Cell Metabolism

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Perspective

Niche-specific control of tissue function by regulatory T cells—Current challenges and perspectives for targeting metabolic disease

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https://doi.org/10.1016/j.cmet.2023.12.019

SUMMARY

Tissue regulatory T cells (Tregs) exert pivotal functions in both immune and metabolic regulation, maintaining local tissue homeostasis, integrity, and function. Accordingly, Tregs play a crucial role in controlling obesity-induced inflammation and supporting efficient muscle function and repair. Depending on the tissue context, Tregs are characterized by unique transcriptomes, growth, and survival factors and T cell receptor (TCR) repertoires. This functional specialization offers the potential to selectively target context-specific Treg populations, tailoring therapeutic strategies to specific niches, thereby minimizing potential side effects. Here, we discuss challenges and perspectives for niche-specific Treg targeting, which holds promise for highly efficient and precise medical interventions to combat metabolic disease.

THE CHALLENGE OF METABOLIC DISEASES

Metabolic diseases such as type 2 diabetes (T2D) are advancing to one of the most significant global health challenges of the 21st century, placing a significant burden on individuals, healthcare systems, and economies worldwide. One of the major contributors to the development of T2D is obesity, with the disease risk rising directly in proportion to an increase in body mass index (BMI). Accordingly, the global rise of T2D can be directly attributed to an increase in the prevalence of obesity. Obesity results from an imbalance between energy intake and expenditure, usually caused by physical inactivity and overnutrition, and is clearly influenced by genetic predisposition. Metabolic disorders, such as obesity and T2D, are associated with specific alterations in metabolic tissues, including chronic low-grade inflammation within fat depots. Exercise not only positively affects the balance between energy intake and expenditure, but it can also contribute to the resolution of local inflammation, such as during obesity. Recent insights into the close interaction between immune cells and metabolic tissues highlight the need for in-depth research to address fundamental questions about how to target this niche-specific immune-tissue crosstalk selectively and effectively. The vision is to pave the way for precision medicines that can not only interfere with, e.g., obesity-induced inflammation and its detrimental consequences as observed in metabolic diseases, but also improve non-classical immune-based control of tissue function, integrity, and regeneration by so-called regulatory T cells (Tregs).

CHARACTERISTICS AND FUNCTIONS OF TREGS IN IMMUNE REGULATION

A specialized immune cell subset, $CD4^+$ Tregs, expresses the master transcription factor Foxp3 that is critical for facilitating and maintaining their identity and immune-regulatory function.[1–3] The fundamental role of classical Tregs in maintaining immune homeostasis is well established because mutations in the *Foxp3* gene lead to fatal autoimmune disorders in mice (scurfy mice)[3] and humans (IPEX).[4] Of note, Tregs control various immune cells via multiple direct and indirect pathways, which are reviewed in detail in comprehensive reviews elsewhere.

In line with their key regulatory function, Tregs have long been considered as promising therapeutic targets to re-establish immune tolerance in autoimmunity, as well as in scenarios of aberrant immune activation and chronic inflammatory diseases. In recent years, specialized Treg populations were described that reside in non-lymphoid tissues and differ remarkably from their lymphoid organ counterparts as detailed below. Based on functional adaptations, these tissue Tregs are essential for maintaining local tissue homeostasis and function under steady-state conditions but also significantly contribute to tissue repair and regeneration in response to damage or injury. Accordingly, in recent years, it became clear that diseases such as obesity and T2D do impact these specialized Tregs in their specific metabolic tissue niches.

In line with the above-mentioned concept of niche- and context-specific functional adaptation, Tregs exert a multitude of distinct roles—these functions not only comprise the control

of immune functions but also relate to the maintenance of tissue function and integrity: (1) classic immune regulation, where tissue Tregs directly regulate other tissue-residing immune cells, such as helper T cells, cytotoxic T cells, innate lymphoid cells, or macrophages, by guiding their differentiation toward either pro- or anti-inflammatory (pro-regenerative) phenotypes. (2) Indirect regulation via responding to soluble mediators, such as interleukin (IL)-33/ST2, adipokines, insulin, local and systemic metabolites, and others that facilitate tissue-immune crosstalk. (3) Secretion of soluble mediators by Tregs, including amphiregulin. For instance, Tregs play a crucial role in enabling the functional adaptation of adipocytes to environmental cues. Tregs also facilitate the integration of signals such as beta-adrenergic stimulation induced by cold and mediated via secretion of catecholamines by the neuronal system to finally drive transcriptional adaptation of the adipose tissue itself.

In addition to niche-specific functional adaptation, we have learned that tissue Tregs differ from their counterparts with respect to their T cell receptors (TCRs). Accordingly, TCR sequencing revealed that non-lymphoid tissue Tregs exhibit expanded TCR clonotypes and/or complementarity-determining regions 3 (CDR3s) as the antigen-recognizing segment of the TCR. This observation suggests that tissue-residing Tregs recognize specific antigens, most likely originating from the tissue they are residing in. With the antigen origins still unknown, this antigen recognition can contribute to the specific expansion of tissue Tregs. Phenotypic features of these tissue Tregs include their predominantly activated CD44^{high} effector memory status[5] when compared with more classical lymphoid tissue Tregs. Concerning expression of costimulatory molecules, CTLA4 and PD1 were found to be one of the higher expressed molecules in these tissue Tregs.[5–8] In contrast to the CTLA4 and PD1, expression profiles of Lag3 and CD28 were not consistently detected in all datasets.[9] Furthermore, tissue Tregs are characterized by an increased expression of tissue-specific transcriptomes, which are critical for enabling them to efficiently fulfill their niche-specific and tissue-protective functions. Next to their master transcription factor *Foxp3*, these specific tissue Treg genes include *Il1rl1* (encoding the IL-33 receptor ST2), *Itgae*, *Gata3*, *Cd69*, *Il10*, and *Klrg1*, among others.[5,6,10–16] Common downregulated transcriptomes in tissue Tregs include *Tcf7* and *Satb1*.[5]

Over the last years, it has been convincingly shown that the elicited tissue-protective functions of tissue Tregs clearly extend beyond their classical tasks as immune regulators.

