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TISSUE-Tregs

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Abstract

The immune system is responsible for defending an organism against the myriad of microbial invaders it constantly confronts. It has become increasingly clear that the immune system has a second major function: the maintenance of organismal homeostasis. Foxp3^{+}CD4^{+} regulatory T cells (Tregs) are important contributors to both of these critical activities, defense being the primary purview of Tregs circulating through lymphoid organs, and homeostasis ensured mainly by their counterparts residing in parenchymal tissues. This review focuses on so-called tissue Tregs. We first survey existing information on the phenotype, function, sustaining factors, and human equivalents of the three best-characterized tissue-Treg populations—those operating in visceral adipose tissue, skeletal muscle, and the colonic lamina propria. We then attempt to distill general principles from this body of work—as concerns the provenance, local adaptation, molecular sustenance, and targets of action of tissue Tregs, in particular.

Keywords

adipose tissue; metabolism; skeletal muscle; tissue repair; mucosal immunology; microbiota

INTRODUCTION

Essentially all immunology textbooks contain a statement to the effect that the major function of the immune system is to fend off microbial challenges. And this declaration is certainly true. But it has become increasingly clear that the immune system has a second critical function: safeguarding organismal homeostasis. That is, immunological cells and molecules contribute to maintaining the activities of diverse organ systems within an optimum range when they are confronted with a genetic or environmental stressor (1). Our initial and continuing appreciation for this second role derives from studies on macrophages (reviewed in 2, 3). It is now well accepted that distinct populations of tissue-resident macrophages are established perinatally; that they are maintained in adults; and that they make specialized responses to local cues, thereby promoting tissue differentiation, function, and homeostasis.

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This review highlights the roles of Foxp3$^+$CD4$^+$ regulatory T cells (Tregs) in promoting organismal homeostasis. Tregs are important regulators of most types of immune responses—including allergy, autoimmunity, inflammation, and reactions to microbes and tumors. As such, they can control the activities of most cell types of the adaptive and innate immune systems (reviewed in 4). But do distinct populations of Tregs reside in nonlymphoid tissues? Do they have tissue-specific phenotypes? And do they regulate the physiology of their home tissue? We first summarize data on the best-characterized nonlymphoid-tissue Foxp3$^+$CD4$^+$ (hereafter referred to as tissue Treg) populations: those residing in visceral adipose tissue (VAT), skeletal muscle, and colonic lamina propria. We then attempt to integrate this body of work to distill some general principles.

VISCERAL ADIPOSE TISSUE TREGS: REGULATORS OF ORGANISMAL METABOLISM

Adipose tissue is a loose connective tissue composed of adipocytes and a stromal vascular fraction, consisting of preadipocytes, fibroblasts, fibro/adipogenic progenitors (FAPs), vascular endothelial cells, and a panoply of immune system cell types (Figure 1) (reviewed in 5). Adipose tissue depots can be found throughout the body: beneath the skin [subcutaneous adipose tissue (SAT)], surrounding various internal organs (VAT), and in bone marrow and marbling skeletal muscle, etc. White, brown, and beige adipose tissues have different developmental origins, distributions, and activities. The main function of white adipose tissue is to store lipids, although it has additional roles in cushioning and insulating the body and as an endocrine organ, secreting a variety of adipokines, cytokines, and other mediators. The primary function of brown adipose tissue, located mainly around the neck and large blood vessels of the thorax, particularly in neonates, is to dissipate chemical energy in the form of heat. Beige adipocytes are brown-like cells induced in white adipose tissue by a variety of activators.

Now-classic studies established that VAT is a site of extensive cellular and molecular cross talk between the metabolic and immune systems (reviewed in 6). In 1993 Hotamisligil et al. demonstrated that synthesis of tumor necrosis factor (TNF)-α is induced in the epididymal fat pad of obese rodents and that neutralization of this cytokine improves their systemic insulin resistance (7). Later, other proinflammatory cytokines, notably IL-6 and IL-1β, were implicated in obesity-induced metabolic dysregulation (8, 9). In 2003, two groups reported a striking accumulation of macrophage-associated gene transcripts and of cells expressing the F4/80 or CD68 marker, presumably macrophages, with increasing adiposity in mice and humans (10, 11). The increase in macrophage representation was accompanied by an altered localization within the adipose tissue, into so-called crown-like structures, and a population drift to a more proinflammatory phenotype (12–14). The functional relevance of macrophages and of their phenotypic changes was established through loss- and gain-of-function experiments (e.g., 15–18). Based on these findings, macrophages emerged as the central players in obesity-associated inflammation, and little thought was given to other types of immunocytes (19, 20).

