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An Eclectic Cast of Cellular Actors Orchestrates Innate Immune Responses in the Mechanisms Driving Obesity and Metabolic Perturbation

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Abstract

The escalating problem of obesity and its multiple metabolic and cardiovascular complications threatens the health and longevity of humans throughout the world. The etiology of obesity and one of its chief complications, insulin resistance, involves the participation of multiple distinct organs and cell types. From the brain to the periphery, cell intrinsic and intercellular networks converge to stimulate and propagate increases in body mass and adiposity, as well as disturbances of insulin sensitivity. This Review focuses on the roles of the cadre of innate immune cells, both those that are resident in metabolic organs and those that are recruited into these organs in response to cues elicited by stressors such as overnutrition and reduced physical activity. Beyond the typical cast of innate immune characters invoked in the mechanisms of metabolic perturbation in these settings, such as neutrophils and monocytes/macrophages, these actors are joined by bone marrow-derived cells such as eosinophils and mast cells and the intriguing innate lymphoid cells (ILCs), which are present in the circulation and in metabolic organ depots. Upon high fat feeding or reduced physical activity, phenotypic modulation of the cast of plastic innate immune cells ensues, leading to the production of mediators that affect inflammation, lipid handling and metabolic signaling. Further, their consequent interactions with adaptive immune cells, including myriad T cell and B cell subsets, compound these complexities. Notably, many of these innate immune cell-elicited signals in overnutrition may be modulated by weight loss, such as that induced by bariatric surgery. Recently, exciting insights into the biology and pathobiology of these cell-type specific niches are being uncovered by state-of-the-art techniques such as single-cell

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None

RNA sequencing. This Review considers the evolution of this field of research on innate immunity in obesity and metabolic perturbation, as well as future directions.

Keywords

obesity; innate immunity; metabolic perturbation; insulin resistance; diabetes

Introduction

The incidence of obesity, a chronic, non-communicable disease, continues to increase in the United States and world-wide; the associated co-morbidities are substantial.[1] Consequences of obesity, such as type 2 diabetes (T2D), cardiovascular disease (CVD), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), musculoskeletal disorders and some types of cancers significantly impact morbidity, quality of life, and mortality in this disease.[1, 2] The complexity of obesity is underscored by multiple observations indicating that perturbations in both the brain and the periphery contribute to this disorder. Roles for adipocyte- and immune cell-intrinsic properties, together with their cross-talk and intercellular communications, characterize the complex milieus brewing in the adipose tissue depots challenged with high fat diets (HFDs), reduced physical activity, reduced energy expenditure and adipocyte expansion in incipient and established obesity. Further, the innate immune cells linked to obesity are not simply confined to the periphery; rather, innate immune cells of the central nervous system (CNS), such as astrocytes and microglia, also contribute to the immunometabolic consequences of overnutrition and obesity. Our understanding of these complex interactions is ever-evolving.

This Review will chronicle the evolution of this field, with focus on historical and recent insights into innate immune cells in the periphery and within the CNS, and their contributions to obesity and metabolic perturbations, such as insulin resistance, the harbinger of T2D. Only by delineating a time-, adipose and metabolic organ depot- and cell type-specific roadmap for protective vs. maladaptive pathways in overnutrition and obesity may effective and safe therapeutic targets be unveiled to target innate immune cell functions gone awry in these disorders.

“Inflammation” and Obesity

In 1993, Hotamisligil, Shargill, and Spiegelman published a seminal study in which they showed that *Tnf* (gene encoding Tumor Necrosis Factor-α) mRNA transcripts were significantly increased in the adipose tissue of T2D mice and rats, but not in the adipose tissue of streptozotocin-induced diabetic mice (a model of type 1 diabetes, T1D).[3] In those T2D models, the upregulation of *Tnf* preceded the development of significant hyperglycemia. In *fa/fa* rats (model of T2D), the administration of soluble TNFα receptor (TNFR)-immunoglobulin G (IgG) chimeric protein did not affect glucose levels, but resulted in the need to administer significantly more glucose to the treated vs. vehicle rats in order to maintain euglycemia during a hyperinsulinemic euglycemic clamp study; those findings suggested that the treated animals were more insulin responsive.[3] It was deduced from those studies that TNFα contributed to impaired sensitivity to insulin in the T2D models.

Subsequent to this discovery, efforts focused on identifying the immune cell composition in the adipose tissue depots in lean vs. obese animal models and human subjects. Experiments testing these concepts placed the early spotlight on the macrophage of the innate immune system.

Macrophages & Complex Roles in Metabolic Perturbation

Macrophage Content in Obese Adipose Tissue—Two reports published in the *Journal of Clinical Investigation* in 2003 associated increased macrophage content in adipose tissue with obesity.[4, 5] Ferrante's group showed that in multiple murine adipose tissue depots, obesity was accompanied by a rise in macrophage content (characterized by the macrophage marker F4/80), which correlated with body mass index (BMI) and adipocyte size.[4] Transcriptomic profiling of adipose tissue showed that approximately 1,000 genes were associated with obesity and body mass; a substantial number of these genes were linked to inflammation.[4] Ferrante and colleagues showed that the majority of TNFα expression in obese adipose tissue was accounted for by adipose tissue macrophages (ATMs).[4] In human obesity, using CD68 to mark macrophages, they showed that obese adipose tissue also displayed increased macrophage content compared to lean adipose tissue.[4] In subsequent work, Ferrante's group showed that deficiency of *Ccr2* (gene that encodes C-C Motif Chemokine Receptor 2) or its pharmacological antagonism in mice fed a HFD resulted in reduced food intake, reduced weight gain and improved insulin sensitivity, in parallel with reduced ATM content.[6] In a parallel study, Chen's group demonstrated increased macrophage content and expression of inflammation-related genes in the adipose tissue of obese mice, which preceded rises in circulating insulin levels.[5] These authors treated T2D *ob/ob* mice with the insulin-sensitizer rosiglitazone for 28 days; in rosiglitazone- vs. vehicle-treated mice, white adipose tissue (WAT) displayed a significant reduction in mRNA levels of *Adam8*, *Ccl3*, *Itgam*, *Emr1* and *Cd68*, suggestive of decreased inflammation.[5] Of note, the Ferrante and Chen groups both showed that whereas macrophage content in adipose tissue was affected by obesity, neutrophil content was not altered, at least under the conditions employed in their studies.[4, 5] Hence, collectively, these studies suggested that macrophage infiltration in adipose tissue contributed to insulin resistance in obesity.

Early studies attempting to classify the inflammatory polarization of macrophages as "inflammatory M1" vs "anti-inflammatory M2" oversimplified the complex and plastic biology of these cells. Nevertheless, it is now clear that beyond differences in the numbers of macrophages in lean vs. obese adipose tissue, their functional properties differ as well. For example, in lean adipose tissue, ATMs may serve as resident sentinels, facilitating lipid buffering, production of anti-inflammatory cytokines such as IL4 and IL10, and pro-remodeling mechanisms, such as removal of dead adipocytes; processes favoring insulin sensitivity.[7–10] However, in obesity, with increasing adipocyte size, processes are set in motion that favor recruitment of monocytes/macrophages derived from the bone marrow and the production of proinflammatory cytokines such as TNFα, generation of reactive oxygen species (ROS), and impaired clearance of dead adipocytes; processes overall favoring insulin resistance.[6, 11] Interestingly, in obesity, beyond lipolysis as a means to stimulate macrophages, intercellular communication via adipocyte-released exosome-type structures

may trigger macrophage differentiation in the adipose tissue, thereby priming these cells to adaptively manage the excess lipid content.[12, 13] If overwhelmed, however, these processes may tip the balance to a proinflammatory ATM phenotype in which insulin resistance may be facilitated.

Beyond recruitment of monocytes/macrophages in obese adipose tissue, roles for immune cell retention factors, such as the guidance cue molecule, Netrin-1, have been suggested. Upregulation of Netrin-1 was observed in ATMs in obese mice and humans; hematopoietic deletion of *Ntn1* reduced adipose tissue inflammation by stimulating macrophage emigration from the adipose tissue; in parallel, insulin sensitivity was restored in mice.[14] In a separate study, using *Ntn1* floxed mice, myeloid-specific deletion resulted in a small decrease in adiposity in the HFD-fed mice. Single-cell RNA-sequencing (RNA-seq) of CD45+ myeloid cells revealed that upon deletion of *Ntn1* in this myeloid population, a significant attrition of ATMs was noted, especially in the resident macrophage population, which was accompanied by improved insulin sensitivity in HFD-fed mice compared with the floxed control mice fed HFD.[15] Deletion of myeloid *Ntn1* also regulated molecules linked to lipid handling, such as fatty acid uptake, intracellular transport of lipid, lipid droplet formation and lipolysis.[15] Also, a decrease in proinflammatory eicosanoids was noted upon the deletion of myeloid *Ntn1*.[15]

In obesity, a particular subset of CD11C-expressing cells, suggested to be dendritic cells (DC), was shown to populate the adipose tissue, thereby triggering processes that further propagate inflammatory mechanisms, with predicted links to insulin resistance.[16–18] Consistent with the premise that these cells are proinflammatory and facilitate insulin resistance, ablation of CD11C+ DCs in obese animals improved insulin sensitivity.[18] Further, in the above-noted study on myeloid deletion of *Ntn1*, the population of CD45+ cells expressing CD11C was also reduced, in parallel with improved metabolic function.[15]

Despite the compelling evidence linking macrophages and a proinflammatory switch to obesity and insulin resistance, other studies have uncovered findings suggesting that a broader perspective is necessary to untangle these complex and chronic perturbations in metabolic dysfunction.

Macrophages Adjust their Metabolic Signatures in Obesity – It's Not just the Numbers—A variety of unbiased proteomic, transcriptomic and metabolomic strategies have been employed to track the origins of adipose inflammation. Kratz and colleagues utilized plasma membrane proteomics approaches in both human and murine obese vs. lean subjects. They found that classical markers of the "M1" macrophage inflammatory state did not define the ATMs in obesity, either in humans or wild-type (WT) male mice fed a HFD.[19] The authors hypothesized that a "metabolically-activated" macrophage might better classify the macrophages embedded in a high glucose/high fat environment. In obesity, cell surface markers indicative of altered lipid metabolism, such as ABCA1, CD36 and PLIN2 were increased compared to the lean state, and although TNFα and IL1β were increased in obese ATMs, markers of the alternatively-activated "M2" ATMs, such as CD163, CD206, and TGFβ1 were suppressed. Interestingly, although inflammatory mediators did not stimulate the development of the metabolically-activated macrophage, p62 and PPARγ were shown to

trigger their development and, mechanistically, these mediators drove overall suppression of the inflammatory signatures in the ATMs.[19]

Robblee and colleagues used a transcriptomic approach in bone marrow-derived macrophages (BMDMs) exposed to saturated fatty acids and showed that saturated but not unsaturated fatty acids activated the endoplasmic reticulum (ER) stress sensor, IRE1α (inositol-requiring enzyme 1 α), which subsequently activated the NLRP3 inflammasome to drive expression of proinflammatory genes.[20] These authors also stimulated BMDMs with lipopolysaccharide (LPS) as a control and found that the transcriptomic profiles were quite different, in that the saturated fatty acids induced only 8% of the genes that were induced by LPS. These findings suggest that there were fundamental differences in the regulation of genes linked to discrete inflammatory triggers.[20] For example, in that study, prolonged treatment of the BMDMs with saturated fatty acids resulted in downregulation of a number of mRNA signatures linked to inflammatory responses. Collectively, this study suggested the critical point that although saturated fatty acid treatment of BMDMs regulated the inflammasome, the inflammatory mediators and time course of expression were overall distinct from those induced by the classical proinflammatory stimulus, LPS. Indeed, these data affirm that “macrophage inflammation” is quite complex and, at least in part, directly impacted by the initiating cues and stimuli.

In a study specifically addressing the primary metabolic routes used by ATMs in the lean vs. the obese state, and how the metabolic pathways contributed to the inflammatory signatures of the ATMs, Boutens and colleagues retrieved ATMs (F4/80+ sorted cells) and showed that obesity was associated with a distinct metabolic rewiring that favored glycolysis, in parallel with higher levels of *Hif1a* (hypoxia inducible factor-1 α), but the inflammatory signatures in the obese ATMs were not identical to those of the classical “M1”-like macrophages.[21] Rather, using adipose tissue cultures, they found the secreted mediators from this tissue, which did not appear to be leptin or lactate, contributed to the rewiring of these ATMs and their metabolic and inflammatory states.[21] An unexpected observation emerged from that study, however, as myeloid-specific deletion of *Hif1a* did not affect the proinflammatory state of the ATMs after 8 weeks of HFD feeding, as for example, levels of IL6 were identical in the adipose tissue from both the *Hif1a* myeloid-deleted and control mice.[21] In that work, however, the *Lysm* cre recombinase strategy was used, which would have deleted *Hif1a* in other cells beyond macrophages, such as neutrophils and microglia. It thus cannot be excluded that non-macrophage/ATM effects contributed to these findings.

