recently, it was believed that sympathetic nerves, which innervate brown and white adipocytes, coordinate metabolic responses to cold exposure [29,34]. However, Nguyen and colleagues demonstrated that increases in sympathetic tone are, in part, transmitted to white and brown adipocytes by alternatively activated macrophages [35]. Importantly, exposure of animals to progressively colder temperatures induces alternative activation of resident brown adipose tissue and white adipose tissue macrophages, resulting in catecholamine biosynthesis and norepinephrine release. These macrophage-derived catecholamines are required for cold-induced lipolysis in white adipose tissue and for inducing brown adipose tissue's oxidative and thermo-genic capacity. Lastly, manipulation of the macrophage set point in brown adipose tissue and white adipose tissue through exogenous IL-4 supplementation or genetic abrogation of alternative activation is sufficient to enhance or impair thermogenesis, respectively [35]. As such, this unexpected interposition of macrophages within the

thermogenic control circuit defines a mechanism by which resident brown adipose tissue and white adipose tissue macrophages support parenchymal cell function.
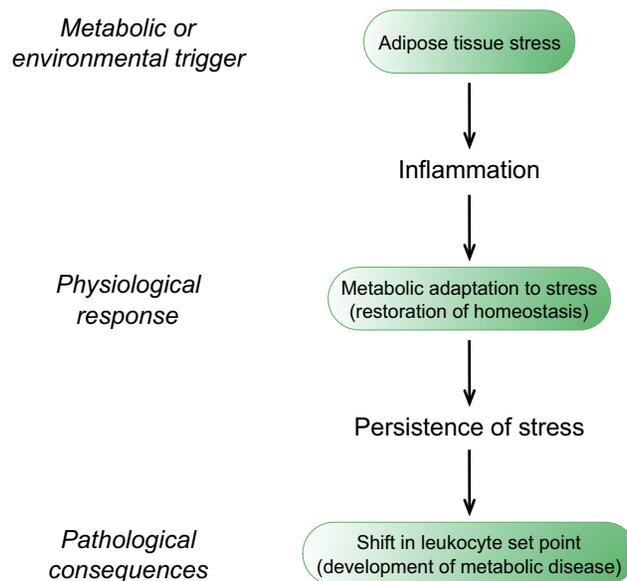
Obesity

While the rigidity and precision of tissue set points are necessitated by the tight physiologic constraints within which vertebrate tissues operate, the organism's survival under varying environmental influences necessitates constant physiologic adaptation (Figure 1). Tissue equilibria must then operate so as to counteract these influences and restore homeostasis. With obesity now an omnipresent global concern, the influence of environmental stress — nutrient excess in particular — on white adipose tissue set points is the object of intense scrutiny. The primary behavioral feature of obesity is excessive dietary intake. As nutrient levels exceed homeostatic requirements, adipocyte hypertrophy and hyperplasia collaborate to buffer excess intake, while signs of cellular stress change the leukocyte set point [18,36]. Under these circumstances, adipose tissue macrophages increase from ~10% of white adipose tissue cellularity in lean animals to up to 50-60% in the obese [20], whereas the representation of eosinophils dramatically decreases in obese white adipose tissue [22]. Accordingly, there is a dramatic shift in the phenotypic set point: adipose tissue macrophages from lean animals demonstrate an alternative (M2) phenotype, whereas those from obese mice are classically (M1) activated, swapping the anti-inflammatory M2 phenotype for production of pro-inflammatory mediators [37]. The resulting phlogistic milieu has two metabolically salient effects. Firstly, it avidly recruits additional macrophages and other leukocytes to white adipose tissue and activates them along similarly inflammatory lines, reinforcing the new numerical and phenotypic set point [18]. Secondly, adipocytes and other metabolically critical cells (e.g. hepatocytes and skeletal muscle myocytes) are rendered insulin-resistant through direct abrogation of insulin signaling pathways in an effort to limit storage of excess nutrients and relieve cellular stress [38].

If nutrient intake returns to homeostatic levels — or metabolic demand increases proportionately — the original tissue equilibrium is reestablished [39]. If nutrient intake and expenditure are chronically mismatched, however, a new white adipose tissue set point is established, characterized by the familiar constellation of metabolic derangements that define the metabolic syndrome (Figure 1). While the wealth of mechanistic detail available is beyond the scope of this review [38,40,41], chronic low-grade inflammation mediated by classical M1 adipose tissue macrophages emerges as the key pathophysiologic nexus responsible for the adverse health consequences of the new obese white adipose tissue set point [7,18].

Figure 1. Leukocyte set points dictate adipose tissue function

Adipose tissues of healthy mice contain a complement of leukocytes whose activation status or numbers change with metabolic or environmental stress. These transient inflammatory responses result in adaptations that restore metabolic homeostasis. However, in the setting of persistent metabolic or environmental stress, a new leukocyte set point is established for adipose tissue that amplifies inflammation, resulting in the various complications associated with obesity.

Tissue set points

The wealth of literature on macrophages and other leukocytes in adipose tissue clearly defines functional roles for these cells in adipose tissue biology and establishes them as *bona fide* tissue constituents rather than immunologic transients. Just as clearly, the literature defines strict numeric and phenotypic relationships between various tissue constituents that correspond to specific physiologic states. The concept of population set points, however, is not limited to states of physiologic normality; the pro-inflammatory milieu of obese adipose tissue itself, once established, demonstrates temporal stability and resistance to change. As in lean adipose tissue, any perturbation of leukocyte set points, whether numeric or phenotypic, disrupts the tissue's new functional parameters [18].

The existence of distinct, defined, and non-arbitrary set points and their correlation with specific tissue phenotypes suggest a mechanism by which the organism can rapidly reprogram tissues to meet emerging environmental requirements. One example of such reprogramming is found in the vertebrate response to significant infectious challenge. This response involves the production, mobilization, and support of an impressive cellular immune response, the majority of which relies heavily on glycolytic metabolism [18]. As such, vertebrates have evolved an adaptive systemic insulin resistance to inflammation; infection-driven inflammation is an organism-wide signal to all non-essential tissues to dampen their insulin sensitivity, thus prioritizing glucose for use by the immune system, while alternative energy sources are mobilized [18]. Because of any given pathogen's inevitable proliferative advantage, the alacrity of this response is of the utmost importance. Therefore, pre-determined set points allow both rapid and accurate tissue recalibration and nutrient re-prioritization.

Conclusion

While significant progress has been made in defining leukocyte set points within adipose tissue with respect to obesity and insulin signaling, our understanding of leukocyte populations, phenotypes, and functional contributions in other tissues remains rudimentary. Indeed, our understanding remains incomplete even within adipose tissue: what defines a stable set point? How are pre-defined set points encoded? How may equilibria be manipulated to combat maladaptive shifts? Addressing these questions within adipose tissue offers the prospect of understanding how other complex tissues may adapt to changing environmental demands.

Abbreviation

IL, interleukin.

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