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More recently, there has been growing interest in the roles of additional members of the innate and adaptive arms of the immune system in regulating organismal metabolism, spawning the field of immunometabolism (21). Besides macrophages, several other types of immunocytes have been implicated: mast cells, eosinophils, neutrophils, dendritic cells (DCs), natural killer (NK) cells, type 2 innate lymphoid cells (ILC2s), $CD4^{-}8^{-}$ γδ T cells, NK T cells, $CD4^{+}$ and $CD8^{+}$ T cells, $Foxp3^{+}CD4^{+}$ Tregs, classical B cells, and regulatory B cells (Bregs) (22–26). This complex set of players can be difficult for one to assimilate; however, such complexity is to be expected. Inflammation is a complicated, highly orchestrated process wherein tissue-resident macrophages and mast cells; recruited neutrophils, monocytes, eosinophils and/or NK cells; and multiple lymphoid cell types have stereotypical functions to perform. In addition, the inflammatory process, especially if it becomes chronic, is kept in check by regulatory cells of diverse types (e.g., M2-type macrophages, Tregs, NKT cells, Bregs).

A unique population of $Foxp3^{+}CD4^{+}$ Tregs was discovered in VAT of lean mice (27) and serves as a paradigm for the tissue-Treg concept. Below we explore their distinct phenotype, their function in maintaining organismal homeostasis, factors that promote their activities, and their human counterparts.

Phenotype

Murine VAT Tregs—more specifically, those residing in the epididymal fat depot—are distinct by a number of criteria. First, $Foxp3^{+}CD4^{+}$ T cells are unusually highly enriched within the VAT $CD4^{+}$ T cell compartment of lean mice, in comparison with the 5–15% Treg representation typical of lymphoid organs such as the spleen and lymph nodes (27). This augmentation is first evident at 10–15 weeks of age in C57BL/6 (B6) mice, and a peak of 40–80% of $CD4^{+}$ T cells is reached by 20–25 weeks. At even later ages, e.g., ~40 weeks, VAT Treg levels unaccountably drop (28). Although these trends in VAT Treg representation occur in multiple mouse colonies, the precise fractions and ages can differ in different colonies, suggesting influences of husbandry and/or the microbiota (D. Kolodin, C. Benoist, and D. Mathis, unpublished data). Histologically, $Foxp3^{+}CD4^{+}$ T cells are found in the spaces between adipocytes, often in the crown-like structures containing macrophage conglomerates (27). Whether or not Tregs accumulate in fat depots surrounding the reproductive organs of female mice as they age has not yet been reported, although it has been suggested that male and female gonadal-fat Tregs respond differently to diet changes (29). Age-dependent accumulation of $Foxp3^{+}CD4^{+}$ cells does not occur in other white adipose tissue depots—notably SAT, of interest because obesity-induced inflammation and metabolic perturbations have not generally been associated with these sites (30).

Second, VAT Tregs display a distinct repertoire of antigen-specific receptors [T cell receptors (TCRs)]. Unlike what is seen in the corresponding lymphoid organs, the VAT $Foxp3^{+}CD4^{+}$ population of individual lean B6 mice exhibits clonal microexpansions (31). In addition, in VAT, but not in lymphoid organs, of mice engineered to express a restricted set of TCRs (a single β chain, and limited α-chain diversity) there are frequent examples of α chains with the same complementarity-determining region 3 (CDR3) protein sequence specified by different nucleotide sequences (27). These two features suggest that VAT Tregs

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might be responding to a specific antigen or antigens in situ. The TCR repertoire of VAT conventional T cells (Tconvs) is much more diverse, akin to that of lymphoid-tissue Tregs or Tconvs.

