Tissular T_{regs}: a unique population of adipose-tissue-resident Foxp3+CD4+ T cells that impacts organismal metabolism

Daniela Cipolletta[1]†, Dmitriy Kolodin[1]†, Christophe Benoist[1], Diane Mathis[1]

[1]Department of Pathology, Harvard Medical School, Boston, MA 02115; and

Address correspondence to: Christophe Benoist and Diane Mathis
Department of Pathology
Harvard Medical School
77 Avenue Louis Pasteur
Boston, MA 02115
E-mail: cbdm@hms.harvard.edu
Phone: (617) 432-7741
Fax: (617) 432-7744

Author contributions:

†These authors contributed equally to this work.

The authors declare no conflict of interest.

Keywords: Regulatory T cells, obesity, type 2 diabetes, inflammation, adipose tissue.

Abstract:

Foxp3+CD4+ regulatory T (T_{reg}) cells are a key population in controlling the immune response. Recently, their roles have been expanded to broader, non-immune, contexts, in particular the metabolic consequences downstream of obesity-induced inflammation, e.g. type-2 diabetes and cardiovascular disease. This review highlights the major innate and adaptive immune cell subsets contributing to adipose-tissue inflammation, the key role played by fat-resident T_{regs}, and the potential of T_{reg}-based therapies for treatment of the metabolic syndrome.

1. Introduction

In recent years, regulatory T (T_{reg}) cells have emerged as one of the most important guardians of the immune response. Usually making up approximately 5-20% of the CD4+ T cell compartment, T_{regs} play a key role in controlling autoimmunity, allergic responses, inflammation, and responses to infection [1]. Traditionally, they were thought to regulate the activities of other T cells, but there has been growing appreciation of their impact on innate immune system cells, e.g. macrophages and neutrophils. Recent data argue for an even broader role, in non-immune contexts such as cardiovascular disease [2-4] and, obesity-induced insulin resistance [5,6], the focus of this review.

Over the past several decades, there has been a world-wide increase in obesity, in parallel with an impressive rise in a cluster of abnormalities termed the "metabolic syndrome", in particular type-2 diabetes (TD2). According to the latest estimates from the Centers for Disease Control, over 25 million people in the United States suffer from diabetes, which is associated with tremendous societal and economic burdens [7]. There is by now a large body of evidence that implicates obesity in provoking chronic, low-grade inflammation which, in turn, promotes metabolic dysregulation and systemic insulin resistance [8,9]. It turns out that the function of adipose tissue is not limited to the storage of excess triglycerides, but that it is also an active endocrine organ playing multiple roles in orchestrating system-wide metabolism. A seminal insight into the pro-inflammatory potential of adipose tissue in response to obesity came from Hotamisligil *et al.*, who demonstrated increased levels of tumor necrosis factor-α (TNF-α) mRNA in adipocytes in visceral adipose tissue (epidydimal, omental, etc.), but not subcutaneous fat, of obese versus lean mice [10]. Since then, an increasing number of adipocyte-derived mediators, collectively called adipokines, have been described. These molecules, which can

increase or decrease in response to extended caloric excess, have multiple effects on a variety of cell populations, both locally in adipose tissue and systemically (reviewed in [11]). They include, but are not limited to, interleukin-1β (IL-1β) [12], IL-6 [13], serum amyloid A3 (SAA3) [14], and macrophage chemoattractant protein-1 (MCP-1) [15]. Obesity, by triggering endoplasmic reticulum (ER) stress [16], hypoxia [17], and oxidative stress [18], results in adipocyte dysfunction, dysregulated expression of adipokines, and inappropriate inflammatory responses [8,9]. There are changes in the cellular composition of adipose tissue, including adjustments in the numbers, phenotypes, and localization of multiple immune cell subsets. There are also systemic metabolic alterations through a complex signaling network that affects insulin signaling in the liver, kidney, and skeletal muscle. Activation of NF-κB [19] and the JUN N-terminal kinase family of serine/threonine protein kinases [20] leads to phosphorylation and inactivation of the insulin receptor substrate (IRS) family, which collectively results in tissues becoming unresponsive to insulin signaling [16,21,22].

Here, we will outline contributions of both the innate and adaptive immune systems to obesity-associated pathologies, highlight the protective role of fat-resident T_{reg} cells, and discuss the possible clinical implications of these findings for treatment of T2D.

2. Immune effector cells in obesity-induced T2D

2.1 Innate immune system:

A number of studies have implicated innate immune system cell types, such as neutrophils, mast cells, and macrophages in obesity-induced pathology. In a mouse model of obesity triggered by a high-fat diet (HFD), neutrophils were early infiltrators of the adipose tissue, detectable already at three weeks of HFD, suggesting a possible role in early phases of

adipose tissue inflammation. However, the importance of neutrophils in downstream metabolic consequences remains to be established [23].

Recently, Shi and colleagues argued for a role for mast cells in obesity and its sequelae [24]. They found that mast cell numbers were increased in the adipose tissue of obese mice and humans compared with those of lean controls. Mice carrying a genetic deficiency in mast cells or treated with mast-cell-stabilizing drugs displayed a significantly reduced body weight and visceral fat pad mass when on HFD through a proposed mechanism of decreased angiogenesis and increased apoptosis of muscle and adipose tissue. It is important to note that the primary effect of mast cell deficiency/stabilization was on obesity-induced weight gain, which would have secondarily altered insulin sensitivity. Finally, using genetic knockout mice, the authors showed that IL-6 and interferon-γ (IFN-γ) produced by mast cells were key cytokines for attenuating weight gain. These findings suggest a role for mast cells in obesity-associated abnormalities through promoting adipose tissue growth.

To date, the largest body of evidence implicates subsets of macrophages/monocytes as major effectors in obesity-induced pathology. Accumulations of large numbers of macrophages around dead adipocytes, forming "crown-like structures," have been observed in visceral fat tissue of obese mice and humans [25,26]. Under normal chow conditions, adipose tissue presents an anti-inflammatory environment, where resident macrophages display a phenotype similar to the anti-inflammatory or alternatively-activated "M2" state, producing factors such as interleukin-10 (IL-10) and transforming growth factor-β (TGF-β)[15,27-31]. In contrast, in obese adipose tissue, resident and infiltrating macrophages appear to be in the pro-inflammatory or classically activated "M1" state, synthesizing numerous inflammatory factors, including TNF-α, IL-6, matrix metalloproteinases, and peroxisome proliferator activated receptor-γ (Pparg),

[15,26,29-31].

