Role of pro- and anti-inflammatory phenomena in the physiopathology of type 2 diabetes and obesity

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Abstract
In obesity, persistent low-grade inflammation is considered as a major contributor towards the progression to insulin resistance and type 2 diabetes while in lean subjects the immune environment is non-inflammatory. Massive adipose tissue (AT) infiltration by pro-inflammatory M1 macrophages and several T cell subsets as obesity develops leads to the accumulation - both in the AT and systemically - of numerous pro-inflammatory cytokines, including interleukin-1β (IL-1β), tumor necrosis factor α, IL-17 and IL-6 which are strongly associated with the progression of the obese phenotype towards the metabolic syndrome. At the same time, anti-inflammatory M2 macrophages and Th subsets producing the anti-inflammatory cytokines IL-10, IL-5 and interferon-γ, including Th2 and T-reg cells are correlated to the maintenance of AT homeostasis in lean individuals. Here, we discuss the basic principles in the control of the interaction between the AT and infiltrating immune cells both in the lean and the obese condition with a special emphasis on the contribution of pro- and anti-inflammatory cytokines to the establishment of the insulin-resistant state. In this context, we will discuss the current knowledge about alterations in the levels on pro- and anti-inflammatory cytokines in obesity, insulin resistance and type 2 diabetes mellitus, in humans and animal models. Finally, we also briefly survey the recent novel therapeutic strategies that attempt to alleviate or reverse insulin resistance and type 2 diabetes via the administration of recombinant inhibitory antibodies directed towards some pro-inflammatory cytokines.

Key words: Type 2 diabetes; Crown-like structures; Adipose tissue inflammation; Macrophages; Eosinophils; Obesity

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Core tip: Low-grade inflammation of adipose tissue (AT) contributes to insulin resistance and type 2 diabetes in obese patients. On the contrary, in lean individuals, the immune environment of AT is non-inflammatory. In obesity,
AT is infiltrated by pro-inflammatory macrophages and T cells leading to the accumulation of interleukin-1β (IL-1β), tumor necrosis factor α, IL-17 and IL-6. On the contrary, M2 macrophages, Th2 and T-regs cells producing anti-inflammatory IL-10, IL-5 and interferon-γ, are present in AT of lean individuals. Here, we discuss the interaction between AT and infiltrating immune cells in the lean vs the obese condition, with emphasis on the contribution of pro- and anti-inflammatory cytokines to the establishment of insulin resistance.

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INTRODUCTION

The steadily increasing incidence of obesity and associated morbidities is recognized as a major public health problem, reaching epidemics proportions both in industrialized and developing countries.

In obesity, adipose tissue (AT) depots are subjected to extensive hypertrophy, with expansion of the visceral AT compartments being a strong predictor of the development of insulin resistance[1]. The AT of obese individuals is in a persistent condition of low-grade inflammation, which is dictated by the infiltration within the AT of several classes of pro-inflammatory immune cells[2], including monocytes, macrophages, natural killer cells, and lymphocytes, resulting in secretion of adipokines and proinflammatory cytokines by both adipocytes and the population of infiltrating immune cells[3]. Here, we discuss the multifaceted interplay existing between the AT and the immune system with an emphasis on the alterations occurring during the transition from the homeostatic state of adipose depots in the lean condition to the AT accumulation experienced throughout the development of obesity.

ANTI-INFLAMMATORY STATE OF THE AT: PEACEFUL TIMES DURING HOMEOSTASIS

The immune environment in the lean AT is predominantly non-inflammatory. In this tissue, eosinophils and innate lymphoid cells drive a bias towards a type 2 immune response, secreting cytokines such as interleukin-4 (IL-4), IL-5 and IL-13, which maintain AT macrophages in an anti-inflammatory, M2-like state. However, this picture is not so simplified. Indeed, IL-10 and IL-33 are also secreted; invariant natural killer (NK)-T cells are involved, as well as newly identified populations of T and B regulatory cells (T-regs and B-regs), some of which appear to be exclusive of AT. Adipocytes are also active regulators of immune responses by means of their own secreted hormones. At the end, research has recently made an intense effort to fully comprehend the nature of the healthy AT, in pursuance of new pathways to successfully treat and win the battle against the expanding epidemic of obesity and type 2 diabetes mellitus (T2DM).

Despite a growing body of evidence linking inflammation and metabolism, the cellular sources of inflammatory mediators in the AT were still unknown at the very beginning of 2000’s. Only in 2003, AT macrophages were pointed out as the culprits, increasing significantly in number and producing a range of inflammatory mediators during obesity[4,5]. In fact, Weisberg et al[4] estimated the percentage of macrophages ranging from 10% in lean AT to almost 50% in obese mice and humans. These infiltrated phagocytes augmented the inflammatory environment in the AT and were demonstrated to be responsible for the increase in local and systemic insulin resistance and metabolic abnormalities associated with obesity[5,6]. It is well known that visceral adipose tissue (VAT) expansion present higher risk for the development of metabolic syndrome and insulin resistance than subcutaneous adipose tissue (SAT) growth[7]. Unsurprisingly, macrophage accumulation in obese VAT tissue is greater than in SAT, as are the levels of the cytokines/chemokines MCP-1, CCR2 and of CD8+ T lymphocytes: These molecules and T cell subsets are essentially pro-inflammatory mediators[8].