CONTROL OF SYSTEMIC METABOLISM BY TREGS AND NICHE-SPECIFIC FEATURES OF TREG-BASED CONTROL

In recent years, tissue Tregs residing in metabolic tissues, such as the adipose tissue, have emerged as key players not only in the safeguarding of proper metabolic tissue function but also in the control of systemic metabolism. From a pathophysiology perspective, it became clear that visceral adipose tissue (VAT) is particularly affected by expansion and metabolic changes during obesity and T2D. The metabolic competence of VAT relies directly on the function of VAT Tregs. Consequently, the manipulation of these locally residing tissue Tregs can distinctly impact adipose tissue function and systemic metabolic indices. In line with this concept, in obesity, a specific loss of the VAT Treg pool in the fat tissues drives metabolic impairments, such as systemic insulin resistance and hyperglycemia.[10] Mechanistically, it was demonstrated that in obese fat specifically, the VAT Treg population expressing the IL-33 receptor ST2 and Pparg is depleted.[10–12] The reasons for this specific loss of Tregs in VAT still remain to be understood.

Concerning immune-metabolic crosstalk, one of the key features of metabolic disease, insulin resistance in obesity, can be prevented and even reverted by specific Treg expansion. Treg expansion has been achieved using independent methods, including anti-IL-2/IL-2 antibody complexes[10,17,18] or administration of recombinant IL-33[15,18–20] in several non-lymphoid tissues (among them adipose tissue and muscles). Such Treg expansion locally in tissues was highlighted to result in improved metabolic characteristics. In addition, it became clear that Treg intrinsic sensing of IL-33 foster T helper (Th) 2 characteristics (e.g., cytokine production) by VAT Tregs.[20]

Vice versa, Treg depletion from the adipose tissue both systemically and specifically results in worsened metabolic parameters and impaired insulin sensitivity. Treg depletion can be achieved by utilizing Tregs that transgenetically express the diphtheria toxin receptor under the *Foxp3* promoter. This results in the specific ablation of Tregs upon the administration of diphtheria toxin. Administered intraperitoneally (i.p.), diphtheria toxin treatment leads to systemic Treg depletion. Similarly, Tregs can be eliminated systemically using depletion with anti-CD25 antibodies.[21] Along those lines, but more specific for all tissue Tregs, anti-ST2 antibodies were used to prove that tissue Tregs are critical for maintaining proper metabolic function of the adipose tissue.[22] Conceptually, it is important to consider that the above-mentioned strategies do not specifically target Tregs in particular tissues. Instead, these approaches target all tissues or all Tregs, thereby highlighting the need to advance into niche-specific Treg targeting for future translational application.

Concerning Treg-metabolic crosstalk, it is of critical relevance that several independent studies and approaches have demonstrated the potential of Treg manipulation to impact local adipose tissue metabolism.[17,23] From a metabolic perspective, it is well accepted that one way to combat obesity is by increasing energy expenditure, which can be achieved through increased physical activity. Another means to increase (steady-state) energy expenditure is promoting programs in adipocytes that consume high amounts of energy by dissipating heat, such as non-shivering thermogenesis. Accordingly, adaptive thermogenesis is usually enforced in response to external stimuli, such as cold exposure or pharmacological β3-adrenergic stimulation. Underscoring the importance of Treg-metabolic tissue crosstalk, the presence of tissue Tregs was crucial for brown and subcutaneous adipose tissues to respond to environmental cues, such as cold exposure, including the upregulation of related metabolic programs in adipocytes.[17,23] Specifically, we showed that Tregs control the beiging of the subcutaneous adipose tissue, as well as the activation of non-shivering thermogenesis in brown adipose tissue upon environmental cold and pharmacological β3-adrenergic stimulation.[17] Activation of brown adipose tissue and beiging of subcutaneous fat depots are evolutionarily conserved mechanisms in homeotherm

animals, necessary for adapting to cold climates. These results highlight the relevance of tissue Tregs in supporting and maintaining metabolic tissue function.

The above-described examples demonstrate the important contribution of fat-residing Tregs in maintaining optimal metabolism and their ability to functionally adapt to environmental and immune-metabolic cues. With regard to highly metabolically active tissues, such as the skeletal muscle, a similar picture is currently emerging. Tissue-residing Tregs are present in skeletal muscles in steady state, with their frequencies varying depending on the predominant muscle fiber types in different muscle subtypes and the respective environment (oxidative versus glycolytic).[24] Furthermore, Tregs in muscle quickly respond to microenvironmental adaptations of the exercising muscle, as well as to (experimentally induced) muscle injury.[6,24–26]

As observed in the healthy adipose tissue, the tissue adaptation and functional maturation of Tregs in exercising muscles involves sensing of the alarmin IL-33 by the receptor ST2 present on the majority of activated and phenotypically matured tissue Tregs. The source of IL-33 in the injured muscle was refined to be of fibroadipogenic progenitor (FAP) cell origin, and their failure to produce sufficient amounts of IL-33 upon aging has direct consequences for muscle regeneration in aged mice,[19] elderly people, and type 2 diabetics.[27] These IL-33 secreting stromal cells are located in close proximity to nerve bundles.[19,28] Amphiregulin secretion by tissue Tregs was demonstrated to be critical for satellite cell and FAP function, and impairments of the Treg-FAP crosstalk via amphiregulin and EGFR are observed in diet-induced obesity.[17,19,26,29]

