Perspective

Immunological Goings-on in Visceral Adipose Tissue

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Chronic, low-grade inflammation of visceral adipose tissue, and systemically, is a critical link between recent strikingly parallel rises in the incidence of obesity and type 2 diabetes. Macrophages have been recognized for some time to be critical participants in obesity-induced inflammation of adipose tissue. Of late, a score of other cell types of the innate and adaptive arms of the immune system have been suggested to play a positive or negative role in adipose tissue infiltrates. This piece reviews the existing data on these new participants; discusses experimental uncertainties, inconsistencies, and complexities; and puts forward a minimalist synthetic scheme.

Classic studies, now decades old, revealed that visceral adipose tissue (VAT) is a site where cells and molecules that control metabolism and immunity interplay, with important consequences for organismal homeostasis. Twenty years ago, Hotamisligil et al. demonstrated that tumor necrosis factor α (TNF-α) messenger RNA (mRNA) and protein were induced in the epididymal fat pad of obese rodents and that neutralization of this cytokine improved their characteristic systemic insulin resistance (Hotamisligil et al., 1993). Ten years later, two groups independently reported an impressive accumulation of macrophage-associated gene transcripts and of cells expressing the F4/80 or CD68 marker, presumed to be macrophages, with increasing adiposity in mice and humans (Xu et al., 2003; Weisberg et al., 2003). These studies catalyzed the emerging notion that chronic, low-grade inflammation is a critical link between obesity and a spectrum of metabolic abnormalities, including insulin resistance, type 2 diabetes, and fatty-liver disease. Other proinflammatory cytokines, notably interleukin-6 (IL-6) and IL-1β, were eventually implicated (Pradhan et al., 2001; Wen et al., 2011). And the increase in macrophage representation was soon found to be accompanied by an altered localization within the adipose tissue and population evolution to a more proinflammatory phenotype (Lumeng et al., 2007a, 2007b; Nguyen et al., 2007). More importantly, the functional relevance of macrophages and their phenotypic changes was established through loss- and gain-of-function experiments (e.g., Patsouris et al., 2008; Weisberg et al., 2006; Kanda et al., 2006; Kamei et al., 2006). A "macrophago-centric" view of obesity-associated inflammation reigned for many years, and was recently reviewed (Osborn and Olefsky, 2012; Chawla et al., 2011).

Of late, there has been growing interest in the roles of additional members of both the innate and adaptive arms of the immune system in regulating organismal metabolism. Besides macrophages, several other immune cell types have been implicated: mast cells, neutrophils, eosinophils, type 2 innate lymphoid cells (ILCs), CD8+ and CD4+ T cells, B cells, Foxp3+ regulatory T (Treg) cells, CD4−8− $\gamma\delta$ T cells, and natural killer T cells (NKT cells). What sense are we to make of this intimidatingly complex cast of characters? This piece will lay out the existing body of data on the participants in obesity-associated inflammation; address sources of uncertainty, inconsistency, and complexity; and suggest a minimalist synthetic scheme.

The Data: Innate Immune Cells

Immune system cells, above all macrophages, accumulate in the VAT (in particular, in the epididymal depot) of both lean and obese individuals. In the lean state, most macrophages have an anti-inflammatory phenotype, often referred to as "M2 like" or "alternatively activated" (Chawla et al., 2011; Osborn and Olefsky, 2012; Lumeng et al., 2007a, 2007b; Nguyen et al., 2007). Their differentiation and/or survival depends on IL-4 and IL-13; they express the cell-surface markers CD11b, F4/80, CD301, and CD206, and they secrete anti-inflammatory cytokines such as IL-10 and IL-1 receptor antagonist (IL-1Ra). This type of macrophage can expand somewhat in the obese state, but there is a much more striking accumulation of a proinflammatory subset, denoted "M1 like" or "classically activated" (Lumeng et al., 2007a, 2007b; Nguyen et al., 2007). These cells, whose differentiation is promoted by agents such as lipopolysaccharide and interferon γ (IFN-γ), display the marker CD11c in addition to F4/80 and CD11b and produce proinflammatory mediators like TNF-α, IL-6, IL-1β, nitric oxide (NO), IL-12, etc. The M1/M2-like dichotomy is certainly an oversimplification in this context, as macrophages with intermediate properties (Shaul et al., 2010; Herrero et al., 2010) or with an extreme phenotype reminiscent of that of foam cells in atherosclerotic lesions (Xu et al., 2003; Prieur et al., 2011) are also found in adipose tissue. As mentioned above, it is by now well established that anti-inflammatory macrophages promote local and systemic insulin sensitivity, while their proinflammatory counterparts induce insulin resistance, primarily via secreted mediators (Osborn and Olefsky, 2012; Chawla et al., 2011). So we will not dwell on this aspect here.