ROLE OF INFILTRATING MACROPHAGES IN LEAN AND OBESE AT

The functional relevance of macrophages and their phenotypic changes was established through loss- and gain-of-function experiments[9,10]. Since the discovery of the increased infiltration of macrophages in the obese AT, the attention of researchers has been focused on the inflammatory type of macrophage easily visualized in the so-called “crown-like structures” (CLS) present around adipocytes and their contribution to metabolic disease. These recently recruited, inflammatory macrophages, were mostly of the “classically activated”, M1-type[11]. However, the role of macrophages in the homeostatic, lean AT, has been left mostly unexplored. In lean AT, macrophages seem to be the major population of immune cells, with most of them belonging to the “alternatively activated” class, often classified as the M2-type, with a ratio of M2:M1 reported to be approximately 4:1[12,13].

M2 macrophages are immunosuppressive cells with a high phagocytic capacity, capable to perform antigen presentation and having the ability to secrete extracellular matrix compounds, angiogenic and chemotactic factors, and anti-inflammatory cytokines. Therefore, they contribute to the resolution of inflammation, tissue repair and remodelling[14]. Despite being adopted here, and within the literature at large, one must bear in mind that the M1/M2 dichotomy seems to be an oversimplification, as macrophages with intermediate or different phenotypes...
may also be found in the AT\textsuperscript{[15]}. Although a definitive standard set of markers for the identification of M2 cells is not available yet, a group of molecules often reported in the literature to be associated with this type of cell has been used, since adopting a single marker would be unrealistic\textsuperscript{[16]}. Arginase-1 (Arg-1) and CD206 are the two most frequently cited markers in AT macrophages classified as M2 cells\textsuperscript{[13]}. Arg-1 participates in amino acid metabolism, being strongly expressed in macrophages exposed to IL-4\textsuperscript{[14]}, Arg-1 metabolizes arginine to ornithine and polyamines, thereby inhibiting the production of nitric oxide (NO)\textsuperscript{[17,18]}. The mannose C-type 1 lectin receptor, CD206, is involved in pathogen recognition by the innate immune system\textsuperscript{[19]}. Other regularly cited markers of M2 macrophages are resistin-like β (Fizz1), CD301, Retnla, Dectin-1, MGL-1, peroxisome proliferator activated receptor (PPAR)\textsubscript{γ} and pSTAT\textsubscript{3}\textsuperscript{[20,21]}. Also, several cytokines, mostly with immunosuppressive characteristics, produced by M2 cells, include IL-10, transforming growth factor β (TGF-β) and some chemokines (CCL17, 18, 22 and 24)\textsuperscript{[16,20,21]}. Most, if not all, of the evidence found so far points out that resident, M2 macrophages, are the primary cells responsible for the homeostatic, anti-inflammatory state in lean AT, ultimately avoiding local and systemic insulin resistance\textsuperscript{[12,22]}. These cells are frequently found in the interstitial spaces between adipocytes in the lean AT. In obese AT, M2 macrophages can expand but not as much as the M1-type; some M2 cells are even localized in CLS, where their suggested role may involve the phagocytosis of dead adipocytes, angiogenesis and tissue remodeling\textsuperscript{[17,18]}. Macrophages recruited into the tip of the gonadal AT promote vascular development during tissue outgrowth\textsuperscript{[23]}. Other functions include a possible role in adipogenesis, suggested by the finding that lectin-binding CD68\textsuperscript{+} F4/80\textsuperscript{+} CD34\textsuperscript{+} macrophage-like cells are present in the adipogenic aggregates in the developing fat pads of young mice\textsuperscript{[24]}. M2 macrophages, expressing high levels of MGL-1 and IL-10, have been demonstrated to participate in iron metabolism and perhaps, iron homeostasis in AT, since up to 25% of the macrophages from lean AT have a twofold increase in iron content, making them, basically, ferromagnetic\textsuperscript{[25]}. Finally, cold exposure can induce alternative activation in macrophages from white AT; promoting tyrosine hydroxylase expression and catecholamine production, factors required for browning of WAT, with expression of uncoupling protein 1 (UCP1) by adipocytes and induction of thermogenic metabolism\textsuperscript{[26]}.

### MOLECULAR MEDIATORS INFLUENCING THE M1/M2 BALANCE

Because of their importance to insulin sensitivity and AT homeostasis, it is interesting to known about the mediators of M2 polarization in AT. Adipokines are substances secreted locally by adipocytes. One of them, adiponectin, appears to work mainly via enhancing insulin sensitivity, particularly by impairing liver gluconeogenesis, increasing fatty acid oxidation and promoting glucose uptake\textsuperscript{[27]}. Adiponectin can also drive M2 polarization in both human and mouse macrophages by increasing the expression of Arg-1, IL-10 and macrophage galactose N-acetyl-galactosamine specific lectin-1 (Mgl-1) molecules\textsuperscript{[28]}, although its effect on already differentiated M1 macrophages is mostly pro-inflammatory\textsuperscript{[29]}. Notwithstanding, there is ample evidence for the role of adiponectin as an anti-inflammatory molecule. Adiponectin production is higher in the lean AT and inversely correlated with obesity and levels of inflammatory markers such as C-reactive protein and IL-6 and can make macrophages secrete more IL-10\textsuperscript{[29]}. Recently, Shimizu et al\textsuperscript{[30]} have found that adiponectin inhibits the production of high mobility group box 1 (HMG1) proteins, an innate pro-inflammatory, damage-associated molecular pattern (DAMP) molecule, in tumor necrosis factor (TNF-α) stimulated 3T3 adipocytes\textsuperscript{[30]}. Adiponectin decreases the expression of NF-κB, inflammatory factors on endothelial cells and diminish monocyte migration to tissues. Through its activated receptors AdipoR1 and AdipoR2, adiponectin can down-regulate TNF-α and MCP-1 gene expression and upregulate interleukin-1 receptor antagonist (IL-1Ra), respectively\textsuperscript{[31]}. Fatty acids are another class of molecules acting on macrophages to switch between the M1/M2 program. In general, saturated fatty acids fuel the development of M1 cells, while the unsaturated types aid the rise of alternatively activated phagocytes. For instance, supplementation of mice with dietary fish oils containing eicosapentanenoic acid (EPA) and docosahexaenoic acid (DHA) can reduce pro-inflammatory gene expression and increase anti-inflammatory gene activity and adiponectin expression\textsuperscript{[32]}. Long chain omega-3 polyunsaturated fatty acids (PUFAs) may induce M2 polarization associated with down-regulation of pro-inflammatory mediators in inflamed AT from obese mice\textsuperscript{[33]}. In addition, omega-3 PUFAs can be metabolized into bioactive molecules: Resolvins, protectins and maresins. Titos et al\textsuperscript{[33]} have also shown that resolvin D1 can decrease IFNγ production, while increasing the expression of Arg-1 in macrophages from AT\textsuperscript{[34]}. Incubation of macrophages in culture with the lipid mediator maresin R1 (MaR1) diminishes ROS and pro-inflammatory cytokine [IL-1, TNFα, IL-6, interferon-γ (IFNγ)] production and induces upregulation of the type 1 mannose receptor mRNA expression, a M2 marker\textsuperscript{[34]}. The role of PUFAs as beneficial nutrients or therapeutic agents is being actively investigated in the prevention/treatment of obesity, T2DM and several inflammation-related diseases and is discussed elsewhere\textsuperscript{[22]}.