In a recent study, Petrus and colleagues examined the releasate from the WAT of 52 obese and 29 non-obese control female human subjects and discovered that levels of glutamine were significantly lower in the obese vs. the lean state, but that there were no significant differences in serum levels of glutamine, thereby reinforcing the importance of the local milieu in mechanisms driving obesity.[22] Upon examining the BMI of the subjects, the authors found a strong inverse association between BMI and the levels of adipose tissue glutamine, which were related to fat mass and adipocyte size. To assess if glutamine exerted effects on ATM inflammation, WT mice were injected with glutamine while consuming a standard low fat diet; although glutamine had no effect on body weight, levels of proinflammatory genes in the epididymal WAT, *Ccl2, Emr1* and *Cd68* were significantly

lower and levels of *Adipoq* (adiponectin) were significantly higher.[22] Glutamine was linked to suppression of proinflammatory genes in human adipocytes; mechanistically, glutamine reduced glycolysis and reduced uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) levels. UDP-GlcNAc is the substrate for the post-translational modification O-linked β-N-acetylglucosamine (O-GlcNAc) mediated by the enzyme O-GlcNAc transferase.[22]

Recently, Drareni and colleagues reviewed the evidence supporting that some of the key transcription factors that regulate macrophage-mediated inflammation in obesity appear to include NF-κB; PPARα and PPARβ; PPARγ; STATs, such as STAT1 and STAT6; AP1; Interferon regulatory factors (IRFs), such as IRF5; Liver X receptors; glucocorticoid receptors (GCs); and Hypoxia inducible factor-1α.[23] Given the diversity of this group of molecules, it is likely that multiple factors, such as the stage of obesity and the presence or absence of T2D (hyperglycemia) may dictate the dominant transcription factors linked to regulation of macrophage-mediated inflammation in discrete settings.

Macrophages & Further Considerations for a Broader View in Obesity and Metabolic Perturbation—Collectively, these data suggest that multiple factors, including an inextricable link between macrophage metabolism, inflammation and the degree of insulin sensitivity must be considered when probing the specific macrophage proteome, transcriptome and/or metabolome species that are operating in obesity.

First, the concept of a positive linear correlation between body mass and adipose tissue macrophage content is not without exception. It was shown that in the earliest days after caloric restriction (CR) to induce weight loss in obese mice, ATM content actually rose in the immediate time frame (approximately days 1-7 of CR), that is, leaner mice displayed higher ATM content. Notably, however, this rise in ATM content did not include increasing content of CD11C+ cells. Lipolysis was identified to be a key process triggering increased ATM content in the adipose tissue through promotion of macrophage migration.[24] This early phase was followed by a gradual reduction in ATM content with ongoing and sustained weight loss in the chronic phase.[24] In separate work, Weinstock and colleagues exposed mice to HFD feeding and, therefore, obesity for 24 weeks (HFD/24 weeks), followed by retrieval of visceral adipose tissue (VAT) for single-cell RNA seq. An additional group of mice underwent 24 weeks of HFD feeding followed by a switch to CR, in which the mice received 70% of the HFD for a further 2 week time course (HFD/CR). The HFD/CR mice demonstrated an approximately 12% loss of body weight with a 25% decrease in VAT mass during that time.[25] Consistent with previous reports, the HFD/CR mice displayed significantly more crown-like structures (CLS) in VAT than the HFD/24 weeks mice. Although the majority of the CD45+ cells/clusters in the HFD/24 weeks group were representative of macrophages (51%), it was found that DCs, T cells, NK cells, monocytes and B cells represented 14%, 11%, 9%, 8% and 6% of the leukocytes, respectively. What was the effect of weight loss? In the HFD/CR group, the authors reported that CR restored T regulatory cells (Tregs) levels to those of lean mice. A major macrophage subpopulation in obese VAT, which expressed genes relevant to lipid binding and metabolism, became more similar to that of lean VAT after HFD/CR.[25] Finally, in the HFD/CR group, unique clusters of phagocytic macrophages, activated macrophages, B cells and resident macrophages were observed, which were not similar to either the HFD/24 weeks or the lean group VAT. Hence,

in the lean state, obesity or in HFD/CR, macrophages may exert a diverse range of functions in the adipose tissue, ranging from adaptive to tissue-damaging properties. Interestingly, HFD/CR contributes to reversal of many but not all of these changes in myeloid cells, along with the appearance of novel non-lean-like functional clusters.[25]

Aleman and colleagues tested these concepts in 10 female human subjects undergoing approximately 10% loss of body weight (and approximately 3.8% loss of fat mass) over an average of 46 days on account of consumption of a very low calorie diet (800 kcal/day).[26] In that study, the authors found on repeat subcutaneous adipose tissue (SAT) biopsy that compared to baseline, CLS increased after weight loss, in parallel with reduced markers of metabolic and inflammatory perturbation, such as levels of glucose, high sensitivity C-reactive protein (CRP) and decreased circulating free fatty acids, glycerol, 25 hydroxyvitamin D and urinary isoprostane M.[26] Hence, in human subjects as well, a disconnect between ATM/CLS and inflammation and metabolic perturbation was evident after weight loss.

Second, in line with the above concepts, the degree of ATM content does not necessarily align with inflammatory gene expression. Global or myeloid (bone marrow transplantation studies) deletion of *Ager* (the gene encoding the receptor for advanced glycation end products (RAGE)) resulted in reduced "FBC" content (F4/80, CD11B+, and CD11C+ cells) in perigonadal adipose tissue (PGAT) compared to WT mice fed HFD, in parallel with improved insulin sensitivity.[27] However, PGAT levels of multiple "proinflammatory" genes, *Tnf*, *Ccl2*, and *Nos2* were not reduced upon deletion of *Ager*; in contrast, in both experimental settings, global or myeloid deletion of *Ager* was associated with trends to or significantly higher levels of "anti-inflammatory" genes, *Cd203d, Dc-Sign, Arg1, Il10 Cd163* compared to the WT control animals.[27]

Third, a key test of concept regarding roles for proinflammatory forces and, specifically, for TNFα in obesity-associated insulin resistance, was not met with definitive conclusions in human subjects. In a study published in 1996, Ofei and colleagues randomized 21 obese T2D subjects to either CDP571, a humanized monoclonal anti-TNFα antibody, or saline vehicle. Subjects received one dose of the treatments; the half-life of the antibody was said to render effective plasma levels throughout the 4-week observation period. At the end of that time, compared to baseline, there were no differences in levels of fasting glucose, insulin or C-peptide. In an insulin sensitivity test, the percentage rates of glucose clearance per minute were not affected in the subjects by CDP571.[28] In a subsequent study in 2000 by Paquot and colleagues, 7 patients with obesity and varying degrees of glucose intolerance underwent two consecutive hyperinsulinemic, euglycemic clamp studies in which they received vehicle in the first study and then, in the second study, a single dose of Ro 45-2081 recombinant human TNF receptor:Fc fusion protein. In both cases, the clamp assays were performed 48 hours after the injections. The authors reported that insulin-mediated glucose disposal and glucose metabolic clearance rate did not differ in the subjects by treatment.[29] Finally, based on the premise that the earlier studies might have been confounded by the short duration of treatment, Stanley and colleagues randomized 40 obese subjects with metabolic syndrome to receive either placebo or the TNFα inhibitor etanercept (Enbrel) over a 6 month period; 34 subjects completed the study. At baseline, the etanercept group was

significantly younger than the placebo group. Although fasting glucose levels at end of study were significantly improved in the etanercept group vs. placebo, there were no treatment-dependent differences in fasting insulin, 2-hour insulin or HOMA-IR (Homeostatic Model Assessment of Insulin Resistance).[30] Levels of high molecular weight adiponectin, however, were higher in the etanercept- vs. placebo-treated group.[30] Hence, firm evidence for improved insulin sensitivity by etanercept treatment was not shown in this study; it is notable, however, that hyperinsulinemic, euglycemic clamp studies were not performed in those subjects.

Hence, the results of these reports suggested that in contrast to the findings in mouse models, TNFα blockade did not incontrovertibly affect insulin sensitivity in obese human subjects with evidence of metabolic dysfunction. In this context, possible roles for other cell types and processes in obesity, insulin resistance and T2D needed to be considered.

Neutrophils: Among the Early Responders in Obesity

Within the first few days of HFD feeding, the migration of neutrophils into adipose tissue commences.[31] In obesity, neutrophils may produce cytokines and chemokines, such as TNFα and MCP1 (CCL2), thereby igniting processes that promote monocyte/macrophage infiltration into the adipose tissue.[32, 33] In human subjects, akin to the observations made with respect to ATMs, at 4 weeks after bariatric surgery, the neutrophil content in obese adipose tissue continued to rise to 15-20 fold above that at the time of surgery and this persisted for months post-surgery, despite improvements in overall metabolic function.[34]

It is speculated that in obesity and metabolic dysfunction, one of the functions of neutrophils is the production of a serine protease elastase, which has the potential to cleave insulin receptor substrate 1 (IRS1), a mechanism to reduce insulin sensitivity in adipocytes.[31, 35] The effects of exercise on elastase expression in obese mice was studied. Mice were fed a standard chow or HFD from 4 to 20 weeks of age and randomized to either a sedentary group or exercise group, five days/week. Exercise training reduced adipose neutrophil content, neutrophil elastase activity and markers of inflammation.[36] However, the authors did not report in that study if these changes were accompanied by improvements in insulin sensitivity.[36]

Recently, neutrophil extracellular traps (NETs), which play roles in cell-cell communication mechanisms involving neutrophils, have been studied for their potential roles in obesity. NETs are structures derived from neutrophils; these cells bear a DNA scaffold that is dotted with nuclear, granular or cytosolic proteins.[37] Beyond roles in anti-microbial functions, NETs may also contribute to the pathogenesis of chronic diseases, such as atherosclerosis.[38] Interestingly, NETs also contain neutrophil elastase, and it was thus hypothesized that these structures may play roles in obesity. Braster and colleagues fed WT mice a 60% HFD and concomitant with the introduction of diet, mice were treated with Peptidyl Arginine Deiminase 4 inhibitor Cl-amidine; a compound that blocks NET release. The authors observed no differences in immune cell infiltration into the adipose tissue or liver and there was no effect on insulin resistance observed in these studies.[39] Others treated mice with HFD-induced obesity with Cl-amidine or with DNAse, and found that although neither affected body mass or glucose/insulin sensitivity, they were associated with improved

endothelial function and reduced inflammation.[40] In human subjects, studies tested the effects of obesity and weight loss on NETs. In obese patients undergoing gastric band surgery, weight loss was associated with decreased neutrophil NETs and decreased evidence of neutrophil activities; interestingly, the authors speculated that such changes might affect the ability to respond to infections, as neutrophil chemotactic accuracy, which was impaired before surgery, was not affected by weight loss.[41] In an observational study, D'Abbondanza and colleagues followed 73 patients with morbid obesity, 55 healthy subjects, and 21 subjects with severe coronary artery disease. NETs levels were higher in the obese vs. control group and correlated with BMI, waist and hip variables, glyco-metabolic variables and systolic blood pressure.[42] After sleeve gastrectomy, there were no definitive changes in NETs.[42] Collectively, the results of these studies suggest that more work will be required to determine if and at what time points during obesity and/or weight loss that NETs contribute to glucose and insulin sensitivity.

Mast Cells

Mast cells, like neutrophils, are bone marrow-derived cells and play seminal roles in immediate responses to stress, as evidenced by the eclectic set of mediators that they produce, including biogenic amines (e.g., histamine and serotonin), enzymes (e.g., proteases), cytokines, lipid metabolites (e.g., leukotrienes and prostaglandins), ATP, and neuropeptides.[43] Studies in mice with obesity have shown increased mast cell content in obese vs. lean adipose tissue.[44] Further, in human subjects, higher levels of serum mast cell-specific tryptase have been demonstrated in obese vs. lean individuals.[45]

Mechanistic evidence of links of mast cells to the pathogenesis of obesity and metabolic perturbation emerged from studies by Liu and colleagues. These researchers used mice deficient in *c-kit*, a growth factor that is essential for mast cell differentiation and survival.[46] Compared to their controls, *c-kit* null mice gained significantly less weight on a HFD, with significant reductions in WAT depots, in parallel with improved glucose tolerance and increased energy expenditure.[46] In other experiments, administration of the mast cell stabilizer, disodium cromoglycate (DSCG), attenuated body weight gain only in the WT but not the mast cell-deficient mice; mast cell content in the WAT was not affected, suggesting that the agent's mechanism of action was via effects on mast cell actions.[46] Other work used *c-kit*-independent means to induce deficiency of mast cells to test the role of these cells in diet-induced obesity. Using $Cpa3^{Cre+}$ mast cell-deficient mice, which are WT for *c-kit* and also display reduction in basophils, it was shown that feeding HFD resulted in no differences in body mass or adiposity compared with control mice; glucose and insulin intolerance were also not altered in these mice.[47] Given these divergent findings, it was important that these concepts were tested in a distinct model of metabolic stress, cold-induced thermogenesis and treatment with β-adrenergic stimuli.

Finlin and colleagues exposed lean and obese human subjects to repeated cold exposure and performed Nanostring array analysis on the SAT; regardless of BMI, mast cell tryptase and CCL26 (a chemokine for mast cells) were upregulated in the cold. Upon cold challenge (repeated cold exposure to unilateral thigh), in both lean and obese subjects, increased mast cell degranulation was evident; however, only in lean but not obese subjects, increases in

mast cell content in the SATs were observed.[48] *In vitro* analysis linked these observations to norepinephrine-stimulated mast cell degranulation.

Zhang and colleagues studied these concepts in mouse models; they employed the *c-kit* deficient mice described above[49] and found that when stimulated by norepinephrine, the metabolic rate was enhanced in these mice compared to controls.[49] Mast cell reconstitution in those mice (in the SAT) reversed these effects of norepinephrine.[49] The results of these findings linked mast cells to suppression of SAT browning in mice, revealing the complex functions of these cells and their diverse released mediators in obesity and in thermogenesis.