In the context of exercise, which induces very mild "muscle injury" as known from muscle soreness, we have demonstrated that Treg-muscle crosstalk relies on signals via the IL-6/IL-6Rα pathway.[24] Physical activity leads to the local release of IL-6 from contracting muscle fibers in mice and humans.[30–32] Furthermore, Tregs present in the muscles express elevated levels of the corresponding high-affinity receptor IL-6Rα, the surface levels of which increase upon exercise.[24] The presence of IL-6Rα is critical for the functional maturation of tissue Tregs, which includes induction of proliferation, upregulation of ST2 and EGFR, and the secretion of amphiregulin. When IL-6Rα signaling is genetically disrupted in T cells through Cre *loxP*-mediated deletion, functional maturation of muscle tissue Tregs is impaired, resulting in detrimental effects upon injury. Mice with a T cell-specific loss of the IL-6Rα exhibit significantly impaired or delayed recovery of the injured muscle fibers and reduced grip strength in models of dextran sodium sulfate-induced sarcopenia. This highlights the importance of IL-6Rα signaling on muscle Tregs for maintaining optimal tissue function.[24]

Similar to adipose tissue-residing Tregs, muscle Tregs also exhibit a clonally expanded TCR repertoire,[6] whereas the antigen they recognize remains elusive. Once again, mirroring the situation observed with adipose tissue-residing Tregs, the depletion of muscle Tregs in scenarios of muscle injury results in impaired muscle regeneration, as evidenced by an increase in fibrotic areas.[6] Even more interesting, Treg depletion directly affects satellite cells—the muscles' "stem cells" that drive myofiber regeneration upon injury.

These niche-specific functional adaptations of Tregs in metabolic tissues argue for their future targeting with the overarching goal to modulate local tissue integrity and function. From a classical immunology perspective, targeting tissue Tregs based on their antigen specificity seems like a reasonable strategy; however, the specific relevant antigens remain currently elusive. In support of the importance of antigen-specific Treg targeting, the capacity of a few antigen-specific Tregs to regulate large populations of infiltrating cells was already described in the last century, underscoring their potent action.[33] In accordance with this concept, Rag1-deficient mice (that lack mature B cells, T cells, and Tregs) present with impaired glucose and insulin tolerance in diet-induced obesity.[34] In this model, the transfer of polyclonal, but not of irrelevant, ovalbumin-specific $CD4^+$ T cells improves metabolic function. This observation suggests that antigen specificity of adipose tissue Tregs is required to optimally support local tissue function.[34] Additionally, the Bluestone lab has demonstrated that the transfer of a small number of antigen-specific Tregs can reverse clinically overt autoimmune type 1 diabetes.[35] Conceptually, a similar scenario pertains to muscle Tregs: it was shown that TCR specificity guides Treg accumulation in injured muscles, significantly enhancing muscle repair.[25] In contrast, conventional T cells with the same TCR specificity do not home to the muscle but accumulate elsewhere.[25]

The question of whether pre-committed subsets of (re-)circulating tissue Treg cells are selectively recruited into an inflammatory niche or whether "any" Treg cell can be locally instructed within a certain tissue to adapt to a distinct inflammatory process remains to be answered. Furthermore, it remains largely unclear how antigenic versus non-antigenic stimuli are integrated into these scenarios. First insights come from a preprint of Adrian Liston's group, which suggests that key transcriptional profiles and TCR sequences are shared between tissue Tregs across different niches.[7]

IMPLICATIONS FOR METABOLIC DISEASE: WHAT DO WE KNOW ABOUT HUMAN TREGS IN METABOLIC TISSUES?

The murine studies described above clearly demonstrate that Tregs isolated from lymphoid organs or the peripheral blood do not necessarily reflect the situation within non-lymphoid tissues and their specific microenvironments. Tissue Tregs are barely detectable in peripheral blood and disease-associated perturbations might be below detection limit or simply not reflected by (re-)circulating Tregs, making many readouts unfeasible. The limited access to human non-lymphoid tissue compartments complicates a comprehensive comparison of the findings obtained in (predominantly male C57BL/6) mice with human disease.

The role of VAT (omental) Tregs in human obesity is less clear compared with studies of male C57BL/6 mice. First, more insights are needed regarding whether the observed loss of Tregs in obese mice is replicated in obese humans. Second, the knowledge about ST2 expression on human tissue Tregs is rather limited. It was reported that obese human VAT contains only 200,000 lymphocytes per gram of adipose tissue, making analyses technically challenging.[36]

Work by Megan Leving's group analyzed omental adipose tissues that were obtained during bariatric surgery.[37] They

observed a negative correlation of FOXP3$^+$ Treg frequencies with BMI, paralleling findings obtained in lean versus obese mice. Notably, further sub-clustering into non-obese and obese but non-T2D and T2D patients revealed trends suggesting the most pronounced decline of VAT Tregs in T2D patients.[37] One study observed a positive correlation of TBET/FOXP3 ratios in omental adipose tissue with increasing BMI; however, whether this is driven by an increase in TBET$^+$ cells or a loss of FOXP3$^+$ cells in stained tissue sections is not indicated.[34] *FOXP3* gene expression is reduced in human VAT in obesity, whereas total CD3$^+$CD4$^+$T cell numbers increase per gram tissue.[38] *FOXP3*/*CD3* transcripts are lower in omental versus subcutaneous adipose tissue and the difference in gene expression ratios negatively correlates with BMI.[10] Furthermore, the expression of *FOXP3* on both activated T cells (low levels) and Tregs (high levels) in the human immune system limits its utility as a sole marker for Tregs.