### IL-4 AND IL-13: KEY CYTOKINES IN THE CONTROL OF M2 POLARIZATION

The differentiation and survival of M2 cells are dependent upon exposure to IL-4 and IL-13\textsuperscript{[10]}. An important
question arises: Where particularly on the context of lean AT surroundings: Where these two cytokines come from? Conditioned medium from 3T3 L1 adipocytes contains IL-13 but not much IL-4[35]. It is well known that both IL-4 and IL-13 are produced by the Th2 lymphocyte, IL-4 being its hallmark[32]. Nonetheless, the role of Th2 CD4+ lymphocytes exerting a protective function and control of AT inflammation is under debate[30]. As explained below, Th2 cells are not the major producers of IL-4 or IL-10 in AT. The major populations expressing the Th2 marker GATA - binding protein 3 in VAT are FoxP3+CD4+ T-reg cells and group 2 innate lymphoid cells (ILC2’s)[16,36]. Thus, although a Th2-type of response is certainly present (see below), more studies must be made to ascertain the specific function of CD4+ Th2 cells in the lean, homeostatic AT.

Recently, it has been demonstrated that innate immune cells such as eosinophils are also a major source of IL-4 in VAT, with their cell numbers inversely correlated with the degree of adiposity[37]. Using Gata1−/− mice (deficient in eosinophils) fed a high-fat diet (HFD), these authors showed animals with increased visceral adiposity, high numbers of M1 macrophages and glucose intolerance/insulin resistance, while IL-4 transgenic mice (enriched in eosinophils) also fed HFD, presented a decrease in these parameters[37]. Eosinophils are granulocytes involved in the combat to helmintfection and in immunopathological processes such as allergies. Not surprisingly, chronic infection of HFD mice with the helmint Schistosoma mansoni triggered strong increases in eosinophil and M2 macrophage numbers in white adipose tissue (WAT) together with improved insulin resistance, better glucose uptake and WAT insulin sensitivity. More importantly, the effects with injections of S. mansoni-soluble egg antigens extract (SEA), instead of the entire helmint, were similar[38]. Eosinophils are not only helpful in decreasing the AT pro-inflammatory milieu and its adverse metabolic effects. Uncoupling protein-1 (UCP1) is critical for non-shivering thermogenesis since it interrupts the mitochondrial electrochemical gradient, creating a proton leak where the excess energy expenditure is dissipated in the form of heat. Precursor-adipocytes from VAT can adopt this cycle, expressing UCP1 and turning into so-called “beige” adipocytes; and this process can protect against obesity[39]. Eosinophil-derived cytokines, signaling through STAT6, are required for the activation of adipocyte “beiging”, since depletion of eosinophils or knockdown of Il4ra in macrophages both result in impaired AT beiging in response to a cold challenge[26]. These authors also showed that treatment with recombinant IL-4 boosts UCP1 expression in both VAT and SAT, resulting in weight loss and improved glucose tolerance and insulin sensitivity.

Eosinophil differentiation and activation is dependent of GM-CSF, IL-3 and IL-5. Moreover, IL-5 is a signal for eosinophil migration and survival in ATs with IL-13 helping to enhance eosinophil’s chemotaxis[40,41]. To keep eosinophils fostering M2 macrophages and an anti-inflammatory milieu in the homeostatic AT, a major source of IL-5 and IL-13 was investigated and zeroed in on a recently discovered subset of non-T cells named group 2 innate lymphoid cells or ILC2’s[41]. These cells resemble Th2 cells in their cytokine production but do not have T-cell receptors. The transcription factors retinoic acid receptor - related orphan receptor and GATA - binding protein 3 are important for ILC2’s development. The role of IL-33 has been associated with tissue repair, parasite elimination, asthma and allergy[42]. Molofsky et al[43] used mice where IL-5- and IL-13-producing cells were eliminated: In this model, they observed the disappearance of ILC2’s, eosinophils and anti-inflammatory macrophages in VAT. Furthermore, ILC2’s displayed a lower cell count in VAT from mice under HFD and IL-33 was identified as the cytokine able to rapidly promote the activation of ILC2’s and the accumulation eosinophils and alternatively activated macrophages in VAT[44].

