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## Review

# Tissular T<sub>regs</sub>: A unique population of adipose-tissue-resident Foxp3+CD4+ T cells that impacts organismal metabolism

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## HIGHLIGHTS

- Inflammation is the link between obesity and type 2 diabetes.
- Innate and adaptive immune systems are implicated in obesity-induced pathology.
- Adipose tissue-resident regulatory T cells control metabolic parameters.

## ARTICLE INFO

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## ABSTRACT

Foxp3+CD4+ regulatory T (T<sub>reg</sub>) 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<sub>regs</sub>, and the potential of T<sub>reg</sub>-based therapies for treatment of the metabolic syndrome.

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## 1. Introduction

In recent years, regulatory T (T<sub>reg</sub>) 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<sub>regs</sub> 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- $\alpha$  (TNF- $\alpha$ ) mRNA in adipocytes in visceral adipose tissue (epididymal, 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-1 $\beta$  (IL-1 $\beta$ ) [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

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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- $\kappa$ B [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 the adaptive immune systems to obesity-associated pathologies, highlight the protective role of fat-resident T<sub>reg</sub> 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 3 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, Liu et al. 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- $\gamma$  (IFN- $\gamma$ ) 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- $\beta$  (TGF- $\beta$ ) [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- $\alpha$ , IL-6, matrix metalloproteinases, and peroxisome proliferator activated receptor- $\gamma$  (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-1 $\beta$  and initiation of a pro-inflammatory immune

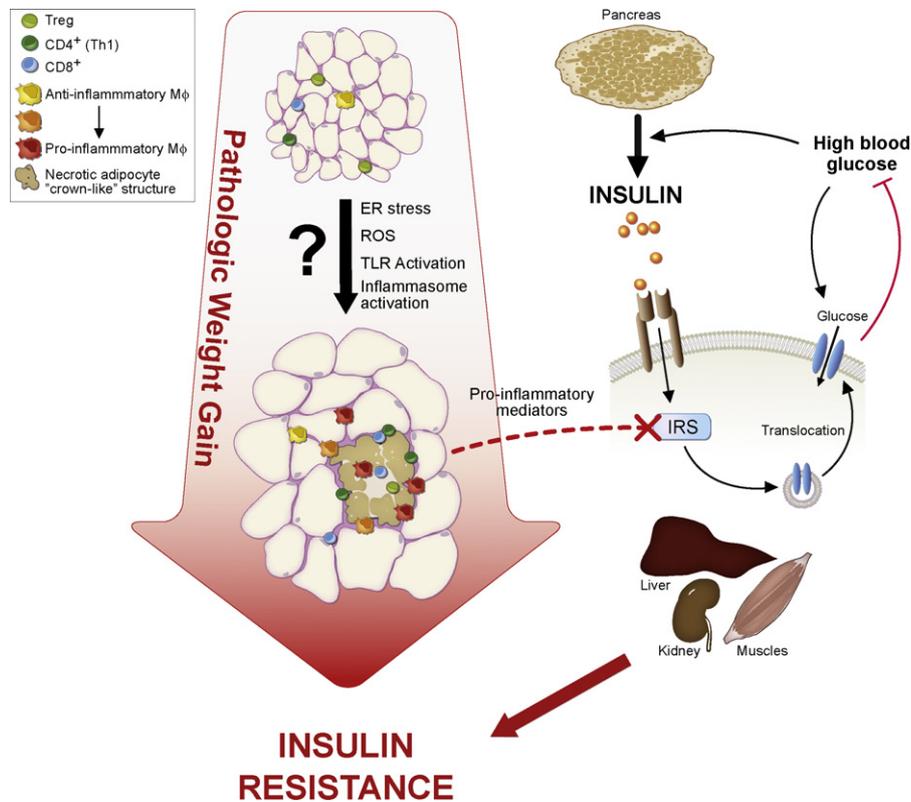
response (reviewed in [35]). Previous studies have demonstrated that cholesterol or similar crystals cause Nlrp3 inflammasome activation in macrophages found in atherosclerotic lesions [36,37]. By analogy, one might speculate that HFD leads to formation of cholesterol crystals activate the Nlrp3 inflammasome, 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 (Fig. 1) [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- $\alpha$ , 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- $\gamma$  [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- $\gamma$  expression can enhance the accumulation of M1 macrophages in obese fat, accompanied by elevated expression of TNF $\alpha$ , 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 IFN $\gamma$  [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].