Third, Tregs in VAT have a transcriptome distinct from that of lymphoid organ Tregs. Whole-genome expression profiling reveals thousands of transcripts significantly up- or downregulated in VAT $Foxp3^{+}CD4^{+}$ T cells vis-à-vis their spleen or lymph node counterparts, which differ from each other by only a few hundred transcripts (27, 28). VAT Tregs are clearly Tregs, as they express diagnostic molecules like Foxp3, CD25, and GITR; but all in all, they transcribe only about 65% of the canonical Treg signature. Multiple classes of genes are differentially regulated in VAT, versus lymphoid organ, Tregs, including those encoding transcription factors (e.g., PPARγ); chemokines and their receptors (e.g., CCR2); cytokines and their receptors (e.g., IL-10, St2); and, most interestingly, a set of molecules implicated in lipid metabolism (e.g., LDLR, Dgat). The VAT Treg “flavor” of the transcriptome can be distinguished already at 5 weeks of age, well before age-dependent expansion of the Treg population in epididymal fat (28). PPARγ, the master regulator of adipocyte differentiation (32), is the major driver of the VAT Treg phenotype (33): Cotransduction of Pparg and Foxp3 cDNAs into $Foxp3^{-}CD4^{+}$ splenic T cells ex vivo yields VAT-like Tregs; mice harboring a Treg-specific knockout of Pparg have a substantially reduced VAT Treg population; and treatment of mice with a PPARγ agonist specifically induces VAT Tregs. Indeed, Foxp3 and PPARγ interact, either directly or as part of a complex. More recent data revealed that the transcriptional regulators IRF4 and BATF are required for the induction of key components of the VAT Treg differentiative and functional programs—upregulating, in particular, PPARγ and the IL-33 receptor, St2 (further discussed below) (34).

It was reported that brown adipose tissue from female B6 mice hosts Tregs with a transcriptome different from that of their splenic counterparts and that a small number of transcriptional changes occur upon cold stress in vivo (35). However, comparison of the transcriptomes of Tregs from brown versus visceral white adipose tissue is complicated by the fact that data on the former came from females and the latter from males.

Function

VAT Tregs function indistinguishably from typical lymphoid-organ Tregs in standard in vitro suppression assays (27). However, their in vivo activities render them distinct. The initial suggestion that VAT Tregs might play a role in metabolic homeostasis came from correlative studies: demonstration that $Foxp3^{+}CD4^{+}$ T cell representation in VAT of male mice, but not in their lymphoid organs, has an inverse correlation with insulin sensitivity in genetically and nutritionally induced models of obesity (27, 36). More directly, punctual ablation of Tregs by injection of diphtheria toxin into mice expressing the diphtheria toxin receptor (DTR) under the dictates of Foxp3 promoter/enhancer elements or by administration of an anti-CD25 monoclonal antibody to standard mice provokes VAT tissue inflammation and metabolic abnormalities (27, 37). Altered metabolic indices have included insulin-induced phosphorylation of the insulin receptor, fasting-glucose and -insulin levels, the homeostatic model assessment of insulin resistance (HOMA-IR) value, the glucose-tolerance test (GTT),

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and/or the insulin-tolerance test (ITT). In addition, and applying similar metabolic read-outs, in vivo expansion of Tregs by injection of IL-2/anti-IL-2 complexes or anti-CD3 improves VAT inflammatory tenor and metabolic health (27, 38, 39).

Ablation of Tregs using a Foxp3-DTR system reduced whole-mouse oxygen consumption and heat generation under 4° cold stress, but not at room temperature (35). This finding suggests that Tregs in brown adipose tissue might regulate the metabolic response to cold stimulation, a notion supported by changes in expression of genes in the thermogenic program at that site.

All of the above-mentioned studies entailed manipulation of Tregs systemically, so it was not possible to attribute the observed effects to adipose-tissue Tregs in particular. Generation of a mouse line with Treg-specific ablation of PPARγ expression solved this problem for VAT Tregs (33). In the lean state, these mice have a striking reduction in VAT, but not lymphoid-organ, Tregs. As a consequence, VAT inflammation worsens and metabolic indices degrade. In addition, injection of PPARγ agonists, such as the thiazolidinedione drug, pioglitazone, into mice fed a high-fat diet expands the VAT Treg population and improves the local inflammatory tenor, as well as local and organismal metabolic health. Mice lacking PPARγ only within Tregs show a substantially muted metabolic response to pioglitazone treatment, arguing that an important component of the insulin-sensitizing effect of thiazolidinedione drugs, employed for many years as first-line agents in the treatment of type 2 diabetes, operates via VAT Tregs.