**ROLES OF ILC’S AND IL-33 IN THE MAINTENANCE OF LEAN AT**

IL-33 is rapidly acquiring growing importance for the maintenance of an anti-inflammatory status in the AT and amelioration of obesity-related insulin resistance. Originally described as a member of the IL-1 cytokine family, IL-33 signals through its receptor, ST2 (suppression of tumorigenicity 2), present in several cell types such as Th2 lymphocytes, mast cells, CD8+ T cells, natural killer (NK) cells and, more importantly, ILC2’s and T-reg cells. Both IL-33 and ST2 are strongly expressed in AT[43-45]. Administration of IL-33 to obese mice improved both adipose-tissue inflammation and systemic insulin resistance, attributed by the authors to this cytokine’s ability to promote polarization of macrophages to an M2-like phenotype and to foster the differentiation of Th2 cells[43]. Furthermore, mice deficient in ST2 and fed HFD developed a high body weight and fat mass, glucose intolerance and impaired insulin sensitivity. Similarly, Il-33−/− mice have aberrant metabolic parameters such as elevated AT mass and insulin/glucose disturbances even when fed a normal diet[46]. Within the same study, Brestoff et al[46] also demonstrated the critical importance of IL-33 for the accumulation and maintenance of ILC2’ s in human WAT and went further to show mechanisms of IL-33/ILC2’s metabolic regulation of homeostasis, such as in vivo beiging of WAT and production of methionine-enkephalin peptides by ILC2’s that can act directly on adipocytes to upregulate the expression of Ucp1[46].

Obesity inversely correlates with the amount of anti-inflammatory T-regs in the AT. In comparison with their lymphoid-tissue counterparts, a unique population of resident Foxp3+CD4+ T-reg cells accumulates in VAT of lean mice[44,48], and they are highly overrepresented in lean individuals (40%-80% vs 5%-15% of the Foxp3 CD4+ T-cell compartment). These T-regs have a distinct transcriptome, particularly the profile of transcripts encoding transcription factors, cytokines/chemokines and their receptors as well as an atypical expression of molecules involved in lipid metabolism. They also
have an unusual, clonally expanded, repertoire of T-cell antigen receptors. Importantly, in rodents where these AT T-regs were experimentally deleted, an increase in AT inflammation (represented by high levels of TNFα, IL-6 and RANTES) and acutely reduced insulin sensitivity was observed\(^{[47]}\). The unique phenotype of this AT T-regs population was emphasized as well by their expression of PPARγ, a transcription factor usually associated with adipocyte differentiation and function\(^{[48]}\); However; PPARγ also drives T-regs cell accumulation, phenotype and function in visceral AT. The injection of pioglitazone, an agonist of PPARγ, could increase the numbers of these AT T-regs in VAT and restore insulin sensitivity of mice under HFD\(^{[49]}\). Interestingly, Han et al\(^{[49]}\) have reported, in comparison with other T-regs populations, higher levels of the ST2 chain of the IL-33 receptor in most AT T-regs. The proportion of these ST2+ T-regs was reduced in obese VAT and their numbers could be restored by injections of recombinant IL-33, which was also able to reduce VAT inflammation and decrease insulin resistance in mice under HFD\(^{[44,49,50]}\). Human omental AT T-regs cells also showed high ST2 expression, suggesting an evolutionarily conserved requirement for IL-33 in VAT-Tregs cell homeostasis\(^{[50]}\). Thus, IL-33 promotes the accumulation and function of both ILC2 and T-regs cells. Interestingly, although AT T-regs can also respond directly to IL-33, in vivo ILC2-intrinsic activation by IL-33 is required before VAT T-regs cells accumulation\(^{[51]}\).

IL-10 is a classical immunosuppressive cytokine, which induces a general anti-inflammatory effect on monocytes/macrophages, T and B cells, mast cells and NK cells\(^{[52]}\). If stimulated with IL-10, macrophages can turn into M2 cells, also secreting IL-10\(^{[13,14]}\). VAT T-regs from 30-wk lean mice showed upregulated IL-10 expression as compared to conventional T-regs. Up to 13.9% of VAT T-regs express IL-10 (contrary to 1.8% of conventional T-regs) as detected by flow cytometry\(^{[47]}\). The high expression of IL-10 by VAT T-regs is altered after HFD since VAT T-regs from obese mice display a significant reduction in IL-10 production\(^{[53]}\). Because IL-10 is necessary for T-reg-mediated suppression of TNFα production from macrophages, this obesity-induced change in VAT T-regs function most likely contributes to inflammation and insulin resistance\(^{[53]}\).

