Published in final edited form as:
Crit Rev Immunol. 2014 ; 34(1): 1–14.

Changes in adipose tissue macrophage and T cell during aging

Sanjay K Garg[1], **Colin Delaney**[1], **Hang Shi**[1], and **Raymond Yung**[1]
[1]Department of Internal Medicine, University of Michigan Medical School, 109 Zina Pitcher Place, Ann Arbor, MI-48109

Abstract

Adipose tissue historically was believed to be an inert tissue, functioning primarily in the storage of energy and thermal homeostasis. However, recent discoveries point toward a critical role for adipocytes in endocrine function as well as immune regulation. Excess body fat, accumulated through aging and/or calorie-rich diet, is associated with many chronic metabolic and inflammatory diseases. Within the stromal vascular fraction of adipose tissue, macrophages and T cells accumulate with increasing tissue mass, secreting pro- or anti-inflammatory cytokines. In this review we discuss the current understanding of immune cell function in both diet-induced and age-related obesity. In both models of obesity, the classically activated, pro-inflammatory (M1) subtype takes precedence over the alternatively activated, anti-inflammatory (M2) macrophages, causing tissue necrosis and releasing pro-inflammatory cytokines like IL-6. Recently, other distinct adipose tissue macrophage (ATM) subtypes have been identified by surface marker expression and their functions characterized. Adipose tissue T cell (ATT) recruitment to adipose tissue is also different between aging and diet-induced obesity. Under both conditions, T cells exhibit restricted T-cell receptor (TCR) diversity and produce higher levels of pro-inflammatory signals like IFN-γ and granzyme B relative to young or healthy mice. However, regulatory T cell numbers are dramatically different between the two models of obesity. Taken together, these findings suggest model of age- and diet-induced obesity may be more distinct than previously thought with many questions yet to be resolved in this multidimensional disease.

Keywords

Adipose tissue; Macrophage; Lymphocytes; Inflammation; Aging

Introduction

Aging refers to the changes in a person's physical, psychological and social ability with the passage of time. The aging mammalian immune system undergoes distinct and comprehensive changes (immune senescence), leading to progressive dysfunction of immune responses.[1] These changes occur at every level of the immune system and affect both innate and adaptive immune responses. Some aspects of the immune response intensify (such as inflammation, regulatory T cells frequency) while others are either unaffected or diminished (such as the decline in Th1-Th2 mediated immunity, less naïve T cell-mediated de novo responses, impaired dendritic cell function).[2–8] Age-related immune dysfunction is believed to be a key factor leading to increased susceptibility to infections, autoimmunity and cancer in elderly people.[9–11]

[1]To Whom Correspondence should be addressed, ryung@umich.edu and sangarg@umich.edu.

Author Disclosure Statement: Authors have no competing financial interests

Adipose tissue or body fat is a loose connective tissue composed mainly of adipocytes. It was earlier believed that the main function of fat was to store energy in the form of lipids as well as cushion and insulate the body. However, it has been shown that it is an active endocrine organ as well, producing a number of hormones such as leptin, estrogen, resistin, visfatin and cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6).[12,13] Through secretion of these factors, adipose tissue plays an important role in mammalian health and diseases, affecting other organ systems of the body through endocrine signaling.[14] Based on its anatomical location, adipose tissue is classified as subcutaneous fat (under skin) or visceral fat (around internal organs). The two main types of adipose tissue in mammals are white adipose tissue (WAT) and brown adipose tissue (BAT). There are two distinct subtypes of BAT, depending on whether they are derived from myf-5 lineage and express the mitochondrial uncoupling protein UCP1. Additionally, investigators recently described CD137-high and UCP1-low expressing 'beige' adipocytes as a distinct type of thermogenic fat cell in mice and humans derived from progenitor cells within WAT.[15] While BAT has been considered a specialized form of adipose tissue with the main role in generating heat by uncoupling the respiratory chain of oxidative phosphorylation, WAT has been associated with quality of life and lifespan in part through sirtuin 1 and peroxisome proliferator-activated receptor-γ (PPAR-γ).[16,17] Excess WAT is associated with cardiovascular disease, type 2 diabetes, insulin resistance and inflammatory diseases.[13,18–20]

Macrophages and T cells are specialized leukocytes that protect the body against foreign invaders, repair tissue damage and clear dead cells from circulation. These cells perform their tasks partly by direct activity (such as phagocytosis in the case of macrophages) or by producing soluble factors (cytokines and chemokines). Macrophages and T cells work in a synergistic manner in order to mount an effective immune response. One of the adverse effects of the immune response is accumulation of inflammatory cytokines and chronic immune cell stimulation. Although acute healthy inflammation is beneficial, an unchecked response can lead to the development of chronic inflammatory diseases. Unfortunately, a state of low-grade chronic inflammation is commonly observed in the elderly. This phenomenon is known as "Inflamm-aging", an immune condition characterized by the presence of greater amounts of pro-inflammatory cytokines compared to those found in young subjects.[1,21]

Advancing age is increasingly accompanied by obesity, accumulation of visceral adipose tissue and inflammation.[22,23] The term obesity is not merely defined by excess weight but also depends on the accumulation of a large amount of fat. While the source of the inflammation in aging has not been definitively identified, the role of macrophages and T cells from adipose tissue has been shown to be associated with higher inflammation in aged mice.[8] Lately, there is increasing interest in the field of age-induced obesity and inflammation; however, how age-induced obesity influences leukocyte-mediated inflammation is not clear. Given the attention paid to the intersection between aging, inflammation and adipose tissue, this current review highlights the changes in macrophage and T cell function during age-induced obesity and their implications for age-related diseases. Due to varying sources of cells (i.e. human or mouse), culture conditions and experimental protocols, it should be noted that there are inconsistencies in the literature and in some cases contradictory results.

Adipose tissue as an immune organ in obesity and aging

Adipose tissue is composed of several different cell types. The cellular composition of adipose tissue varies depending on locale and the metabolic health of the organism. Composition of the adipose tissue also depends on the lean state or physiological/

pathological condition of the organism.[8,24,25] Physiologically, adipocytes are the major component of adipose tissue, but non-adipocytic cells, which make up the "stromal vascular cell fraction" (SVCF), also account for a significant proportion of adipose tissue. Separation of fat cells from the more dense SVCF has been described many years ago in pioneering protocol by Rodbell.[26] Using collagenase digestion and low speed centrifugation, adipocytes could be separated in the floating fraction from the dense SVCF pellet.[26] The dominant cell types in the SVCF are fibroblasts, macrophages, lymphocytes, pericytes and endothelial cells that are increasingly shown to perform a number of important functions for adipose tissue homeostasis. For example, monocytes and macrophages that reside in multiple fat depots, commonly known as adipose tissue macrophages (ATMs), help in the clearance of necrotic adipocytes, a role that appears to be of importance in obesity.[27] Several qualitative and quantitative phenotypic changes in ATMs that impact the inflammatory state of fat have been reported.[8] The total percentage of SVCF consisting of ATMs increases during age-induced obesity.[8,28] Increase in the adipose tissue mass with aging is also due to differentiation of an existing pool of pre-adipocytes into adipocytes. Deferential gene and protein expression between pre-adipocytes and adipocytes revealed that major changes in adipose tissue are due to changes in pre-adipocytes which occur in parallel with an increase in inflammatory cytokines.[8,28,29]