It is important to note, however, that, while adipose tissue macrophages have been historically subdivided into M1 and M2 subsets [30,31], and this is an attractively simple dichotomy, recent studies have challenged this binary classification, instead arguing in favor of a spectral classification [27,32,33]. A recent study demonstrated the importance of the NLRP3 (nucleotide-binding domain leucine-rich repeat containing family, pyrin domain containing 3) inflammasome in activation of adipose-tissue macrophages and subsequent development of insulin resistance [34]. The inflammasome is a key player in sensing various "danger" stimuli, leading to secretion of IL-1β and initiation of a pro-inflammatory immune response (reviewed in [35]). Previous studies have demonstrated that cholesterol or similar crystals cause Nlrp3 inflammasome activation in macrophages found in atherosclerotic lesions [36,37]. By analogy, one might speculate that HFD leads to formation of cholesterol crystals activate the Nlrp3 inflammasome, in particular in adipose tissue macrophages, resulting in a pro-inflammatory activation state and ultimately resulting in systemic inflammation and insulin resistance. Additionally, activation of adipose tissue macrophages has been shown to result from recognition of free fatty acids by Toll-like receptors (TLR) 2 and 4 in mice on HFD [38].

2.2 Adaptive immune system:

The contributions of the innate immune system, in particular of macrophages, to obesity-induced inflammation have been the central focus of by far most of the relevant studies, but of late there has been a growing interest in the role of the adaptive immune system as well. A T cell implication in T2D would not be so surprising given their crucial roles in various other inflammatory contexts. Early studies noted an increased frequency of CD3+ cells in the adipose tissue of obese humans and mice [39]. Since then, accumulations of both CD4+ and CD8+ T

cells have been reported, and evidence of a significant role presented, but there remains substantial controversy over the extent to which the two compartments contribute to disease progression.

Several recent studies have evaluated the role of CD8+ T cells in adipose tissue inflammation. They were found infiltrate visceral adipose tissue in response to HFD, reportedly prior to the characteristic increase in macrophages [40]. Depletion of CD8+ T cells via either antibody treatment or genetic ablation resulted in reduced macrophage infiltration into visceral fat depots, decreased production of key pro-inflammatory mediators such as TNF-α, IL-1, IL-6, and MCP-1, and improved insulin sensitivity. *In vitro* experiments demonstrated that CD8+ T cells isolated from obese adipose tissue had a highly activated phenotype and produced large quantities of pro-inflammatory mediators known to function in macrophage recruitment and activation [40,41]. The frequency of CD44+CD62L- effector-memory cells was significantly higher, while naïve CD44-CD62L+ CD8+ T cells was decreased, in obese compared with lean adipose tissue, consistent with the notion of an activated CD8+ T cell phenotype in obese fat [41]. Together, these findings suggest that obesity promotes activation of CD8+ T cells in fat, which, in turn, leads to the recruitment, differentiation and activation of activated macrophages via pro-inflammatory mediators.

Other studies have characterized the pro-inflammatory properties of adipose-resident effector CD4+ T cells, especially given their well-established ability to promote inflammation and recruit macrophages. A significant increase in CD4+ T cells residing in adipose tissue of obese humans and mice was reported [41,42]. The fat-resident CD4+ T cell compartment of obese mice was enriched for CD44+CD62L- effector-memory cells [41], phenotype characterized by production of IFN-γ [5,42,43]. T cell receptor (TCR) sequence analysis

revealed a repertoire bias among T cells isolated from obese compared with lean adipose tissue, suggestive of antigen-driven T cell activation, expansion, and/or infiltration [41,42]. An increase in IFN-γ expression can enhance the accumulation of M1 macrophages in obese fat, accompanied by elevated expression of TNFα, and MCP-1 [44]. Finally, enflamed adipose tissue may further propagate Th1 cell polarization through the production of leptin, which can act on T cells directly and indirectly to promote increased proliferation and cytokine production, specifically of IL-2 and IFNγ, [45]. The proposal that Th1 polarization is at the expense of Th2 cells [42] is poorly substantiated as Gata3+CD4+ and Th2 cells cannot be equated [46].

One report has proposed a protective role for effector CD4+ T cells in obesity-induced insulin resistance [42]. CD4+ T cells were adoptively transferred into recombination-activating genes (RAG)-null mice, which have no T or B cells [47], and a decreased body weight and fat pad mass, and an improved insulin sensitivity were observed 2-4 weeks later. The authors proposed that the improved metabolic parameters reflected protection by Th2 cells. However, an alternative interpretation is that the improvement is due to a lower body weight in the recipient mice coupled with the insulin-sensitizing effect of T_{reg} cells. Transfer of CD4+ T cell populations depleted of Foxp3+ T_{regs} or CD4+ T cells from IL-10-deficient mice into immunodeficient recipients are both well-established animal models of colitis [48,49]. Therefore, the decreased weight gain of the recipient RAG-null mice following CD4+ T cell transfer was likely due to the initiation of colitis, and the improved metabolic parameters were merely a reflection of decreased body weight. The presence of colitis is further substantiated by the fact that there was no attenuated weight gain or improvement in metabolic parameters following the transfer of CD8+ T cells [49] or OT2 TCR-transgenic CD4+ T cells [50], because neither of these cell types is able to transfer colitis. In addition, the authors proposed that the protective effect of CD4+ T cells was

a result of their Th2 polarization by showing that transferred CD4+ T cells produced IL-4 and IL-13 following restimulation and that there was a lower frequency of Gata3+ T cells in visceral adipose tissue of obese compared with lean animals. Under lymphopenic conditions, CD4+ T cells become activated and differentiate into Th2 cells, as suggested by the authors, but they also differentiate into Th1 cells; therefore, upon restimulation, CD4+ T cells from a lymphopenic host may produce IL-4 and IL-13, as seen in the paper, but they would also produce IFNγ, which was not measured. Furthermore, it was recently shown that T_{regs} can express high levels of Gata-3 [46]. Therefore, it is possible that the loss of Gata3+ CD4+ T cells from abdominal adipose tissue following HFD is actually due to loss of T_{reg} cells. Thus, the root of the observed protection bears re-examination.

There are substantial contradictions among the aforementioned studies on the contributions of CD4+ and CD8+ T cells to obesity-associated inflammation and insulin resistance. Several groups reported an increase in the fraction of CD8+ T cells in response to HFD [40,41,51]; however, a more recent study found no such difference [43]. Similarly, some groups described significant CD4+ T cell accumulation in adipose tissue of obese humans and HFD-fed mouse models [42,51], while another study did not see this increase [43], and still another saw a decrease [40]. In interpreting and comparing these seemingly contradictory results, it needs to be kept in mind that methods used to quantify T cells by flow cytometry differed in various studies. In addition, there were multiple divergences in the HFD protocols used, including the age of the mice at introduction of the enriched diet, length of time on the diet, and the composition, in particular the fat content, of the HFD and normal chow comparator.

With the recognition of an important role for T cells in obesity-associated pathologies, there is an increased interest in the function of B cells in these abnormalities. Recent studies on

HFD-fed mice revealed that B cells infiltrated adipose tissue within the first three weeks of diet changes [52,53], and that there was a rise in serum immunoglobulin (Ig) levels. Analysis of B-cell-deficient mice on HFD revealed that the absence of B cells led to increased insulin sensitivity compared with wild-type controls. Additionally, transfer of B cells or serum IgG isolated from HFD-fed mice into B-cell-deficient recipients resulted in transfer of insulin resistance; however, there was no change in insulin resistance when transferred B cells or IgG were isolated from lean normal-chow-fed mice. Finally, the authors showed that insulin resistant human patients had a distinct IgG profile compared with age- and weight-matched insulin sensitive subjects. While it is clear from this study that Igs can have an effector function in promoting insulin resistance, perhaps through adipocyte death and the generation of antibodies that inhibit insulin signaling molecules, their role in more upstream events is more cloudy, given the well-known T cell aberrancies in B-cell deficient mice [54-56].