**Fig. 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 as 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.

One report has proposed a protective role for effector CD4<sup>+</sup> T cells in obesity-induced insulin resistance [42]. CD4<sup>+</sup> 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<sub>reg</sub> cells. Transfer of CD4<sup>+</sup> T cell populations depleted of Foxp3<sup>+</sup> T<sub>regs</sub> or CD4<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> T cells [49] or OT2 TCR-transgenic CD4<sup>+</sup> 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<sup>+</sup> T cells was a result of their Th2 polarization by showing that transferred CD4<sup>+</sup> T cells produced IL-4 and IL-13 following restimulation and that there was a lower frequency of Gata3<sup>+</sup> T cells in visceral adipose tissue of obese compared with lean animals. Under lymphopenic conditions, CD4<sup>+</sup> 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<sup>+</sup> T cells from a lymphopenic host may produce IL-4 and IL-13, as seen in the paper, but they would also produce IFN $\gamma$ , which was not measured. Furthermore, it was recently shown that T<sub>regs</sub> can express high levels of Gata-3

[46]. Therefore, it is possible that the loss of Gata3<sup>+</sup>CD4<sup>+</sup> T cells from abdominal adipose tissue following HFD is actually due to loss of T<sub>reg</sub> cells. Thus, the root of the observed protection bears re-examination.

There are substantial contradictions among the aforementioned studies on the contributions of CD4<sup>+</sup> and CD8<sup>+</sup> T cells to obesity-associated inflammation and insulin resistance. Several groups reported an increase in the fraction of CD8<sup>+</sup> 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<sup>+</sup> 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 3 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<sub>reg</sub> cells in obesity-induced T2D

Foxp3+ T<sub>reg</sub> cells are important regulators of essentially every category of immune response. While Foxp3 is a critical orchestrator of the T<sub>reg</sub> 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<sub>conv</sub>) 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<sub>conv</sub> cells with Foxp3 can only partially recapitulate the canonical “T<sub>reg</sub> signature” [57,58]. Furthermore, Foxp3 is not necessary for expression of all of the suppressive activities characteristic of T<sub>reg</sub> cells. Disruption of the Foxp3 gene in mice by insertion of the green fluorescent protein (GFP) results in the generation of so-called T<sub>reg</sub> “wannabes,” cells expressing a number of the canonical T<sub>reg</sub> markers (such as CTLA4, ICOS, IL-2ra), but lacking any suppressive function [59,60]. These findings suggest that the regulatory activities of T<sub>regs</sub> are subject to a level of regulation upstream of Foxp3. Finally, the gene-expression profiles of different subtypes of Foxp3+ cells (thymus-derived T<sub>regs</sub>, splenic, converted, etc.) [57,58,61] coupled with findings on transcription factor knockout mice [62–64], have revealed that T<sub>reg</sub> cells come in “different flavors”, determined by distinct origins and anatomic locations.

So far, the most striking example of a distinct subset of T<sub>reg</sub> 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<sub>regs</sub> 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<sub>reg</sub> 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<sub>reg</sub> 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<sub>regs</sub>); and CCL5 and CXCR3 (under-represented in fat T<sub>regs</sub>). In addition, the repertoire of TCRs displayed by adipose T<sub>regs</sub> was distinct from that of fat T<sub>convs</sub> as well as T<sub>reg</sub> and T<sub>convs</sub> 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<sub>regs</sub> may undergo a selective pressure favoring the display of a TCR with a particular antigenic specificity. Moreover, the fact that fat T<sub>regs</sub> shared almost no TCR sequences with the T<sub>conv</sub> cells in fat or LN, suggested that the accumulation of Foxp3+ T<sub>regs</sub> in the abdominal adipose tissue is unlikely to be due to a local conversion of T<sub>conv</sub> cells [5].

Interestingly, fat T<sub>regs</sub> 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<sub>reg</sub> cells in modulating adipose tissue inflammation was also demonstrated via gain- and loss-of-function experiments. It is possible to expand T<sub>regs</sub> *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<sub>regs</sub> 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<sup>ob/ob</sup>*), mice heterozygous for the yellow spontaneous mutation (*A<sup>y/a</sup>*) and mice chronically fed a high-fat diet (HFD). In all of these models, the percentage and number of fat T<sub>regs</sub> 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, Winer et al. did not see a dramatic decrease in fat T<sub>reg</sub> 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<sub>reg</sub> activity in these mice in the context of autoimmune and inflammatory diseases, including in the fat (unpublished data).

Why the T<sub>reg</sub> 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<sub>regs</sub> 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<sub>regs</sub> 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<sub>reg</sub> cells [73,74]. The elevated levels of leptin accompanying obesity could dampen T<sub>reg</sub> proliferation, perhaps explaining why their numbers decrease. But, this speculation is difficult to reconcile with the striking loss of fat T<sub>regs</sub> in the leptin-deficient mouse model [5]. Alternatively, Deiluiis et al. have argued that the reduced accumulation of fat T<sub>regs</sub> in obese individuals is a direct consequence of inflammatory signals produced by macrophages [6]. However, their arguments assumed that fat T<sub>regs</sub> result from peripheral conversion of T<sub>conv</sub> cells, for which there are few supporting data but several lines of evidence in contradiction [5]. Further, while this group suggested that peripheral T<sub>regs</sub> 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<sub>reg</sub> cells [5,6].