Collectively, although the findings linking mast cells to obesity may be model-dependent, they nevertheless underscore that distinct experimental modalities should be employed, where feasible, to test hypotheses regarding roles for specific cell types in metabolic dysfunction.

Eosinophils – Flipping the switch to anti-inflammatory / protective roles for innate immune cells in obesity

Eosinophils, like monocytes, neutrophils and mast cells are also bone marrow-derived cells.[50] To respond to allergic stimulation, differentiation mediated by Interleukin-5 (IL5) is critical and roles for IL3 and GM-CSF (granulocyte-macrophage colony stimulating factor) have also been described. Upon terminal differentiation, eosinophils in the circulation may then infiltrate target tissues in response to chemokines, such as eotaxin.[51] Activated eosinophils may release mediators to aid in allergic responses and, akin to neutrophils, eosinophils may release EETs or eosinophil extracellular traps. Eosinophils exert anti-parasite and anti-viral roles.[50]

Early evidence linking eosinophils to adipose tissue homeostasis emerged from mouse models and showed that the number of eosinophils in adipose tissue in obesity was significantly reduced compared to that observed in the lean state. It was also shown that these reductions in eosinophil numbers in adipose tissue might have been linked to obesity, as, when mice lacked eosinophils, they were more prone to diet-induced obesity and glucose intolerance when fed a HFD. Further, hypereosinophilic mice were protected from diet-induced obesity.[52] However, despite these compelling associative studies, it was shown that simply artificially buoying the number of eosinophils in the adipose tissue of obese mice to homeostatic levels by administering IL5 failed to improve insulin signaling or to boost energy expenditure.[53] However, in IL5-transgenic mice, whose local tissues were long bathed in IL5, their basally elevated numbers of eosinophils rendered the mice protected from diet-induced obesity, adiposity and glucose intolerance.[52] These findings highlight potential confounding issues that might arise in experiments probing acute vs. chronic administration of cytokines or other inflammatory mediators vs. chronic transgene-mediated expression in discrete mouse models.

Do eosinophils play roles in weight loss? Bolus and colleagues showed that in mice with HFD-induced obesity, reduced adipose tissue eosinophils could be restored by weight loss.[54] In weight loss (switching mice from HFD to low fat diet), the eosinophil content in adipose tissue rose, in parallel with reduced ATM content, reduced inflammation, and improved

tissue remodeling.[54] However, these authors reported that eosinophils might exert unique effects in distinct tissues, as levels of eosinophils did not vary in the liver during various phases of weight gain and weight loss.

Studies considering cross-talk between eosinophils and other cells within the adipose tissue suggested that one function of eosinophils is to maintain adipogenic maturation.[55] Eosinophils also contribute to the development of alternatively-activated “M2”-like macrophages via IL4 and IL13-dependent mechanisms.[56] It was shown that mice deficient in *Ccr2* displayed increased numbers of eosinophils, which was sustained and further increased during obesity mediated by HFD.[57] This increase in numbers of eosinophils in all WAT depots was not observed in other organs such as liver or bone marrow. In that study, there were no differences in insulin sensitivity, body mass, or adiposity between the *Ccr2* null and WT mice, perhaps suggesting that the specific milieu in which eosinophils are born and thrive and migrate is not typically devoid of *Ccr2*, which undoubtedly will exert distinct effects that were not accounted for in that study. Indeed, those authors reported that eosinophils in that model were present in the CLS, typically associated with macrophage accumulation around dead/dying adipocytes.[57]

In this context, innate lymphoid cells (ILCs) and ILC2, in particular, have intimate relationships with eosinophils and alternatively-activated macrophages. The three major members of the ILC family (ILC1, ILC2, and ILC3) have been linked to adipose tissue inflammation and provide a relatively recent lens into adipose inflammation.

Innate Lymphoid Cells – Key Roles in Inflammation and Metabolism

Innate lymphoid cells (ILCs) lack adaptive antigen receptors and are the innate counterparts of T lymphocytes.[58–62] ILC function begins in development and they contribute to tissue homeostasis in the intestine, lung and adipose tissues.[60] Earlier classification placed ILCs into three major groups. Recently, Vivier and colleagues suggested that these complex tissue resident cells ILCs be divided into five major subsets of the following: ILC1s and NK cells, which respond to intracellular pathogens and tumors and secrete molecules such as IFNγ and perforin; ILC2s, which respond to parasites and allergens and secrete molecules such as IL4, IL5, IL13 and IL9; lymphoid tissue inducer (LTi) cells, which are mesenchymal organizer cells linked to the formation of secondary lymphoid structures and secrete molecules such as RANK, TNFα, IL17 and IL22; and ILC3s, which respond to extracellular microbes such as bacteria and fungi and secrete molecules such as IL22, IL17, GM-CSF and lymphotoxin.[60] In functional terms relevant to T cells, ILCs contribute to distinct types of immunity (Type 1, Type 2 and Type 3; See Reference [63]). Specifically, NK/ILC1s contribute to Type 1 immunity; ILC2s contribute to Type 2 immunity; and ILC3s contribute to Type 3 immunity.[60–62] Increasingly, there is evidence linking ILCs to obesity. It has been suggested that one of the important functions of ILCs in adipose tissue relates to the regulation and “polarization” of macrophage inflammation.[64–66] In the sections to follow, we summarize what is currently known regarding these cells in obesity and metabolic disorders.

NK cells and ILC1s—In human and murine subjects, research has been performed examining the number and activation state of NK cells and the relationship to obesity.[67] It is

established that ILC1s are present in both human and murine adipose tissue and using parabiosis techniques, it was shown that ILC1s are predominantly tissue resident in mouse models.[64] Lynch and colleagues found that compared to lean controls, obese patients had fewer circulating NK cells and cytotoxic T cells. Among the obese subjects specifically, metabolically healthy obese had higher levels of NK cells and cytotoxic T cells compared to the unhealthy obese independent of age and BMI; the NK cells were also less activated in the metabolically healthy vs. the unhealthy obese subjects.[68] In a study in children, Tobin and colleagues found that circulating NK cells were reduced in obese vs. lean children, which inversely correlated with BMI and insulin resistance and the NK cells in the obese children were more activated than those from lean children.[69]

Other studies examined NK cells in the adipose tissue itself. O'Rourke and colleagues examined stromal vascular fractions (SVF) from VAT and SAT from obese human subjects undergoing bariatric surgery. VAT demonstrated higher IFNγ mRNA transcript levels, and NK cells, which constitutively express IFNγ, were also higher in the VAT vs. SAT; SVF-derived NK cells expressed IFNγ on activation, which was associated with TNFα expression by macrophages.[70] These findings suggest depot-dependent differences in NK cell content in obese adipose tissue. The altered profiles of NK cells in obesity may be affected by weight loss. Diet and exercise-induced weight loss over a three month period in obese individuals resulted in increased production of IFNγ along with increased NK cell functions.[71] Barra and colleagues showed that 6 weeks of high intensity interval training increased NK cell frequency in both obese women and in mice; in mice, this change in NK cells was associated with a reduced lung tumor burden, suggesting that the NK cells were more functionally competent.[72]

Wang and colleagues examined the effect of obesity on ILC1s; in their study, they characterized these cells as $Lin^{-}CD45^{+}$ $CD127^{+}$ $CD117^{-}CRTH2^{-}NKP44^{-}$ and showed that the number of circulating and omental adipose tissue ILC1s was higher in obese vs. lean individuals.[73] The adipose tissue ILC1 content positively correlated with adipose tissue fibrosis in these subjects. In obese subjects who underwent weight loss surgery, at the three months follow-up point, the numbers of circulating ILC1s were reduced compared to the pre-operative levels and the reduction of ILC1s correlated with the reduction in fasting blood glucose levels and HbA1C, suggesting that higher levels of the ILC1s were associated with poorer metabolic health.[73]

Wang and colleagues tested these concepts in a mouse model. They adoptively transferred adipose ILC1s ($Lin^{-}NK1.1^{+}NKP46^{+}T\text{-}bet^{+}Eomes^{-}DX5^{-}$)[66] from HFD-fed WT mice into *Prkdc*$^{-/-}$*IL2rg*$^{-/-}$ mice and fed them HFD for an additional 4 weeks. Compared to controls not receiving the ILC1s (phosphate buffered saline, PBS), there were no differences in body weight but those mice receiving the ILC1s were more glucose intolerant and showed more IFN-γ-dependent interstitial fibrosis in the VAT.[73] Further, the transfer of ILC1s resulted in more CD11C+ cells and more CLS vs. PBS. Since it was shown that IL12 was required for proliferation of the adipose ILC1s during HFD-induced obesity, inhibition of IL12 using antibodies was tested in *Rag1*$^{-/-}$ mice fed a HFD. Compared to controls, there were no differences in body weight or fasting glucose, but the number of adipose ILC1s and CD11C + cells was reduced in anti-IL12-treated mice, as was adipose fibrosis.[73]

In another study, O'Sullivan and colleagues performed adoptive transfer of purified adipose iNK or ILC1 from HFD-fed WT mice into *Rag2*$^{-/-}$ *Il2rg*$^{-/-}$ mice maintained on a HFD for 12 weeks. In both cases, the number of "M1"-type macrophages was higher in the adipose tissue of the recipients, but especially upon the adoptive transfer of ILC1s. Further, in both iNK or ILC1 transfers, the recipients were more glucose and insulin intolerant vs. controls, which was independent of the amount of weight gained on the HFD.[66]

Wensveen and colleagues also linked NK cells to adipose tissue inflammation and the induction of insulin resistance in obesity; the authors showed that in mice fed a HFD, obesity resulted in upregulation of the ligands of NK-cell activating receptor (NCR1) on adipocytes, which stimulated an increase in NK cell proliferation and their production of IFNγ; which, in turn, led to an increase in proinflammatory macrophage content in the adipose tissue along with exacerbation of insulin resistance.[67] These investigators also induced deficiency of NK cells, NCR1 or IFNγ and showed that the accumulation of proinflammatory macrophages was greatly reduced, in parallel with reduced insulin resistance.[67] Furthermore, Lee and colleagues tested the role of NK cells in obesity using two major strategies.[65] First, they depleted NK cells with neutralizing antibodies or by their genetic ablation in *E4bp4*(+/−) mice (E4bp4 is essential for NK cell development[74]), and found improvements in insulin resistance and decreased numbers of ATM and adipose tissue inflammation. Second, when they expanded NK cells through IL15 administration or by reconstitution of NK cells into *E4bp4*(−/−) mice, they found that upon HFD feeding, ATM numbers and adipose tissue inflammation increased, in parallel with increased insulin resistance.[65]

Boulenouar treated mice with anti-asialo GM1 antibody to deplete NK cells and found increased numbers of ATMs that were more "M2"-like in nature; mice deficient in *Klrk1* (lacking a major NK cell-activating receptor (or mice deficient in perforin (*Prf1*), which is an important pore-forming cytolytic protein), also showed increased ATMs that were "M2"-like in nature in diet-induced obesity.[64]

Collectively, these data suggest that NK cells and ILC1s have less impact on HFD-induced obesity but are more relevant for the metabolic consequences of HFD and obesity, potentially through modulation of macrophage survival and their pro- vs. anti-inflammatory properties. From the above studies, IFNγ was shown to be a leading candidate mediating the effects of these cells on macrophage inflammatory polarization. Studies in human subjects argue that the situation in long-term human obesity may be more complex and critically dependent on the time course (onset, duration, and/or reversal) of obesity, as well as on whether or not obesity was associated with metabolic consequences, such as T2D.

ILC2s—ILC2s are resident cells that mediate Type 2 immune functions in response to varied types of cues that cull dietary, metabolic, circadian, neural and mechanical inputs.[75] In adipose tissue, these cells are defined as Lin^{-}c-KIT^{+} Sca1^{+} immune cells that are highly expressed in human and murine adipose tissues.[32] These cells express GATA3 and are characterized by their ability to produce Type 2 T helper cytokines, specifically IL5 and IL13 and, in adipose tissue, they also express the IL33 receptor.[32, 76] Studies in mice revealed that with prolonged HFD feeding, the numbers of ILC2s and eosinophils decrease

in VAT/epididymal VAT.[77, 78] Similar observations were made in human subjects, in which it was found that the abdominal SAT of obese subjects revealed a decreased frequency of ILC2s compared to the lean controls.[78] These considerations suggested that ILC2s might play adaptive/protective roles in HFD-induced obesity.