3. Role of fat-resident T_{reg} cells in obesity-induced T2D

Foxp3+ T_{reg} cells are important regulators of essentially every category of immune response. While Foxp3 is a critical orchestrator of the T_{reg} phenotype, it, alone, is not sufficient to drive the full phenotype program [57,58]. For example, the induction of Foxp3 expression through "conversion" of conventional T (T_{conv}) cells (by *in vitro* culture with TGF-β, *in vivo* exposure to agonist peptide, or *in vivo* homeostatic proliferation) or by retroviral transduction of T_{conv} cells with *Foxp3* can only partially recapitulate the canonical "T_{reg} signature" [57,58]. Furthermore, Foxp3 is not necessary for expression of all of the suppressive activities characteristic of T_{reg} cells. Disruption of the *Foxp3* gene in mice by insertion of the green fluorescent protein (GFP) results in the generation of so-called T_{reg} "wannabes," cells expressing

a number of the canonical T_{reg} markers (such as CTLA4, ICOS, IL-2ra), but lacking any suppressive function [59,60]. These findings suggest that the regulatory activities of T_{regs} are subject to a level of regulation upstream of Foxp3. Finally, the gene-expression profiles of different subtypes of Foxp3+ cells (thymus-derived T_{regs}, splenic, converted, etc.) [57,58,61] coupled with findings on transcription factor knockout mice [62-64], have revealed that T_{reg} cells come in "different flavors", determined by distinct origins and anatomic locations.

So far, the most striking example of a distinct subset of T_{reg} cells came from the identification of an adipose-tissue-resident population that seems to impact metabolic parameters, in particular of insulin resistance secondary to obesity [5]. Such cells accumulated with age in lean mice to eventually represent more than half of the CD4+ T lymphocytes residing in the visceral fat. This is a much greater fraction than the 15% or so normally found in lymphoid tissues. Interestingly, T_{regs} were not enriched in subcutaneous adipose depots – interesting because changes in visceral, but not a subcutaneous, fat have been associated with insulin resistance [65].

Characterization of fat T_{reg} cells via gene-expression profiling and TCR repertoire analysis revealed a population distinct from counterparts in lymphoid organs. Fat-resident Foxp3+CD4+ cells retained about 60% of the canonical T_{reg} signature; however, they specifically over- or under-expressed many genes, especially those coding for molecules involved in leukocyte migration and extravasation, such as CCR1, CCR2, CCR9, CXCL10 (over-represented in fat T_{regs}); and CCL5 and CXCR3 (under-represented in fat T_{regs}). In addition, the repertoire of TCRs displayed by adipose T_{regs} was distinct from that of fat T_{convs} as well as T_{reg} and T_{convs} from lymphoid tissues. Intriguingly, there were multiple examples, in the same or in different mice, of a particular CDR3 amino-acid sequence encoded by different nucleotide sequences. This finding

suggests that in adipose tissue, T_{regs} may undergo a selective pressure favoring the display of a TCR with a particular antigenic specificity. Moreover, the fact that fat T_{regs} shared almost no TCR sequences with the T_{conv} cells in fat or LN, suggested that the accumulation of Foxp3+ T_{regs} in the abdominal adipose tissue is unlikely to be due to a local conversion of T_{conv} cells. [5]

Interestingly, fat T_{regs} expressed a very high level of the anti-inflammatory cytokine, IL-10 [5]. In an *in vitro* experimental model that reproduces the main mechanism of induction of cellular insulin resistance (treatment of a cultured pre-adipocyte line with TNF-α), IL-10 could suppress markers of inflammation and restore the expression of the transporter for glucose, GLUT4 [5]. However, other mechanisms of suppression are also possible, even likely, given that they express other molecules like Granzyme-b and CTLA-4 that could be responsible for their regulatory activity [66].

The potential role of fat T_{reg} cells in modulating adipose tissue inflammation was also demonstrated via gain- and loss-of-function experiments. It is possible to expand T_{regs} *in vivo* using IL-2/anti-IL-2 complexes or, conversely, deplete them by injecting diphtheria toxin (DT) in a mouse model where *Foxp3* promoter/enhance elements drive the expression of the diphtheria toxin receptor (*Foxp3*-DTR mice) [67,68]. Both *in vivo* manipulations impacted metabolic parameters, improving (by IL-2/anti-IL-2 complexes) or compromising (by DT) their insulin sensitivity. [5] Moreover, an interesting, but indirect, indication of the involvement of T_{regs} in the control of metabolic functions came from studies performed using several animal models of obesity and insulin resistance, such as leptin-deficient mice ($Lep^{ob/ob}$), mice heterozygous for the yellow spontaneous mutation (A^y/a) and mice chronically fed a high-fat diet (HFD). In all of these models, the percentage and number of fat T_{regs} was substantially reduced

compared with that of lean controls [5,69-71]. Similar to the mouse data, a reduction in *Foxp3* transcripts was also observed in the omental vis-à-vis subcutaneous fat of obese humans [5].

However, Dosch and colleagues did not see a dramatic decrease in fat T_{reg} numbers in mice fed an HFD [42]. We attribute this difference to their use of the "Foxp3-GFP" mouse line, in which the GFP reporter is fused near the N terminus of the Foxp3 protein. Our group, as well as other laboratories, has found abnormal T_{reg} activity in these mice in the context of autoimmune and inflammatory diseases, including in the fat (unpublished data).

Why the T_{reg} fraction in abdominal adipose tissue decreases during obesity is still not clear. Even though only a few years have passed since the first report on fat T_{regs} and their role in guarding against insulin resistance, several groups have already contributed to this discussion. An interesting model to explain such a dramatic reduction in fat T_{regs} with obesity has been proposed by Matarese *et al.* [71]. Leptin is an adipokine that controls food intake and promotes the activation of T lymphocytes, enhancing the proliferation of Th1 cells and their production of pro-inflammatory cytokines [72]. Conversely, leptin inhibits the proliferation of T_{reg} cells [73,74]. The elevated levels of leptin accompanying obesity could dampen T_{reg} proliferation, perhaps explaining why their numbers decrease. But, this speculation is difficult to reconcile with the striking loss of fat T_{regs} in the leptin-deficient mouse model [5]. Alternatively, Deiuliis J. *et al*. have argued that the reduced accumulation of fat T_{regs} in obese individuals is a direct consequence of inflammatory signals produced by macrophages [6]. However, their arguments assumed that fat T_{regs} result from peripheral conversion of T_{conv} cells, for which there are few supporting data but several lines of evidence in contradiction [5]. Further, while this group suggested that peripheral T_{regs} may home to the adipose tissue through the expression of CXCR3

and CCR7, it has been reported that these chemokine receptors are down-regulated in fat T_{reg} cells [5,6].