Hormones, cytokines, and chemokines produced in adipose tissue, collectively known as adipokines, play a prominent role in the attraction of immune cells to adipose tissue. Adipokines such as adiponectin, leptin, resistin, visfatin, TNF-α, IL-6, IL-1, plasminogen activator inhibitor 1 (PAI-1) and selected complement factors impact the inflammatory status of adipose tissue by affecting the quantity and action of immune cells that extravasate to the tissue.[12] Adiponectin, which is associated with lean states and insulin sensitivity, has been hailed as an anti-inflammatory force in adipose tissue via regulation of the production of cytokines in adipocytes and polarization of ATMs toward an M2 anti-inflammatory phenotype.[30,31] Leptin, produced in states of abundant adipose tissue and systemic inflammatory distress, induces the production of pro-inflammatory cytokines (such as TNF-α, IL-6, and IL-12) in monocytes.[32,33] Additionally, leptin promotes neutrophil chemotaxis and causes dysregulation of cell-mediated immunity, perhaps contributing to autoimmune diseases.[34] Resistin, made nominally in adipocytes and in greater quantities by circulating monocytes, increases expression of pro-inflammatory cytokines and endothelial cell adhesion molecules.[35] The pro-inflammatory adipocytokine profile (low adiponectin, and high leptin, TNF-a, and IL-6) observed in obese states has been hypothesized to be along a continuum of adipocyte dysregulation, stress, cell death, and immune system reactivation that is also seen in aging.[36] However, studies of healthy centenarians with low adipose tissue mass and high insulin sensitivity do not demonstrate elements of the pro-inflammatory profile, suggesting that adipocyte dysfunction may be a greater determinant of inflammation than aging.[36]

Infiltration of macrophages in the adipose tissue is also shown to be responsible for obesity-associated inflammation and obesity-induced insulin resistance. For example, adipocytes secrete cytokines that act on endothelial cells to enhance vascular permeability, thereby allowing bone marrow-derived monocytes to migrate across vascular walls to localize to adipose tissue and differentiate into ATMs.[37] Although the complete spectrum of factors attracting the monocytes/macrophages is not fully understood, leptin in adipocyte-conditioned media may upregulate adhesion molecules on endothelial cells, permitting increased chemotaxis of blood monocytes.[37,38] Myeloid-specific deletions of inhibitor of nuclear factor kappa-b (IKKb), CC chemokine receptor-2 (CCR2), toll like receptor-4 (TLR4), Cbl-asscociated protein, fatty acid binding protein 4, or depletion of CD11c+ cells in mice have been shown to ameliorate obesity-induced inflammation, raise glucose tolerance and lower insulin resistance.[39–44] Moreover, germline deletion of *Ccr2* gene

resulted in reduced macrophage infiltration, adipose tissue inflammation, and improved insulin sensitivity in a mouse model fed a high fat diet (HFD).[45] Other studies have shown that PPAR-γ and PPAR-δ serve a protective role in immune and metabolic homeostasis, as deletion of these genes worsen inflammation and switch the native M2 (anti-inflammatory) macrophage into M1 (pro-inflammatory) phenotype.[46,47] In addition to macrophages, the number of mast cells and natural killer T (NKT) cells also increase in obese adipose tissue compared with lean tissue and may contribute to the inflammatory and metabolic pathophysiology.[48,49]

Adipose tissue T cells (ATT) contribute to the pro- inflammatory environment in visceral fat during normal aging.[8] The role of ATT-cells in obesity-induced inflammation has also been reported. Recent works report altered T cell homeostasis in obesity, likely due to a decrease in Th2 cells and an increase in CD8+ T cells.[50–52] Moreover, the number of regulatory T cells ($CD4^+CD25^+$, Tregs) decrease as a function of obesity, which may contribute to a hyper immune activation. In fact, depletion of $CD8^+$ cells, NKT, or mast cells or enhancement of $CD4^+$ cells or Treg cells decreased macrophage recruitment and inflammation in the adipose tissue and improved glucose homeostasis.[48–52] These data clearly show that adipose tissue-resident immune cells are important contributors to obesity-induced inflammation and metabolic syndrome. Thus, adipose tissue can be thought of as an immune organ in the context of both aging and obesity.

Macrophage subtypes in adipose tissue

Macrophages are highly heterogeneous hematopoietic cells produced by the differentiation of monocytes in tissue. They are specialized phagocytic cells that clear foreign substances, infectious microbes, and cancer cells by ingestion and destruction. In addition to their role in innate immunity, macrophages are important sentinels of the adaptive immune response, wound healing, and tissue repair. Not surprisingly, there is no uniformly accepted classification of the many macrophage subtypes. Mouse macrophages can be identified by the expression of several surface markers such as CD14, CD40, CD11b, F4/80, and CD68. The human ortholog of F4/80 is EMR1. Historically, macrophages were classified under the prototypical dichotomy of M1 “classically” activated macrophages and M2 “alternatively” activated macrophages (Table 1).[53,54] Undifferentiated macrophages exposed to lipopolysaccharide (LPS, a cell wall component of gram negative bacteria) or to interferon-γ (IFN-γ) give rise to M1 macrophages ($CD11c^+CD206^-$). These cells have high phagocytic and bactericidal potential, secrete pro-inflammatory cytokines and activate Th1 lymphocytes. In contrast, alternative activation occurring in the presence of IL-4, IL-13, or parasitic infection generates M2 macrophages ($CD11c^-CD206^+$). These macrophages interact with Th2 lymphocytes to promote anti-parasitic activity, wound healing and tissue repair as well as produce anti-inflammatory cytokines (e.g. IL-10) that prevent excessive immune responses (Table 1).[7,55] The M2 population is further subdivided into M2a (initiates type II inflammation and fibrosis), M2b (immunoregulation/immunosuppression), M2c (participate in matrix remodeling and tissue repair), and M2d (tumor-associated).[56,57] It has been proposed that some macrophages may have regulatory functions.[58] However, a recent report of Foxp3-expressing regulatory macrophages (Mregs) that secrete large amounts of PGE2 was retracted.[59]

Adipose tissue macrophages (ATMs; $CD11b^+F4/80^+$) are the greatest proportion of leukocytes in adipose tissue. While the M1/M2 paradigm has been a useful and straightforward classification of tissue macrophages in previous studies, ATMs exist in a spectrum of inflammatory and activation states, thus requiring an expanded definition of ATM classification. One proposed classification of ATMs in mice by Morris *et al* renames M1 ATMs as Type 1a ATMs ($CD11c^+CD206^-$ $MGL1^-$), maintains M2 ATMs as Type 2

ATMs ($CD11c^{-}CD206^{+}$ $MGL1^{hi}$), and introduces the two new subtypes Type 1b ATMs ($CD11c^{+}CD206^{+}$ $MGL1^{mid}$) and Type 3 ATMs ($CD11c^{-}CD206^{-}$) (Table 2). Morris *et al* also introduces a similar classification of ATM subtypes for humans as Type 1a ATMs ($CD11c^{+}$ $CD206^{-}$), Type 1b ATMs ($CD11c^{+}$ $CD206^{+}$), and Type 2 ATMs ($CD11c^{-}$ $CD206^{+}$). However, the double negative Type 3 ATM phenotype has not been observed in humans.[60] Lumeng et al proposed a similar classification of ATMs in mice that maintains the M1 and M2 ATM subtypes and names the inflammatory double-negative macrophage ($CD11c^{-}CD206^{-}$) as a type 4 ATM.[8]

It is generally accepted that inflammatory M1 ATMs are more prevalent as a percentage of total macrophages in the adipose tissue of obese animals,[8] while protective M2 ATMs are strongly associated with the adipose tissue of lean animals (Table 3, 4). Similarly, M2 ATMs seem to decrease and DN Type 3 ATMs seem to increase in quantity with increasing age.[8] However, transcriptional profiles of ATM subtypes did not always reveal the expected subset associated prototypical markers and cytokines. Type 1a and Type 1b ATMs isolated in obese mice by Shaul *et al* expressed a mixed profile of M1 and M2 traits.[61] The $CD11b^{+}$ ATMs had upregulated pro-inflammatory genes such as *IL1β* and Th1-priming *IL12p40* as well as M2-profile tissue homeostasis protein arginase 1 (*Arg1)* and *IL1Ra* (IL-1 receptor antagonist).[61] All ATMs identified in this study, which included Type 1a, Type 1b, and Type 2 ATMs, had a tendency toward M2 anti-inflammatory and tissue homeostatic traits. Similarly, Zeyda and coworkers also identified Type 1, Type 2, and Type 3 ATMs in diet-induced obese mice but Type 2 ATMs isolated in this study did not exhibit the typical anti-inflammatory profile of alternatively activated M2 macrophages.[62] Rather, Type 2 ATMs expressed the most chemokines (*Ccl2*, *Ccl5*, *Ccl8*, *Ccl11*, and *Cx3cl1*) out of the three types of ATMs, indicating they may play a significant role attracting immune cells to adipose tissue during obesity and infection, rather than attenuating inflammatory responses. Interestingly, Type 3 ATMs demonstrated M2 ATM traits of anti-inflammatory action and tissue repair based on its expression of *Ym1/Chi313* (YM1/chitinase 3-like 3) and *Arg1* to a greater extent than Type 2 ATMs, which minimally expressed those proteins in this study. These observations of complex properties of Type 1a, Type 1b, Type 2, and Type 3 ATMs suggest a phenotypic plasticity of macrophages, expose the limitations of the previous M1/M2 dichotomy label of ATMs, and demonstrate the need for further investigation to more clearly elucidate subtype characteristics and functions.