Additional innate immune cell types, whether of a pro- or anti-inflammatory nature, have more recently been implicated in obesity-induced metabolic aberrancies. Mast cells, once mature, reside in connective and mucosal tissue, where they are part of an "early-warning" system that infection or injury has occurred in the vicinity. They contain numerous granules loaded with preformed mediators, e.g., histamine, serotonin, heparin, serine proteases, eicosanoids, and cytokines (notably TNF-α and IL-1β). Upon mast cell activation, all or a select subset of these mediators are released in a rapid burst, activating the surrounding vasculature and promoting recruitment of additional inflammatory cell types. Liu et al. reported that there is a greater

representation of mast cells in the adipose tissue of obese than lean mice and humans (Liu et al., 2009). Genetic mutations at the *Kit* locus, which lead to a mast cell deficiency, or pharmacological stabilization of mast cell granules resulted in less weight gain and VAT mass on a high-fat diet (HFD), reduction in VAT-resident macrophages and both adipose-tissue and circulating inflammatory mediators, and improvement in several indices of insulin sensitivity. IL-6 and IFN-γ, but not TNF-γ, were found to be important mediators of these effects.

Neutrophils are also early participants in inflammatory reactions, recruited from the circulation by activated endothelium and/or by tissue-resident macrophages or mast cells. Upon appropriate stimulation, these short-lived cells release reactive oxygen species and nitrogen intermediates; degranulate, expelling serine proteases and other enzymes; and lay down extracellular traps, or NETs, constituted of extruded webs of chromatin and serine proteases. Besides being antimicrobial, these activities amplify inflammatory responses, through recruitment of circulating monocytes, for example. Neutrophils begin to accumulate in the VAT within days after mice are placed on a HFD, an increase that is sustained for at least 3 months on this diet (Talukdar et al., 2012; Elgazar-Carmon et al., 2008). Genetic ablation of the gene encoding the critical neutrophil protease, elastase, or pharmacological inhibition of its activity substantially improved the inflammatory tone and metabolic indices of HFD-fed mice; conversely, injection of recombinant neutrophil elastase into mice on normal chow (NC) provoked glucose intolerance (Talukdar et al., 2012). These alterations did not rely on body weight and VAT mass changes.

Just as anti-inflammatory macrophages predominate in the benign infiltrates of VAT in lean mice, particular populations of anti-inflammatory innate immune cells have been found in these fat depots. Eosinophils circulate in the immature state and lodge in a limited range of tissues once mature, a range that is extended by parasite infection. Their differentiation and activation are critically dependent on IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF). They are granulocytes, releasing a broad array of pro- and anti-inflammatory mediators, in particular IL-4 and IL-13, known to be crucial for the differentiation and survival of anti-inflammatory, M2-like macrophages, including those residing in adipose tissue (Odegaard et al., 2007; Kang et al., 2008). Wu et al.'s attention was drawn to eosinophils because they found them to be the major producers of IL-4 in VAT, and their representation in VAT to be inversely correlated with adiposity (Wu et al., 2011). Mice impoverished in eosinophils (a *Gata1*$^{-/-}$ strain), and thereby anti-inflammatory macrophages, exhibited an increase in body and VAT weight, as well as more marked glucose intolerance and insulin resistance, when fed a HFD. Conversely, animals enriched in eosinophils (an *IL-5* transgenic strain), and thus M2-like macrophages, showed a decline in these parameters.