Lastly, there seems to be an interesting parallelism in the cellular mechanisms underlying insulin resistance and atherosclerosis. T_{regs} were prominent in the atherosclerotic lesion of Apolipoprotein-E-deficient mice, a standard model, and they decreased with age [2,75]. Moreover, depletion of T_{regs} through genetic ablation of CD80/86, CD28 or ICOS, or via anti-CD25 mAb treatment, enhanced atherogenesis and lesion inflammation. Conversely, adoptive transfer of T_{regs} prevented the development of atherosclerotic plaques [3,76]. In humans, impairment in the number and function of circulating T_{regs} was observed in patients with acute coronary syndrome [77]. Interestingly, no T cells were found in normal vessel fragments while a few T_{regs} (0.5–5%) were detected in the intima during all stages of plaque development [77]. It has not been clear whether the loss/lack of T_{regs} is the cause or the effect of the atherosclerotic lesions.

4. Regulatory T cells as potential immunotherapy

As illustrated using many rodent disease models, the therapeutic potential of manipulating T_{reg} cells is enormous. T_{reg} activity can be attenuated to improve anti-tumor or anti-microbe responses [78,79], or enhanced to treat allergic, inflammatory or autoimmune diseases [80-87]. Treatment with anti-CD3 mAb is a promising approach, already in the clinic: this agent seems to evacuate T effectors cells while concomitantly enhancing the representation of T_{reg} cells [88].

Potential application of T_{regs} to therapy of T2D has only recently been explored. Winer *et al.*, using the non-mitogenic $F(ab^1)_2$ fragment of anti-CD3 mAb, described a long-term

normalizing effect on insulin resistance and glucose tolerance in mice treated with a short regime of 5 days [42]. They highlighted a correlation between the improved metabolic profile and the increase in numbers of adipose-tissue T_{regs} and anti-inflammatory macrophages.

Ilan *et al.*, on the other hand, employed oral administration of anti-CD3-mAb in combination with β-glucosylceramide (GC) [89]. GC, an intermediate of glycosphingolipid metabolism, interacts with CD1d, a molecule recognized by NK-T cells. Oral administration of GC led to decreased intra-hepatic NK-T cell numbers and amelioration of the metabolic syndrome in $Lep^{ob/ob}$ mice [90]. This protocol results in anti-CD3's uptake by the gut-associated lymphoid tissue, and the induction of CD4+CD25- T_{reg} cells expressing TGF-β1 latency-associated peptide (LAP) [91]. The suppressive capacity of such cells has been studied in several diseases, like autoimmune diabetes, experimental encephalomyelitis and systemic erythematosus lupus [91-93]. Body weight did not change after oral treatment of $Lep^{ob/ob}$ mice with anti-CD3 mAb. There was an induction of CD4+ LAP+ T cells, and a concomitant reduction of NK-T cells, in the mesenteric lymph nodes, blood and spleen, but, surprisingly, there was an increase of T_{regs}, but not of CD4+LAP+T cells, in the adipose tissue. The authors suggested that the increase in fat T_{regs} may have resulted from increased production of TGF-β by LAP-positive T cells, which correlates with a decrease in adipose tissue inflammation. Metabolic parameters were impaired by oral anti-CD3 mAb treatment: a reduction in blood-glucose, cholesterol and aspartate amino transferase levels.

Interestingly, none of effects on the metabolic and pathologic abnormalities could be recapitulated when $Lep^{ob/ob}$ mice were treated singly with either anti-CD3 or GC. The authors hypothesized that T_{regs} activation was induced through engagement of the TCR by anti-CD3, and that tolerogenic DCs were promoted by NK-T cells, further inducing T_{reg}. In conclusion, this

report made the novel suggestion that a previously described interaction between T_{regs} and NK-T cells may play an important role in controlling the inflammation associated with T2D [94]. However, transfer of the induced CD4+LAP+ (but not CD4+LAP-) T cells to *Lep*$^{ob/ob}$ mice ameliorated blood glucose and aspartate amino transferase levels and reduced levels of inflammatory mediators like IL-6, IFN-γ and IL-17 produced by stimulated splenocytes, suggesting that the main factor in controlling insulin resistance may rather be the expansion of CD4+LAP+ T cells in the periphery rather than the induction of T_{reg} cells. Finally, it would be useful to supplement these results with data on the commonly accepted measures of insulin resistance, including levels of blood insulin, the glucose tolerance test and HOMA-IR (Homeostatic Model Insulin Resistance) to better situate them in the context of T2D in humans and animal models.

To date, approaches to T2D therapy have been focused on sulfonylureas, biguanides, and thiazolidinediones. However, the aforementioned studies all support the idea that T_{regs} and their products may represent novel targets. Similarly, the use of anti-CD3 mAb has been proposed to explore the potential beneficial effect of *in vivo* T_{reg} induction in the context of atherosclerosis. Preventive therapy with anti-CD3 reduced plaque development and its therapeutic use decreased lesion progression in mice with established atherosclerosis. [95]

Cellular therapy based on the administration of *ex vivo* expanded T_{regs} is currently the object of extensive research for the treatment of autoimmune and graft-versus-host disease. However, several issues must be addressed before their clinical use. First of all, large numbers of customized T_{regs} will be needed for transfer into humans. Presently, much effort is directed at developing protocols to achieve their *ex-vivo* expansion and to ensure the maintenance of their phenotypic and functional purity. Moreover, the possibility for contamination of T_{reg}

preparations by effector T cells must be carefully addressed because this could result in exacerbation of inflammatory responses on adoptive transfer. Achieving the goal of a pure preparation of patient-derived T_{reg} cells will require the identification of a more specific surface marker of natural T_{regs} or, as proposed by Battaglia *et.al*., the use of drugs like rapamycin which appear to induce T_{regs} while blocking the proliferation of effector T cells. [96]

In conclusion, T_{reg} numbers, purity, diversity, allo-reactivity and antigen specificity are all factors that render their translation from the bench to the bedside very difficult. The identification/design of compounds that can selectively expand T_{reg} cells *in vivo* would be an elegant solution to the aforementioned concerns. Given our recent appreciation of T_{reg} heterogeneity based on anatomic location and physiologic functions, this goal is simultaneously made more complex and more attractive with regard to the specificity of targets.

5. Concluding remarks and future prospects

Inflammation is a critical link between obesity and T2D. Recent studies have painted a picture in which the inflammation of adipose tissue is correlated with alterations in the balance between different T cell populations. In lean mice, production of IL-10 by regulatory T cells and anti-inflammatory macrophages ensures the maintenance of adipocyte insulin sensitivity. Conversely, in obese mice, the equilibrium is shifted in the direction of CD8+ T cells and pro-inflammatory macrophages (Fig.2).