THE ROLE OF NUCLEAR RECEPTORS IN THE INDUCTION OF M2 DIFFERENTIATION

M2 cells preferentially use fatty acids and oxidative metabolism, while M1 cells utilize glucose\(^{[34]}\), which is comprehensible since the latter needs increased levels of reactive oxygen species (ROS) and NO to better perform their microbicial activities. Interestingly, pushing oxidative metabolism into M1 macrophages seems to change their phenotype towards a M2-type\(^{[55]}\). On the other hand, after IL-4 stimulation, STAT6 activation on macrophages can induce the co-activator protein PPARγ-coactivator-1β (PGC1-β), which promotes mitochondrial respiration and biogenesis. PGC1-β is considered an important metabolic trigger for the switch towards the M2 profile\(^{[55]}\). STAT6 activation also induces the transcriptional regulators PPARγ and PPARδ, both helping in the maintenance of the M2 phenotype: PPARγ induces the expression of MGL-1, a marker often found on M2 cells\(^{[13,56]}\). In addition, knock-down of PPARδ can lead to insulin resistance\(^{[56]}\), demonstrating that its function is important for the expression of anti-inflammatory mediators by M2 macrophages. Another marker of M2 cells, arginase-1, is highly responsive to agonists of both PPARγ and PPARδ and the arginine metabolism is a relevant feature of M2 cells\(^{[55,57]}\). Disruption of PPARγ impairs the maturation of M2 macrophages and leads mice towards diet-induced obesity, glucose intolerance and insulin resistance\(^{[58]}\). Treatment of macrophages from ob/ob mice with a thiazolidinedione (rosiglitazone), a pharmacological activator of PPARγ, can induce anti-inflammatory M2 markers such as Arg1 and reduce the number of M1 macrophages even in ob/ob mice\(^{[59]}\). Therefore, both PPARγ and PPARδ are important activators of M2 differentiation. Another regulator of the arginase-1 gene (Arg1), the hypoxia inducible factor-2α (HIF-2α), seems an important driver of M2 phenotype\(^{[60]}\). However, in this context, the function of HIF-2α still needs to be better elucidated since it also induces NFκB, a pro-inflammatory transcription factor\(^{[61]}\). Macrophage metabolism seems to be important for insulin sensitivity and new investigations on this area will most certainly bring a better understanding of the role of macrophages in the AT on its homeostatic state.

LOW-GRADE CHRONIC INFLAMMATORY OF THE AT: T CELLS AT PLAY DURING OBESITY

The establishment of a pro-inflammatory phenotype is viewed as the link between the development of obesity and the evolution of obesity towards insulin resistance and ultimately T2DM and its associated cardiovascular burden\(^{[62]}\). As discussed above, during the development of obesity, hypertrophied AT experiences a stronger infiltration by macrophages and other immune cells and, critically, these infiltrating immune cells are mainly pro-inflammatory, as opposed to the milder infiltration in AT of the lean which is chiefly constituted by anti-inflammatory lineages\(^{[3]}\). Infiltration of the AT by proinflammatory M1 macrophages occurs at an advanced stage of AT hypertrophy, and, while being necessary to promote inflammation, can be viewed as a secondary event\(^{[4,5]}\). On the contrary, recent research promotes the idea that the initial events leading to the regulation of obesity-induced inflammation can be attributed to T cell lineages\(^{[63]}\). The lean AT is populated by resident anti-inflammatory CD4+ Foxp3+ T-regs and Th2 cells. These T cells secrete IL-10, an anti-inflammatory cytokine known to improve adipocyte insulin sensitivity of adipocytes\(^{[47]}\), but also systemically as mice overexpressing

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IL-10 in skeletal muscle subjected to a high fat diet did not develop insulin resistance albeit becoming markedly obese[64]. During the transition from a lean phenotype to obesity, infiltrating anti-inflammatory CD4^+ decline, and pro-inflammatory T lineages Th1 and Th17 become predominant. Pro-inflammatory T cell infiltration precedes macrophage infiltration and, via the secretion of pro-inflammatory IL-17 and TNFα, drive the expansion of the inflammatory state[65]. In addition to this inflammatory amplification driven by Th1/Th17 cells and IL-1β-producing M1 macrophages, the adipocytes are also secreting several pro- and anti-inflammatory adipokines that participate to the regulation of metabolic homeostasis. Notably, adipocytes of lean individuals mainly secrete anti-inflammatory adiponectin, while obese AT secretes pro-inflammatory IL-6[66]. Therefore, a crosstalk between adipocytes and the immune cell populations infiltrating AT maintains an anti-inflammatory state in physiological conditions, but can switch to a state of sub-clinical inflammation characterized by an IL-1β, IL-6 and IL-17- rich environment, a prerequisite for insulin resistance, during the development of obesity (Table 1).

**IL-17: A NOVEL PLAYER IN OBESITY-INDUCED INSULIN RESISTANCE**

The contribution of pro-inflammatory cytokines IL-1β and TNFα in mediating insulin-resistance in the obese state is now widely accepted and has been comprehensively reviewed elsewhere[67,68]. Similarly, IL-6, which is often increased in pro-inflammatory settings, is likewise viewed as a pro-inflammatory cytokine, although such notion is now in part disputed and IL-6 might serve both in pro- and anti-inflammatory context depending on the global environment and the balance with other pro- or anti-inflammatory mediators[69,70].

More recent data have also called into action Th17 cells - and their secreted cytokine IL-17 - in the establishment of inflammation associated to obesity[71]. Studies in a mouse model indicated that T-cells derived from diet-induced obese mice accumulated the Th17 subset, thereby releasing IL-17 in an IL-6-dependent fashion[72].

In a more clinically relevant setting, a 3 to 10-fold accumulation of IL-17 and IL-22 secreting Th17 cells was observed in AT from insulin-resistant obese subjects[73], in VAT from morbibly obese women[74] and in peripheral blood from obese children. Also, increased plasmatic levels of IL-17 have been observed in obese women[75].

From a mechanistic point of view, several mechanisms, perhaps not mutually exclusive, have been proposed as participating into the polarization of T cells towards the Th17 lineage in obesity. Purinergic signaling resulting from the activation of the P2X7 receptor by ATP have been shown to promote Th17 polarization within the AT microenvironment[76]. Also, co-culture of mature adipocytes derived from obese donors with peripheral blood mononuclear cells promoted increased release of IL-17 and IL-22 by the latter, and this cytokine production exacerbated inflammation by amplifying IL-1β secretion by macrophages.