One step further removed is a recently discovered subset of innate lymphoid cells termed ILC2s. Like all ILCs, these cells are characterized by dependence on the transcription factor Id2 and the cytokine receptor common gamma chain (γc). They are widely distributed in mammalian tissues, and are reminiscent of T helper 2 (Th2) cells in their production of the cytokines IL-5 and IL-13. As IL-13 promotes differentiation of M2-like macrophages and IL-5 fosters the maturation and

activities of eosinophils, which make most of the IL-4 required by M2 macrophages, Molofsky et al. were prompted to address their role in adipose tissue inflammation (Molofsky et al., 2013). Indeed, they found ILC2s to be the major source of both IL-5 and IL-13 in VAT and their representation in VAT to decline under a HFD regime. Genetically engineered or cytokine-promoted increases or decreases in ILC2s led to an enhancement or reduction, respectively, in VAT eosinophils and anti-inflammatory macrophages. These cellular changes were accompanied by the expected alterations in body weight, VAT weight, and measures of insulin resistance and glucose intolerance when the mice were put on a HFD.

Together, these results implicate multiple members of the innate immune system in controlling adipose tissue inflammation and its downstream consequences—either positively (proinflammatory macrophages, mast cells, and neutrophils) or negatively (anti-inflammatory macrophages, eosinophils, and ILC2s). A concern that needs to be kept in mind when considering these findings is that in most cases—even for macrophages—optimum tools for precise manipulation of the cell type of interest are not currently available. Loss- or gain-of-function experiments often rely on manipulation of a factor thought to be required for differentiation or maintenance of the population (e.g., cKit for mast cells [Liu et al., 2009] or GATA1 or IL-5 for eosinophils [Wu et al., 2011; Molofsky et al., 2013]) or an element thought to mediate one of the population's critical effector mechanisms (e.g., degranulation for mast cells [Liu et al., 2009], elastase for neutrophils [Elgazar-Carmon et al., 2008], or IL-5 and IL-13 for ILCs [Molofsky et al., 2013]). But these manipulations are seldom exquisitely specific, and so many of them impact other cells or processes. Neither, in general, do they encompass the entire potential influence of the targeted cell type. So it is important to consider to what extent these imperfections might compromise the critical conclusions concerning obesity-associated inflammation. It is also important to be cognizant of the fact that the list of innate immune cell types contributing to obesity-associated inflammation might not be complete. A few classic cell types (e.g., basophils and NK cells) have not yet been adequately evaluated, and the quite new field of ILC biology is yielding more and more innate lymphocyte groupings with activities astonishingly parallel to those of adaptive lymphocyte subsets (Klose et al., 2013).

The Data: Adaptive Immune Cells
While it was recognized some time ago that T and B lymphocytes infiltrate adipose tissue and that their representation correlates with adiposity (Caspar-Bauguil et al., 2005; Wu et al., 2007; Rausch et al., 2008; Kintscher et al., 2008; Rocha et al., 2008), the demonstration that they play important roles, either effector or regulatory, in organismal metabolism came only quite recently. Nishimura et al. reported that CD8$^+$ T cells are critical participants in obesity-induced adipose tissue and systemic inflammation (Nishimura et al., 2009). Their accumulation after introduction of a HFD appeared to precede that of macrophages, beginning within 2 weeks. Genetic ablation or monoclonal-antibody-mediated neutralization of CD8$^+$ T cells in HFD-fed mice improved VAT inflammatory tone and systemic insulin sensitivity, under both protective and curative regimes, while not impacting body or VAT pad weight. Adoptive transfer of

splenic CD8$^+$ T cells largely reversed the effects of a $CD8^{-/-}$ mutation. Coculture experiments showed that CD8$^+$ T cells can provoke macrophage migration and differentiation and, in turn, that adipocytes from obese, but not lean, mice can stimulate CD8$^+$ cells. Consistent with the latter finding, HFD feeding resulted in a restriction of the repertoire of CD8$^+$ T cells within VAT. Several of these findings were confirmed in an independent study (Yang et al., 2010).