Some of the effects of VAT (and brown adipose tissue) Tregs are likely to be on local macrophages and other immunocytes. Indeed, culturing Tregs isolated from VAT of lean mice with perinatal macrophages provokes the expression of anti-inflammatory markers in the latter (23). Yet, almost certainly, VAT Tregs also have direct influences on their adipocyte neighbors. For example, these Tregs express exceptionally high levels of IL-10; adipocytes display the corresponding receptor; and culture of fully differentiated 3T3-L1 cells, a preadipocyte cell line, with IL-10 (a) restrains its ability to synthesize IL-6, RANTES, and other inflammatory mediators in response to TNF-α; (b) down-modulates insulin-dependent tyrosine phosphorylation of insulin receptor substrate-1; and (c) inhibits glucose uptake via the glucose transporter, Glut4 (12, 27).

Factors

Much effort is now being devoted to identifying factors that drive the specific accumulation and functions of VAT Tregs. Several such factors have already been identified. The importance of local TCR:MHC-II interactions for the buildup of VAT Tregs was initially suggested, as mentioned above, by the ready detection of clonal microexpansions, coupled with the observation of TCRs with the same CDR3 protein sequence specified by different nucleotide sequences (27, 31). Indeed, Tregs and MHC-II-expressing antigen-presenting cells (APCs) (both $CD11b^{+}c^{+}$ macrophages and $CD11b^{-}c^{+}$ DCs) are found in close proximity in VAT, and this colocalization can be extinguished by treating mice with anti-MHC-II monoclonal antibodies (31). $Foxp3^{+}CD4^{+}$ T cells do not accumulate in VAT of mice genetically lacking MHC-II molecules (31, 40), further indicating that TCR:MHC-II interactions are needed for their differentiation and/or accumulation. The relative importance

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of MHC-II expression on APCs versus adipocytes for CD4+ T cell activities, whether in the lean or obese state, remains the subject of active debate (31, 40–42).

The critical contribution of PPARγ to VAT Treg accumulation and function has already been highlighted. This transcription factor, a member of the nuclear receptor family, imposes much of what is unique about the phenotype of VAT Tregs from lean mice (33). In obese animals, VAT Tregs not only die or evacuate but also lose their characteristic transcriptional signature, while gaining an additional set of over- or underrepresented transcripts (compared with lean VAT Treg transcripts) (28). This new signature depends on phosphorylation of the serine residue at position 273 of PPARγ, parallel to a pathway recently elucidated in adipocytes (43–45). Nutritionally induced obesity activates cyclin-dependent kinase 5 (Cdk5) and Erks in adipocytes, leading to phosphorylation of PPARγ's Ser273 residue, which in turn results in dysregulation of a set of genes abnormally expressed in the obese state. Certain PPARγ ligands, e.g., rosiglitazone, are antidiabetic because they block Cdk5-induced phosphorylation of PPARγ. The ligands that naturally trigger PPARγ in VAT (and elsewhere) have not been identified.