Two studies reported that ILC2s may regulate adipocyte differentiation to drive beige fat growth.[78–80] Using gain- and loss-of-function approaches, Lee and colleagues showed that IL33-mediated activation of ILC2s promoted the growth of functional beige fat in mice under thermoneutral conditions. These authors showed that the activation of ILC2s stimulated the proliferation of bipotential adipocyte precursors and their subsequent commitment to the beige fat lineage.[80] In a different study, Brestoff and colleagues discovered an additional mechanism by which ILC2s contributed to beiging of fat; they found that ILC2s produce methionine-enkephalin peptides, which act directly on adipocytes, the consequences of which include the upregulation of UCP1 expression *in vitro* and beiging *in vivo*.[78, 79]

In addition to ILC2 effects on adipocytes, ILC2s may also exert their effects in adipose tissue via modulating the functions of other immune cells, particularly eosinophils and “M2”-like ATMs. IL4 and IL13, secreted by ILC2s, are critical regulators of eosinophil migration and their beneficial activities in adipose tissue in HFD feeding.[52, 77] Treatment of mice with IL25 increased ILC2 and invariant (i)NKT cells in obesity to impact weight loss and modulate glucose homeostasis.[81] Hams and colleagues showed that treatment of obese mice with recombinant helminth-derived *Schistosoma mansoni* egg-derived ω1 (ω1) stimulates the recruitment /accumulation of ILC2s, eosinophils and alternatively activated macrophages (“M2”-like) into adipose tissue, which resulted in improved metabolism.[82] In HFD-induced obese mice, treatment with the helminth *S. mansoni* and helminth egg-derived antigens reduced obesity, adiposity and insulin intolerance and increased type 2 immune responses in WAT, together with increased eosinophils and “M2”-like alternatively activated macrophages.[83] Furthermore, ILC2s may directly affect the properties of Tregs; IL-33 activates ILC2s and Treg cells in adipose tissue in homeostasis, but also after helminth infection or treatment with IL2. However, ILC2-intrinsic IL33 activation was necessary for the accumulation of Treg cells and was independent of ILC2 type 2 cytokines, but partially dependent on direct co-stimulatory interactions via ICOSL-ICOS (inducible T cell CoStimulator).[84]

In turn, other cell types may modulate the functions of ILC2s in obesity. For example, ILC1-derived IFNγ production may repress the activation of ILC2s in adipose tissue, as IFNγ inhibited the activation of ILC2s and, therefore, Treg cell accumulation by IL33 in adipose tissue in HFD-induced obesity.[84]

In addition to the different types of ILCs, the specific tissues distinct from adipose tissue in which ILC2s reside may also have impact on adipose tissue ILC2s. Sasaki and colleagues recently reported the results of a comprehensive study in which they made a number of key observations on lymphocytes and ILCs in obesity. First, they showed that mice devoid of all lymphocytes ($Il2^{-/-}/Rag2^{-/-}$) were resistant to HFD-induced obesity. Second, they showed that when bone marrow cells from $Rag2^{-/-}$ mice (which lacked only the acquired immune

cells) were transplanted into the *Il2*$^{-/-}$/*Rag2*$^{-/-}$ mice, the benefits on protection from HFD-induced obesity were lost, specifically indicating roles for ILCs in these processes.[85] Third, these authors found that the ILC2 effects on obesity resided in the small intestine, as the adoptive transfer of ILC2s from the small intestine and not the adipose tissue restored the development of HFD-induced obesity in the *Il2*$^{-/-}$/*Rag2*$^{-/-}$ mice.[85] They traced the specific mechanisms by transcriptional profiling of the small intestine- vs. adipose-derived ILC2s and found that the former expressed higher levels of IL2 than the latter; they implicated IL2 in the pathogenesis of diet-induced obesity by showing that blockade of IL2 signaling attenuated weight gain and reduced the populations of both ILC2s and ILC3s in the small intestine.[85] These findings solidify that ILC2s are imbued with extensive plasticity and flexibility, not only on account of the host stress to which they are responding, but, also dependent on the tissue organ/depot in which they reside.

Taken together, these considerations indicate roles for ILC2s in obesity and suggest that their functions may be more complex than previously appreciated. More research is needed using gain- and loss-of-function strategies to unravel their complex stress condition- and tissue-dependent roles in obesity.

ILC3s—ILC3s regulate “type 3 immunity”, which is regulated in part by the production of mediators such as IL22, IL17, lymphotoxin and GM-CSF.[60] ILC3s require the nuclear hormone retinoic acid receptor-related orphan receptor γt (RORγt) as a major regulatory factor for their development and function.[86] Studies in mouse models of HFD suggested that ILC3 content increased in the adipose tissue with obesity.[87] Wu and colleagues[88] determined that in overweight children with asthma, there were higher frequencies of ILC3s, determined by expression of Lin^{-}CD127^{+}IL-23R^{+} cells[89] in the peripheral blood of obese vs. lean asthmatic children, in parallel with higher mRNA levels of *IL17* and *IL22*.[88] However, there were no correlations between the frequencies of ILC3s and the expression of IL17A, and IL22 and the severity of asthma. The authors deduced from these results that childhood obesity was an independent factor associated with the elevated levels of ILC3s and these two key cytokines.[88]

Studies in animal models to examine the potential roles of ILC3s and, specifically, their associated mediators in diet-induced obesity are now coming to light. For example, Upadhyay and colleagues showed that mice devoid of *Ltbr* or lymphotoxin, which lacked IL23 and IL22, were resistant to HFD-induced obesity.[90] Roles for IL22 were studied in other models. For example, in Winnie mice, which are predisposed to colitis, and display impaired intestinal barriers, upon HFD, treatment with IL22 improved the integrity of the intestinal barrier.[91] In other studies, Hasnain and colleagues examined the cytokines that affected glucose intolerance through pancreatic beta cell stress in T2D and reported that in obese mice, IL22 administration modulated oxidative stress regulatory genes in pancreatic islets, suppressed ER stress and inflammation, promoted secretion of insulin and fully restored glucose homeostasis, which was followed by restitution of insulin sensitivity.[92] In other studies testing IL22, Wang and colleagues showed that mice deficient in *Il22* displayed increased metabolic abnormalities upon HFD feeding; in contrast, treatment of T2D *db/db* mice or obese HFD-fed mice with IL22 resulted in reversal of hyperglycemia and insulin resistance.[93] Hence, in such studies, it is important to distinguish effects of full body

deletion of a mediator such as *Il22* versus the effects of its administration it adult mice. Further, it is important to note that no specific experiments have been performed, to date, to elucidate the specific role(s) of the ILC3s themselves in obesity and metabolic dysfunction. Rather, experiments have focused on the mediators produced by these cells and their roles in obesity. As these mediators may be produced by other cells, any specific roles of ILC3s in obesity require further interrogation. Furthermore, given the links between ILC3s and the lamina propria, it will also be important to discern if ILC3s modulate components of the gut microbiota, as has been shown in settings such as colitis.[94–96] If so, then their putative effects in obesity might be more complex and beyond the production of mediators such as IL22.

Collectively, the discovery of ILCs and, more recently, their complex roles in obesity, underscore the potentially powerful influence of these cells in tipping the balance between inflammatory and metabolism-perturbing properties vs. anti-inflammatory and metabolo-protective mechanisms in obesity and, in particular, in the adipose tissue depots.

Beyond the cadre of peripheral cell types that regulate the response to HFDs and obesity, the innate immune cells of the brain, the microglia and astrocytes, also may contribute to obesity and its metabolic consequences. In the section to follow, we consider the roles of the microglia and astrocytes in obesity and metabolism.

The Innate Immune Cells of the Central Nervous System (CNS), Microglia & Astrocytes and their links to obesity and metabolism

Astrocytes and microglia are considered the innate immune cells of the CNS; their cell intrinsic and intercellular communications with other immune cells, such as T cells, and other cell types within the CNS, such as neurons, oligodendrocytes and vascular cells contribute to shaping the organism's response to nutrient and metabolic stresses.[97–99] Evidence supporting roles for these cells in obesity and its metabolic consequences continue to be uncovered; these findings highlight the complexities involved in solving the problem of obesity.

Microglia and Astrocytes: Origins and the Effects of Obesity—Astrocytes are glial cells of the CNS that are of ectodermal/neuroepithelial origin.[100] Among their major roles in maintaining homeostasis and support to neurons in the CNS is the regulation of ions, such as potassium, chloride and calcium; pH; water transport and neurotransmitters, such as glutamate, GABA (γ-amino butyric acid), adenosine and monoamines.[100] Astrocytes participate in extensive cell-cell communications in the CNS with neurons, vascular cells and microglia, as examples. Microglia are the yolk sac-derived primary myeloid/macrophages of the CNS;[101–103] recent evidence suggests extensive region-to-region heterogeneity of microglia transcriptomic signatures in the CNS, as well as sexual dimorphism.[104] The implications of both of these facets of microglia biology/pathobiology are not yet understood.

In obesity, there is growing evidence that both astrogliosis and microgliosis characterize this state.[105] In rodents fed a HFD, increased hypothalamic astrocytosis was observed, in parallel with increased blood vessel diameter, suggestive of changes in the blood-brain barrier

(BBB) properties.[105] In this context, astrocytes envelop blood vessels in the CNS and their products and properties may affect vascular function. For example, altered expression of astrocyte connexins may directly impact the integrity of the BBB.[106] Others showed that HFD feeding in rats resulted in astrocytosis and that chronic leptin exposure affected hypothalamic neuron-astrocyte interactions.[107] Gao and colleagues showed that HFD increased the total number of arcuate nucleus microglia in the hypothalamus with evidence of increased microglial activity.[108] Furthermore, akin to the often-cited controversies regarding whether or not peripheral immune cells infiltrate the brain in neurodegenerative disorders, so, too, in HFD feeding and obesity has this question been raised. Indeed, multiple studies suggest that in HFD conditions, peripheral immune cells may enter the CNS and, perhaps, contribute to the overall "inflammatory" state.[109, 110] For example, Buckman and colleagues used a GFP+ bone marrow chimera model to show that feeding mice a HFD resulted in an approximately 30% increase in the number of GFP+ cells in the CNS when compared to the control diet mice; more than 80% of the GFP+ cells in the CNS bore CD45+ CD11B+ signature, indicating that these GFP+ cells were analogous to microglia and macrophages.[109] Indeed, given that studies have suggested alterations in the BBB in obesity,[111–115] it is plausible that the obese state favors the influx of peripheral inflammatory cells. Interestingly, these effects of HFD may differ between male and female organisms.[111] Furthermore, even if peripheral immune cells themselves do not infiltrate the CNS in HFD feeding and obesity, their released mediators/factors may cross the BBB to exert paracrine effects on glial and/or neuronal properties. Finally, evidence is accruing that HFD affects the gut microbiome, the consequences of which may feed back into the brain to alter neuroinflammatory states.[116–119] It is important to note, however, that not all studies demonstrated links between the gut microbiome, obesity and neuroinflammation.[120, 121] In summary, evidence suggests that HFD feeding and obesity are associated with a neuroinflammatory state, at least in part characterized by astrocytosis and/or microgliosis. Whether or not peripheral immune cell infiltration into the CNS in these conditions is substantial and, if so, if such infiltration contributes to the overall neuroinflammatory state remains unclear. As well, potential roles for alterations in the gut microbiome and the changes in neurotransmitters, for example, likely contribute as well to the overall adverse consequences of HFD feeding and obesity. In order to settle these questions, as well as those of the role of the gut microbiome in neuroinflammation, well-controlled, sex-matched, diet-matched and time course- synchronized experiments, including a consideration of the role of circadian rhythms, as examples, will need to be designed for testing in multiple different species (eg, mice and rats and other mammals) and strains, using standardized protocols for microbiome and metabolite assessments.

Diving into the Transcriptional Regulators of Neuroinflammation in Obesity and HFD Feeding—In the CNS, neurons, glia and other cell types express the transcription factor, NF-κB.[122] Its roles in both homeostasis and disease processes have been described. For example, in neurons, NF-κB signaling contributes to synaptic plasticity and learning and memory; in glia, NF-κB signaling is important for responses to injury and immune challenge, as well as for glutamate clearance and regulation of metabolism.[122] In pathological settings, however, activation of NF-κB may unleash its pro-injury side, contributing to chronic inflammation, failure of repair, and loss of tissue functions.

Numerous studies have tested roles for NF-κB in obesity and HFD feeding, which have unveiled its potentially deleterious effects in these settings.

Yan and colleagues showed evidence of excess TGFβ activity in the CNS in obesity and aging. Brain TGFβ caused an increase in glucose intolerance and hepatic glucose production, which was localized in part to astrocytes using astrocyte-specific glial fibrillary acidic protein (GFAP)-driven genetic approaches in mice models.[123] Further, excess brain TGFβ induced an ER stress response in the hypothalamus, which resulted in accelerated mRNA decay of an inhibitor of NF-κB, IκBα, which thus unleashed a neuroinflammatory response in obesity.[123]

Andre and colleagues showed that feeding excess fats and excess carbohydrates to mice resulted in obesity and, in the arcuate nucleus of the hypothalamus, a rapid and significant increase in the number of microglia.[124] These researchers attributed these effects to the expansion of the microglia pool in the hypothalamus. When they administered AraC (arabinofuranosyl cytidine), an anti-mitotic agent which blocked proliferation, they observed reduced food intake, and reduced body weight gain and adiposity. In parallel, this treatment also prevented the rise in microglia number and activation and reduced the expression of TNFα in the microglia, along with reduced NF-κB activation. Further, AraC resulted in restoration of leptin sensitivity in the hypothalamus.[124]

Douglass and colleagues employed tamoxifen/temporal regulation for deletion of the NF-κB essential cofactor, namely IKKβ in astrocytes in mice fed a HFD.[125] When IKKβ was deleted in astrocytes prior to the HFD feeding, there were no effects on body weight or glucose intolerance compared to control animals. However, mice treated with HFD for 6 weeks prior to the tamoxifen treatment (i.e., deletion of IKKβ in mice with established obesity), the mice bearing astrocyte deletion of IKKβ revealed a protection from further gain of body weight, reduced food intake, increased energy expenditure, reduced glucose intolerance and insulin resistance, and reduced hypothalamic inflammation and astrocytosis.[125] Hence, these data suggesting time-dependent roles for astrocyte NF-κB signaling in obesity unveil the complexities of mediating mechanisms at various stages of early obesity, progressive obesity and weight loss.

Zhang and colleagues tested the physiological roles of astrocyte NF-κB and found that astrocytic process plasticity and IKKβ/NF-κB are critical to the central control of blood glucose, blood pressure, and body weight in homeostasis and in chronic overnutrition states.[126] Hence, even in physiological settings, the actions of NF-κB in astrocytes may favor metabolic perturbation in body mass and glucose tolerance.