Role of ATMs in obesity associated inflammation

ATMs infiltrate visceral adipose tissue in greater numbers than subcutaneous adipose tissue during obesity.[63] In both lean and obese states in mice, Type 1a, Type 1b, and Type 3 ATMs localize to adipose tissue as crown-like structures (CLS) of up to 15 macrophages surrounding one adipocyte.[27,60] However, CLS are rare in the adipose tissue of lean animals as pro-inflammatory ATMs do not infiltrate adipose tissue in significant numbers until the onset of obesity or pathology in which adipocytes are more prone to necrosis.[62] CLS have been observed to increase 30-fold in obese mice and humans, accounting for more than 90% of the ATMs in adipose tissue. If adipocyte death is persistent, as exhibited in obesity, the ATMs of CLS can recruit more macrophages and develop into multinucleated giant cells that augment the chronic inflammation seen in obesity.

ATMs are major contributors to the chronic pro-inflammatory state of fat in obese individuals.[28] Feeding mice a HFD increases obesity and shifts the ATM profile from anti-inflammatory, protective Type 2 ATMs to pro-inflammatory, destructive Type 1 ATMs.[64] During obesity, the inflammatory genes that Type 1 ATMs express are *IL-6* and *Nos2*, *IL-1β*, *Ccr2*, *Cx3cr1*, *Ccr5*, *Ccr7* PPAR-γ; Type 1 and Type 2 ATMs express equal amounts of *TNF-α*; and Type 2 ATMs express more *ApoE*.[45,62,64–70] Lean mice ATMs are

predominantly the Type 2 variety, and they express the characteristic anti-inflammatory genes *IL-10*, *Arg1*, *Mrc2* (mannose receptor C type 2, also known as CD206), *Ym1/Chi313* (YM1/chitinase 3-like 3), and *Mgl1/2* (macrophage galactose N-acetyl-galactosamine-specific lectins 1 and 2) (Table 3, 4).[62,64] Greater numbers of Type 1 ATMs and loss of Type 2 ATMs in obesity result in the loss of the anti-inflammatory, protective functions of Type 2 ATMs in maintaining adipose tissue homeostasis and repair. Rather, Type 1 ATMs have *carte blanche* to secrete pro-inflammatory cytokines and perpetuate the inflammatory state.

Role of ATMs in Inflamm-aging

Natural selection favors a strong immune system that protects humans well into their reproductive years, but the dramatic increase in lifespan in recent centuries has exposed negative consequences of a persistent immune response in the elderly. Thus, although an acute inflammatory response to infection and injury is critical to life, the chronic low grade inflammatory state seen in the elderly increases the individual's susceptibility to age-related diseases. In aging, high levels of inflammatory cytokines such as IL-3, IL-6, IL-8, IL-10, IL-15, and TNF-α are coupled with a decrease in naïve T cell compartment, leading to chronic low-level inflammation yet decreased ability to fight off novel infections.[71] NK cells increase in number, and macrophages demonstrate increased secretion of pro-inflammatory signals like prostaglandins.[72] The negative consequences of overactive innate immunity, chronic inflammation, and T cell senescence are referred to as 'inflamm-aging.'

While the source of the low grade elevated inflammatory cytokines in aging has not been defined, it is believed that fat tissue inflammation in aging-associated obesity may play a role. Diverse types of leukocytes are present in fat tissue and act as cellular mediators of inflamm-aging and contribute to insulin resistance. Normal aging often leads to an increase in visceral and subcutaneous adiposity. This buildup of adipose tissue has been implicated as the source for elevated inflammatory cytokines observed in obese individuals.[73,74] Indeed, it has been suggested that 30% of circulating IL-6 may be derived from adipose tissue.[67] Comparing lean old horses with fat old horses demonstrated that increased adiposity led to greater numbers of T cells and monocytes producing inflammatory cytokines IFN-γ and TNF-α, a phenotype which could be mitigated by feeding the obese animals a calorie-restricted diet for several weeks.[68] Therefore, normal aging leads to a more pro-inflammatory profile,[75,76] further accentuated by increasing adiposity. The M1/M2 paradigm of macrophage polarization explains the phenotypic switch to this inflammatory pathway. Concomitant with greater body fat percentage, aged mice also have a greater number of ATTs and ATMs than young mice.[8] These macrophages were also more likely to possess a pro-inflammatory phenotype than their younger counterparts, as aging decreases M2 while increasing the pool of M1 and double negative (DN) macrophage subsets, and they produce greater quantities of TNF-α, IL-6, IL-1β and monocyte chemoattracting protein-1 (MCP-1), *Ccr2*, *Ccr5*, *Cxcr3*, Ym1/Chi3/3.[8,77] In contrast to obesity versus lean model, aging is manifested with decrease in *Cx3cr1*, *Ccr7* and PPAR-γ expression in respect to young mice (Table 4).[8,77] The contribution of adipose tissue to inflamm-aging is supported by numerous studies demonstrating aged individuals who exercise regularly and are leaner have less senescent T cells and lower circulating pro-inflammatory cytokines.[78] Acute exercise also can repolarize ATMs toward the anti-inflammatory M2 phenotype in rats fed high fat diet.[79] Regular exercise can maintain skeletal muscle and prevent accumulation of visceral fat, resulting in a more 'youthful' body composition.[80] Therefore, higher levels of circulating inflammatory signals derived from greater body fat percentage in aged individuals suggest adipose tissue-resident immune cells may be key contributors toward the sub-clinical phenomenon of inflamm-aging.

Contribution of T cells subtypes in age- and obesity-related inflammation

T cell homeostasis (number, subset ratio, function and phenotype) changes during normal aging and obesity. There are several subtypes of T cells that play a role in cell-mediated immunity- T helper cells or $CD4^+$ cells; cytotoxic T cells or $CD8^+$ cells; regulatory T cell or $CD4^+CD25^+$ cells; memory T cells; NKT cells; and γδ T cells- each with distinct number, phenotype and function under physiological conditions. A vast array of literature suggests that aged spleens and lymph nodes, both from humans and mice, show an increase in the proportion of memory T cells and a reciprocal decrease in naïve T cells.[81–83] A longitudinal study from genetically heterogeneous mouse peripheral blood leukocytes (PBLs) reports that aging led to an increase in the total number of $CD3^+$ cells, $CD4^+$ and $CD8^+$ memory T cells and declines in the proportion of $CD4^+$ and $CD8^+$ naïve T cells.[84] While changes in the T cell repertoire occur during normal aging, changes in the chemokine and cytokine profiles contribute to a pro-inflammatory environment.[85,86] Freshly isolated $CD4^+$ cells from aged mice were shown to express a higher level of several T cell chemokine receptors (such as CCR1, 2, 4, 5, 6, and 8; CXCR2,3,4 and 5) and lower level of CCR7 and CCR9 in relation to young mice.[87] T cells from old mice also showed higher chemotaxis to stromal cell-derived factor-1 and monocyte inhibitory protein-1α (MIP-1α). Repeated observations have shown that T cells from healthy elderly people secrete higher level of several cytokines including TNF-α, IL-6 and C-reactive protein (CRP) relative to their younger counterparts[6,88] that correlate with increasing overall mortality and/or frailty.[89]