Whether ST2 expression on human adipose tissue Tregs is of similar relevance as in the murine setting is not yet fully conclusive. Although one study does not observe any ST2$^+$ Tregs in human VAT Tregs,[37] another study reports (at least some) ST2[18] expression and, additionally, shows that IL-33 is expressed by human adipocytes and stromal cells upon tumor necrosis factor alpha (TNF-α) stimulation.[39,40] It was revealed in other studies that human obese fat presents with high Treg frequencies and an increase of IL-33.[40,41] Currently, comprehensive data on tissue Tregs in human (obese versus lean) VAT remain limited. However, mechanisms of the response of T cells to cold environments or β3-adrenergic stimulation are conserved between mice and humans. Specifically, it was shown that defined individual cold exposure on human study subjects had conserved positive effects on *in vitro* Treg induction and Treg frequencies in subcutaneous adipose tissue biopsies.[42]

Despite the technical challenges of analyzing human VAT Tregs, the above-mentioned findings suggest (at least some) conservation in their function and the involved signaling pathways. This highlights the importance of future research efforts in dissecting the heterogeneity and niche-specific functional adaptation of human tissue Tregs in the setting of metabolic disease.

Regardless of the first insights concerning tissue Tregs in human adipose tissue, comprehensive studies of muscle Tregs using immune fluorescence stainings or fluorescence-activated cell sorting (FACS) analyses of muscle biopsies are currently lacking. Nevertheless, indirect evidence suggests similarities between human and murine muscle Tregs. In T2D patients, impairments are evident not only in muscle function but also in the FAP compartment of skeletal muscles, which are associated with muscle degeneration.[27] These results indicate that IL-33 release by FAPs may be crucial for human tissue Tregs to reach their full potential. As observed in mice, physical activity increases serum IL-6 without altering TNF-α levels.[32,43] However, no analyses of Treg frequency alterations were conducted upon exercise, neither in muscle biopsies nor by FACS.[31,32,43,44] Some data are available from patients with Duchenne's muscular dystrophy, showing that Tregs get recruited to inflamed human muscles, and their numbers increase significantly at the injured site.[45]

If future studies can strengthen the importance of tissue Tregs for maintaining tissue homeostasis in metabolic diseases also in humans, targeting these Tregs specifically offers tremendous potential for the personalized prevention and treatment of metabolic diseases. To approach this goal experimentally, further research in this area is urgently needed to obtain a clearer understanding of human Treg-metabolic tissue crosstalk and how it is affected in metabolic disease.

CHALLENGES AND PERSPECTIVES FOR NICHE-SPECIFIC TARGETING OF LOCAL TREGS

The exciting findings of non-canonical roles of tissue Tregs and their contribution in maintaining and regulating tissue homeostasis and function, as discussed above, highlight the potential of niche-specific targeting of these Tregs in metabolic disease. Such niche- and/or context-specific Treg targeting has the prospect to not only support tissue regeneration in settings of injury, inflammation, and autoimmunity but also to advance metabolic tissue function in obesity and diabetes.

An important prerequisite for such niche-specific Treg targeting will be insights into the molecular underpinnings that permit their local adaptation and execution of their niche-specific functions. Such mechanistic details on local tissue Treg signaling requirements are likewise of key importance to understanding the side effects of systemic immune targeting (Figure 1). One example in this context relates to tocilizumab, an anti-IL-6 receptor (IL-6R) antibody. Application of tocilizumab can lead to muscle weakness as a side effect in the clinics when it is applied systemically to treat autoinflammatory disorders.[46] As detailed above, we discovered recently that muscle tissue Tregs exhibit a high expression of the IL-6Rα in response to muscle activity.[24] Accordingly, we show that IL-6Rα expression on T cells is required for the Treg-mediated control of muscle function.[24] Therefore, muscle-residing Tregs are particularly susceptible to the treatment of systemic IL-6R blockade, and these findings can offer a possible mechanistic explanation for this side effect in response to anti-IL-6R treatment regimens.

In line with this concept and from a muscle Treg-targeting perspective, we have shown that exercise training fosters a stable and highly functional population of muscle Tregs that positively affects metabolic parameters. Importantly, exercise did not only induce the production of IL-6 locally in the muscle but also specifically enhanced L6Rα expression on Tregs.[24] The positive impact of exercise training on muscle Tregs has very recently been confirmed in an independent study and experimental setup.[47] These results underscore the relevance of understanding the molecular determinants of niche-specific Treg adaptation also with a prospect of future targeting opportunities.

Although the specific fostering of muscle Tregs by exercise training supports the concept of a niche-specific Treg-targeting effect in muscle, the exploitation of tissue Tregs for therapeutic use in metabolic disease is currently still largely impeded due to a lack of efficient and specific targeting strategies. Therefore, this perspective discusses challenges and opportunities of niche-specific targeting of these Tregs in metabolic diseases (Figure 2).

As detailed above, one means to specifically induce Tregs relates to their antigen-specific stimulation. Accordingly, one significant knowledge gap hindering the development of niche-specific antigen-related Treg-targeting strategies awaits the