Valdearcos and colleagues also addressed the role of NF-κB in microglia in obesity.[127] These authors used PLX5622, a CSF1R inhibitor, to selectively deplete microglia from the CNS but not macrophages from the adipose tissue and showed that such depletion of microglia protected the mice from HFD-induced body weight gain and reduced total body fat mass, but not lean mass.[127] There were no effects of PLX5622 in mice fed a standard chow. To test if microglia NF-κB contributed to these effects, the authors used tamoxifen-inducible *Cx3cr1* ERT2 mice to specifically delete IKKβ from the microglia. HFD was then

begun 4 weeks after tamoxifen; deletion of IKKβ resulted in reduced gain of body mass, reduced food intake, no change in energy expenditure, reduced microgliosis and reduced microglial activation, the latter effects were localized to the microglia of the mediobasal hypothalamus (MBH).[127] When these authors deleted A20 in microglia, which is an inhibitor of NF-κB, they found that the benefits of reducing microglia NF-κB in HFD feeding were reversed, thereby illustrating the importance of microglia NF-κB in obesity in HFD using two distinct genetic approaches.[127] Finally, using multiple tracking experiments, Valdearcos and colleagues showed that peripheral myeloid cells that are recruited to the MBH in HFD feeding contribute to the observed microgliosis and that this recruitment of the peripheral myeloid cells is controlled by the resident microglia of the MBH.[127]

It is important to note that other transcriptional regulatory factors, such as STAT3, AKT and JNK signaling have been implicated in glial inflammation in HFD feeding and obesity;[128] the aforementioned experiments testing NF-κB illustrate the importance of this factor in these processes. It will be important, therefore, to consider in the experimental design of these studies the hierarchical dependence, or independence, of NF-κB with these distinct pathways in the immunometabolic responses spurred by glia in HFD feeding and obesity. This is especially relevant since generalized inhibition of these canonical signaling cascades, which play important roles in health and homeostasis, may be a double-edged sword in the armamentarium of obesity-tackling therapeutic agents.

Emerging lessons on adipose tissue immune cell content/functions in obesity - before and after significant weight loss

This Review focuses on innate immune cells in obesity and its metabolic consequences. Of note, not discussed here are the roles of adaptive immune cells, such as T cells, B cells and their multiple subsets, as well as Natural Killer T cells (NKT cells) and others in obesity and metabolic dysfunction. Thus, a key challenge that emerges is how to integrate these multiple and distinct innate immune cell types and their functions into a hierarchical schema for understanding the mechanisms underlying obesity and metabolic dysfunction. In this context, insights from studies of weight loss may be a good place to gather hints as to what cell types/mediators are actually playing pathogenetic roles. Hence, in this context, single-cell RNA-seq of adipose tissue depots may be a beneficial strategy to accomplish these goals. Data reported to date are restricted to but a few publications; however, with more work, especially considering the time course of obesity as well as “before and after” bariatric surgery and other means to induce significant weight loss, the pieces of this complex puzzle may begin to come together. For starters, Vijay and colleagues recently published the results of single-cell RNA-seq from multiple depots (SVF VAT and SAT) of 25 adipose tissue samples (12 VAT and 13 SAT) from 14 human subjects with obesity undergoing bariatric surgery. Although there are no post-operative sample analyses provided in this report, the results, nevertheless, provide state-of-the-art and novel insights into cell type specifications in human obese adipose tissue.[129] The authors identified that 34% of the cells from their merged clusters expressed markers consistent with different immune cell populations; they subsetted these immune cell groupings to identify 14 new clusters of immune cells (termed IS1-IS14), which were present in both VAT and SAT depots. IS1, IS4, I6 and IS8 clustered together largely and bore transcriptome signatures reminiscent of NK

and T cells (including naïve T cells and activated T cells). IS2, IS3, IS7, IS9 and IS12 were identified as ATMs; the cell types within these clusters expressed varied amounts of CD68 and were also shown to express genes linked to lipid metabolism in obesity. IS2, in particular, bore signatures relevant to "metabolically-activated macrophages." IS5 and IS10 were identified as $CD16^-$ monocytes, which bore high expression of *S100A8*, *S100A9* and *S100A12*.[129] Notably, these S100s, especially S100A12, are ligands of RAGE and toll-like receptors (TLRs).[130–132] S100s are considered as damage-associated molecular pattern molecules (DAMPs), which may be released by stressed cells and, thereby, amplify and perpetuate pro-damage responses, at least in part through interactions with receptors such as RAGE and TLRs, which have been described as pattern-associated molecular pattern receptors or PAMPs.[133–135] Vijay and colleagues further showed that IS5 are most likely representative of DCs in these populations. Finally, they linked IS11 to B cells and noted that the remaining cluster, IS14, revealed mixed signatures of various cell types.[129] Overall, this data set provides a promising starting point for which next steps include expansion of cell type-specific markers to capture a broader swath of cell types, such as the eclectic ILC1s, ILC2s and ILC3s; examination of lean subjects; examination of peripheral blood; and, where feasible, examination of SAT, at least, in the "before and after" bariatric surgery scenarios.

Indeed, for obese patients, bariatric surgery has induced significant weight loss and improvements in such metabolic and cardiovascular parameters as glycemic control, serum lipid levels, blood pressure, and renal function.[136] Although the underlying mechanisms are complex and relate, in part, to the presence or absence of T2D and the type of surgical intervention employed to induce weight loss, irrespective of these factors, studies are now beginning to report the effects of weight loss on immune cell content and properties in the peripheral circulation and in the accessible adipose tissue depots.

Poitou and colleagues performed bulk RNA-seq on adipose tissue from 22 obese women before and at three months after bariatric surgery (15 Roux-en-Y gastric bypass and 7 adjustable gastric banding).[137] In these women, significant reductions in body mass occurred in all 22 of them, along with improvement in multiple metabolic markers. Using a FDR (false discovery rate) of 5%, the authors reported that 7.6% of genes (1214 total) were differentially expressed post-surgery. There were trends in greater numbers of differentially-expressed genes in the Roux-en-Y vs. the adjustable gastric banding procedures.[137] Gene Set Enrichment Analyses (GSEA) revealed that some of the top hits in the upregulated group were in the following categories "Ribosomes," "mRNA splicing," "IFNγ signaling," and "antigen processing/presentation." In the down-regulated group, significant hits included "ECM organization," "biosynthesis of cholesterol," "biosynthesis of unsaturated fatty acids," "packaging telomere ends," "metabolism of porphyrin and chlorophyll," and "metabolism of starch and sucrose." Specifically, among immune cell modules, first, a macrophage module was highly conserved post- vs. pre-operatively with the exception of two down-regulated genes, *DUSP2* and *CD300C.* Second, within a T cell module, there were differences in genes involved in T cells signaling such as *CXCR1*, *CXCR2*, *GPR97*, *CCR7* and *IL7R.* Third, neutrophil-mediated inflammation was reduced post-operatively (reflective of changes in *S100A8*, *S100A12*, *CD300E*, *VNN2*, *TUBB1* and *FAM65B*). Finally, 19 genes linked to the interferon signaling pathways were highly preserved in the

post-operative vs. the pre-operative state, except for *DDX60*.[137] The authors concluded that overall improvements in the inflammatory state in the post-operative condition might provide insight into novel targets for therapeutic interventions.

In other studies supportive of these general conclusions, Wang and colleagues showed that ILC1s were reduced in the peripheral circulation post-bariatric surgery;[73] Latorre and colleagues showed that *TLR3* as well as other members of the TLR family were reduced in SAT post-operatively vs. baseline;[138] and Nijhuis and colleagues showed that multiple markers of neutrophil activation were reduced in the periphery after bariatric surgery.[139]

Perspectives, Gaps and Next Steps

In summary, innate immune cells play important roles in metabolic homeostasis, perturbation and resolution in the adipose tissue depots. The innate immune cell contribution to these processes is not confined to the periphery, but extends to the brain, in which cells such as microglia and astrocytes respond to peripheral cues in overnutrition and attenuated physical activity, thereby contributing to obesity and metabolic dysfunction. Tables 1 and 2 depict representative pioneering studies described in this Review with respect to role for innate immune cells in metabolic perturbation in human subjects and animal models, respectively.

Recent research has underscored that a cadre of innate immune cell types, both cell-intrinsic and intercellular communication mechanisms, drive the formation of a range of pro-inflammatory vs. anti-inflammatory mediators, whose expression appears to relate to the degree of, or protection from, metabolic perturbation. Hence, the expression of individual cytokines may not be ascribed to a single innate immune cell type. Figure 1 summarizes these concepts with respect to the consequences of overall innate immune cell function and their cross-talk with adaptive immune cells in a key organ affected by metabolic perturbation, the adipose tissue. Specifically, Figure 1A illustrates that homeostatic mediators, such as IL10, IL5, IL4, and IL13, produced by a diverse set of innate immune cells, override proinflammatory and pro-damage forces in the lean and metabolically healthy state; thus, the functions of adaptive immune cells, such as Tregs, may prevail. Beneficial interactions with the immune cells of the brain, skeletal muscle, small intestine (and the microbiome) and the liver join forces to block obesity and foster insulin sensitivity. In overnutrition and decreased physical activity, however, Figure 1B illustrates that the innate immune cells seem to become part of the problem! Specifically, when the adipose tissue depots assume a pro-DAMP signature, the innate immune cells produce mediators that block homeostasis and amplify pro-damage and pro-metabolic perturbants, such as IL6, IL1β, TNFα, MCP1, NOS2, IFNγ and IL2. Together with cell-intrinsic and intercellular cross-talk with innate immune cells of the brain, small intestine, skeletal muscle and liver, the environment becomes one of pro-damage inflammation and insulin resistance. Upon weight loss, Figure 1C, there is evidence of reducing DAMPs, and suppression of the pro-damage mediators with return of at least some of the homeostatic factors. The content of innate immune cells fluctuates; intriguingly, in the early phases of weight loss, for example, ATM content increases, perhaps in an effort to phagocytose and engulf dying adipocytes and their sequelae. More, though, needs to be discovered about the effects of weight loss on immune

cells in the brain, gut, liver and skeletal muscle. Certainly, however, even in weight loss, seemingly unexpected things happen. For example, as discussed above, in weight loss, the IFN signaling pathways in the adipose tissue are not down-regulated. Such considerations reinforce that the chief function of the innate immune system is to react promptly to acute damage, such as infection or trauma. To summarize, the depictions in Figure 1A–B–C suggest that it is unlikely that a particular cytokine or inflammatory mediator is produced exclusively by one type of innate immune cell. Rather, the balance of the products of these cells, heavily influenced by the overall microenvironment, emerges from cell-intrinsic and intercellular communications, not solely with other innate immune cells, but also with those of the adaptive immune system, vascular cells and adipocytes.

In these contexts, the discovery of ILCs and their roles in tissue resident functions is likely to exert significant impact on our understanding of innate immunity and the connection to adaptive immune networks in adipose tissue and other metabolic organs. The findings that these cells contribute to Th1-, Th2- and Th3-like type immunity bridges the classically termed “innate” and “adaptive” immune cell denominations. Hence, it is not surprising that in adipose tissue, these cells may cross talk with their neighbors (other immune cells, adipocytes and vascular cells, to name a few), thereby influencing the net balance output of proinflammatory, anti-inflammatory, profibrotic and metabolism-perturbing species. More surprising, though, is the possibility that ILCs in distinct organs, such as the intestine, may influence the biology and pathobiology of ILCs in adipose tissue.[85]

Collectively, these exciting advances in the presence and roles of these diverse immune cell actors in the adipose tissue depots and in other metabolic organs support the very likely notion that it is not just about TNFα! Indeed, recent insights from RNA-seq suggested that classical pathways, such as type 1 interferons, may well be preserved after metabolically-beneficial weight loss, suggesting potential duality of this pathway in the “good” vs. “bad” effects on immunity and metabolism.[137] Hence, it is not surprising that Wieser and colleagues showed that adipocyte expression of IFN receptors in HFD feeding was endogenously protective.[140] Results such as these underscore that the innate (and adaptive) immune systems evolved to protect and defend; even if these functions go awry in chronic disease, they nevertheless remain staunchly relevant for distinct forms of acute injury to the organism, such as infection or wounding.

How do we integrate such complex data into a hierarchical scheme to delineate where, when and how to intervene safely in combatting the ever-growing epidemic of obesity? Some suggestions to glean as much insightful information as possible from our experiments include: (1) meticulous consideration and reporting of the type of obesity-provoking diets in use; (2) the study of both male and female subjects in both animal model and human studies; (3) varied time courses of diet induction, that is, the study of onset and interruption of obesity; (4) reporting of the vivarium conditions – pathogen free vs. conventional, as such considerations may affect multiple factors, including the microbiome; (5) reporting of the vivarium temperatures, as well as consideration of study performance at room temperature and at thermoneutrality, where feasible; (6) reporting of genetic background; the study of multiple backgrounds such as in rodents may be of value; and (7) reporting the age of the animals. Lessons learned from murine manipulation studies must be acknowledged as well:

(1) multiple means to test roles of a specific cell type need to be considered; for example, it is unlikely that modulation of a single transcription factor will impact solely a single cell type; (2) adoptive transfers and lethal irradiation/transplantation need to consider the effect of the homing and niche re-settling nuances imbued by the distinct groups of transferred cells; (3) global and embryonic deletion approaches for hypothesis-testing models, such as in mice, need to be considered carefully, with respect to the possibility that cells of interest, which developed and matured in a gene-specific-deleted environment, may demonstrate altered epigenetic imprinting, and, therefore, they (and their precursors) may respond uniquely to various insults during maturity; (4) in using cell-type specific approaches, the use of temporally-regulated gene modulation may be useful to avoid developmental confounding issues; (5) the careful inclusion of controls when using tamoxifen or doxycycline, and the study of cre and floxed controls, for example, in gene deletion models must be considered; (6) meticulous consideration of off-target effects in all modes of gene modulation; (7) the testing of animals of multiple age ranges, including young, middle-aged and aged, where possible, given the plethora of effects of aging on adipose tissue, immune cell biology and metabolism;[141] and (8) where feasible and practical, the use of pharmacological agents to stimulate or antagonize pathways of interest to provide independent means of support in experimental paradigms.