The role of conventional CD4$^+$ Th cells in organismal metabolism seems less clear-cut. Several groups have noted an enrichment of IFN-γ-producing Th1 cells in the VAT of obese individuals (Feuerer et al., 2009; Zúñiga et al., 2010; Cheng et al., 2012). And certainly there are abundant cells at that site potentially capable of antigen presentation to CD4$^+$ T cells because they express both major histocompatibility complex (MHC) class II and costimulatory molecules—whether they be macrophages (Morris et al., 2013) or adipocytes themselves (Deng et al., 2013; Meijer et al., 2011). Given that IFN-γ is an important proinflammatory cytokine in obesity-induced inflammation and consequent metabolic abnormalities (Rocha et al., 2008), Th1 cells are likely to join CD8$^+$ T cells as important effector lymphocytes. On the other hand, Winer et al. have argued that Th2 cells exert a protective function in these processes (Winer et al., 2009). However, such a role remains questionable because of certain experimental uncertainties in this study. First, the markers used to identify Th2 cells were not the best. GATA3 was taken to delineate Th2 cells, but it was subsequently demonstrated that the major VAT T cell population expressing this transcription factor is actually Foxp3$^+$CD4$^+$ Treg cells (Cipolletta et al., 2012). In addition, the Th2-defining cytokine, IL-4, was not assayed, and it was later shown that by far the major IL-4 producers in VAT are eosinophils (Wu et al., 2011). Second, the functional assay used to support a protective role for Th2 cells, transfer of Foxp3$^-$CD4$^+$ cells into lymphopenic RAG$^{-/-}$ mice, is known to induce phenotypic changes (in activation, homing, etc.) in the transferred cells as an accompaniment to homeostatic proliferation induced by the lymphopenia (Goldrath et al., 2004). Thus, a potential role for Th2 cells requires further substantiation.

Given the demonstrated importance of T lymphocytes in obesity-associated inflammation, it was logical to explore a role for B lymphocytes. B cells are present in the VAT of lean mice and are early participants in the more aggressive infiltrate induced by HFD (Duffaut et al., 2009; Winer et al., 2011). In particular, there is an enrichment of cells secreting antibodies of the immunoglobulin G (IgG) isotype. Winer et al. reported that B cells exert a potent proinflammatory influence: on HFD, mice lacking this cell type exhibited the usual weight gain, but had less inflammatory VAT and improved metabolic indices (Winer et al., 2011). Reintroduction of splenic B cells from HFD-fed, but not NC-fed, mice complemented the differences in B cell null vis-à-vis wild-type animals, e.g., they induced insulin resistance in the former case. Strikingly, transfer of serum IgGs from mice on HFD, but not NC, also provoked insulin resistance in B cell null mice. In humans, a distinct repertoire of serum IgG antibodies correlated with a state of insulin resistance, but the IgGs from both insulin-resistant and -sensitive individuals recognized mostly intracellular proteins from a broad range of tissues. A more recent study confirmed the proinflammatory impact of B

cells in the HFD setting but did not address a function for IgGs (DeFuria et al., 2013). It seems clear, then, that B cells play a proinflammatory role in obesity-induced insulin resistance. How they exert their impact, in particular the precise function of IgG antibodies, requires further elucidation. Given that the "pathogenic" antibodies appeared late in the disease process and that they recognized intracellular proteins likely released upon cell death, one is tempted to suggest that they are downstream players, e.g., by blocking molecules in critical metabolic pathways or stabilizing anti-inflammatory mediators.

Foxp3$^+$CD4$^+$ Tregs are important negative regulators of VAT inflammation and metabolic indices. This cell lineage, delineated by the Foxp3 transcription factor, controls many types of immune responses, including autoimmunity, allergy, inflammation, infection, and antitumor immunity (Josefowicz et al., 2012). It can operate by a variety of mechanisms, such as secretion of inhibitory molecules, sequestration of growth factors, metabolic interference, induction of cell death, etc. Feuerer et al. described a unique population of Tregs residing in epididymal adipose tissue of lean mice—special in its transcriptome and clonally expanded TCR repertoire. In insulin-resistant mouse models of obesity, there was a striking reduction in VAT-resident, but not lymphoid-organ, Tregs (Feuerer et al., 2009). Experimental reduction or augmentation of the Treg compartment led to an increase or decrease, respectively, of adipose tissue inflammation and insulin resistance, likely by a combined effect on macrophages, other T cells, and adipocytes. Several of these findings were later confirmed (Ilan et al., 2010; Eller et al., 2011; Deiuliis et al., 2011). The unique phenotype of VAT Tregs is driven by their unusual expression of PPARγ (Cipolletta et al., 2012). Treg-specific ablation of $Pparg$ resulted in a depletion of VAT-resident, but not lymphoid-organ, Tregs in mice on NC; conversely, injection of the PPARγ agonist, pioglitazone, into HFD-fed animals specifically expanded the VAT Treg population. Surprisingly, much of the insulin-sensitizing effect of pioglitazone disappeared in mice lacking PPARγ specifically in Tregs.