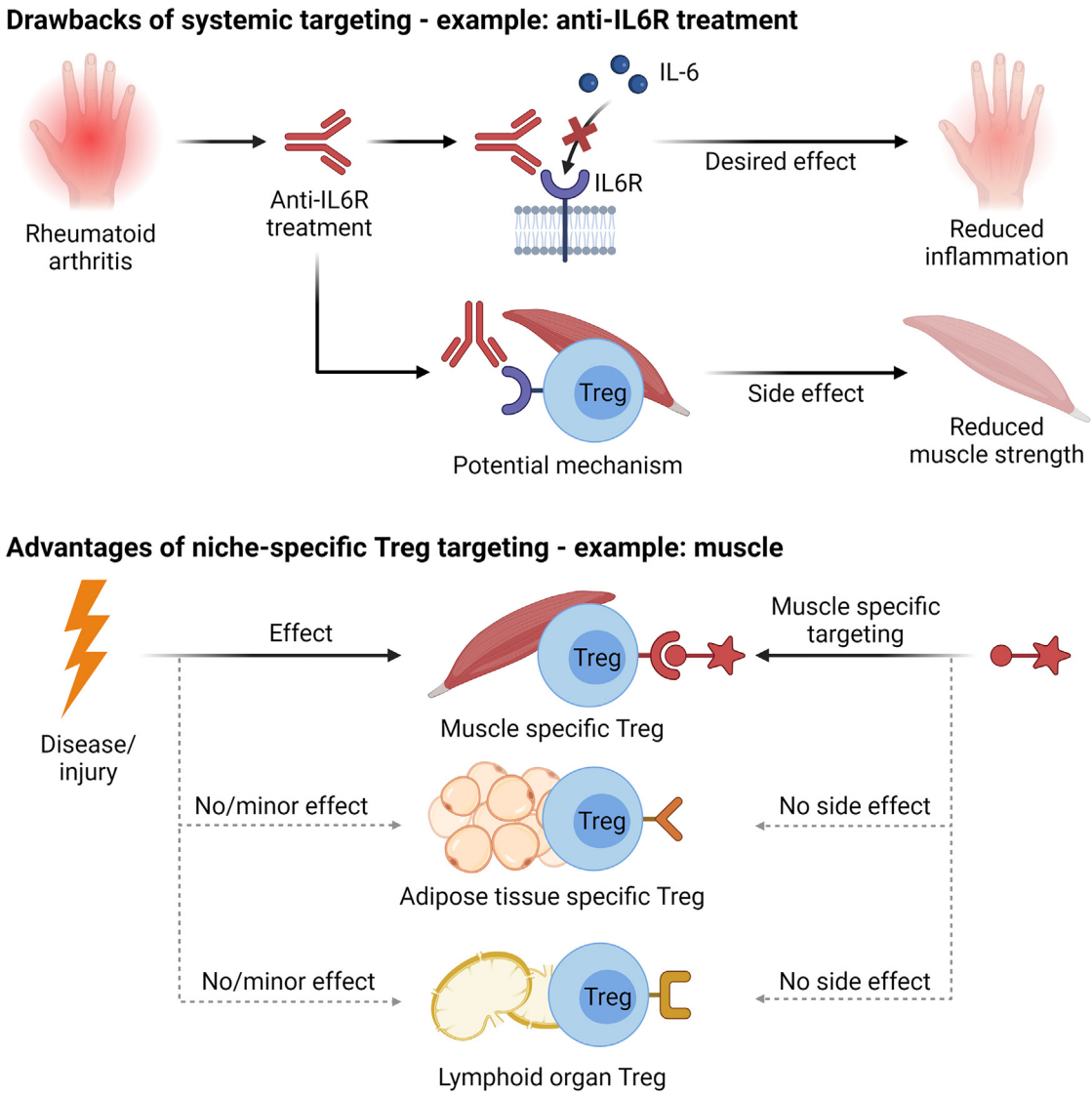

Figure 1. Comparison of systemic targeting strategies and niche-specific Treg targeting strategies

Systemic targeting strategies (top), e.g., anti-IL-6R application to treat autoinflammatory disorders can have side effects such as muscle weakness. By specifically targeting the affected tissue/tissue-specific cell population, niche-specific Treg targeting (bottom) can improve treatment efficiency and specificity to minimize potential side effects. Figure created with BioRender.com.

identification of endogenous antigens recognized selectively by VAT or muscle Tregs. Although it is known that, depending on the resident tissue, Tregs exhibit specific transcriptomes, distinct growth and survival factors, and unique TCR repertoires, the TCR diversity in tissue-resident Tregs varies across the different tissues and significantly differs from those of peripheral Tregs. In general, Tregs recognize peptide (as segments of antigens)-major histocompatibility complex (MHC) molecules by their highly specialized TCR. That tissue-derived antigens drive local Treg expansion is indicated because Tregs residing in the VAT exhibit a significantly limited TCR repertoire with prominent clonal expansions. Consequently, local VAT Tregs react to one or more antigens present in the local tissue microenvironment. Importantly, Diane Mathis and colleagues were able to identify one specific Treg clone (vTreg53) enriched in the VAT Treg population.[48] This vTreg53 clone efficiently homes to the VAT, but the trigger of this accumulation remains unknown. One possible explanation is retention at the site where the cognate antigen can be found and induction of corresponding TCR signaling. To shed light on this, Fernandes et al. conducted a peptide-library screen to pinpoint potent agonists specifically for this VAT Treg clone.[49] The identified surrogate peptides facilitated clonal Treg cell proliferation of vTreg53 cells without undermining crucial aspects of the VAT Treg transcriptome. Of relevance, the identified surrogate peptides were able to suppress high-fat-diet-induced inflammation within the VAT while simultaneously enhancing insulin sensitivity. Thus, vTreg53 cells were enriched in the VAT, but not conventional T cells, indicating that application of the antigenic peptides targets primarily Tregs. However, the challenge of identifying the endogenous antigen responsible