The two IL-17 isoforms, IL-17A and IL-17F, are central mediators of inflammation and contribute to the development of multiple autoimmune disorders and are thus...
attractive therapeutic targets[57]. The IL-17 receptors IL-17RA and IL-17RC are ubiquitously expressed, explaining the large spectrum of activities of these two cytokines[58]. In addition to its pro-inflammatory action, IL-17 might affect metabolic homeostasis by inhibiting adipogenesis[59]. The inhibition of adipogenesis, in the context of an hypercaloric diet, would hamper lipid storage in the AT and favor the increase in circulating levels of free fatty acids, contributing to the worsening of insulin resistance.

TREATING METABOLIC INFLAMMATION WITH TARGETED THERAPIES

The current understanding that inflammatory events in obesity and T2D are mediated by multiple cytokines, including IL-1β, IL-6, TNFα and IL-17 produced by various cell types within the AT has lent support to the idea that inhibition of these cytokines by specifically designed inhibitory antibodies might curb the progression of the obes phenotype towards insulin resistance and diabetes[80]. Several inhibitory antibodies acting on the IL-1 system, IL-6 and TNFα[81] have been tested in indications related to obesity and the metabolic syndrome[82]. Canakinumab, an IL-1β inhibitory antibody, originally used to treat pro-inflammatory diseases, has more recently been used in several clinical trials aiming at treating T2D and has been shown to induce mild improvements in glycated hemoglobin and beta cell functioning in patients with T2D[83].

Undoubtedly, with the improved understanding of the anti- and pro-inflammatory phenomena playing a role in the development of obesity and T2D that we tried to summarize here, more efforts will be done in the near future to try to bring to clinical fruition targeted therapies aiming to treat metabolic inflammation via the inhibition of pro-inflammatory mediators or the activation of anti-inflammatory pathways.

REFERENCES

1. Gustafson B, Hedjaziﬁr S, Goggi S, Hammarstedt A, Smith U. Insulin resistance and impaired adipogenesis. Trends Endocrinol Metab 2015; 26: 193-200 [PMID: 25703677 DOI: 10.1016/j.tem.2015.01.006]
2. Ferrante AW. Macrophages, fat, and the emergence of immuno-metabolism. J Clin Invest 2013; 123: 4992-4993 [PMID: 24292661 DOI: 10.1172/JCI73658]
3. Osborn O, Olefsky JM. The cellular and signaling networks linking the immune system and metabolism in disease. Nat Med 2012; 18: 363-374 [PMID: 22395709 DOI: 10.1038/nm.2627]
4. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003; 112: 1796-1808 [PMID: 14697176]
5. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 2003; 112: 1821-1830 [PMID: 14697177]
6. Wensveen FM, Valentijn S, Sestan M, Turk Wensveen T, Poltorak I. The “Big Bang” in obese fat: Events initiating obesity-induced adipose tissue inflammation. Eur J Immunol 2015; 45: 2446-2456 [PMID: 26220361 DOI: 10.1002/eji.201545502]
7. Tchernev A, Despré JP. Pathophysiology of human visceral obesity: an update. Physiol Rev 2013; 93: 359-404 [PMID: 23303913 DOI: 10.1152/physrev.00033.2011]
Obesity alters adipose tissue macrophage iron content and tissue iron distribution. *Diabetes* 2014; 63: 421-432 [PMID: 24130337 DOI: 10.2337/db13-0213]

Qiu Y, Nguyen KD, Odegaard JJ, Cui X, Tian X, Locksley RM, Palmer TD, Chawla A. Eosinophils and type 2 cytokine signaling in macrophages orchestrate development of functional beige fat. *Cell* 2014; 157: 1292-1308 [PMID: 24906148 DOI: 10.1016/j.cell.2014.03.066]

Okada-Iwabu M, Iwabu M, Ueki K, Yamauchi T, Kadowaki T. Perspective of Small-Molecule AdipoR Agonist for Type 2 Diabetes and Short Life in Obesity. *Diabetes Metab J* 2015; 39: 363-372 [PMID: 26566694 DOI: 10.4093/dmj.2015.39.5.363]

Ohashi K, Parker JL, Ouchi N, Higuchi A, Vita JA, Golce N, Pedersen AA, Kalthoff C, Tullin S, Samau S, Sumner R, Walsh K. Adiponectin promotes macrophage polarization toward an anti-inflammatory phenotype. *J Biol Chem* 2010; 285: 6153-6160 [PMID: 20028977 DOI: 10.1074/jbc.M108078870]

van Stijin CM, Kim J, Lusis AJ, Barish GD, Tangirala RK. Macrophage polarization phenotype regulates adiponectin receptor expression and adiponectin anti-inflammatory response. *FASEB J* 2015; 29: 636-649 [PMID: 25302268 DOI: 10.1096/fj.14-253831]

Shimizu T, Yamakuchi M, Biswas KK, Aryan B, Yamada S, Hashiguchi T, Manuyama Y. HMGB1 is secreted by T3-L1 adipocytes through JNK signaling and the secretion is partially inhibited by adiponectin. *Obesity* (Silver Spring) 2016; 24: 1913-1921 [PMID: 27430164 DOI: 10.1002/oby.21549]

Tian L, Luo N, Zha X, Chung BH, Garvey WT, Fu Y. Adiponectin-AdipoR1/2-APPL1 signaling axis suppresses human foam cell formation: differential ability of AdipoR1 and AdipoR2 to regulate inflammatory cytokine responses. *Atherosclerosis* 2012; 221: 66-75 [PMID: 22227293 DOI: 10.1016/j.atherosclerosis.2011.12.014]

Im DS. Functions of omega-3 fatty acids and FFAR4 (GPR120) in macrophages. *Eur J Pharmacol* 2016; 785: 36-43 [PMID: 29587421 DOI: 10.1016/j.ejphar.2015.03.094]