Finding a unique population of regulatory T lymphocytes in the abdominal adipose tissue of lean, but not obese, mice gives rise to many interesting questions. First, where do fat T_{regs} come from? When and where do they acquire their unique phenotype? Why and how do they accumulate in the visceral fat with age? Do they respond to an antigen or antigens and, if so,

what is it? What chemokine/chemokine receptors are necessary for their homing to/retention in the fat? What precise functions do they perform in the adipose tissue? By what mechanisms? Do they operate directly on adipocytes, on macrophages or both? Why do T_{regs} evacuate obese adipose tissue?

Studies aimed to address these questions ultimately may result in treatments to modulate fat T_{reg} cell numbers and function in order to address the inflammation of the adipose tissue that promotes the metabolic syndrome.

Acknowledgements:

We would like to thank the National Science Foundation for providing DK with a Graduate Research Fellowship.

References

[1] Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell 2008; 133:775-87.

[2] Mor A, Planer D, Luboshits G, Afek A, Metzger S, Chajek-Shaul T ,et al. Role of naturally occurring CD4+ CD25+ regulatory T cells in experimental atherosclerosis. Arterioscler Thromb Vasc Biol 2007; 27:893-900.

[3] Ait-Oufella H, Salomon BL, Potteaux S, Robertson AK, Gourdy P, Zoll J ,et al. Natural regulatory T cells control the development of atherosclerosis in mice. Nat Med 2006; 12:178-80.

[4] Xie JJ, Wang J, Tang TT, Chen J, Gao XL, Yuan J ,et al. The Th17/Treg functional imbalance during atherogenesis in ApoE(-/-) mice. Cytokine 2010; 49:185-93.

[5] Feuerer M, Herrero L, Cipolletta D, Naaz A, Wong J, Nayer A ,et al. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat Med 2009; 15:930-9.

[6] Deiuliis J, Shah Z, Shah N, Needleman B, Mikami D, Narula V ,et al. Visceral adipose inflammation in obesity is associated with critical alterations in tregulatory cell numbers. PLoS ONE 2011; 6:e16376.

[7] Center for Disease Control and Prevention. 2011 National Diabetes Fact Sheet 2011.

[8] Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Invest 2006; 116:1793-801.

[9] Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006; 444:860-7.

[10] Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993; 259:87-91.

[11] Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol 2011; 11:85-97.

[12] Jager J, Gremeaux T, Cormont M, Le Marchand-Brustel Y, Tanti JF. Interleukin-1beta-induced insulin resistance in adipocytes through down-regulation of insulin receptor substrate-1 expression. Endocrinology 2007; 148:241-51.

[13] Rotter V, Nagaev I, Smith U. Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-alpha, overexpressed in human fat cells from insulin-resistant subjects. J Biol Chem 2003; 278:45777-84.

[14] Han CY, Subramanian S, Chan CK, Omer M, Chiba T, Wight TN ,et al. Adipocyte-derived serum amyloid A3 and hyaluronan play a role in monocyte recruitment and adhesion. Diabetes 2007; 56:2260-73.

[15] Kamei N, Tobe K, Suzuki R, Ohsugi M, Watanabe T, Kubota N ,et al. Overexpression of monocyte chemoattractant protein-1 in adipose tissues causes macrophage recruitment and insulin resistance. J Biol Chem 2006; 281:26602-14.

[16] Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E ,et al. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science 2004; 306:457-61.

[17] Halberg N, Khan T, Trujillo ME, Wernstedt-Asterholm I, Attie AD, Sherwani S ,et al. Hypoxia-inducible factor 1alpha induces fibrosis and insulin resistance in white adipose tissue. Mol Cell Biol 2009; 29:4467-83.

[18] Minamino T, Orimo M, Shimizu I, Kunieda T, Yokoyama M, Ito T ,et al. A crucial role for adipose tissue p53 in the regulation of insulin resistance. Nat Med 2009; 15:1082-7.

[19] Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M ,et al. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. Science 2001; 293:1673-7.

[20] Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K ,et al. A central role for JNK in obesity and insulin resistance. Nature 2002; 420:333-6.

[21] Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL ,et al. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Science 1999; 283:1544-8.

[22] Feinstein R, Kanety H, Papa MZ, Lunenfeld B, Karasik A. Tumor necrosis factor-alpha suppresses insulin-induced tyrosine phosphorylation of insulin receptor and its substrates. J Biol Chem 1993; 268:26055-8.

[23] Elgazar-Carmon V, Rudich A, Hadad N, Levy R. Neutrophils transiently infiltrate intra-abdominal fat early in the course of high-fat feeding. J Lipid Res 2008; 49:1894-903.

[24] Liu J, Divoux A, Sun J, Zhang J, Clement K, Glickman JN ,et al. Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. Nat Med 2009; 15:940-5.

[25] Cinti S, Mitchell G, Barbatelli G, Murano I, Ceresi E, Faloia E ,et al. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. J Lipid Res 2005; 46:2347-55.

[26] 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; 112:1796-808.

[27] Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nat Rev Immunol 2008; 8:958-69.

[28] Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, Morel CR, Subramanian V, Mukundan L ,et al. Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. Nature 2007; 447:1116-20.

[29] Fujisaka S, Usui I, Bukhari A, Ikutani M, Oya T, Kanatani Y ,et al. Regulatory mechanisms for adipose tissue M1 and M2 macrophages in diet-induced obese mice. Diabetes 2009; 58:2574-82.

[30] Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 2007; 117:175-84.

[31] Lumeng CN, Deyoung SM, Bodzin JL, Saltiel AR. Increased inflammatory properties of adipose tissue macrophages recruited during diet-induced obesity. Diabetes 2007; 56:16-23.

[32] 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; 59:1171-81.

[33] Herrero L, Shapiro H, Nayer A, Lee J, Shoelson SE. Inflammation and adipose tissue macrophages in lipodystrophic mice. Proc Natl Acad Sci U S A 2010; 107:240-5.

[34] Vandanmagsar B, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL ,et al. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nat Med 2011; 17:179-88.

[35] Schroder K, Tschopp J. The inflammasomes. Cell 2010; 140:821-32.

[36] Duewell P, Kono H, Rayner KJ, Sirois CM, Vladimer G, Bauernfeind FG ,et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. Nature 2010; 464:1357-61.

[37] Rajamaki K, Lappalainen J, Oorni K, Valimaki E, Matikainen S, Kovanen PT ,et al. Cholesterol crystals activate the NLRP3 inflammasome in human macrophages: a novel link between cholesterol metabolism and inflammation. PLoS ONE 2010; 5:e11765.

[38] Nguyen MT, Favelyukis S, Nguyen AK, Reichart D, Scott PA, Jenn A ,et al. A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids via Toll-like receptors 2 and 4 and JNK-dependent pathways. J Biol Chem 2007; 282:35279-92.

[39] Kintscher U, Hartge M, Hess K, Foryst-Ludwig A, Clemenz M, Wabitsch M ,et al. T-lymphocyte infiltration in visceral adipose tissue: a primary event in adipose tissue inflammation and the development of obesity-mediated insulin resistance. Arterioscler Thromb Vasc Biol 2008; 28:1304-10.