Several studies have highlighted the role of IL-33 and its receptor, St2, in VAT Treg biology (31, 34, 46, 47). IL-33, made mostly by nonhematopoietic cells, is a member of the IL-1 family of cytokines; St2, encoded by Il1rl1, is expressed primarily on various types of hematopoietic-lineage cells. It associates with IL-1RAcP to signal through MyD88, IRAK-4, and TRAF6; the MAP kinases Erk1/2, p38, and JNK; and the AP-1 and NF-κB transcription factors (reviewed in 48). IL-33 is best known for promoting type-2 immunity by acting on ILC2s, T helper 2 (Th2) cells and anti-inflammatory macrophages. It can also serve as an alarmin, released by necrotic or struggling cells to signal recent or impending tissue damage (e.g., from hypoxia or mechanical stress). Its release mechanisms are rather obscure. But it is now clear that IL-33 can also affect certain Treg populations, promoting their impact on populations of Tregs, revealing their expansion in lymphoid organs and in the colonic lamina propria, for example (49, 50). Il1rl1 transcripts are overrepresented in the VAT, versus lymphoid-organ, Treg transcriptome, and St2 is expressed on an unusually large fraction of VAT Tregs, more than 80% in lean mice at 20–25 weeks of age (31, 34). St2- or IL-33-deficient mice kept on a diet of normal chow show a substantial reduction in the VAT Treg population, with only a minor or no effect on Tregs circulating through lymphoid organs of the same animals (31, 34, 46). Injection of exogenous IL-33 into lean or obese mice can expand VAT Tregs by at least an order of magnitude, while lymphoid-organ Tregs increase by less than a few fold; as a consequence, VAT inflammation is reduced and metabolic indices improve (31, 34, 46, 47, 51).

The identity of the major IL-33-expressing cell types in VAT, and their response to physiologic and environmental changes, is controversial. One group has highlighted expression by fibroblastic cells in lean mice, especially those associated with the capsule encasing VAT lobules (31), whereas another has emphasized endothelial cell expression (46). It is possible that the different impressions reflect the fat depot areas that were examined—i.e., whether or not they included the capsular regions—or, alternatively, the different methods of IL-33 detection employed (an antibody versus a transgenic reporter). It may be relevant that constitutive endothelial cell expression of IL-33 has been thought to be

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a feature of human but not mouse tissues (52). It will be important to obtain a more global and quantitative view through development of a cytofluorometric method of IL-33 detection.

Also controversial is the extent to which IL-33 directly affects VAT Tregs. Molofsky et al. (46) have argued that this cytokine's major influence on Tregs in VAT is through an ICOS/ICOSL-mediated interaction with ILC2s in the vicinity. Their evidence is, first, that ablation of IL-5-expressing cells, taken to be ILC2s, leads to a substantial reduction in expansion of the VAT Treg compartment with age or after IL-33 injection; and, second, that Treg-specific deletion of MyD88 (through which St2 signals) has little effect on VAT Treg induction in response to IL-33. In contrast, several groups have shown direct influences of IL-33 on Tregs in vitro, whether taken from VAT or other tissues (34, 49, 50, 53). And Vasanthakumar et al. have used an elegant mixed–bone marrow chimera approach to evidence substantial VAT-Treg-intrinsic effects of ablating St2 or MyD88, as well as the putatively upstream transcription factors, IRF4 and BATF (34). In considering these results, it might be worth keeping in mind that directed gene knockouts can be less specific than originally anticipated. For example, VAT Tregs express readily detectible levels of Il5 transcripts, especially in young mice (28, 33; Figure 2)—the level need not be very high to affect their ablation in an Il5-cre-driven system, especially given the highly restrained turnover of the VAT Treg compartment (31). On the other hand, silencing of MyD88 in Tregs could influence signals other than those mediated by IL-33. Recent studies on the lung suggest that an IL-33/mast cell/Treg axis might also be worth exploring (54).

Other factors reported to influence VAT Treg accumulation include IL-21 (55) and Stat3 (56), both as negative regulators. Interestingly, Helicobacter pylori colonization of lean mice augments the VAT Treg population, diminishes the VAT inflammatory macrophage population, and improves systemic metabolic indices, via a PPARγ-dependent mechanism (57).

Humans

Most of the findings mentioned above pertain to mice, raising the obvious question of how they translate to humans. Certainly, similar associations between VAT—in this case omental fat—inflammation and metabolic indices in lean and obese humans have been documented (58). Concerning the role of VAT Tregs, in particular, it has been reported that human omental fat has measurable levels of Foxp3 (normalized to Cd3) transcripts, which have an inverse correlation with body mass index, as well as demonstrable $Foxp3^{+}CD4^{+}$ T cells (27, 34, 36, 37). Il33 and Il1rl1 transcripts have also been detected in human omentum (59), and a high fraction of Tregs at that site display St2 (34).