While the underlying mechanism for the observed aging-associated changes in immune function is unclear, a shift in memory/naïve profile and Th1/Th2 ratio may account partly for some of the aging-associated changes in immune function. However, based on the recent work on changes in chemokine and cytokine systems in aged individuals, other mechanisms may also contribute to the aging phenotype. For example, the *Ccr2* gene has a binding site for C/EBP, a transcription factor that interacts with p50 subunit of nuclear factor kappa-B (NF-kB) and activates CCR5 expression.[90–92] NF-kB is also shown to be an important regulator of a number of chemokine receptor genes.[93–95] Since NF-kB activity is shown to diminish with age, it is unlikely that observed age-associated changes in the chemokine system are entirely due to changes in the transcriptional activity.[96,97] Maintenance of epigenetic marks declines with age;[98] thus, it is possible that epigenetic alteration could play a crucial role in greater inflammation in the elderly. Several chemokine/cytokine pathways implicated in obesity-induced inflammation have been shown to be regulated by epigenetic modulation.[99,100] DNA methylation can regulate gene expression at proximal promoters, upstream and downstream enhancers, and can alter chromatin structures which may regulate gene expression over large distances. Our lab has shown previously that *Ccr2, Cxcr3, Ccr5* messages and TNF-α, IFN-γ, IL-4 and IL-2 cytokines are sensitive to global epigenetic perturbation. The levels of these chemokines and cytokines were significantly lower in T cells exhibiting hypermethylated DNA derived from mice born from dams fed with a methyl-donor rich diet. This intervention also protects against atherosclerosis in $ApoE^{-/-}$ mice fed a high fat diet.[101] In addition, histone modification also changes the higher order structure of chromatin, often working in tandem with DNA methylation patterning.

Given that ATMs and ATTs work synergistically and ATM are responsible for inflammation in the adipose tissue and T cell-derived cytokines are required for migration and activation of macrophages, it is plausible to ascribe to T cells a role in adipose tissue inflammation and insulin resistance.[102] It has been shown that diet-induced obesity activates ATTs that in turn alter ATM phenotype. Moreover, it has been shown that changes in ATMs are parallel with the similar changes in the ATTs.[8] In addition, Lumeng et al have demonstrated that aging is associated with a 2 fold induction of ATT cells in visceral fat tissue but not in the spleen, suggesting that this change is unique to adipose tissue (Table

5).[8] While studying the changes in ATT subpopulations, the investigators observed a significant increase in the percentage of both $CD4^+$ and $CD8^+$ cells in adipose tissue in old mice. They also report that the expansion of $CD4^+$ T cells in the aged adipose tissue parallels an increase in the size of fat-associated lymphoid clusters (FALCs). In the case of diet-induced obesity, it was demonstrated that total $CD3^+$, $CD4^+$ and $CD8^+$ T cells were dramatically increased in the epididymal tissue (Table 5).[103] Furthermore, obesity reduced the frequency of adipose-resident naïve T cells in the subcutaneous fat and increased the effector memory population in the visceral fat. ATTs from obese mice produce higher pro-inflammatory mediators such as IFN-γ and granzyme B relative to lean mice. Similar to peripheral T cells from aged mice, adipose resident T cells also exhibit restricted TCR diversity, which is further compromised by obesity.[8]

Regulatory T cells (Treg) which constitute about 5–20% of the $CD4^+$ T cells are thought to maintain peripheral tolerance and keep autoreactive cells at bay. Treg can be defined by the expression of the forkhead/winged-helix family of transcription factor (Foxp3) that is both necessary and sufficient for Treg development.[104] Two major populations of Tregs exist: so-called natural (nTreg) and inducible (iTreg) Tregs. nTregs mature in the thymus and iTreg are induced extrathymically in the secondary lymphoid organs in response to antigen exposure.[105] Normally, Tregs control the activity of other T cell populations, maintain lymphoid homeostasis and prevent auto-reactivity. However, excess Treg activity may lead to increased susceptibility to infection, neurodegenerative diseases and cancer.[9–11] This is particularly important during normal aging where increased frequency of Treg might contribute to immune senescence.

Tregs have also been found in adipose tissue. It is believed that Treg cells in the fat provide anti-inflammatory signals to block adipose tissue inflammation. While comparing fat from lean and obese animals, several studies revealed that the latter adipose tissue had decreased numbers of Tregs, perhaps contributing to higher levels of inflammation and decreased insulin sensitivity (Table 5).[50,52,103] Boosting Treg number increased the insulin sensitivity partly through secretion of IL-10 which also controls ATM-mediated inflammation.[50] TNF-α and IL-6 are higher in circulation in the obese mice. TNF-α signal adversely affects adipocyte and decreases insulin sensitivity. Feurer *et al* also show that Treg-mediated IL-10 not only blocks adipocyte-derived inflammatory mediators but also protects adipocytes from the negative effect on insulin signaling induced by TNF-α.[50] Moreover, recent work done by our group showed an 11-fold induction in adipose tissue Treg number as a function of aging (Table 5).[8] These data clearly suggest that obesity decreases the number of Treg frequency in the adipose tissue while normal aging increases it. The difference in adipose tissue Treg frequency in aging and diet-induced model of obesity could be attributable to either a differential inflammatory or nutrient metabolism environment. However, our understanding about an underlying mechanism that promotes an increase in adipose tissue Treg in one case versus decrease in the other is incomplete and requires further investigation.

Summary

The role of adipose tissue in regulating systemic inflammation is only now beginning to be elucidated. Novel macrophage cell types and sites of lymphoid localization have been described in the fat. Within the context of obesity, whether diet-induced or aging-related, excess body fat creates a pro-inflammatory environment, sustaining chronic low level inflammation and decreased immune cell plasticity, the hallmarks of inflamm-aging. While macrophages seem to behave similarly in the two models of obesity, T cell subsets are different, especially the Treg compartment. Since T cells are responsible for chemotactic signals that recruit monocytes to the fat to differentiate into ATMs, the observed difference suggests the aging model of obesity may not be as similar to the diet-induced model as is

currently understood. More investigation into the role of adipose tissue function is needed to clarify the phenotype, function and number of macrophage subsets, the role of T cell subsets in mediating signals that recruit immune cells, and the relationship between diet and aging in the development of obesity-induced systemic inflammation.

Acknowledgments

This work was supported by National Institutes of Health National Institute on Aging (AG020628, AG028268), National Institute of Environmental Health Science (P30 ES017885), University of Michigan (Claude D. Pepper Older American Independence Center, Nathan Shock Center for the Basic Biology of Aging, Rheumatic Disease Clinical Center, Caner Center Microarray Core, Michigan Diabetes and Research Training Center Animal Phenotyping Core), Geriatrics Research, Education and Clinical Care Center (GRECC) and the VA Ann Arbor Healthcare System. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Abbreviations

TCR	T cell receptor
ATM	adipose tissue macrophage
ATT	adipose tissue T cell
SVCF	stomal vascular cell fraction
SVF	stromal vascular fraction
HFD	high fat diet
CD	cluster of differentiation
MHC	major histocompatibility complex
IFN	interferon
TNF	tumor necrosis factor
IL	interleukin
LPS	lipopolysaccharide
ROS	reactive oxygen species
RNS	reactive nitrogen species
PDGF	platelet-derived growth factor
VEGF	vascular endothelial growth factor
EGF	epidermal growth factor
Th	helper T cell
NKT	natural killer T cell
Tregs	regulatory T cell
nTreg	naturally occurring regulatory T cells
Nos	nitric oxide synthetase
TGF	transforming growth factor
Arg	arginase
Ccr	CC chemokine receptor
Cxcr	CXC chemokine receptor