[40] Nishimura S, Manabe I, Nagasaki M, Eto K, Yamashita H, Ohsugi M ,et al. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nat Med 2009; 15:914-20.

[41] Yang H, Youm YH, Vandanmagsar B, Ravussin A, Gimble JM, Greenway F ,et al. Obesity increases the production of proinflammatory mediators from adipose tissue T cells and compromises TCR repertoire diversity: implications for systemic inflammation and insulin resistance. J Immunol 2010; 185:1836-45.

[42] Winer S, Chan Y, Paltser G, Truong D, Tsui H, Bahrami J ,et al. Normalization of obesity-associated insulin resistance through immunotherapy. Nat Med 2009; 15:921-9.

[43] Strissel KJ, DeFuria J, Shaul ME, Bennett G, Greenberg AS, Obin MS. T-cell recruitment and Th1 polarization in adipose tissue during diet-induced obesity in C57BL/6 mice. Obesity (Silver Spring) 2010; 18:1918-25.

[44] Rocha VZ, Folco EJ, Sukhova G, Shimizu K, Gotsman I, Vernon AH ,et al. Interferon-gamma, a Th1 cytokine, regulates fat inflammation: a role for adaptive immunity in obesity. Circ Res 2008; 103:467-76.

[45] Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. Nature 1998; 394:897-901.

[46] Wang Y, Souabni A, Flavell RA, Wan YY. An intrinsic mechanism predisposes Foxp3-expressing regulatory T cells to Th2 conversion in vivo. J Immunol 2010; 185:5983-92.

[47] Mombaerts P, Iacomini J, Johnson RS, Herrup K, Tonegawa S, Papaioannou VE. RAG-1-deficient mice have no mature B and T lymphocytes. Cell 1992; 68:869-77.

[48] Powrie F, Leach MW, Mauze S, Menon S, Caddle LB, Coffman RL. Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RBhi CD4+ T cells. Immunity 1994; 1:553-62.

[49] Davidson NJ, Leach MW, Fort MM, Thompson-Snipes L, Kuhn R, Muller W ,et al. T helper cell 1-type CD4+ T cells, but not B cells, mediate colitis in interleukin 10-deficient mice. J Exp Med 1996; 184:241-51.

[50] Feng T, Wang L, Schoeb TR, Elson CO, Cong Y. Microbiota innate stimulation is a prerequisite for T cell spontaneous proliferation and induction of experimental colitis. J Exp Med 2010; 207:1321-32.

[51] Rausch ME, Weisberg S, Vardhana P, Tortoriello DV. Obesity in C57BL/6J mice is characterized by adipose tissue hypoxia and cytotoxic T-cell infiltration. Int J Obes (Lond) 2008; 32:451-63.

[52] Duffaut C, Galitzky J, Lafontan M, Bouloumie A. Unexpected trafficking of immune cells within the adipose tissue during the onset of obesity. Biochem Biophys Res Commun 2009; 384:482-5.

[53] Winer DA, Winer S, Shen L, Wadia PP, Yantha J, Paltser G ,et al. B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. Nat Med 2011; 17:610-7.

[54] Ngo VN, Cornall RJ, Cyster JG. Splenic T zone development is B cell dependent. J Exp Med 2001; 194:1649-60.

[55] Baumgarth N, Jager GC, Herman OC, Herzenberg LA. CD4+ T cells derived from B cell-deficient mice inhibit the establishment of peripheral B cell pools. Proc Natl Acad Sci U S A 2000; 97:4766-71.

[56] Linton PJ, Harbertson J, Bradley LM. A critical role for B cells in the development of memory CD4 cells. J Immunol 2000; 165:5558-65.

[57] Hill JA, Feuerer M, Tash K, Haxhinasto S, Perez J, Melamed R ,et al. Foxp3 transcription-factor-dependent and -independent regulation of the regulatory T cell transcriptional signature. Immunity 2007; 27:786-800.

[58] Feuerer M, Hill JA, Kretschmer K, von Boehmer H, Mathis D, Benoist C. Genomic definition of multiple ex vivo regulatory T cell subphenotypes. Proc Natl Acad Sci U S A 2010; 107:5919-24.

[59] Lin W, Haribhai D, Relland LM, Truong N, Carlson MR, Williams CB ,et al. Regulatory T cell development in the absence of functional Foxp3. Nat Immunol 2007; 8:359-68.

[60] Gavin MA, Rasmussen JP, Fontenot JD, Vasta V, Manganiello VC, Beavo JA ,et al. Foxp3-dependent programme of regulatory T-cell differentiation. Nature 2007; 445:771-5.

[61] Feuerer M, Hill JA, Mathis D, Benoist C. Foxp3+ regulatory T cells: differentiation, specification, subphenotypes. Nat Immunol 2009; 10:689-95.

[62] Zheng Y, Chaudhry A, Kas A, Deroos P, Kim JM, Chu TT ,et al. Regulatory T-cell suppressor program co-opts transcription factor IRF4 to control T(H)2 responses. Nature 2009; 458:351-6.

[63] Chaudhry A, Rudra D, Treuting P, Samstein RM, Liang Y, Kas A ,et al. CD4+ regulatory T cells control TH17 responses in a Stat3-dependent manner. Science 2009; 326:986-91.

[64] Koch MA, Tucker-Heard G, Perdue NR, Killebrew JR, Urdahl KB, Campbell DJ. The transcription factor T-bet controls regulatory T cell homeostasis and function during type 1 inflammation. Nat Immunol 2009; 10:595-602.

[65] Tran TT, Yamamoto Y, Gesta S, Kahn CR. Beneficial effects of subcutaneous fat transplantation on metabolism. Cell Metab 2008; 7:410-20.

[66] Vignali DA, Collison LW, Workman CJ. How regulatory T cells work. Nat Rev Immunol 2008; 8:523-32.

[67] Thorburn J, Frankel AE, Thorburn A. Apoptosis by leukemia cell-targeted diphtheria toxin occurs via receptor-independent activation of Fas-associated death domain protein. Clin Cancer Res 2003; 9:861-5.

[68] Boyman O, Kovar M, Rubinstein MP, Surh CD, Sprent J. Selective stimulation of T cell subsets with antibody-cytokine immune complexes. Science 2006; 311:1924-7.

[69] Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T ,et al. Effects of the obese gene product on body weight regulation in ob/ob mice. Science 1995; 269:540-3.

[70] Klebig ML, Wilkinson JE, Geisler JG, Woychik RP. Ectopic expression of the agouti gene in transgenic mice causes obesity, features of type II diabetes, and yellow fur. Proc Natl Acad Sci U S A 1995; 92:4728-32.

[71] Matarese G, Procaccini C, De R, V, Horvath TL, La CA. Regulatory T cells in obesity: the leptin connection. Trends Mol Med 2010; 16:247-56.

[72] Schwartz MW, Woods SC, Porte D, Jr., Seeley RJ, Baskin DG. Central nervous system control of food intake. Nature 2000; 404:661-71.