SKELETAL MUSCLE TREGS: FACILITATORS OF REPAIR

The basic unit of skeletal muscle is a long cylindrical cell with multiple acentric nuclei called the myofiber, which is formed during development by the fusion of immature myocytes, termed myoblasts (Figure 3). Under the myofibers' basal lamina are muscle progenitor cells (MPCs), often called satellite cells, and coursing between the fibers are nerves and blood vessels. Myofibers are bundled together and surrounded by connective tissue, which is attached to bone by tendons.

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Regeneration of injured skeletal muscle follows the same orchestrated plan no matter what insult occasioned the damage. It is driven largely by the quiescent pool of satellite cells located in close apposition to the fibers (60). In response to injury, these cells become activated, proliferate, differentiate, migrate, and fuse to form new myofibers. This series of events is controlled by the sequential activation and repression of specific transcription factors—for example, Pax7, MyoD, MyoG, and MEF2 (61). With muscular dystrophies, wherein chronic myofiber loss is due to a genetic defect, the satellite cell pool is called on repeatedly, resulting in its exhaustion or loss of function over time, thereby dampening the repair process (62).

Skeletal muscle regeneration is influenced by inflammatory events that accompany repair (63–67). Following an early, transient recruitment of neutrophils, myeloid-lineage mononuclear cells, mainly derived from a pool of circulating monocytes, infiltrate acutely injured muscle. Within days, the myeloid infiltrate transitions from a pro- to an anti-inflammatory phenotype, a shift that is critical for proper repair. An initial population of proinflammatory, or M1-type, macrophages is required for clearance of apoptotic or necrotic cells and derivative debris; a subsequent population of anti-inflammatory, or M2-type, macrophages has various proregenerative functions, such as matrix remodeling and promotion of angiogenesis. Ablation or impaired recruitment of macrophages severely compromises muscle repair. Chronic muscle injury entails analogous shifts in innate immune system cell populations (68–70).

Though far less markedly, lymphocytes also accumulate in skeletal muscle after acute injury, as well as in the dystrophin-deficient muscles of mice harboring the mdx mutation or humans with Duchenne's muscular dystrophy (DMD) (63, 68, 71). Their function has not been well studied, although both $CD4^+$ and $CD8^+$ T cells seem to promote the mdx pathology, and blocking their activities with immunosuppressants can ameliorate disease (72). For example, local or systemic treatment with rapamycin improves the mdx phenotype, with concomitant decreases in infiltrating $CD4^+$ and $CD8^+$ effector T cell populations, while Tregs remain stable (73). Even less is known about the composition and function of infiltrating T cell populations in models of acute muscle injury. Interestingly, male nu/nu mice were reported to have considerably smaller limb muscles with a far higher proportion of collagen compared with their wild-type counterparts, but only before 12 weeks of age (74). This observation was taken as evidence that T lymphocytes somehow promote skeletal muscle development, but this hypothesis needs to be tested in mice more specifically deficient in T cells.

A distinct population of $Foxp3^+CD4^+$ Tregs residing in acutely and chronically injured skeletal muscle was uncovered in 2013 (75), serving to extend the concept of tissue Tregs. Again, we explore this population's distinct phenotype, unique functions, sustaining factors, and human equivalent.

Phenotype

Skeletal muscle Tregs can be distinguished from their lymphoid-organ counterparts by the same three criteria that set VAT Tregs apart from classical Tregs: their representation, TCR repertoire, and transcriptome (75). A small population of $Foxp3^+CD4^+$ T cells resides in

normal muscle, and it rapidly expands after mild cryoinjury or subsequent to the more severe damage induced by injecting cardiotoxin (ctx), reaching levels as high as 60% of the $CD4^+$ T cell compartment. This rise coincides with the switch in macrophage phenotype from pro- to anti-inflammatory. An elevated muscle Treg fraction and number can be detected at least a month after acute injury. Tregs are located both within the inflammatory infiltrate of injured muscle and between remote myofibers. The $Foxp3^+CD4^+$ population is also enriched in mice with chronic muscle injury, i.e., the mdx or Dysf knockout models (75, 76).