MMGL	macrophage galactose N-acetyl galactosamine-specific lectins
YM1/Chi3/3	chitinase 3-like 3 protein
CLS	crown-like structure
MCP	monocyte chemoattractant protein
PPAR	peroxisome proliferator-activated receptor
ApoE	apoliporotein E
FALCs	fat-associated lymphoid clusters

References

1. Desai A, Grolleau-Julius A, Yung R. Leukocyte function in the aging immune system. J Leukoc Biol. 2010; 87:1001–1009. [PubMed: 20200405]
2. Raynor J, Lages CS, Shehata H, Hildeman DA, Chougnet CA. Homeostasis and function of regulatory T cells in aging. Curr Opin Immunol. 2012; 4:482–487. [PubMed: 22560294]
3. Grolleau-Julius A, Harning EK, Abernathy LM, Yung RL. Impaired dendritic cell function in aging leads to defective antitumor immunity. Cancer Res. 2008; 68:6341–6349. [PubMed: 18676859]
4. Caligiuri G, Rudling M, Ollivier V, Jacob MP, Michel JB, Hansson GK, Nicoletti A. Interleukin-10 deficiency increases atherosclerosis, thrombosis, and low-density lipoproteins in apolipoprotein E knockout mice. Mol Med. 2003; 9:10–17. [PubMed: 12765335]
5. Roubenoff R, Harris TB, Abad LW, Wilson PW, Dallal GE, Dinarello CA. Monocyte cytokine production in an elderly population: effect of age and inflammation. J Gerontol A Biol Sci Med Sci. 1998; 53:M20–26. [PubMed: 9467429]
6. O'Mahony L, Holland J, Jackson J, Feighery C, Hennessy TP, Mealy K. Quantitative intracellular cytokine measurement: age-related changes in proinflammatory cytokine production. Clin Exp Immunol. 1998; 113:213–219. [PubMed: 9717970]
7. Lloberas J, Celada A. Effect of aging on macrophage function. Exp Gerontol. 2002; 37:1325–1331. [PubMed: 12559402]
8. Lumeng CN, Liu J, Geletka L, Delaney C, Delproposto J, Desai A, Oatmen K, Martinez-Santibanez G, Julius A, Garg S, Yung RL. Aging is associated with an increase in T cells and inflammatory macrophages in visceral adipose tissue. J Immunol. 2011; 187:6208–6216. [PubMed: 22075699]
9. Richardson BC. Role of DNA methylation in the regulation of cell function: autoimmunity, aging and cancer. J Nutr. 2002; 132:2401S–2405S. [PubMed: 12163700]
10. Mendez S, Reckling SK, Piccirillo CA, Sacks D, Belkaid Y. Role for CD4(+) CD25(+) regulatory T cells in reactivation of persistent leishmaniasis and control of concomitant immunity. J Exp Med. 2004; 200:201–210. [PubMed: 15263027]
11. Sharma S, Dominguez AL, Lustgarten J. High accumulation of T regulatory cells prevents the activation of immune responses in aged animals. J Immunol. 2006; 177:8348–8355. [PubMed: 17142731]
12. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab. 2004; 89:2548–2556. [PubMed: 15181022]
13. Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. Am J Physiol Endocrinol Metab. 2001; 280:E745–751. [PubMed: 11287357]
14. Trujillo ME, Scherer PE. Adipose tissue-derived factors: impact on health and disease. Endocr Rev. 2006; 27:762–778. [PubMed: 17056740]
15. Wu J, Bostrom P, Sparks LM, Ye L, Choi JH, Giang AH, Khandekar M, Virtanen KA, Nuutila P, Schaart G, Huang K, Tu H, van Marken Lichtenbelt WD, Hoeks J, Enerback S, Schrauwen P, Spiegelman BM. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell. 2012; 150:366–376. [PubMed: 22796012]

16. Guarente L, Picard F. Calorie restriction--the SIR2 connection. Cell. 2005; 120:473–482. [PubMed: 15734680]

17. Picard F, Guarente L. Molecular links between aging and adipose tissue. Int J Obes (Lond). 2005; 29(Suppl 1):S36–9. [PubMed: 15711582]

18. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, Lisheng L. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet. 2004; 364:937–952. [PubMed: 15364185]

19. Montague CT, O'Rahilly S. The perils of portliness: causes and consequences of visceral adiposity. Diabetes. 2000; 49:883–888. [PubMed: 10866038]

20. Marette A. Molecular mechanisms of inflammation in obesity-linked insulin resistance. Int J Obes Relat Metab Disord. 2003; 27 (Suppl 3):S46–48. [PubMed: 14704744]

21. Fagiolo U, Cossarizza A, Scala E, Fanales-Belasio E, Ortolani C, Cozzi E, Monti D, Franceschi C, Paganelli R. Increased cytokine production in mononuclear cells of healthy elderly people. Eur J Immunol. 1993; 23:2375–2378. [PubMed: 8370415]

22. Horber FF, Gruber B, Thomi F, Jensen EX, Jaeger P. Effect of sex and age on bone mass, body composition and fuel metabolism in humans. Nutrition. 1997; 13:524–534. [PubMed: 9263233]

23. Pascot A, Lemieux S, Lemieux I, Prud'homme D, Tremblay A, Bouchard C, Nadeau A, Couillard C, Tchernof A, Bergeron J, Despres JP. Age-related increase in visceral adipose tissue and body fat and the metabolic risk profile of premenopausal women. Diabetes Care. 1999; 22:1471–1478. [PubMed: 10480511]

24. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell. 2002; 13:4279–4295. [PubMed: 12475952]

25. Villena JA, Cousin B, Penicaud L, Casteilla L. Adipose tissues display differential phagocytic and microbicidal activities depending on their localization. Int J Obes Relat Metab Disord. 2001; 25:1275–1280. [PubMed: 11571587]

26. Rodbell M. Metabolism of Isolated Fat Cells. Effects of Hormones on Glucose Metabolism and Lipolysis. J Biol Chem. 1964; 239:375–380. [PubMed: 14169133]

27. Cinti S, Mitchell G, Barbatelli G, Murano I, Ceresi E, Faloia E, Wang S, Fortier M, Greenberg AS, Obin MS. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. J Lipid Res. 2005; 46:2347–2355. [PubMed: 16150820]

28. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest. 2003; 112:1796–1808. [PubMed: 14679176]

29. Urs S, Smith C, Campbell B, Saxton AM, Taylor J, Zhang B, Snoddy J, Jones Voy B, Moustaid-Moussa N. Gene expression profiling in human preadipocytes and adipocytes by microarray analysis. J Nutr. 2004; 134:762–770. [PubMed: 15051823]

30. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun. 1999; 257:79–83. [PubMed: 10092513]

31. Ohashi K, Parker JL, Ouchi N, Higuchi A, Vita JA, Gokce N, Pedersen AA, Kalthoff C, Tullin S, Sams A, Summer R, Walsh K. Adiponectin promotes macrophage polarization toward an anti-inflammatory phenotype. J Biol Chem. 2010; 285:6153–6160. [PubMed: 20028977]

32. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature. 1998; 395:763–770. [PubMed: 9796811]

33. Sarraf P, Frederich RC, Turner EM, Ma G, Jaskowiak NT, Rivet DJ 3rd, Flier JS, Lowell BB, Fraker DL, Alexander HR. Multiple cytokines and acute inflammation raise mouse leptin levels: potential role in inflammatory anorexia. J Exp Med. 1997; 185:171–175. [PubMed: 8996253]

34. La Cava A, Matarese G. The weight of leptin in immunity. Nat Rev Immunol. 2004; 4:371–379. [PubMed: 15122202]

35. Fargnoli JL, Sun Q, Olenczuk D, Qi L, Zhu Y, Hu FB, Mantzoros CS. Resistin is associated with biomarkers of inflammation while total and high-molecular weight adiponectin are associated with biomarkers of inflammation, insulin resistance, and endothelial function. Eur J Endocrinol. 2010; 162:281–288. [PubMed: 19920090]