Titos E, Xu B, González-Pérez A, López-Vicario C, Morán-Salvador E, Martínez-Clemente M, Arroyo V, Claria J. Resolvin D1 and its precursor docosahexaenoic acid promote resolution of adipose tissue inflammation by eliciting macrophage polarization toward an M2-like phenotype. *J Immunol* 2011; 187: 5408-5418 [PMID: 22013115 DOI: 10.4049/jimmunol.1100225]

Marcon R, Bento AF, Dutra RC, Bica MA, Leite DF, Calixto JB. Eosinophils sustain visceral adipose tissue eosinophils and alternatively activated macrophages associated with beige fat phenotype. *J Immunol* 2013; 191: 4288-4298 [PMID: 24038901 DOI: 10.4049/jimmunol.1300743]

Kang K, Reilly SM, Karabacak V, Gangi 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 [PMID: 18522830 DOI: 10.1016/j.cmet.2008.04.002]

Chalubinski M, Luzak E, Wódkan J, Gorzelak-Pabius P, Brzonec M. Invasive lymphoid tissue type 2 - emerging immune regulators of obesity and atherosclerosis. *Immunol Lett* 2016; 179: 43-46 [PMID: 27466628 DOI: 10.1016/j.imlet.2016.09.007]

Wu D, Molofsky AB, Li A, Kamei N, Lee J, Shoelser SE, Benoist C, Mathis D. Antigen- and cytokine-driven accumulation of regulatory T cells in visceral adipose tissue of lean mice. *Cell Metab* 2015; 21: 543-557 [PMID: 25863247 DOI: 10.1016/j.cmet.2015.03.005]

Liew FY, Girard JP, Turnquist HR. Interleukin-33 in health and disease. *Nat Rev Immunol* 2016; 16: 676-689 [PMID: 27640624 DOI: 10.1038/nri.2016.95]

Brestoff JR, Kim BS, Saenz SA, Stine RR, Monticelli LA, Sonnenberg GF, Thome JJ, Darber DL, Lutfy K, Seale P, Artis D. Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. *Nature* 2015; 519: 242-246 [PMID: 25533952 DOI: 10.1038/nature14115]

Feuerer M, Herrero L, Cipolletta D, Naaz A, Wong J, Nayer A, Lee J, Goldfine AB, Benoist C, Shoelser 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 [PMID: 19633656 DOI: 10.1038/nm.2002]

Cipolletta D, Feuerer M, Li A, Kamei N, Lee J, Shoelser SE, Benoist C, Mathis D. PPAR-γ is a major driver of the accumulation and phenotype of adipose tissue Treg cells. *Nature* 2012; 486: 549-553 [PMID: 22722857 DOI: 10.1038/nature11312]

Han JM, Wu D, Demroche HC, Yao Y, Verchere CB, Levison MK. IL-33 Reverses an Obesity-Induced Deficit in Visceral Adipose Tissue ST2+ T Regulatory Cells and Ameliorates Adipose Tissue Inflammation and Insulin Resistance. *J Immunol* 2015; 194: 4777-4783 [PMID: 25870243 DOI: 10.4049/jimmunol.1500020]

Vasanthakumar A, Moro K, Xin A, Liao Y, Glorey R, Kawamoto S, Fagarasan S, Mielke LA, Afifar-Sterle S, Masters SL, Nakaes S, Saito H, Wentworth JM, Li P, Liao W, Leonard WJ, Smyth GK, Shi W, Nutt SL, Koyasu S, Kalissies A. The transcriptional regulators IRF4, BAF5 and IL-33 orchestrate development and maintenance of adipose tissue-resident regulatory T cells. *Nat Immunol* 2015; 16: 276-285 [PMID: 25599561 DOI: 10.1038/ni.3085]

Molofsky AB, Van Gool F, Liang HE, Van Dyken SJ, Nussbaum JC, Lee J, Bluestone JA, Locksley RM. Interleukin-33 and Interferon-γ Counter-Regulate Group 2 Inflammatory Cell Activation during Immune Perturbation. *Immunity* 2015; 43: 161-174 [PMID: 26092469 DOI: 10.4049/jimmunol.2015.05.019]

Moore KW, de Waal Malefyt R, Coffman RL, O’Garra A. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* 2001; 19: 683-765 [PMID: 11144051 DOI: 10.1146/annurev.immunol.19.1.683]

Han JM, Patterson SJ, Speck M, Ehles JA, Levison MK. Insulin inhibits IL-10-mediated regulatory T cell function: implications for obesity. *J Immunol* 2014; 192: 623-629 [PMID: 24323581 DOI: 10.4049/jimmunol.1302181]

Freemaner AJ, Johnson AR, Sacks GN, Milner JJ, Kirk EL, Troester MA, Macintyre AN, Goraksha-Hicks P, Rathmell JC, Makowski L. Metabolic reprogramming of macrophages: glucose transporter 1 (GLUT1)-mediated glucose metabolism drives a proinflammatory phenotype. *J Biol Chem* 2014; 289: 7884-7896 [PMID: 24490265 DOI: 10.1074/jbc.M113.220307]
polarization. Front Immunol 2014; 5: 420 [PMID: 25228902 DOI: 10.3389/fimmu.2014.00420]

56 Olfsky JM, Glass CK. Macrophages, inflammation, and insulin resistance. Ann Rev Physiol 2010; 72: 219-246 [PMID: 2048674 DOI: 10.1146/annurev-physiol-021909-135846]

57 Gallardo-Soler A, Gómez-Nieto C, Campo ML, Marathe C, Tontonoz P, Caso A, Corraliza I. Arginase I induction by modified lipoproteins in macrophages: a peroxisome proliferator-activated receptor-gamma/delta-mediated effect that links lipid metabolism and immunity. Mol Endocrinol 2008; 22: 1394-1402 [PMID: 18323470 DOI: 10.1210/me.2007-0525]