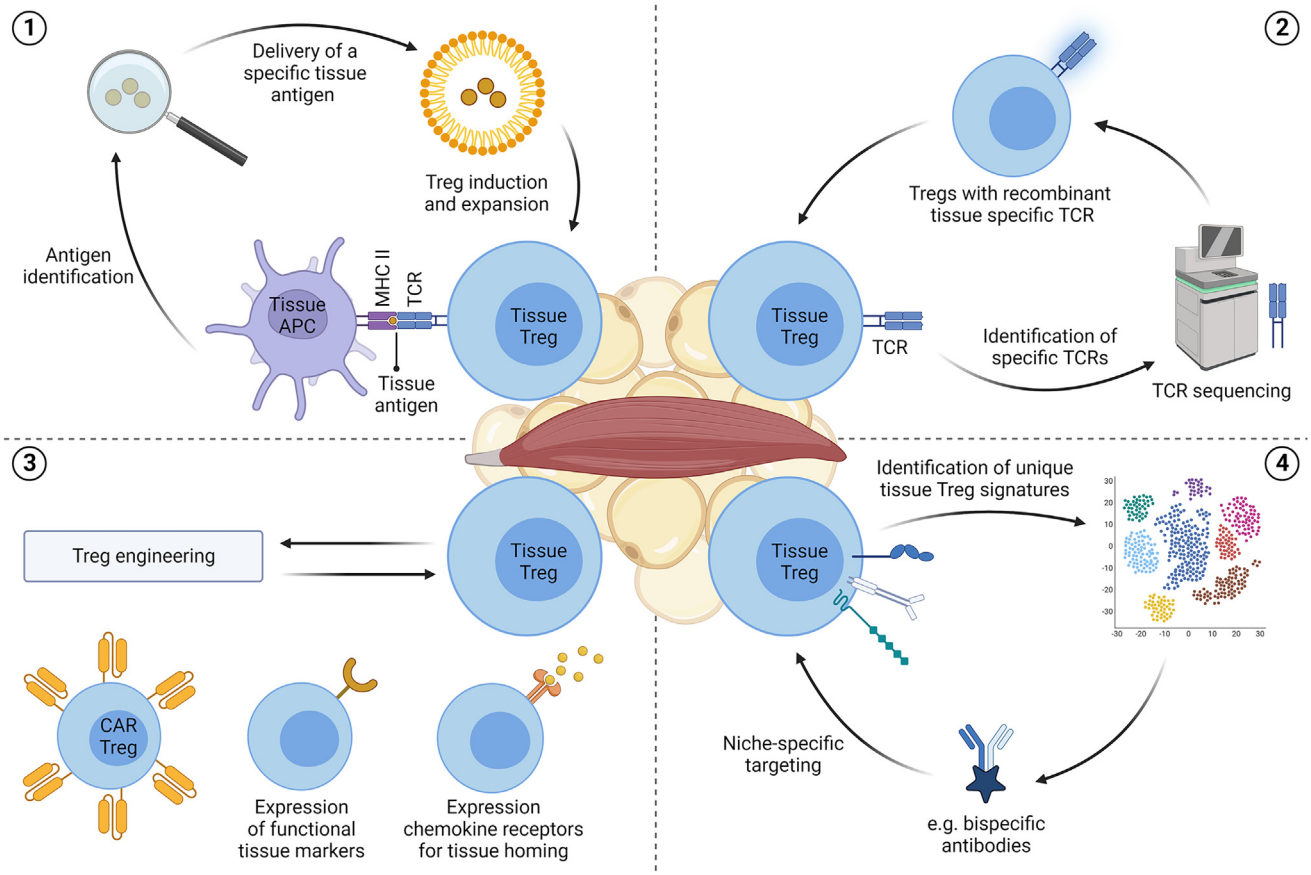

Figure 2. Perspectives for niche-specific Treg targeting

Obstacles limiting niche-specific tissue Treg targeting could be overcome with strategies such as (1) the identification and delivery of specific tissue antigens to induce and expand tissue Tregs, (2) the identification of specific tissue Treg TCRs and design of engineered Tregs with recombinant tissue-specific TCRs, (3) the engineering of tissue Tregs using CAR Tregs or the forced expression of functional tissue markers and/or chemokine receptors for tissue homing, and (4) the identification of specific tissue Treg surface signatures and targeting via bispecific antibodies or antibody drug conjugates. MHC II, major histocompatibility complex class II; APC, antigen-presenting cell; TCR, T cell receptor; CAR, chimeric antigen receptor. Figure created with BioRender.com.

for deorphanizing the vTreg53 TCR persists. The source of the relevant peptides in murine VAT has not yet been identified.[49]

It can be envisioned that the future identification of these tissue antigens will permit the targeting of Tregs via their corresponding TCR. Such antigen-specific Treg targeting has been successfully employed in previous studies, primarily focusing on the canonical functions of Tregs, such as the induction of immune tolerance during autoimmune diseases. It will be of high interest to address the relevance of such antigen-specific Treg targeting with the goal to modulate non-canonical Treg functions, e.g., in metabolic tissues in the future. Again, from a classical immune regulation perspective, many pre-clinical studies in mice have observed an increase in Treg numbers after administering disease relevant antigen in a combination therapy with cytokines or immunomodulators. One example is the administration of (pre)proinsulin as the main autoantigen in type 1 diabetes.[50] Based on these findings, these approaches are now translated into to clinical trials, where antigens are administered via different administration routes. In a phase 1b clinical trial (NCT0228367), engineered dendritic cells loaded with peptides from various myelin proteins as autoantigens in multiple sclerosis were administered intravenously. This approach led to an increase in the frequency of a Treg, called Tr1.[51] However, whether such Tregs are able to home to a certain tissue of interest to exert their niche-specific functions remains to be learned from future studies.

Because TCR sequencing and single-cell RNA sequencing (scRNA-seq) of human tissue Tregs is straightforward once the tissue can be freshly processed, the research community eagerly awaits advancements in the field of bioinformatics that will enable the prediction of T cell specificity based on TCR sequencing. As recently reviewed,[52] machine learning techniques have made significant progress, yet they still fall short of predicting the relevant peptide in the particularly complex context of TCR-peptide-MHC interactions. As soon as antigen identification is within reach, it is important to note that antigen-specific Treg-targeting strategies offer several advantages over polyclonal Tregs. Although adoptive cell therapy (ACT) using polyclonal Tregs is currently being investigated in several phase 1 and 2 trials for the treatment of autoimmune diseases[53–55] and in the setting of transplantation,[56–58] it has become apparent from pre-clinical studies that ACT using antigen-specific Tregs is more potent for the treatment of autoimmunity[59–61] and in the context of transplantation.[62–65] However, the

very low frequencies of (auto)antigen-specific Tregs in peripheral blood makes the isolation and expansion for transfer difficult. Similar considerations regarding very low cell numbers in peripheral blood is relevant for tissue Tregs and within the context of metabolic disease.