Finally, a potential role for adipose-tissue-resident γδ T cells in organismal metabolism was recently evoked (Zúñiga et al., 2010). IL-17a-deficient mice showed increased adiposity on both NC and HFD, as well as enhanced glucose intolerance and insulin resistance. By far the most frequent IL-17-producing cell type in adipose tissue was found to be CD4$^-$CD8$^-$ γδ T cells. In vitro experiments suggested that γδ cells can indeed impact metabolic processes as they suppressed differentiation of the 3T3-L1 preadipocyte cell line and impaired glucose uptake by mature 3T3-L1 adipocytes. However, mice devoid of γδ T cells did not phenocopy those lacking IL-17a—by way of explanation, it was argued that IL-17-producing Th (Th17) cells fulfilled their functional niche. It is necessary to more directly confirm (or infirm) a regulatory role for γδ T cells and to delineate more precisely which members of this diverse lineage are involved.

In short, proinflammatory (CD8$^+$ T cells, CD4$^+$ Th1 cells, and B cells) and anti-inflammatory (Foxp3$^+$CD4$^+$ Tregs) VAT-resident adaptive immune cells also control inflammatory and metabolic processes in the VAT and systemically. Further progress awaits elucidation of their relative importance, how they interplay, and how they impact innate immune cells, adipocytes and their precursors, and supporting stromal cells and vasculature.

Perspective

The Perplexing Case of NKT Cells

NKT cells straddle innate and adaptive immunity (Rossjohn et al., 2012). They display cell-surface markers typical of NK cells (e.g., NK1.1) but also express the antigen-specific receptors characteristic of T cells (TCRs). Unlike classical T cells, however, they generally recognize glycolipid antigens in the context of CD1d, an MHC class Ib molecule. Type 1 or "invariant" NKT cells employ a restricted set of TCRs consisting of a single α chain (Vα14Jα18) and one of a limited number of β chains. Type 2 cells exploit a broader repertoire of TCRs. NKT cells may be either pro- or anti-inflammatory, rapidly secreting large amounts of IFN-γ or IL-4 upon stimulation.

Given NKT cell recognition of glycolipids and their ready detection in the VAT of lean mice and humans (Lynch et al., 2009; Caspar-Bauguil et al., 2005), a flood of investigators assessed their role in obesity-induced inflammation and its downstream consequences over the past few years (Ohmura et al., 2010; Mantell et al., 2011; Kotas et al., 2011; Satoh et al., 2012; Wu et al., 2012; Ji et al., 2012a, 2012b; Schipper et al., 2012; Lynch et al., 2012; Strodthoff et al., 2013) (Table 1). Loss-of-function experiments generally entailed examination of mice with null mutations in *Cd1d*, which impacts all NK cells, or *Jα18*, which more specifically targets the type 1 population; gain-of-function studies relied on injection of alpha-galactosylceramide (αGalCer), a ligand for type-1 cells. Conclusions from this set of studies have spanned the gamut from NKT cells positively impacting organismal metabolism, to having no apparent influence, to negatively affecting metabolic processes.

How can we reconcile these conflicting outcomes? Close examination of the details of these studies reveals multiple experimental issues that could substantially impact results, including the following:

- Employment of different strategies to manipulate NKT cell levels—*Cd1d*⁻/⁻ versus *Jα18*⁻/⁻ versus αGalCer-injected mice. Note that the *Cd1d*⁻/⁻ mutation compromises all NKT cells, while the *Jα18*⁻/⁻ mutation and injection of αGalCer target only type 1 cells. It may also be relevant that CD1d is expressed by certain parenchymal cells, notably adipocytes.
- Nonuse of littermate controls in some cases—critical in light of recent observations of the impact of the microbiome on adiposity/inflammation/metabolism (Nicholson et al., 2012), as well as on NKT cells (Olszak et al., 2012).
- Occasional use of both sexes (versus only males)—it is well known that gender influences inflammation and local and systemic metabolic indices (e.g., Macotela et al., 2009).
- Utilization of different feeding regimes—variable HFD compositions, ages at HFD introduction, HFD durations (ranging from 4 days to 26 weeks).
- Quantification of different readouts—for example, inflammation might be assessed by flow cytometry of leukocytes, macrophages, and/or T cells or by PCR titration of cell-type-specific or inflammatory transcripts. Or, different sites might be examined (VAT versus subcutaneous adipose tissue versus blood), problematic because different fat depots are known to have inherently variable association with insulin sensitivity, inflammatory tenors,

transcriptomes/proteomes, and adipocyte precursor cells, for example (Perrini et al., 2008; Macotela et al., 2009, 2012; Alvehus et al., 2010; Tran et al., 2008).