58 Odegaard JJ, Ricardo-Gonzalez RR, Goforth MH, Morel CR, Subramanian V, Mukundan L, Red Eagle A, Vais D, Bronnbacher F, Ferrante AW, Chawla A. Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. Nature 2007; 447: 1116-1120 [PMID: 17515919 DOI: 10.1038/nature05894]

59 Prieur X, Mol CY, Velagapudi VR, Núñez F, Ventes J, Montaner D, Ishikawa K, Camacho A, Barbarroja N, O’Rahilly S, Sethi JK, D’Orazio SA, Fabbrini E, Cellis M, McCartney SA, Fuchs A, Abumrad NA, Baffy N, Falus A, Fulop AK. The major inflammatory IL-1 family members in the pathogenesis and treatment of metabolic disease: Focus on adipose tissue inflammation and insulin resistance. Cytokine 2015; 75: 280-290 [PMID: 26194067 DOI: 10.1016/j.cyto.2015.05.005]

60 Guerrier A, Virtanen J, Purk V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nat Rev Mol Cell Biol 2008; 9: 367-377 [PMID: 18401346 DOI: 10.1038/nrm2391]

61 Mazer J, Denson JL, Brüning JC. Versatile functions for IL-6 in metabolism and cancer. Trends Immunol 2015; 36: 92-101 [PMID: 25616716 DOI: 10.1016/j.it.2014.12.008]

62 Covarrubias AJ, Horng T. IL-6 strikes a balance in metabolic inflammation. Cell Metab 2014; 19: 898-899 [PMID: 24896536 DOI: 10.1016/j.cmet.2014.05.009]

63 Ahmed M, Gaffen SL. IL-17 in obesity and adipogenesis. Cytokine Growth Factor Rev 2010; 21: 449-453 [PMID: 21084215]

64 Winer S, Paliaras G, Chan Y, Tsai H, Engelmann E, Winer D, Dosh HM. Obesity predisposes to Th17 bias. Eur J Immunol 2009; 39: 2629-2635 [PMID: 19662632]

65 Fabbrini E, Cellis M, McCartney SA, Fuchs A, Abumrad NA, Pietka TA, Chen Z, Finck BN, Han DH, Magkos F, Conte B, Bradley D, Fraterrigo G, Eagon JC, Patterson BW, Colonna M, Klein S. Association between specific adipose tissue CD4+ T-cell populations and insulin resistance in obese individuals. Gastroenterology 2013; 145: 366-374.e1 [PMID: 23997726 DOI: 10.1053/j.gastro.2013.04.010]

66 Zapata-Gonzalez F, Auguet A, Aragonés G, Gùirau-Jurado E, Berlanga A, Martinez S, Martí A, Sabench F, Hernandez M, Aguilar C, Sirvent J, Horba R, Del Castillo D, Richtar C. Interleukin-17A Gene Expression in Morbidly Obese Women. Int J Mol Sci 2015; 16: 17469-17481 [PMID: 26236971 DOI: 10.3390/ijms16081749]

67 Sumarac-Dumanovic M, Stevanovic D, Ljubicic A, Jorga J, Simic M, Stanenkovic-Pejkovic D, Starcevic V, Trajkovic V, Micic D. Increased activity of interleukin-23/interleukin-17 proinflammatory axis in obese women. Int J Obes (Lond) 2009; 33: 151-156 [PMID: 18982006]

68 Pandolfi JB, Ferraro AA, Sananee I, Gancedo MC, Baz P, Billordo LA, Fainboim L, Arruvito L. ATP-Induced Inflammation Drives Tissue-Resident Th17 Cells in Metabolically Unhealthy Obesity. J Immunol 2016; 196: 3287-3296 [PMID: 26951799 DOI: 10.4049/jimmunol.1502506]

69 Beringer A, Noack M, Miossee P. IL-17 in Chronic Inflammation: From Discovery to Targeting. Trends Mol Med 2016; 22: 230-241 [PMID: 26837266 DOI: 10.1016/j.molmed.2016.01.001]

70 Gaffen SL. Recent advances in the IL-17 cytokine family. Curr Opin Immunol 2011; 23: 613-619 [PMID: 21852080]

71 Ahmed M, Gaffen SL. IL-17 inhibits adipogenesis in part via C/EBPα, PPARγ and Krüppel-like factors. Cytokine 2013; 61: 899-905 [PMID: 23332504]

72 Donath MY. Targeting inflammation in the treatment of type 2 diabetes: time to start. Nat Rev Drug Discov 2014; 13: 465-476 [PMID: 24854413]

73 Esser N, Paquot N, Schein AJ. Anti-inflammatory agents to treat or prevent type 2 diabetes, metabolic syndrome and cardiovascular disease. Expert Opin Investig Drugs 2015; 24: 283-307 [PMID: 25345753 DOI: 10.1515/13543784.2015.974800]

74 Bönni-Schuetzler M, Donath MY. How biologics targeting the IL-1 system are being considered for the treatment of type 2 diabetes. Br J Clin Pharmacol 2013; 76: 263-268 [PMID: 22506644 DOI: 10.1111/1365-2125.120429.x]

75 Rissanen A, Howard CP, Botha J, Thuren T. Effect of anti-IL-1β antibody (canakinumab) on insulin secretion rates in impaired glucose tolerance or type 2 diabetes: results of a randomized, placebo-controlled trial. Diabetes Obes Metab 2012; 14: 1088-1096 [PMID: 22726220 DOI: 10.1111/j.1463-1326.2012.01637.x]

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