Finally, techniques such as single-cell RNA-seq and single-nucleus RNA-seq are unveiling the promise of broad-scale and unbiased approaches for many settings, including obesity. However, it will be essential to ensure that bioinformatics approaches are enabled to capture the full gamut of innate (and adaptive) immune cells that populate the metabolic organs in homeostasis and in disease. We look forward to the many new discoveries using these and other state-of-the-art approaches as we enter this new decade of obesity research.

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Non-standard Abbreviations and Acronyms

AraC	arabinofuranosyl cytidine
ATM	adipose tissue macrophage
BBB	blood-brain barrier
BMDM	bone marrow-derived macrophage
BMI	body mass index
CLS	crown-like structures
CNS	central nervous system

CR	caloric restriction
CRP	C-reactive protein
CVD	cardiovascular disease
DAMPs	damage-associated molecular patterns
DC	dendritic cell
DSCG	disodium cromoglycate
EETs	eosinophil extracellular traps
ER	endoplasmic reticulum
FBC	F4/80, CD11B+, and CD11C+
FDR	false discovery rate
GABA	γ-amino butyric acid
GC	glucorticoid receptor
GFAP	glial fibrillary acidic protein
GM-CSF	granulocyte-macrophage colony stimulating factor
GSEA	Gene Set Enrichment Analyses
HFD	high fat diet
HIF-1α	hypoxia inducible factor-1 α
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
ICOS	Inducible T cell Costimulator
Ig	immunoglobulin
IL	interleukin
ILC	innate lymphoid cell
IRE1α	inositol-requiring enzyme 1 α
IRF	interferon regulatory factor
IRS1	insulin receptor substrate 1
LPS	lipopolysaccharide
LTi	lymphoid tissue inducer cells
MBH	mediobasal hypothalamus
NAFLD	non-alcoholic fatty liver disease

NASH	non-alcoholic steatohepatitis
NCR1	NK-cell activating receptor
NETs	neutrophil extracellular traps
NK	Natural killer cell
NKT	Natural killer T cells
O-GlcNAc	O-linked β-N-acetylglucosamine
PAMP	pattern-associated molecular pattern
PBS	phosphate buffered saline
PGAT	perigonadal adipose tissue
RAGE	receptor for advanced glycation end products
RNA-seq	RNA-sequencing
RORγt	retinoic acid receptor-related orphan receptor γt
ROS	reactive oxygen species
SAT	subcutaneous adipose tissue
SVF	stromal vascular fraction
T1D	type 1 diabetes
T2D	type 2 diabetes
TLR	toll like receptor
TNFα	tumor necrosis factor alpha
TNFR	tumor necrosis factor receptor
Treg	T regulatory cells
UDP-GlcNAc	uridine diphosphate N-acetylglucosamine
VAT	visceral adipose tissue
WAT	white adipose tissue
WT	wild type

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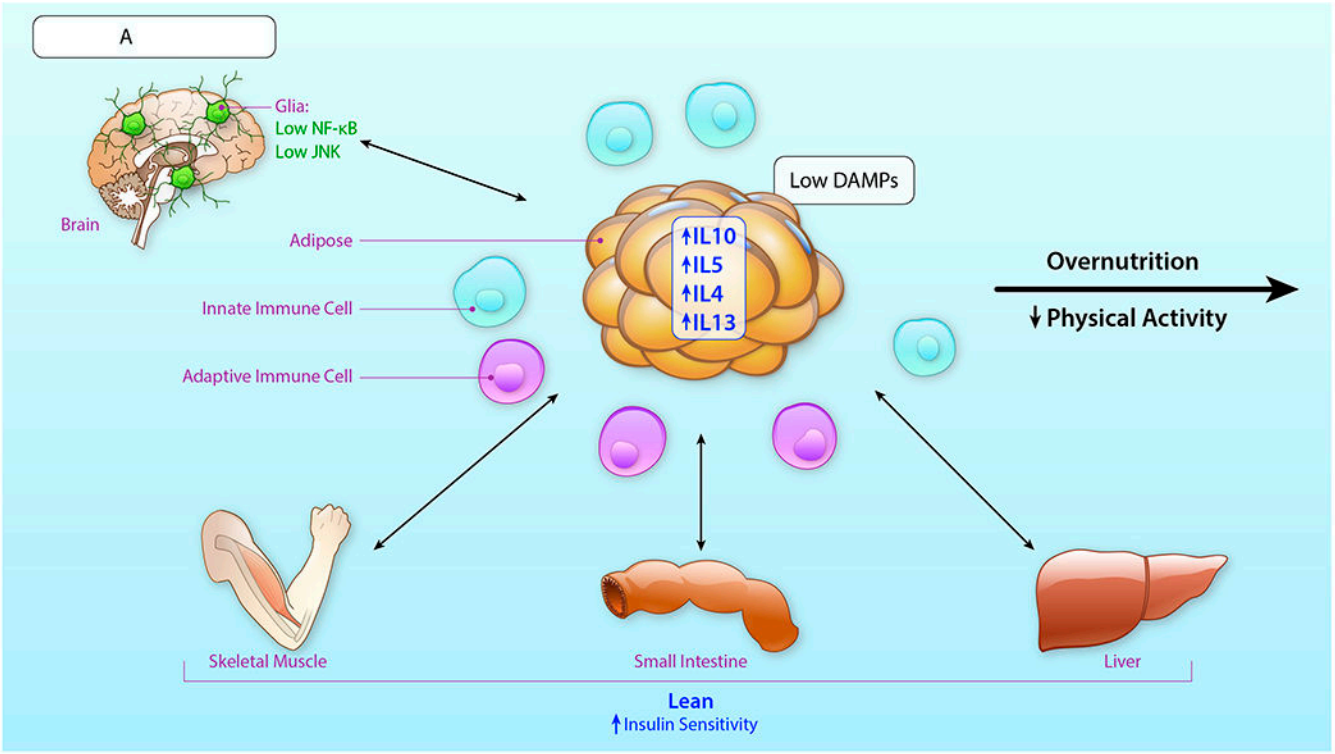

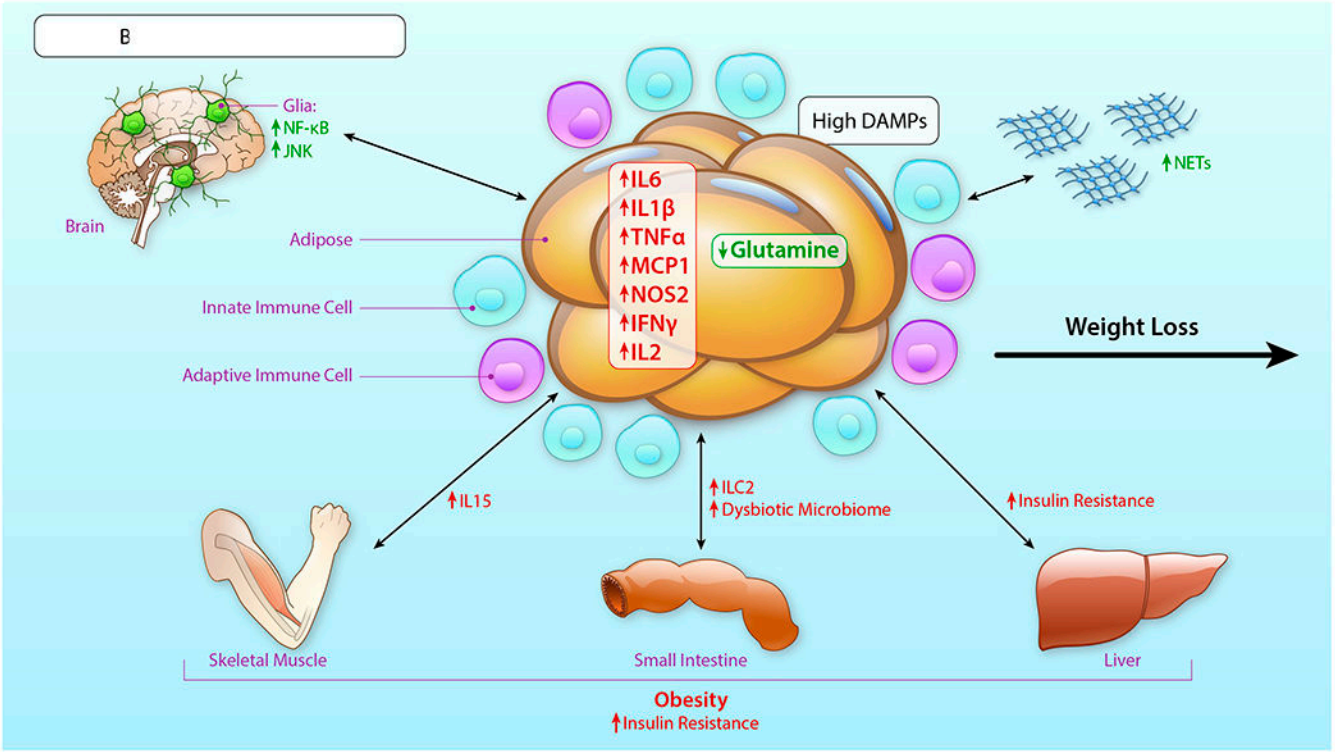

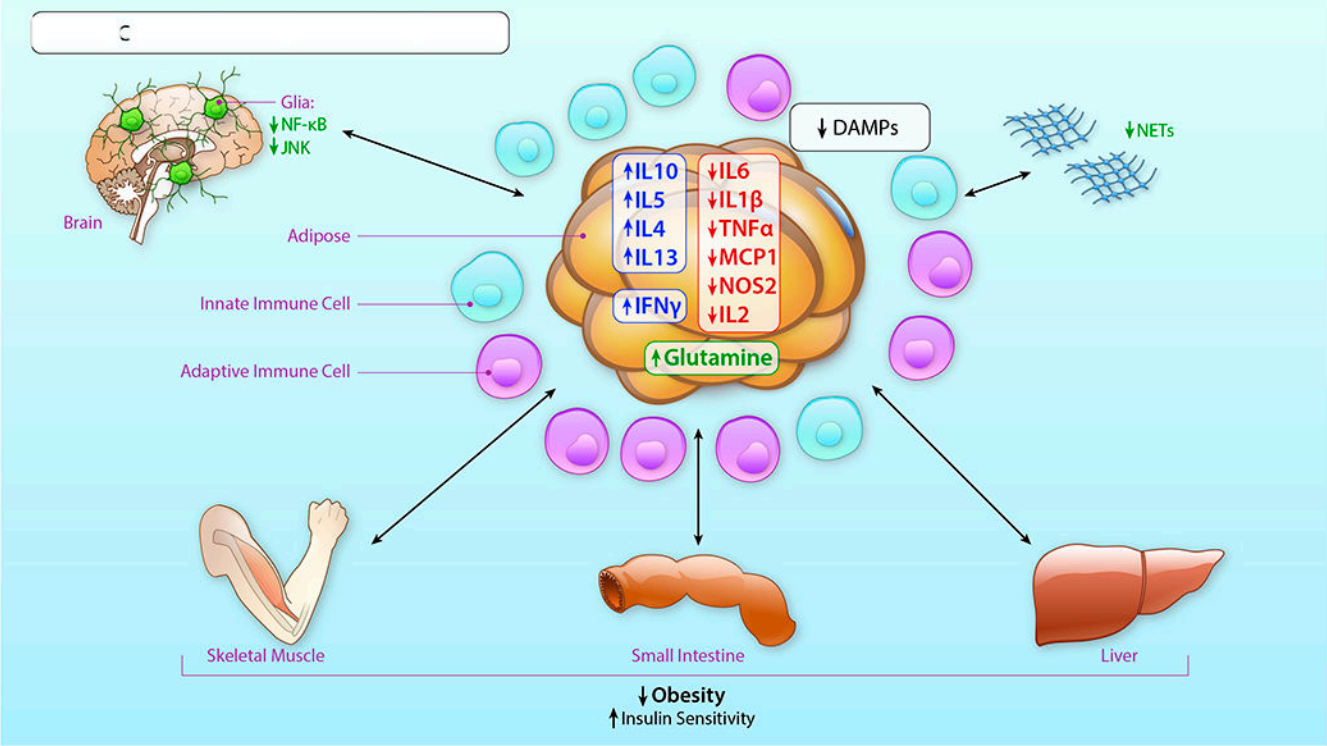

Figure 1. Obesity and Multi-Organ Metabolism: Roles of Innate Immunity in Lean, Homeostatic State, Obesity and Weight Loss.

This Review considers the driving roles for innate immune cells in the adipose tissue in the lean state (A), obesity (B) and upon weight loss (C) with respect to the mediators produced by these cells, their cross-talk with the adaptive immune system as well as the influence of intercellular communications with the brain, skeletal muscle, the gut, particularly the small intestine, and the liver. As these inflammatory mediators are most likely produced/released by multiple different types of innate immune cells, as well as cells of the adaptive immune system, this Figure does not attribute roles for one cell type to expression of specific mediators in the overall environment of metabolic perturbation triggered by obesity.