[73] Matarese G, Di GA, Sanna V, Lord GM, Howard JK, Di TA ,et al. Requirement for leptin in the induction and progression of autoimmune encephalomyelitis. J Immunol 2001; 166:5909-16.

[74] De R, V, Procaccini C, Cali G, Pirozzi G, Fontana S, Zappacosta S ,et al. A Key Role of Leptin in the Control of Regulatory T Cell Proliferation. Immunity 2007; 26:241-55.

[75] Wigren M, Bengtsson D, Duner P, Olofsson K, Bjorkbacka H, Bengtsson E ,et al. Atheroprotective effects of Alum are associated with capture of oxidized LDL antigens and activation of regulatory T cells. Circ Res 2009; 104:e62-e70.

[76] Gotsman I, Grabie N, Gupta R, Dacosta R, MacConmara M, Lederer J ,et al. Impaired regulatory T-cell response and enhanced atherosclerosis in the absence of inducible costimulatory molecule. Circulation 2006; 114:2047-55.

[77] de Boer OJ, van der Meer JJ, Teeling P, van der Loos CM, van der Wal AC. Low numbers of FOXP3 positive regulatory T cells are present in all developmental stages of human atherosclerotic lesions. PLoS ONE 2007; 2:e779.

[78] Baecher-Allan C, Anderson DE. Regulatory cells and human cancer. Semin Cancer Biol 2006; 16:98-105.

[79] Belkaid Y, Rouse BT. Natural regulatory T cells in infectious disease. Nat Immunol 2005; 6:353-60.

[80] Miyara M, Amoura Z, Parizot C, Badoual C, Dorgham K, Trad S ,et al. Global natural regulatory T cell depletion in active systemic lupus erythematosus. J Immunol 2005; 175:8392-400.

[81] Baecher-Allan C, Viglietta V, Hafler DA. Human CD4+CD25+ regulatory T cells. Semin Immunol 2004; 16:89-98.

[82] Taylor PA, Noelle RJ, Blazar BR. CD4(+)CD25(+) immune regulatory cells are required for induction of tolerance to alloantigen via costimulatory blockade. J Exp Med 2001; 193:1311-8.

[83] Borish L, Aarons A, Rumbyrt J, Cvietusa P, Negri J, Wenzel S. Interleukin-10 regulation in normal subjects and patients with asthma. J Allergy Clin Immunol 1996; 97:1288-96.

[84] Viglietta V, Baecher-Allan C, Weiner HL, Hafler DA. Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. J Exp Med 2004; 199:971-9.

[85] Ehrenstein MR, Evans JG, Singh A, Moore S, Warnes G, Isenberg DA ,et al. Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNFalpha therapy. J Exp Med 2004; 200:277-85.

[86] Tang Q, Henriksen KJ, Bi M, Finger EB, Szot G, Ye J ,et al. In Vitro-expanded Antigen-specific Regulatory T Cells Suppress Autoimmune Diabetes. J Exp Med 2004; 199:1455-65.

[87] Tarbell KV, Yamazaki S, Olson K, Toy P, Steinman RM. CD25+ CD4+ T Cells, Expanded with Dendritic Cells Presenting a Single Autoantigenic Peptide, Suppress Autoimmune Diabetes. J Exp Med 2004; 199:1467-77.

[88] Chatenoud L, Bluestone JA. CD3-specific antibodies: a portal to the treatment of autoimmunity. Nat Rev Immunol 2007; 7:622-32.

[89] Ilan Y, Maron R, Tukpah AM, Maioli TU, Murugaiyan G, Yang K ,et al. Induction of regulatory T cells decreases adipose inflammation and alleviates insulin resistance in ob/ob mice. Proc Natl Acad Sci U S A 2010; 107:9765-70.

[90] Margalit M, Shalev Z, Pappo O, Sklair-Levy M, Alper R, Gomori M ,et al. Glucocerebroside ameliorates the metabolic syndrome in OB/OB mice. J Pharmacol Exp Ther 2006; 319:105-10.

[91] Ochi H, Abraham M, Ishikawa H, Frenkel D, Yang K, Basso AS ,et al. Oral CD3-specific antibody suppresses autoimmune encephalomyelitis by inducing CD4+. Nat Med 2006; 12:627-35.

[92] Ishikawa H, Ochi H, Chen ML, Frenkel D, Maron R, Weiner HL. Inhibition of autoimmune diabetes by oral administration of anti-CD3 monoclonal antibody. Diabetes 2007; 56:2103-9.

[93] Wu HY, Center EM, Tsokos GC, Weiner HL. Suppression of murine SLE by oral anti-CD3: inducible CD4+CD25-LAP+ regulatory T cells control the expansion of IL-17+ follicular helper T cells. Lupus 2009; 18:586-96.

[94] La CA, Van KL, Fu DS. CD4+CD25+ Tregs and NKT cells: regulators regulating regulators. Trends Immunol 2006; 27:322-7.

[95] Steffens S, Burger F, Pelli G, Dean Y, Elson G, Kosco-Vilbois M ,et al. Short-term treatment with anti-CD3 antibody reduces the development and progression of atherosclerosis in mice. Circulation 2006; 114:1977-84.

[96] Battaglia M, Stabilini A, Roncarolo MG. Rapamycin selectively expands CD4+CD25+FoxP3+ regulatory T cells. Blood 2005; 105:4743-8.

Figure legends

Figure 1. Obesity results in systemic, chronic, low-grade inflammation and insulin resistance. Excess weight gain leads to necrotic and apoptotic death of adipocytes, thereby activating various inflammatory responses such TLR and inflammasome activation, release of reactive oxygen species (ROS), and endoplasmic reticulum (ER) stress. In response to these inflammatory stimuli, adipocytes and infiltrating leukocytes release a variety of pro-inflammatory mediators that act locally in adipose tissue and distally in liver, muscle, and kidneys to inactivate insulin receptor substrates (IRS), leading to insulin resistance and persistent high blood glucose.

Figure 2. **Cellular and metabolic alterations in adipose tissue during obesity**. In lean mice, the abdominal adipose tissue hosts anti-inflammatory macrophages, as well as an elevated fraction of T_{regs}. In obese mice, by contrast, there is a switch in cellular equilibrium: more CD8+ than Th1 CD4+ T cells, fewer regulatory T cells and a preponderance of pro-inflammatory macrophages. These effector cells promote inflammation and exacerbate adipose tissue dysfunction through the production of inflammatory cytokines.