It is anticipated that engineering Tregs for transfer will be one option to overcome the hurdle of low-frequent Treg numbers for antigen-specific and/or niche-/context-specific targeting. Possibilities of engineering include altering Treg functional properties, for example, by expression of functional tissue markers, such as ST2. Importantly, Megan Levings' group was able to induce a tissue program, including AREG secretion, in human Tregs by engineered expression of ST2.[66] Of note, the AREG secretion by the engineered human Tregs was induced by IL-33 sensing via ST2 but independent of TCR signaling. This amphiregulin release by engineered Tregs was sufficient to guide human macrophages toward an anti-inflammatory/pro-regenerative phenotype,[66] which is desirable also with regard to adipose tissues and muscles in metabolic disease and injury.

However, it remains to be determined whether such altered Tregs would efficiently home to the tissue of interest, e.g. the VAT or muscle, just by "adding" ST2. To overcome the hurdle of tissue homing and niche-specific adaptation, further engineering to additionally express chemokine receptors that induce homing to the desired tissue might be an option. In line with this concept, it was demonstrated that the induction of CCR9 or $\alpha 4\beta 7$ integrin expression on human Tregs by retinoic acid and subsequent transfer into recipient mice results in efficient homing to the gut.[67] However, the chemokine receptors expressed by Tregs for homing to different tissues are not entirely specific and partially overlap. For example, CCR6 was shown to be important for Treg homing to the colon in colitis models,[68] whereas it was, likewise, demonstrated to be involved in Treg homing to the skin[69] and even the brain after ischemic injury.[70] Additionally, although it has been demonstrated that Tregs from VAT and muscle express CCR2 in the mouse, little is known about specific homing markers of human Tregs for metabolic tissues, such as VAT and muscle.

Alternatively, the expression of specific TCRs instructs Tregs to home to the site of expression of their cognate antigen. The identification of specific TCRs for human tissue Tregs would potentially enable the design of engineered human Tregs with recombinant tissue-specific TCRs. Importantly, the feasibility of this approach was studied in several pre-clinical studies in various disease models, including heart transplantation,[71] EAE,[72] rheumatoid arthritis,[73] and type 1 diabetes.[74] One important limitation of this approach relates to the human leukocyte antigen (HLA) restriction of antigen recognition by the TCR, meaning that the specific TCRs required would have to be defined individually for every HLA type. In order to potentially simplify the identification of TCRs, a better understanding of common TCR traits that govern Treg functions within a given tissue is a prerequisite that is currently not sufficiently understood. Importantly, a study by Sprouse et al. indicates that TCR affinity can elicit specific Treg functions.[75] Specifically, they demonstrate that Tregs with both high- and low-affinity TCRs enter the pancreas, whereas high-affinity TCR expressing Tregs acquire signatures associated with canonical suppressive function, and interestingly, low-affinity TCR expression on Tregs elicits programs related to tissue repair. It will be of particular interest to dissect the possibility of such TCR-related signaling aspects in the context of niche-specific Tregs in metabolic tissues such as fat or muscle.

TCRs and CARs have emerged nearly simultaneously for the purpose of redirecting T cell specificity thereby inspiring similar strategies in the field of Treg targeting. Specifically, such synthetic immune receptors, known as CARs, have now the potential to offer a more advanced approach of engineered Treg therapies that awaits its future exploitation in the field of metabolic disease. These CAR Tregs offer the big advantage in that they are no longer MHC restricted, broadening the therapeutic potential to patients irrespective of their MHC/HLA. In the field of organ transplantation, the use of HLA-A2-specific human CAR Tregs has made significant progress to prevent alloimmune organ rejection. The application of autologous HLA-A2 CAR Tregs is currently being investigated in first-in-human clinical trials for kidney transplantation (Steadfast trial, NCT04817774)[76] and liver transplantation (LIBERATE trial, NCT05234190). Although initial insights in these studies support the safety and feasibility of CAR Treg therapy in the context of transplantation medicine, it remains to be determined whether CAR Treg therapy can be used to treat autoimmune diseases or to stop aberrant (chronic) inflammation. The efficacy of CAR Treg therapies is currently addressed in other studies of pre-clinical mouse models for type 1 diabetes,[77,78] multiple sclerosis (MS),[79] and inflammatory bowel disease.[80–83] These ongoing studies testing CAR Treg therapies will assess important implications for targeting inflammation and aberrant immune activation as observed in tissues affected by metabolic disease.

With the goal to achieve niche-specific Treg targeting in the future, it is becoming more and more clear that most surface signatures that were initially thought to be specific for tissue Tregs residing in a certain tissue might be shared between tissue Tregs residing in different tissues. Hence, the identification of a single specific surface molecule to target tissue Tregs in specific niches remains a challenge. However, it can be envisioned that targeting two surface molecules in combination on the tissue Treg at once might solve this problem. Consequently, bispecific antibodies (bsAbs) offer a solution by binding to two different epitopes and thereby significantly improve the chances to target the right Treg population residing in a certain tissue such as fat or muscle. Such targeting approaches have been proven effective, especially in cancer treatment in both human and mice (as summarized in Zhang et al.[84]). Most bsAbs are engineered to target distinct epitopes on different cells, typically immune and cancer cells. This design fosters immune crosstalk between these cells, thereby enhancing cancer clearance. The feasibility and success of this approach is shown by Catumaxomab, a trifunctional bsAb targeting both the tumor cell (by their expression of Ep-CAM) and T cells by their expression of CD3. Catumaxomab was approved for the European market in 2009 to treat malignant ascites resulting from metastasizing cancer. The antitumor effect arises from T cell-mediated lysis, antibody-dependent cell-mediated cytotoxicity, and phagocytosis.[85] MEDI5752, binding dual-positive PD-1^+ CTLA-4^+ cells, has shown promising antitumor activity in patients with advanced renal cell carcinoma (NCT03530397).[86] Coupled to delivery agents, such as nanoparticles, bsAbs will offer an additional

means of exploitation for specific targeting and delivery of payloads toward tissue Tregs in metabolic tissues.