Table 1.

Representative Human Subject Studies

Human subjects	Experimental Methods	Major Findings	Reference
Macrophages			
Healthy lean, overweight, and obese subjects were fed a liquid formula diet of 40% fat (corn oil), 45% carbohydrate (glucose polymer), and 15% protein (casein hydrolysate).	CD68 marker was used to determine the macrophage content in lean vs. obese human adipose tissues.	Adipose tissue macrophage numbers increase in obesity and participate in inflammatory pathways that are activated in adipose tissues of obese individuals.	4
Adipose tissue from human obese and non-obese subjects was collected by abdominoplasty and laparoscopic intra-abdominal surgeries.	Samples were analyzed by proteomic, gene expression and flow-cytometric approaches.	Cell surface markers indicative of altered lipid metabolism and inflammatory markers were increased in adipose tissue macrophages of obese subjects compared to the lean controls. Markers of classical activation are absent on ATMs from obese humans.	19
52 obese and 29 non-obese control female human subjects were recruited and their abdominal subcutaneous white adipose tissue (WAT) biopsies and serum samples were collected.	Metabolome released from subcutaneous abdominal WAT in 81 obese and non-obese women was assessed for glutamine levels (measured in the releasate and in the serum).	In WAT, glutamine was downregulated in obesity. However, there were no differences in the levels of serum glutamine between the groups. Glutamine was linked to suppression of proinflammatory genes in human adipocytes.	22
10 female human subjects underwent weight loss by consumption of a very low calorie diet .	Subcutaneous adipose tissue (SAT) biopsy samples were obtained and stained for CLS and markers of metabolic and inflammatory perturbation, such as levels of glucose, high sensitivity C-reactive protein (hsCRP).	CLS increased after weight loss and markers of metabolic and inflammatory perturbation, such as levels of glucose, hsCRP, circulating fatty acids and glycerol were decreased.	26
Obese T2D subjects were treated with either CDP571, a humanized monoclonal anti-TNFα antibody, orrecombinant human TNF receptor: Fc fusion protein or TNFα inhibitor etanercept.	Insulin sensitivity test and/or hyperinsulinemic, euglycemic clamp assays were performed.	TNFα blockade did not incontrovertibly affect insulin sensitivity in obese human subjects.	28–30
Neutrophils			
Patients with obesity who underwent bariatric/metabolic surgery were recruited.	Fasting blood and subcutaneous abdominal adipose tissue were obtained before (n=14), at one month (n=9), and 6-12 months (n=14) after bariatric/ metabolic surgery from subjects who were not on insulin or anti-diabetes medication.	The neutrophil content in obese adipose tissue continued to rise to 15-20 fold above that at the time of surgery and this persisted for months post-surgery, despite improvements in overall metabolic function.	34
Twenty-three obese patients undergoing gastric band surgery were recruited to a longitudinal intervention study, along with non-obese, healthy gender- and age-matched controls.	Peripheral blood neutrophil (PBNs) were isolated by density centrifugation and a comprehensive analysis of PBN function was undertaken at various stages of the patients' bariatric surgical care pathway.	Obese patients exhibited exaggerated PBN activity in response to various stimuli, with higher reactive oxygen species (ROS) generation and release of pro-inflammatory cytokines and lower PBN extracellular trap (NET) formation. PBN chemotactic accuracy was also impaired prior to surgery. Weight loss was associated with normalized NET production and lower ROS production and cytokine release relative to healthy controls.	41
Seventy-three patients with morbid obesity and 55 healthy subjects, and 21 subjects with severe coronary artery disease were recruited before and after bariatric surgery.	Anthropometric parameters, peripheral blood pressure, biochemical and serum analysis at the enrollment and at twelve months after surgery were measured along with assessment of plasmatic levels of MPO-DNA complexes by ELISA.	NETs levels were higher in obese than in controls and correlated with the anthropometric variable (BMI, waist, hip), glyco-metabolic variables and systolic blood pressure. After surgery, there were no definitive changes in NETs.	42
Mast cells			

Human subjects	Experimental Methods	Major Findings	Reference
A general population of 1,216 persons, aged 15-69 years, was recruited.	Mast cell-specific serum tryptase was determined using ImmunoCAP Tryptase assay.	Higher levels of serum mast cell-specific tryptase were demonstrated in obese vs. lean individuals.	45
Human subjects were recruited from the Lexington, KY area in the summer; mean temperature 20-24 °C.	Baseline biopsies of thigh adipose were performed, the subjects then applied an icepack to one leg for 30 minutes each day for 10 consecutive days, and thigh biopsies were performed on the cold treated leg and the contralateral leg. Nanostring ncounter multiplex system was performed along with immunohistochemical analysis of the mast cell marker, anti-tryptase.	Regardless of BMI, mast cell tryptase and CCL26 (a chemokine for mast cells) were upregulated in the cold. Upon repeated cold exposure to unilateral thigh, in both lean and obese subjects, increased mast cell degranulation was evident; however, only in lean but not obese subjects, increases in mast cell content in the SATs were observed.	48
NK cells and ILC1s			
120 consecutive male and female obese subjects and 11 lean healthy controls were recruited	Blood samples were collected and BMI and blood pressure were measured. Peripheral blood mononuclear cells were prepared	Compared to lean controls, obese patients had fewer circulating NK cells and cytotoxic T cells	68
A cohort of 90 children (45 obese/45 non-obese) aged between 6 and 17 years was recruited.	Blood samples were collected for Peripheral blood mononuclear cells and various immune cell population was measured. Clinical assessments and biochemical measurements were performed.	Circulating NK cells were reduced in obese vs. lean children, which inversely correlated with BMI and insulin resistance and the NK cells in the obese children were more activated than those from lean children.	69
Stromovascular cell fractions (SVFs) from VAT and SAT from obese humans undergoing bariatric surgery were studied *in vitro*.	Transcriptional profiling, flow cytometric phenotyping, enzyme-linked immunosorbent assay and intracellular cytokine staining were performed.	VAT demonstrated higher IFNγ mRNA transcript levels, and NK cells, which constitutively express IFNγ, were also higher in the VAT vs. SAT; SVF-derived NK cells expressed IFNγ on activation, which was associated with TNFα expression by macrophages.	70
Thirty-two healthy adults with obesity were divided into control and experimental groups. Participants in the experimental group performed a 3-month program of exercise training and nutrition.	Peripheral blood mononuclear cells were analyzed by flow cytometry and Western blot assay.	Diet and exercise-induced weight loss over a three month period in obese individuals resulted in increased production of IFNγ, along with increased NK cell functions.	71
A total of 85 individuals, including 49 obese patients underwent laparoscopic Roux-en-Y gastric bypass (RYGB) surgery, and 36 age- and sex-matched non-obese non-T2D controls received elective abdominal surgery (e.g., hernia or hemangioma resection) were enrolled.	Peri-umbilical adipose tissue was collected during surgery and blood samples were collected before and 3 months after surgery.	The number of circulating and omental adipose tissue ILC1s was higher in obese vs. lean individuals and higher levels of the ILC1s were associated with poorer metabolic health.	73
ILC2s			
Subcutaneous white adipose tissue (S-WAT) from the abdominal region was obtained from lean and obese subjects.	ILC2 was measured from all subjects by flow cytometry and their frequencies were also compared for all characteristics	The abdominal SAT of obese subjects revealed a decreased frequency of ILC2s compared to the lean controls	78
ILC3s			
Fifty asthmatic children who were diagnosed with mild to severe asthma based on commonly-accepted clinical laboratory guidelines were recruited for the study and their blood samples were collected.	PBMCs were isolated from the blood and the levels of ILC3 was measured by flow cytometry and qPCR.	Overweight children with asthma had higher frequencies of ILC3s in the peripheral blood vs. lean asthmatic children, in parallel with higher mRNA levels of *IL17* and *IL22*.	88
Twenty-five subcutaneous and visceral adipose tissues from 14 human obese subjects undergoing	Stromal vascular fraction was isolated from mature adipocytes and subjected to single-cell RNA sequencing.	34% of the cells expressed markers consistent with different immune cell populations, specifically of NK cells, T-cells	129

Human subjects	Experimental Methods	Major Findings	Reference
bariatric surgery were collected along with tissue from healthy non-obese subjects.		and macrophages that were indicative of “metabolically-activated macrophages.”	
Twenty-two obese women before and at three months after bariatric surgery were included.	Adipose tissues were collected during and three months after surgery and were subjected to bulk RNA-sequencing.	Significant reductions in body mass occurred in all 22 subjects, along with improvement in multiple metabolic markers; macrophages, T-cells and neutrophil signatures showed overall improvements in the inflammatory state in the post-operative condition.	137

Table 2.

Representative Animal Subject Studies

Animal model	Experimental Methods	Major Findings	Reference
Inflammation and obesity			
Obese (*ob/ob*) mice, Diabetic (*db/db*) mice and Zucker (*fa/fa*) rats.	*Tnf* (gene encoding Tumor Necrosis Factor-α) mRNA transcripts were measured in adipose tissue of T2D mice and rats, and in streptozotocin-induced diabetic mice (a model of type 1 diabetes, T1D).	TNF-α mRNA transcripts were significantly increased in the adipose tissue of T2D mice and rats, but not in the adipose tissue of streptozotocin-induced T1D mice. In T2D mice, the upregulation of *Tnf* preceded the development of significant hyperglycemia. TNFα contributed to impaired sensitivity to insulin in the T2D models.	3
Macrophages			
Obesity was induced in C57BL/6J mice by a high-fat diet (diet induced obesity).	F4/80 was used to determine the macrophage content in lean vs. obese adipose tissues in mice.	Obese adipose tissue displayed increased macrophage content compared to lean adipose tissue and TNFα was increased in obese adipose tissue with increased macrophage content.	4
T2D (*ob/ob*) obese mice	T2D (*ob/ob*) mice were treated with rosiglitazone for 28 days. mRNA levels of inflammatory markers were measured.	Rosiglitazone- vs. vehicle-treated mice showed decreased inflammation.	5
Netrin1$^{fl/fl}$ LysMCre$^{+/-}$ (*Ntn1*$^{\Delta mac}$) mice were studied.	*Ntn1* was deleted in myeloid cells and subjected to high fat feeding. Single cell RNA-sequencing of CD45+ myeloid cells was performed.	Single-cell RNA-sequencing (RNA-seq) of CD45+ myeloid cells revealed that upon deletion of *Ntn1*, a significant attrition of ATMs was noted. Increased insulin sensitivity, improved lipid handling and metabolic function and decreased proinflammatory eicosanoids were observed.	15
C57BL/6J and *Hif1α*$^{fl/fl}$ LysMCre $^{-/-}$ were subjected to high fat feeding.	High-fat feeding of wild-type mice and myeloid-specific *Hif1α −/− mice* was used to examine the role of hypoxia-inducible factor-1α (HIF-1α) in ATMs of obese adipose tissue.	Transcriptome analysis and real-time flux measurements showed that HIF-1α did not alter the proinflammatory state of the ATMs after 8 weeks of HFD feeding.	21
C57BL/6J mice on a chow diet received daily intraperitoneal injection of glutamine (1 g/kg body weight) or PBS (20 mL/kg body weight) for 14 days.	qPCR analyses of Glul mRNA expression in eWAT samples was performed.	Glutamine had no effects on body weight but levels of proinflammatory genes in the eWAT were significantly lower and levels of *Adipoq* (adiponectin) gene were significantly higher.	22
C57BL/6J mice were fed high-fat diet and their average daily food intake was measured for a month. When they reached 40-41g of weight, caloric restriction (CR) was initiated.	Abdominal subcutaneous, epididymal, perirenal, and mesenteric adipose tissues were analyzed for macrophage content by qPCR and immunohistochemistry for F4/80.	In the earliest days after CR to induce weight loss in obese mice, ATM content rose in the immediate time frame (approximately days 1-7 of CR), that is, leaner mice displayed higher ATM content and Lipolysis was identified to be the key process triggering increased ATM content in the adipose tissue through the process of increased macrophage migration.	24
C57BL/6J mice were fed high-fat diet for 24 weeks and then switched to CR @ 70% of high-fat diet for two weeks.	VAT was subjected to single-cell RNA sequencing and stained for crown-like structures (CLS) by immunohistochemistry.	A major macrophage subpopulation in obese VAT expressed genes relevant to lipid binding and metabolism and the HFD/CR mice displayed significantly more CLS in VAT.	25
C57BL/6J mice and mice with global or myeloid specific deletion of *Ager* (gene encoding RAGE) were fed high-fat diet.	Insulin sensitivity, the FBC content and the levels of pro and anti-inflammatory markers in the eWAT were measured.	The *Ager* deleted mice showed improved Insulin sensitivity, with decreased FBC content and higher levels of anti-inflammatory markers in the eWAT.	27
Neutrophils			