Lastly, there seems to be an interesting parallelism in the cellular mechanisms underlying insulin resistance and atherosclerosis. T<sub>regs</sub> 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<sub>regs</sub> 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<sub>regs</sub> prevented the development of atherosclerotic plaques [3,76]. In humans, impairment in the number and function of circulating T<sub>regs</sub> was observed in patients with acute coronary syndrome [77]. Interestingly, no T cells were found in normal vessel fragments while a few T<sub>regs</sub> (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  $T_{reg}$ s 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 effector cells while concomitantly enhancing the representation of  $T_{reg}$  cells [88].

Potential application of  $T_{reg}$ s to therapy of T2D has only recently been explored. Winer et al., using the non-mitogenic  $F(ab)_2$  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  $T_{reg}$ s and anti-inflammatory macrophages.

Ilan et al., on the other hand, employed oral administration of anti-CD3-mAb in combination with  $\beta$ -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-CD3s uptake by the gut-associated lymphoid tissue, and the induction of  $CD4+CD25- T_{reg}$  cells expressing TGF- $\beta$ 1 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  $T_{reg}$ s, but not of  $CD4+LAP+ T$  cells, in the adipose tissue. The authors suggested that the increase in fat  $T_{reg}$ s may have resulted from increased production of TGF- $\beta$  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.

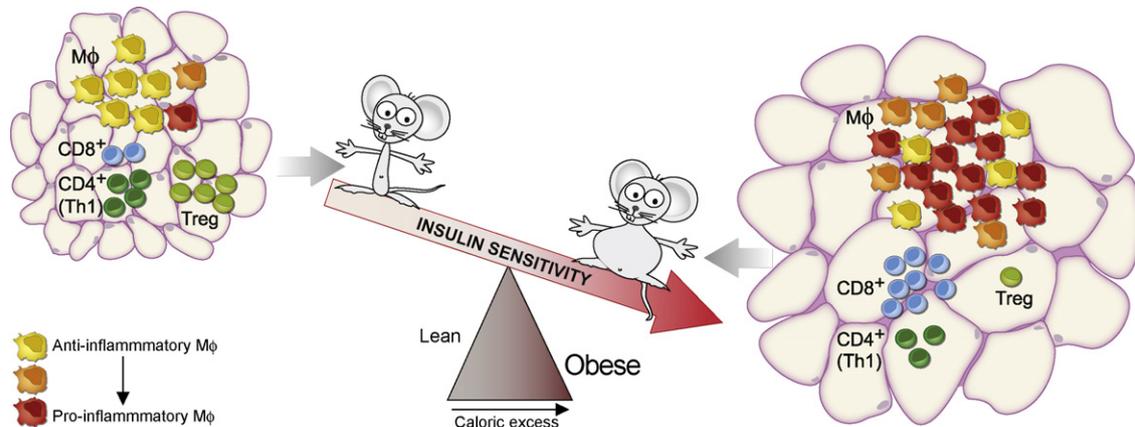
Interestingly, none of effects on the metabolic and pathologic abnormalities could be recapitulated when  $Lep^{ob/ob}$  mice were treated singly with either anti-CD3 or GC. The authors hypothe-

sized that  $T_{reg}$ s 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  $T_{reg}$ s 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- $\gamma$  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  $T_{reg}$ s 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  $T_{reg}$ s 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  $T_{reg}$ s 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  $T_{reg}$ s or, as proposed by Battaglia et al., the use of drugs like rapamycin which appear to induce  $T_{reg}$ s 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



**Fig. 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  $T_{reg}$ s. 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.

be an elegant solution to the aforementioned concerns. Given our recent appreciation of T<sub>reg</sub> 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<sub>regs</sub> 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<sub>regs</sub> evacuate obese adipose tissue?

Studies aimed to address these questions ultimately may result in treatments to modulate fat T<sub>reg</sub> cell numbers and function in order to address the inflammation of the adipose tissue that promotes the metabolic syndrome.

## Conflict of interest statement

The authors declare no conflict of interest.

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## 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] Deiliiis J, Shah Z, Shah N, Needleman B, Mikami D, Narula V, et al. Visceral adipose inflammation in obesity is associated with critical alterations in regulatory 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, Gremaux T, Cormont M, Le Marchand-Brustel Y, Tanti JF. Interleukin-1 $\beta$ -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 1 $\alpha$  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 I $\kappa$ B $\beta$ . *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 Jr AW. 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 PPAR $\gamma$  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] Dweil 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, Shaub 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] Pellemounter 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, Proccaccini C, De RV, 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 Jr D, 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 RV, Proccaccini 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:e62–70.
- [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, Rumblyrt 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. Glucocorticoid 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.