36. Tchkonia T, Morbeck DE, Von Zglinicki T, Van Deursen J, Lustgarten J, Scrable H, Khosla S, Jensen MD, Kirkland JL. Fat tissue, aging, and cellular senescence. Aging Cell. 2010; 9:667–684. [PubMed: 20701600]

37. Curat CA, Miranville A, Sengenes C, Diehl M, Tonus C, Busse R, Bouloumie A. From blood monocytes to adipose tissue-resident macrophages: induction of diapedesis by human mature adipocytes. Diabetes. 2004; 53:1285–1292. [PubMed: 15111498]

38. Ito A, Suganami T, Yamauchi A, Degawa-Yamauchi M, Tanaka M, Kouyama R, Kobayashi Y, Nitta N, Yasuda K, Hirata Y, Kuziel WA, Takeya M, Kanegasaki S, Kamei Y, Ogawa Y. Role of CC chemokine receptor 2 in bone marrow cells in the recruitment of macrophages into obese adipose tissue. J Biol Chem. 2008; 283:35715–35723. [PubMed: 18977759]

39. Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM, Wynshaw-Boris A, Poli G, Olefsky J, Karin M. IKK-beta links inflammation to obesity-induced insulin resistance. Nat Med. 2005; 11:191–198. [PubMed: 15685170]

40. Baccarelli A, Wright RO, Bollati V, Tarantini L, Litonjua AA, Suh HH, Zanobetti A, Sparrow D, Vokonas PS, Schwartz J. Rapid DNA methylation changes after exposure to traffic particles. Am J Respir Crit Care Med. 2009; 179:572–578. [PubMed: 19136372]

41. Saberi M, Woods NB, de Luca C, Schenk S, Lu JC, Bandyopadhyay G, Verma IM, Olefsky JM. Hematopoietic cell-specific deletion of toll-like receptor 4 ameliorates hepatic and adipose tissue insulin resistance in high-fat-fed mice. Cell Metab. 2009; 10:419–429. [PubMed: 19883619]

42. Lesniewski LA, Hosch SE, Neels JG, de Luca C, Pashmforoush M, Lumeng CN, Chiang SH, Scadeng M, Saltiel AR, Olefsky JM. Bone marrow-specific Cap gene deletion protects against high-fat diet-induced insulin resistance. Nat Med. 2007; 13:455–462. [PubMed: 17351624]

43. Furuhashi M, Fucho R, Gorgun CZ, Tuncman G, Cao H, Hotamisligil GS. Adipocyte/macrophage fatty acid-binding proteins contribute to metabolic deterioration through actions in both macrophages and adipocytes in mice. J Clin Invest. 2008; 118:2640–2650. [PubMed: 18551191]

44. Patsouris D, Li PP, Thapar D, Chapman J, Olefsky JM, Neels JG. Ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin resistant animals. Cell Metab. 2008; 8:301–309. [PubMed: 18840360]

45. Weisberg SP, Hunter D, Huber R, Lemieux J, Slaymaker S, Vaddi K, Charo I, Leibel RL, Ferrante AW Jr. CCR2 modulates inflammatory and metabolic effects of high-fat feeding. J Clin Invest. 2006; 116:115–124. [PubMed: 16341265]

46. Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, Morel CR, Subramanian V, Mukundan L, Red Eagle A, Vats D, Brombacher F, Ferrante AW, Chawla A. Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. Nature. 2007; 447:1116–1120. [PubMed: 17515919]

47. Kang K, Reilly SM, Karabacak V, Gangl MR, Fitzgerald K, Hatano B, Lee CH. Adipocyte-derived Th2 cytokines and myeloid PPARdelta regulate macrophage polarization and insulin sensitivity. Cell Metab. 2008; 7:485–495. [PubMed: 18522830]

48. Liu J, Divoux A, Sun J, Zhang J, Clement K, Glickman JN, Sukhova GK, Wolters PJ, Du J, Gorgun CZ, Doria A, Libby P, Blumberg RS, Kahn BB, Hotamisligil GS, Shi GP. Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. Nat Med. 2009; 15:940–945. [PubMed: 19633655]

49. Ohmura K, Ishimori N, Ohmura Y, Tokuhara S, Nozawa A, Horii S, Andoh Y, Fujii S, Iwabuchi K, Onoe K, Tsutsui H. Natural killer T cells are involved in adipose tissues inflammation and glucose intolerance in diet-induced obese mice. Arterioscler Thromb Vasc Biol. 2010; 30:193–199. [PubMed: 19910631]

50. Feuerer M, Herrero L, Cipolletta D, Naaz A, Wong J, Nayer A, Lee J, Goldfine AB, Benoist C, Shoelson S, Mathis D. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat Med. 2009; 15:930–939. [PubMed: 19633656]

51. Nishimura S, Manabe I, Nagasaki M, Eto K, Yamashita H, Ohsugi M, Otsu M, Hara K, Ueki K, Sugiura S, Yoshimura K, Kadowaki T, Nagai R. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nat Med. 2009; 15:914–920. [PubMed: 19633658]
52. Winer S, Chan Y, Paltser G, Truong D, Tsui H, Bahrami J, Dorfman R, Wang Y, Zielenski J, Mastronardi F, Maezawa Y, Drucker DJ, Engleman E, Winer D, Dosch HM. Normalization of obesity-associated insulin resistance through immunotherapy. Nat Med. 2009; 15:921–929. [PubMed: 19633657]
53. Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2003; 3:23–35. [PubMed: 12511873]
54. Martinez FO, Sica A, Mantovani A, Locati M. Macrophage activation and polarization. Front Biosci. 2008; 13:453–461. [PubMed: 17981560]
55. Lumeng CN, Saltiel AR. Inflammatory links between obesity and metabolic disease. J Clin Invest. 2011; 121:2111–2117. [PubMed: 21633179]
56. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol. 2004; 25:677–686. [PubMed: 15530839]
57. Li Y, Cai L, Wang H, Wu P, Gu W, Chen Y, Hao H, Tang K, Yi P, Liu M, Miao S, Ye D. Pleiotropic regulation of macrophage polarization and tumorigenesis by formyl peptide receptor-2. Oncogene. 2011; 30:3887–3899. [PubMed: 21499310]
58. Fleming BD, Mosser DM. Regulatory macrophages: setting the threshold for therapy. Eur J Immunol. 2011; 41:2498–2502. [PubMed: 21952805]
59. Manrique SZ, Correa MA, Hoelzinger DB, Dominguez AL, Mirza N, Lin HH, Stein-Streilein J, Gordon S, Lustgarten J. Foxp3-positive macrophages display immunosuppressive properties and promote tumor growth. J Exp Med. 2011; 208:1485–1499. [PubMed: 21670203]
60. Morris DL, Singer K, Lumeng CN. Adipose tissue macrophages: phenotypic plasticity and diversity in lean and obese states. Curr Opin Clin Nutr Metab Care. 2011; 14:341–346. [PubMed: 21587064]
61. Shaul ME, Bennett G, Strissel KJ, Greenberg AS, Obin MS. Dynamic, M2-like remodeling phenotypes of CD11c+ adipose tissue macrophages during high-fat diet--induced obesity in mice. Diabetes. 2010; 59:1171–1181. [PubMed: 20185806]
62. Zeyda M, Gollinger K, Kriehuber E, Kiefer FW, Neuhofer A, Stulnig TM. Newly identified adipose tissue macrophage populations in obesity with distinct chemokine and chemokine receptor expression. Int J Obes (Lond). 2010; 34:1684–1694. [PubMed: 20514049]
63. Cancello R, Tordjman J, Poitou C, Guilhem G, Bouillot JL, Hugol D, Coussieu C, Basdevant A, Bar Hen A, Bedossa P, Guerre-Millo M, Clement K. Increased infiltration of macrophages in omental adipose tissue is associated with marked hepatic lesions in morbid human obesity. Diabetes. 2006; 55:1554–1561. [PubMed: 16731817]
64. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest. 2007; 117:175–184. [PubMed: 17200717]
65. Chen A, Mumick S, Zhang C, Lamb J, Dai H, Weingarth D, Mudgett J, Chen H, MacNeil DJ, Reitman ML, Qian S. Diet induction of monocyte chemoattractant protein-1 and its impact on obesity. Obes Res. 2005; 13:1311–1320. [PubMed: 16129712]
66. Bassaganya-Riera J, Misyak S, Guri AJ, Hontecillas R. PPAR gamma is highly expressed in F4/80(hi) adipose tissue macrophages and dampens adipose-tissue inflammation. Cell Immunol. 2009; 258:138–146. [PubMed: 19423085]
67. Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, Klein S, Coppack SW. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. J Clin Endocrinol Metab. 1997; 82:4196–4200. [PubMed: 9398739]
68. Adams AA, Katepalli MP, Kohler K, Reedy SE, Stilz JP, Vick MM, Fitzgerald BP, Lawrence LM, Horohov DW. Effect of body condition, body weight and adiposity on inflammatory cytokine responses in old horses. Vet Immunol Immunopathol. 2009; 127:286–294. [PubMed: 19097648]
69. Kitade H, Sawamoto K, Nagashimada M, Inoue H, Yamamoto Y, Sai Y, Takamura T, Yamamoto H, Miyamoto K, Ginsberg HN, Mukaida N, Kaneko S, Ota T. CCR5 plays a critical role in