In addition, the use of antibodies as targeting agents represents a new class of targeted therapies, in use mainly for antibody-drug conjugates (ADCs) that await future testing in the field of niche-specific Treg targeting. ADCs are composed of three components: a monoclonal antibody, traditionally a small cytotoxic drug, and a linker group connecting both components. (Monoclonal) antibodies are utilized as a powerful tool for the selective targeting of cellular antigens on the surface of the target cells, enabling a precise and efficient cell-specific delivery of the cytotoxic drug and thereby minimizing off-target effects.[87] In recent years, however, the use of ADCs has been expanded beyond conventional small cytotoxic drugs. Different cargo options, such as oligonucleotides or proteins, are currently under investigation. Avidity Bioscience is the first company that has progressed to clinical trials (NCT05027269 and NCT05479981) by using siRNAs as payloads, reducing the levels of disease-related *DMPK* mRNA in the treatment of myotonic dystrophy type 1, a neuromuscular disease. These novel developments can be utilized to broaden the potential applications of ADC technology toward the delivery of oligonucleotides or proteins to tissue Tregs in metabolic niches, such as muscle or adipose tissue. In line with this concept, one protein of interest as potential payload to improve tissue Treg function in metabolic tissues is the cytokine IL-33 because it only targets (engineered) $ST2^+$ cells, with ST2 being a marker expressed by tissue Tregs. In further studies, a hybrid cytokine (called IL-233) was created to effectively target murine Tregs by utilizing the synergy between IL-33 and IL-2, a cytokine essential for Treg function and survival. IL-233 expanded the Treg population in mice, enhanced their immunosuppressive function, and ultimately induced remission of the autoimmune disease lupus glomerulonephritis.[88,89] Coupled to a bsAb as a combinatorial approach between ADCs and bsAbs, delivery of IL-233 provides an interesting approach that awaits further testing in future studies. Possible challenges of this strategy that need to be overcome include the requirement to engineer a protein cargo (as IL-233) in such a way to allow for binding to the intended cell surface receptor in the appropriate formation to promote downstream signaling and effects.

In the end and from a conceptual perspective, it might be worthwhile to set these opportunities of niche-specific Treg targeting for metabolic disease in the perspective of recent advances of next-generation anti-obesity drugs, including GLP1R agonists, such as semaglutide, which appear capable of achieving significantly improved body weight loss.[90] Importantly, such a significant weight loss has been identified as the key driver of improved metabolic indices upon such treatment regimens with GLP1R agonists in patients with metabolic disease. From an immunological perspective, it will be of interest to learn from future studies how these novel anti-obesity drugs will impinge on immune cells and especially tissue Tregs in metabolic tissues such as VAT. Does adipose tissue inflammation resolve as well upon application of these drugs? Additionally, and as discussed above, the dynamic nature of Treg-metabolic crosstalk requires constant responsiveness of Tregs to immunometabolic cues. This Treg-metabolic tissue crosstalk allows for fine-tuning of tissue Treg adaptation and their optimal functional specialization. For that, Tregs possess an extensive molecular interface, allowing them to respond to local and systemic signals.[17,24,42] It can be envisioned that various immunometabolic transmitters, including endocrine hormone signaling molecules,[91] are of relevance for tissue adaptation and the execution of their non-canonical features. From a precision targeting perspective and in line with the niche-specific adaptation of these tissue Tregs, it is important to highlight that their broad spectrum of canonical and non-canonical features, and the future targeting thereof is not limited to controlling local inflammation and improving systemic metabolic parameters but will include restoration of tissue function, regeneration, and repair.

In sum, Tregs in metabolic tissues differ significantly from their counterparts in lymphoid organs, displaying unique characteristics based on their homing tissues, such as a distinct transcriptome and TCR repertoire. They play a critical role in maintaining metabolic balance, preventing inflammation, and controlling tissue function in metabolic diseases. Hence, metabolic tissue-residing Tregs display an attractive target for the future development of personalized prevention strategies for metabolic diseases. Despite this promise and dedicated research efforts, developing such niche-specific Treg targeting strategies currently still faces critical challenges. In the future, intensified efforts need to study the niche-specific functional adaptations of Tregs in metabolic tissues in fine resolution. Specifically, one of the most important knowledge gaps to fill includes the identification of the antigens recognized by these Tregs in the desired tissue and/or the identification of stable and tissue-specific markers on the surface of the Tregs. Despite these challenges, available strategies such as bsAbs or ADCs provide a foundation for future advancements in this important field of research. The possibilities that open up in this research field obviously justify the tremendous effort required with the future vision of developing personalized medicines targeting Tregs in distinct tissue niches to combat metabolic disease.

ACKNOWLEDGMENTS

C.D. holds a professorship grant from the Excellence Program for Outstanding Female Scientists from the Helmholtz Association and is supported by a Research Group at Helmholtz Zentrum München, through a membership in the CRC1054 of the Deutsche Forschungsgemeinschaft Project-ID 210592381–SFB 1054 (B11), and through an award of the EFSD/JDRF/Lilly Program on Type 1 Diabetes Research 2020. C.D. and M.B. received funding by the Helmholtz Munich Innovation and Translation Fund 2023. The authors are supported by a membership of the German Center for Diabetes Research (DZD) with grants to C.D. and M.B. S.M.D. is supported by a membership of iTarget2.0 of the Ludwig-Maximilians-Universität München. The Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) supports I.S. under project number 490846870-TRR355/1 TPB02. Part of the research discussed here was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) project number 490846870-TRR355/1 TPA02 to C.D.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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