Animal model	Experimental Methods	Major Findings	Reference
C57BL/6J mice were fed a chow or HFD from 4 to 20 weeks of age and randomized to either a sedentary group or exercise group five days/ week.	Neutrophil content, neutrophil elastase activity and markers of inflammation were measured in the adipose tissue.	Exercise training reduced adipose neutrophil content, neutrophil elastase activity and markers of inflammation.	36
C57BL/6J mice were fed a 60% HFD treated with Peptidyl Arginine Deiminase 4 inhibitor Cl-amidine; a compound that blocks neutrophil extracellular traps (NET) release.	Flow cytometry of adipose tissue and liver, immunohistological analysis and glucose and insulin tolerance tests were performed.	No differences in immune cell infiltration into the adipose tissue or liver were noted and there was no effect on insulin resistance.	39
Mast cells			
C57BL/6 mice were fed a high-fat diet for 20 weeks.	Prevalence and distribution of dead adipocytes, mast cells, macrophages (F4/80), and apoptotic cells were measured in the eWAT along with gene and/or protein expression of several adipocytokines, F4/80, mMCP6 and cleaved caspase-3.	Increased mast cell content in obese vs. lean adipose tissue was observed.	44
WT (C57BL/6 and WBB6F1/J) and *c-Kit deficient, $Kit^{W\text{-}sh/W\text{-}sh}$* mice were fed a western diet for 12-13 weeks following which the mast cell stabilizer, disodium cromoglycate (DSCG) was administered.	Body weight, glucose tolerance and energy expenditure and the weight of the WAT depots were measured.	The *c-Kit* deficient mice on a western diet gained less weight with significant reductions in WAT depots, in parallel with improved glucose tolerance and increased energy expenditure. Administration of DSCG, attenuated body weight gain only in the WT but not the mast cell-deficient mice; mast cell content in the WAT was not affected.	46
Kit-dependent B6.$Kit^{W/Wv}$ and Kit-independent B6.$Cpa3^{Cre/+}$ mast-cell-deficient mouse strains were fed either chow diet or high-fat diet for 16 weeks.	Body weight, glucose tolerance, energy expenditure and adiposity were measured.	No differences in body mass or adiposity vs. control mice were observed and glucose and insulin intolerance were not altered in these mice.	47
WT and $Kit^{w\text{-}sh/w\text{-}sh}$, c-Kit deficient mice at 12-weeks of age were injected with norepinephrine to stimulate thermogenesis and mast cells were reconstituted by bone marrow transplant.	Body weight, core body temperature, and indirect calorimetry were measured.	When stimulated by norepinephrine, the metabolic rate was enhanced in these mice compared to controls. Mast cell reconstitution in those mice (in the SAT) reversed these effects of norepinephrine.	49
Eosinophils			
WT (C57BL/6) and *Il4*-deficient male mice were subjected to chow diet or high-fat feeding for 15-24 weeks.	Body weight, glucose tolerance and insulin sensitivity were measured.	Number of eosinophils in adipose tissue in obesity was significantly reduced compared to that observed in the lean state and when mice lacked eosinophils, they were more prone to diet-induced obesity and glucose intolerance.	52
C57BL/6J mice fed a high fat diet (HFD) were simultaneously given recombinant interleukin-5 (rIL5) for 8 weeks to increase adipose tissue (AT) eosinophils.	Weight gain, AT inflammation, glucose, lipid, and mixed-meal tolerance, AT insulin signaling, energy substrate utilization, energy expenditure, and white AT beiging capacity were measured.	Eosinophils were increased ~3-fold in AT of obese HFD-fed mice treated with rIL5, and thus were restored to levels observed in lean healthy mice. However, there were no significant differences in rIL5-treated mice among the comprehensive set of metabolic assays, despite the increased AT eosinophils.	53
C57BL/6J mice were either fed a chow diet or a high fat diet (HFD) for 12 weeks and later switched to chow diet to induce weight loss for up to 6 weeks.	Body weight, individual tissue weights, and the levels of adipose eosinophil and macrophages were measured.	Weight loss (switching mice from HFD to low fat diet) led to increased eosinophil content in adipose tissue, in parallel with reduced ATM content, reduced inflammation, and improved tissue remodeling.	54
Male WT (C57BL/6J) and CCR2 deficient ($CCR2^{-/-}$) mice were subjected to high-fat diet.	Insulin sensitivity, body mass, or adiposity and the number of eosinophils in different tissues were measured.	No differences in insulin sensitivity, body mass, or adiposity were observed. Increase in numbers of eosinophils in all WAT	57

Animal model	Experimental Methods	Major Findings	Reference
		depots was observed with no differences in other organs such as liver or bone marrow.	
NK cells and ILC1s			
WT (C57BL/6), Rag1 deficient *Rag1*$^{-/-}$, IFNγ deficient *Ifng*$^{-/-}$ and *Prkdc*$^{-/-}$*IL2rg*$^{-/-}$ (mice lacking mature T, B, and natural killer lymphocytes) were fed either a chow diet or high-fat diet.	In adoptive transfer experiments, adipose ILC1s were purified by flow cytometry from C57BL/6 or *Ifng*$^{-/-}$ mice fed a HFD and transferred into *Prkdc*$^{-/-}$*IL2rg*$^{-/-}$ mice on HFD. After HFD feeding for 4 weeks, recipient peripheral organs were harvested, and adoptively transferred cells were analyzed by flow cytometry.	Compared to controls not receiving the ILC1s, there were no differences in body weight but those mice receiving the ILC1s were more glucose intolerant and showed more IFN-γ-dependent interstitial fibrosis in the VAT with more CD11C+ cells and more CLS vs. PBS.	73
WT (C57BL/6) and *Ifng*$^{-/-}$ mice were fed high-fat diet for 12 weeks .	In adoptive transfer experiments, purified adipose iNK or ILC1 from HFD-fed WT and *Ifng*$^{-/-}$ mice were transferred into *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice and further subjected to high-fat feeding for 2 weeks. Recipient peripheral organs were harvested and analyzed by flow cytometry 2 or 4 weeks after final transfer and additional HFD feeding.	In both cases, the number of “M1”-type macrophages was higher in the adipose tissue of the recipients, but especially upon the adoptive transfer of ILC1s. In both iNK or ILC1 transfers, the recipients were more glucose and insulin intolerant vs. controls, which was independent of the amount of weight gained on the HFD.	66
WT (C57BL/6) and *E4bp4*$^{+/-}$ mice were fed high-fat diet for 8 weeks and injected with NK cell neutralizing antibody to deplete NK cell population. To expand NK cells *in vivo*, mice fed chow diet or HFD for 6 weeks were intraperitoneally injected with IL-15. For the reconstitution experiments *E4bp4*$^{-/-}$ homozygous knockout mice were used.	To reconstitute *E4bp4*$^{-/-}$ homozygous knockout mice with NK cells, splenic NK cells were isolated from C57BL/6 WT mice that had been fed HFD for 8 weeks by negative sorting using a mouse NK cell isolation kit were used.	When NK cells were depleted with neutralizing antibodies or using *E4bp4*$^{+/-}$ mice, improvements in insulin resistance and decreased numbers of ATM and adipose tissue inflammation were observed. When NK cells were expanded through IL15 administration or by reconstitution of NK cells into *E4bp4*$^{-/-}$ mice, ATM numbers and adipose tissue inflammation increased, in parallel with increased insulin resistance.	65
WT (C57BL/6) were fed high-fat diet for 8 weeks and injected with NK cell neutralizing antibody to deplete NK cell population. For other experiments *Klrk1*$^{-/-}$ (mice lacking a major NK cell-activating receptor) and *Prf1*$^{-/-}$ (mice lacking perforin) knockout mice were subjected to high-fat feeding.	Adipose tissues were harvested for M1/M2 macrophage markers.	There were increased numbers of Adipose tissue macrophages that were more “M2”-like in nature in both models.	64
ILC2s			
WT (C57BL/6) and IL33-deficient *Il33*$^{-/-}$ mice were subjected to prolonged high-fat diet feeding and administered carrier-free recombinant murine IL-33.	Visceral adipose tissues were collected and the numbers of ILC2s and eosinophils were measured.	With prolonged HFD feeding, the numbers of ILC2s and eosinophils decrease in VAT/epididymal VAT.	78
WT (C57BL/6) mice and heterozygous Red5 (*Il5*$^{Red5/+}$, R5) mice were maintained at thermoneutrality and administered various doses of IL-33 for over 8 days.	Subcutaneous WAT was collected at the end of the study and the levels of UCP1 expression was measured by immunoblotting technique.	IL33-mediated activation of ILC2s promoted the growth of functional beige fat in mice under thermoneutral conditions.	80
WT (C57BL/6), IL1RL1-deficient (*Il1rl1*$^{-/-}$) *Il5*$^{tdtomato\text{-}cre/+}$ and *Il33*$^{-/-}$mice were used.	IL33 was induced by either helminth infection or treatment with IL2 on these mice and the levels of ILC2s and Tregs were measured by flow cytometry.	IL-33 activates ILC2s and Treg cells in adipose tissue in homeostasis, but also after helminth infection or treatment with IL2 and ILC2-intrinsic IL33 activation was necessary for the accumulation of Treg cells.	84
Male WT (C57BL/6), *Rag2*$^{-/-}$, and *Il2rg*$^{-/-}$ *Rag2*$^{-/-}$ mice were fed a normal chow diet or high-fat diet (HFD) and analyzed over a period of several weeks.	Body weight, glucose and insulin tolerance tests and indirect calorimetry were measured in addition to bone marrow transplantation and gut microbiome studies.	Mice devoid of all lymphocytes (*Il2rg*$^{-/-}$ *Rag2*$^{-/-}$) were resistant to HFD-induced obesity; bone marrow transplant indicated roles for ILCs in protection from diet induced obesity; the ILC2 effects on obesity resided in the small intestine.	85

Animal model	Experimental Methods	Major Findings	Reference
ILC3s			
Male WT (C57BL/6), *Rag2*$^{-/-}$ and leptin deficient Lep$^{ob/ob}$ *(ob/ob)* mice were fed either chow diet or high-fat diet.	Lung and adipose tissues were harvested and the presence of ILC3 was detected by flow cytometry.	ILC3 content increased in the adipose tissue with obesity.	87
Wild type (WT) C57BL/6 and Winnie mice were fed either a chow diet or a high-fat diet and administered recombinant IL-22.	Body weights of mice and diarrhea and rectal bleeding scores were recorded weekly.	Winnie mice, which are predisposed to colitis, displayed impaired intestinal barriers, upon HFD. However, treatment with IL22 improved the integrity of the intestinal barrier.	91
Wild type (WT) C57BL/6 mice, were fed either a chow diet or a high-fat diet and injected with control Ig or IL22.	Glucose and insulin tolerance tests were performed and pancreatic islets were isolated and cultured overnight for rt-qPCR analysis.	In obese mice, IL22 administration modulated oxidative stress regulatory genes in pancreatic islets, suppressed ER stress and inflammation, promoted secretion of insulin and fully restored glucose homeostasis, which was followed by restitution of insulin sensitivity.	92
Wild type (WT) C57BL/6, T2D (*db/db)* mice and *IL-22r1* deficient mice fed a high-fat diet were used.	Body weights of mice were monitored and glucose and insulin tolerance tests were performed.	Mice deficient in *Il22* displayed increased metabolic abnormalities upon HFD feeding; in contrast, treatment of T2D (*db/db*) mice or obese HFD-fed mice with IL22 resulted in reversal of hyperglycemia and insulin resistance.	93
Microglia			
Wild type (WT) C57BL/6 and Lep$^{ob/ob}$ (*ob/ob)* and fed either a chow diet or a high fat diet and were treated with leptin for 5 days.	Body weights of mice were monitored and brains were collected at the end of the study for qPCR and immunohistochemistry.	HFD increased the total number of arcuate nucleus microglia in the hypothalamus with evidence of increased microglial activity.	108
Wild type (WT) C57BL/6 and Global green fluorescent protein (C57BL/6-Tg (CAG-GFAP)) mice on C57BL/6 background were used.	Bone marrow from GFP mice were transplanted into WT recipients and fed either high-fat diet or chow diet following which adipose tissues and brain were extracted for qPCR and flow cytometry .	Feeding mice a HFD resulted in an approximately 30% increase in the number of GFP+ cells in the CNS when compared to the control diet; more than 80% of the GFP+ cells in the CNS bore CD45+ CD11B+ signature, indicating that these GFP+ cells were analogous to microglia and macrophages.	109
Wild type (WT) C57BL/6 and *Tlr4*$^{-/-}$ mice were used.	Mice were injected with either vehicle or TGF-β1 in the brain by intracerebroventricular surgery and fed either chow diet or a high-fat diet and their body weights were monitored and glucose and insulin tolerance tests were performed.	Brain TGF-β1 caused an increase in glucose intolerance and hepatic glucose production, which was localized in part to astrocytes using astrocyte-specific glial fibrillary acidic protein (GFAP)-driven genetic approaches.	123
Wild type (WT) C57BL/6 were used.	All mice were subjected to intracerebroventricular surgery and injected with artificial or aCSF containing 4.5 μg/μL BrdU with or without AraC 15 μg/μL and maintained on either chow diet or switched to an HFD.	Feeding excess fats and excess carbohydrates to mice resulted in obesity and, in the arcuate nucleus of the hypothalamus, a rapid and significant increase in the number of microglia was observed.	124
GFAP expressing GFAP/^{CA}*Ikkβ*$^{+/-}$ mice and their wild-type (WT) littermates were fed either high-fat diet or chow diet.	Body weights of mice were monitored and glucose and insulin tolerance tests were performed.	Astrocytic process plasticity and IKKβ/NF-κB are critical to the central control of blood glucose, blood pressure, and body weight in homeostasis and in chronic overnutrition states.	126
Wild type (WT) C57BL/6 fed either control or high-fat diet containing PLX5622.	PLX5622, a CSF1R inhibitor, selectively depleted microglia from the CNS but not macrophages from the adipose tissue and the body mass and composition were measured .	Depletion of microglia protected the mice from HFD-induced body weight gain and reduced total body fat mass, but not lean mass.	127