Figure 1

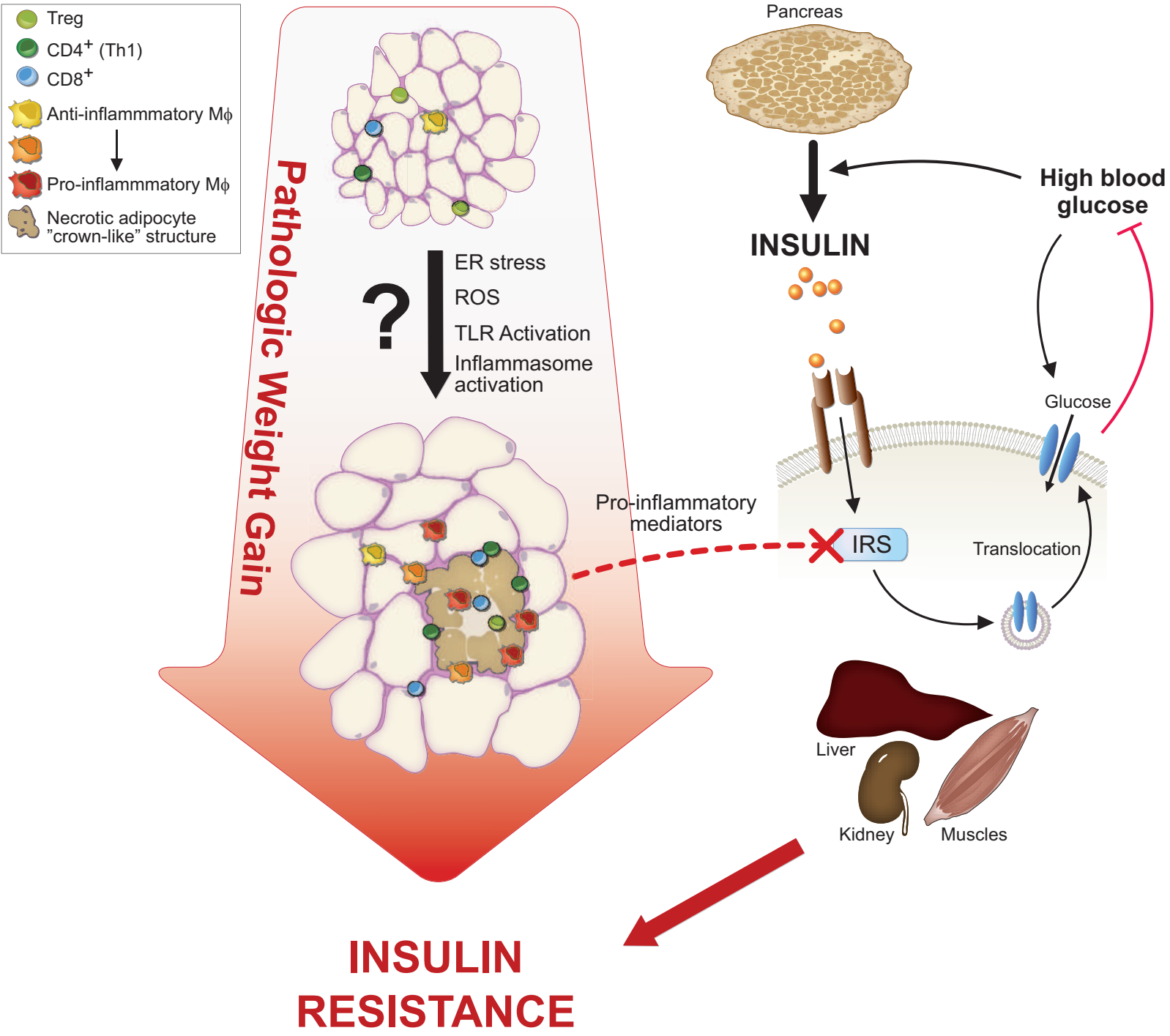
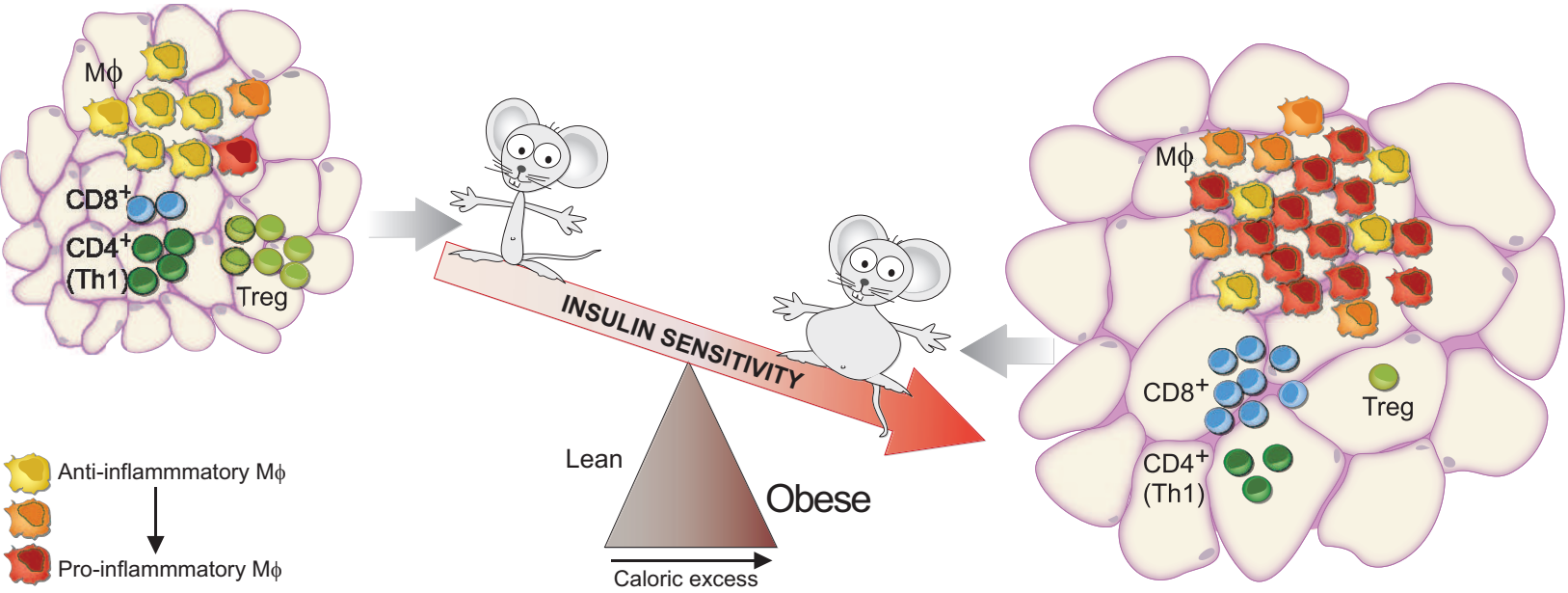

Treg
CD4+ (Th1)
CD8+
Anti-inflammmatory Mϕ
Pro-inflammmatory Mϕ
Necrotic adipocyte
"crown-like" structure
Pathologic Weight Gain
?
ER stress
ROS
TLR Activation
Inflammasome activation
Pro-inflammatory mediators
Pancreas
INSULIN
High blood glucose
Glucose
IRS
Translocation
Liver
Kidney
Muscles
INSULIN RESISTANCE

Figure 2


Mϕ
CD8+
CD4+
(Th1)
Treg
INSULIN SENSITIVITY
Lean
Obese
Caloric excess
Mϕ
CD8+
Treg
CD4+
(Th1)
Anti-inflammmatory Mϕ
Pro-inflammmatory Mϕ