Given these diverse issues, it seems best to reserve judgment on the role of NKT cells in obesity-associated inflammation and its consequences, while being open to a potentially important role. It would be helpful to the research community if in the future investigators would employ, and journal editors would insist on the use of, cohoused single-sex littermates of stable genetic background for critical comparisons.

Synthesis

The complex cast of cellular characters implicated in obesity-associated inflammation and attendant metabolic abnormalities, and their diverse activities, are difficult for one (especially one on the "metabolism" side of immunometabolism) to assimilate. The goings-on in certain Hieronymus Bosch paintings—for example "The Garden of Earthly Delights" (Figure 1)—spring to mind. What is the root of this complexity?

First, it is important to recognize that complexity is to be expected. Inflammation is a complicated, highly orchestrated process, wherein tissue-resident macrophages and mast cells; recruited neutrophils, monocytes, and, in some contexts, eosinophils; and eventually multiple lymphoid cell types have stereotyped functions to perform. In addition, the inflammatory process, especially if it becomes chronic, is kept in check by regulatory cells of diverse types (e.g., M2-type macrophages and Tregs). A clear understanding of how VAT inflammation unfolds awaits definitive identification of its trigger(s). The initiating stimulus for the benign inflammation of lean individuals remains largely unexplored. Lipotoxicity, hypoxia, endoplasmic reticulum stress, necrosis, and the microbiota have all been implicated as initiators of the more aggressive inflammation provoked by obesity (Donath and Shoelson, 2011; Henao-Mejia et al., 2012). The NLRP3-containing inflammasome seems to be a critical driver (Vandanmagsar et al., 2011; Stienstra et al., 2011; Henao-Mejia et al., 2012). Another important issue that needs to be resolved is precisely when T and B cells come in. It is generally thought that innate immune cells are first responders to inflammatory stimuli, eventually calling in adaptive immune cells—but the opposite certainly occurs (e.g., Ji et al., 2002)—and several of the studies reviewed above implicate changes in T cell populations as a primordial event. The skewed TCR repertoires of VAT-infiltrating T cell subsets (Nishimura et al., 2009; Yang et al., 2010; Winer et al., 2009; Feuerer et al., 2009) suggest that recognition of an antigen (or antigens) might be a spark.

Second, the inventory of immune cell types implicated in obesity-associated inflammation is expanded by the participation of both effector and regulatory cells (summarized in Figure 2). Logically, the more of the former, the more of the latter: different regulators might be called into play at specific junctures to control specific activities.

Third, the development of insulin resistance and type 2 diabetes is a multistage process. For several immune cell types it is not entirely clear where they come into play. For example, manipulations of mast cells, eosinophils, ILCs, and γδ T cells all impact body and/or VAT weight. This, in and of itself, could promote adipose-tissue inflammation and downstream