obesity-induced adipose tissue inflammation and insulin resistance by regulating both macrophage recruitment and M1/M2 status. Diabetes. 2012; 61:1680–1690. [PubMed: 22474027]

70. Morris DL, Oatmen KE, Wang T, DelProposto JL, Lumeng CN. CX3CR1 deficiency does not influence trafficking of adipose tissue macrophages in mice with diet-induced obesity. Obesity (Silver Spring). 2012; 20:1189–1199. [PubMed: 22252034]
71. Franceschi C, Bonafe M, Valensin S. Human immunosenescence: the prevailing of innate immunity, the failing of clonotypic immunity, and the filling of immunological space. Vaccine. 2000; 18:1717–1720. [PubMed: 10689155]
72. Malaguarnera L, Ferlito L, Imbesi RM, Gulizia GS, Di Mauro S, Maugeri D, Malaguarnera M, Messina A. Immunosenescence: a review. Arch Gerontol Geriatr. 2001; 32:1–14. [PubMed: 11251234]
73. Park HS, Park JY, Yu R. Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-alpha and IL-6. Diabetes Res Clin Pract. 2005; 69:29–35. [PubMed: 15955385]
74. Sewter CP, Digby JE, Blows F, Prins J, O'Rahilly S. Regulation of tumour necrosis factor-alpha release from human adipose tissue in vitro. J Endocrinol. 1999; 163:33–38. [PubMed: 10495404]
75. Masternak MM, Bartke A. Growth hormone, inflammation and aging. Pathobiol Aging Age Relat Dis. 2012; 2:17293.
76. De Angulo A, Faris R, Cavazos D, Jolly C, Daniel B, Degraffenried L. Age-related alterations in T-lymphocytes modulate key pathways in prostate tumorigenesis. Prostate. 2013; 73:855–864. [PubMed: 23532664]
77. Wu D, Ren Z, Pae M, Guo W, Cui X, Merrill AH, Meydani SN. Aging up-regulates expression of inflammatory mediators in mouse adipose tissue. J Immunol. 2007; 179:4829–4839. [PubMed: 17878382]
78. Simpson RJ, Lowder TW, Spielmann G, Bigley AB, LaVoy EC, Kunz H. Exercise and the aging immune system. Ageing Res Rev. 2012; 11:404–420. [PubMed: 22465452]
79. Oliveira AG, Araujo TG, Carvalho BM, Guadagnini D, Rocha GZ, Bagarolli RA, Carvalheira JB, Saad MJ. Acute exercise induces a phenotypic switch in adipose tissue macrophage polarization in diet-induced obese rats. Obesity (Silver Spring). 2013; 10:20402.
80. Strasser B. Physical activity in obesity and metabolic syndrome. Ann N Y Acad Sci. 2012; 1281:141–159. [PubMed: 23167451]
81. Nagelkerken L, Hertogh-Huijbregts A, Dobber R, Drager A. Age-related changes in lymphokine production related to a decreased number of CD45RBhi CD4+ T cells. Eur J Immunol. 1991; 21:273–281. [PubMed: 1671835]
82. De Paoli P, Battistin S, Santini GF. Age-related changes in human lymphocyte subsets: progressive reduction of the CD4 CD45R (suppressor inducer) population. Clin Immunol Immunopathol. 1988; 48:290–296. [PubMed: 2456868]
83. Ernst DN, Hobbs MV, Torbett BE, Glasebrook AL, Rehse MA, Bottomly K, Hayakawa K, Hardy RR, Weigle WO. Differences in the expression profiles of CD45RB, Pgp-1, and 3G11 membrane antigens and in the patterns of lymphokine secretion by splenic CD4+ T cells from young and aged mice. J Immunol. 1990; 145:1295–1302. [PubMed: 1974562]
84. Miller RA. Age-related changes in T cell surface markers: a longitudinal analysis in genetically heterogeneous mice. Mech Ageing Dev. 1997; 96:181–196. [PubMed: 9223120]
85. Yamamoto J, Adachi Y, Onoue Y, Adachi YS, Okabe Y, Itazawa T, Toyoda M, Seki T, Morohashi M, Matsushima K, Miyawaki T. Differential expression of the chemokine receptors by the Th1- and Th2-type effector populations within circulating CD4+ T cells. J Leukoc Biol. 2000; 68:568–574. [PubMed: 11037980]
86. Sallusto F, Lenig D, Mackay CR, Lanzavecchia A. Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. J Exp Med. 1998; 187:875–883. [PubMed: 9500790]
87. Mo R, Chen J, Han Y, Bueno-Cannizares C, Misek DE, Lescure PA, Hanash S, Yung RL. T cell chemokine receptor expression in aging. J Immunol. 2003; 170:895–904. [PubMed: 12517955]
88. Johnson TE. Recent results: biomarkers of aging. Exp Gerontol. 2006; 41:1243–1624. [PubMed: 17071038]

89. Krabbe KS, Pedersen M, Bruunsgaard H. Inflammatory mediators in the elderly. Exp Gerontol. 2004; 39:687–699. [PubMed: 15130663]

90. Rosati M, Valentin A, Patenaude DJ, Pavlakis GN. CCAAT-enhancer-binding protein beta (C/EBP beta) activates CCR5 promoter: increased C/EBP beta and CCR5 in T lymphocytes from HIV-1-infected individuals. J Immunol. 2001; 167:1654–1662. [PubMed: 11466389]

91. LeClair KP, Blanar MA, Sharp PA. The p50 subunit of NF-kappa B associates with the NF-IL6 transcription factor. Proc Natl Acad Sci U S A. 1992; 89:8145–8149. [PubMed: 1518839]

92. Yamamoto K, Takeshima H, Hamada K, Nakao M, Kino T, Nishi T, Kochi M, Kuratsu J, Yoshimura T, Ushio Y. Cloning and functional characterization of the 5′-flanking region of the human monocyte chemoattractant protein-1 receptor (CCR2) gene. Essential role of 5′-untranslated region in tissue-specific expression. J Biol Chem. 1999; 274:4646–4654. [PubMed: 9988701]

93. Huber MA, Denk A, Peter RU, Weber L, Kraut N, Wirth T. The IKK-2/Ikappa Balpha/NF-kappa B pathway plays a key role in the regulation of CCR3 and eotaxin-1 in fibroblasts. A critical link to dermatitis in Ikappa Balpha -deficient mice. J Biol Chem. 2002; 277:1268–1275. [PubMed: 11694538]

94. Yang M, Sang H, Rahman A, Wu D, Malik AB, Ye RD. G alpha 16 couples chemoattractant receptors to NF-kappa B activation. J Immunol. 2001; 166:6885–6892. [PubMed: 11359849]