Table 1. Variable Results Concerning NKT Cells

| NKT Cell Study | NKT Effect on: | | | NKT Manipulation | | | Experimental Mice | | | HFD Regime | | | |
	Adiposity	Inflammation	Insulin Sensitivity	$Cd1d^{-/-}$	$J\alpha18^{-/-}$	αGal Cer	Clean Genetics?	Littermate Controls?	Gender	Starting Age	Duration	Comments
Ohmura et al., 2010	no change	worsened	negative	no	no	yes	yes	no	M	8 weeks	13 weeks	Relied on $\beta2\mu^{-/-}$ mice, which also have CD8^{+} T cell deficiency
Mantell et al., 2011	no change	no change	no change	yes	no	no	probably (N7 backcross)	yes	?	?	26 weeks	
Kotas et al., 2011	no change	little	slightly positive $(Cd1d^{-/-})$ or no change $(J\alpha18^{-/-})$	yes	yes	no	yes $(Cd1d^{-/-})$, probably $(J\alpha18^{-/-})$ (6 backcrosses)	yes	M	6–8 weeks	8–16 weeks	
Satoh et al., 2012	enhanced $(Cd1d^{-/-})$, no change $(J\alpha18^{-/-}$ or αGalCer)	worsened $(Cd1d^{-/-})$, no change $(J\alpha18^{-/-})$	negative $(Cd1d^{-/-})$, no change $(J\alpha18^{-/-})$	yes	yes	yes	?	no	M + F	8 weeks	14–18 weeks	
Wu et al., 2012	no change (M) or slightly enhanced (F)	worsened	negative	yes	yes	yes	yes	yes	M + F	5–6 weeks	variable	
Ji et al., 2012b	no change	no change $(Cd1d^{-/-})$, improved (αGalCer)	no change $(Cd1d^{-/-})$, positive (αGalCer) on glucose tolerance	yes	no	yes	yes	no	M	6 weeks	8 weeks	Mediated through IL-4/STAT6
Ji et al., 2012a	no change	improved	positive	yes	no	no	yes	no	M	6 weeks	4 days	Mediated through IL-4
Schipper et al., 2012	little change	mixed	positive	yes	yes	no	yes	no	M	10–11 weeks	18–19 weeks	Greater effects on NC: NKT cells improve metabolic indices
Lynch et al., 2012	diminished	improved	positive	yes	yes	yes	yes	no	M + F	6 weeks	10 weeks	
Strodthoff et al., 2013	enhanced	no change	no change	no	yes	no	?	no	M	5–8 weeks	10–14 weeks	Effects on lipid metabolism mainly mediated by liver

Figure 1. "The Garden of Earthly Delights" by Hieronymus Bosch
An image that springs to mind when attempting to synthesize data on the participants and goings-on in obesity-associated inflammation.

metabolic abnormalities. Such a scenario would not diminish the interest of these cell types, but begs the question of whether their primary effect might not rather be on nonimmunological processes such as satiety, intestinal food absorption, macronutrient metabolism, etc, some of which have already been associated with IL-4, for example (Ricardo-Gonzalez et al., 2010; Orihara et al., 2009).

Lastly, some of the complexity might reflect experimental artifacts—inadequate cell-lineage markers, segregating genes in genetically impure mouse lines, microbiome effects, nonuse of littermate controls, housing conditions, etc. How impactful these issues can be was detailed above for the case of NKT cells.

With these thoughts in mind, we offer the minimalist scheme of Figure 2. It is clear that both pro- and anti-inflammatory cells of both the innate and adaptive arms of the immune system are important participants in the obesity-induced inflammation that promotes local and systemic metabolic aberrancies. Imperative at this point is a critical, data-based integration of these cells and their products into a synthetic scenario. In order to
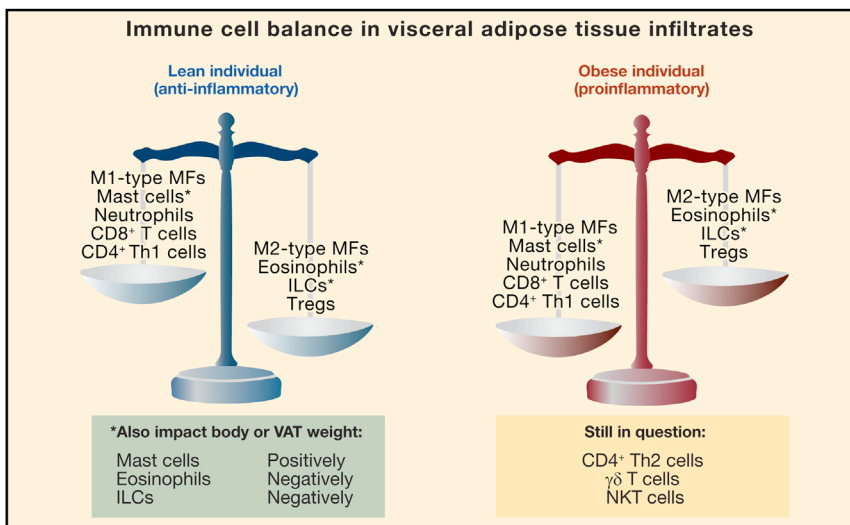

Figure 2. Immune Cell Players in Organismal Metabolism
The outcome of obesity-associated adipose-tissue inflammation reflects a balance between pro- and anti-inflammatory elements of the innate and adaptive immune systems.

accomplish this task, we must go beyond simple demonstrations that a particular cell type or molecule is an important player to systems-level dissections of its upstream, downstream, and lateral interactions.

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