95. Ganju RK, Brubaker SA, Meyer J, Dutt P, Yang Y, Qin S, Newman W, Groopman JE. The alpha-chemokine, stromal cell-derived factor-1alpha, binds to the transmembrane G-protein-coupled CXCR-4 receptor and activates multiple signal transduction pathways. J Biol Chem. 1998; 273:23169–23175. [PubMed: 9722546]

96. Whisler RL, Chen M, Beiqing L, Carle KW. Impaired induction of c-fos/c-jun genes and of transcriptional regulatory proteins binding distinct c-fos/c-jun promoter elements in activated human T cells during aging. Cell Immunol. 1997; 175:41–50. [PubMed: 9015187]

97. Ginn-Pease ME, Whisler RL. Redox signals and NF-kappaB activation in T cells. Free Radic Biol Med. 1998; 25:346–361. [PubMed: 9680181]

98. Fraga MF, Esteller M. Epigenetics and aging: the targets and the marks. Trends Genet. 2007; 23:413–418. [PubMed: 17559965]

99. Delaney C, Hoeltzel M, Garg SK, Warner R, Johnson K, Yung R. Maternal micronutrient supplementation suppresses T cell chemokine receptor expression and function in f1 mice. J Nutr. 2012; 142:1329–1335. [PubMed: 22649261]

100. Scotet E, Schroeder S, Lanzavecchia A. Molecular regulation of CC-chemokine receptor 3 expression in human T helper 2 cells. Blood. 2001; 98:2568–2570. [PubMed: 11588056]

101. Delaney C, Garg SK, Fernandes C, Hoeltzel M, Allen RH, Stabler S, Yung R. Maternal Diet Supplemented with Methyl-Donors Protects against Atherosclerosis in F1 ApoE(−/−) Mice. PLoS One. 2013; 8:e56253. [PubMed: 23437105]

102. Lumeng CN, Maillard I, Saltiel AR. T-ing up inflammation in fat. Nat Med. 2009; 15:846–847. [PubMed: 19661987]

103. Deiuliis J, Shah Z, Shah N, Needleman B, Mikami D, Narula V, Perry K, Hazey J, Kampfrath T, Kollengode M, Sun Q, Satoskar AR, Lumeng C, Moffatt-Bruce S, Rajagopalan S. Visceral adipose inflammation in obesity is associated with critical alterations in tregulatory cell numbers. PLoS One. 2011; 6:e16376. [PubMed: 21298111]

104. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science. 2003; 299:1057–1061. [PubMed: 12522256]

105. Huehn J, Polansky JK, Hamann A. Epigenetic control of FOXP3 expression: the key to a stable regulatory T-cell lineage? Nat Rev Immunol. 2009; 9:83–89. [PubMed: 19114986]

Table 1

Macrophage ($CD11b^{+}F4/80^{+}$) subtypes in mice.

	M1 (classically activated macrophages)	M2 (alternatively activated macrophages)
Marker	$CD11c^{+}CD206^{-}$, MHC-II^{high}, CD86	$CD11c^{-}CD206^{+high}$
Stimuli	IFN-γ, LPS, TNF-α	IL-4, IL-13, IL-10, TGF-β
Secrete/Produce	ROS, RNS, TNF-α, IL-1, IL-6, IL-12, IL-23	IL-10, TGF-β, PDGF, VEGF, EGF, Arg-1
Function	help Th1 cells, anti-microbial, inflammation, cytotoxicity, tissue injury, phagocytosis, tumor suppression	help Th2 cells, anti-parasitic (all M2). type II inflammation and fibrosis (M2a); anti-inflammation and immune suppression (M2b); matrix remodeling, wound healing and tissue repair (M2c); tumor promoting (M2d).
Reference	[53, 54]	[7, 8, 53, 54, 56]

CD: cluster of differentiation, MHC: major histocompatibility complex, IFN: interferon, TNF: tumor necrosis factor, IL: interleukin, TGF: transforming growth factor, LPS: lipopolysaccharide, ROS: reactive oxygen species, RNS: reactive nitrogen species, PDGF: platelet-derived growth factor, VEGF: vascular endothelial growth factor, EGF: epidermal growth factor, Th: helper T cell,

Table 2

Adipose tissue macrophage (ATMs; CD11b+F4/80+) subtypes in mice.

	Type 1a ATMs	Type 1b ATMs	Type 2 ATMs	Type 3 ATMs
Marker	$CD11c^{+}CD206^{-}$ $MGL1^{-}$	$CD11c^{+}CD206^{+}MGL1^{mid}$	$CD11c^{-}CD206^{+}$ $MGL1^{hi}$	$CD11c^{-}$ $CD206^{-}$
Gene expression	IL-6, TNF-α, Nos2, Ccr-7	IL-12, Arg-1	IL-10, Arg-1, Ccl-2, Mgl1/2, YM1/Chi3/3	Arg-1, Chi3/3, Ccl-2, Ccr-1, Ccr-9
Change with obesity/lean	Increase	Increase	decrease or no	Increase
Change with aging/young	no change or increase	no change or increase	decrease	increase
Localization	CLS	CLS	Interstitial	CLS
Function	Inflammatory	Inflammatory	Anti-inflammatory	Inflammatory
Resemblance	classical M1 type	classical M1 type	classical M2 type	Mix of M1 and M2
Reference	[8,60,61,62]	[8,60,61,62]	[8,60,61,64]	[8,60,61,62]

CD: cluster of differentiation, TNF: tumor necrosis factor, IL: interleukin, Arg: arginase, CCr: CC chemokine receptor, MMGL: macrophage galactose N-acetyl-galactosamine-specific lectins, YM1/Chi3/3: chitinase 3-like 3 protein, CLS: crown-like structure.

Table 3

ATMs in obese versus lean mice

	ATMs from obese mice	**ATMs from lean mice**
Markers	$CD11c^{+}CD206^{-}$	$CD11c^{-}CD206^{+high}$, $MGL1^{+high}$
Expression	IL-1β, IL-6, Nos2, TNF-α	Arg-1, Ym-1
Function	Inflammatory	Anti-inflammatory, insulin sensitivity
Localization	CLS	interstitial

Table 4

Changes in ATMs proteins expression due to either obesity or aging in respect to lean or young state respectively.

	Obesity/Lean	Old/Young
IL-6	increase [65,66,67]	increase [8]
MCP-1	increase [66]	increase [8]
TNF-α	increase [64,66,68]	increase [8]
Ccr-2	increase [45,62,69]	increase [8]
Cx3cr-1	no change or increase [62,66,70]	decrease [8]
Ccr-5	increase [62,69]	increase [8]
Ccr-7	increase [62]	decrease [8]
Cxcr-3	nd	increase [8]
PPAR-γ	increase [66]	decrease [77]
NOS2	increase [62,64]	nd
ApoE	decrease [64]	nd
Ym1/Chi3/3	decrease [62,64]	decrease
IL-10	decrease [64]	no change [8]
IL-1β	Increase [60]	increase [77]

IL: interleukin, MCP: monocyte chemoattractant protein, TNF: tumor necrosis factor, Ccr: CC chemokine receptor, Cxcr: CXC chemokine receptor, PPAR: peroxisome proliferators-activated receptor, ApoE: apoliporotein E, YM1/chi3/3: chitinase 3-like 3 protein, nd: not determined.

Table 5

Quantitative changes in T cell populations as a function of aging and obesity

	Naïve $CD3^+$	**Naïve $CD4^+$**	**Naïve $CD8^+$**	**$nCD4^+CD25^+$**
Aged SVF [8]	increase	no change or increase	no change or increase	increase
Aged spleen [8]	decrease	decrease	decrease	no change
Obese SVF [103]	increase	increase	increase	decrease
Obese spleen [103]	nd	decrease	nd	increase

SVF: stromal vascular fraction, $nCD4^+Cd25^+$: naturally occurring $CD4^+CD25^+$, nd: not determined.