Aging of skeletal muscle, like that of most mammalian tissues, is associated with a steady decline in bulk, function, and regenerative capacity (60). Given the increasing representation of older adults in the populations of many modern societies, propensities toward muscle wasting and musculoskeletal injury are substantial public health problems. The defect in regeneration is due at least in part to an age-associated decrease in satellite cell frequency and function (77). The molecular mechanisms underlying reduction of the satellite pool are the focus of ongoing investigation, with studies on heterochronic parabiotic mice suggesting an important contribution of circulating factors and/or migratory immune system cells (78–81). The representation of $Foxp3^+CD4^+$ T cells in lymphoid organs increases with age, in both humans and mice (82); however, they are strikingly impoverished in injured skeletal muscle of aged mice (53). The reduction reflects age-dependent effects on Treg recruitment to muscle, their proliferation, and their retention within muscle.

Clonal expansions of skeletal muscle Tregs occur in response to both acute and chronic injury and can be even more pronounced than those typical of VAT Tregs (compare 31, 75). Interestingly, one clone (with identical TCR-α and -β chains) was found in 9 of 11 mice examined 2 or 4 days after ctx injury; in the other 2 animals, a clone with a conservative valine-lysine substitution for alanine-valine in the CDR3α emerged. These features again argue that such tissue Tregs may be responding to a local antigen. Tconvs in injured muscle can also exhibit clonal repetition, but with some delay in comparison with the Treg expansions.

The transcriptome of skeletal muscle Tregs is readily distinguishable from that of lymphoid-organ Tregs, although it is perhaps less distinct than that of VAT Tregs (compare 28, 75). Again, genes encoding transcription factors, chemokines and their receptors, and cytokines and their receptors stand out as differential, certain of the same ones as seen for VAT versus lymphoid-organ Tregs, e.g., Ccr2, Il10, and Il1rl1. Yet, the transcriptomes of VAT and muscle $Foxp3^+CD4^+$ T cells are also dissimilar, significantly different for hundreds of transcripts (further discussed below).

Function

Diphtheria-toxin-induced ablation of Tregs in mice carrying a Foxp3-DTR allele compromises muscle regeneration by a number of criteria (53, 75). The pro- to anti-inflammatory shift in infiltrating myeloid cells is muted; histological markers of healthy regeneration, notably centrally nucleated myofibers and increased fiber width, are less evident; fibrosis is exacerbated; and the usual succession of whole-muscle transcriptomic changes is disturbed, notably signatures associated with homeostasis/function and repair/regeneration. There are also clear effects on MPCs, including a more sparse representation

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and lower clonal efficiency (53, 83). It has been reported that in vitro differentiated Tregs (iTregs) can improve the ability of satellite cells to proliferate in culture, while inhibiting their differentiation (83), but the relevance of this observation is not clear because true skeletal muscle Tregs are not iTregs and have a very different phenotype. At least part of the influence on MPCs is mediated by amphiregulin (Areg), a member of the epidermal growth factor family whose receptor (epidermal growth factor receptor, Egfr) is expressed on satellite cells. Muscle Foxp3$^+$CD4$^+$ T cells overexpress Areg; injection of this growth factor into Treg-deficient or -sufficient mice improves muscle repair; and addition of Areg to MPC cultures increases their capacity to differentiate. Yet, it is not known to what extent the Areg produced specifically by Tregs is required or contributory. A potential role for IL-10 has also been highlighted (76), largely because of this cytokine's high-level expression by muscle Tregs and known effects on myeloid-lineage cells in regenerating muscle (69, 70, 75).

The case for Tregs playing an important role in maintaining skeletal muscle homeostasis is strengthened by data on dystrophic and geriatric muscle. As mentioned above, the Foxp3$^+$CD4$^+$ T cell populations in muscles, but not in the lymphoid organs, of mdx or Dysf knockout mice are elevated (75, 76). Systemic augmentation of Tregs in mdx mice by injection of IL-2/anti-IL-2 complexes dampens muscle damage in short-term experiments, whereas their systemic reduction (or inhibition) by administration of anti-CD25 monoclonal antibodies or via genetically enabled ablation heightens the damage (75, 76). Also mentioned above was that skeletal muscle from aged mice shows an impoverishment of the Foxp3$^+$CD4$^+$ population, accompanying its reduced regenerative capacity (53). As detailed below, experimental augmentation of aged muscle Tregs by injection of recombinant IL-33 improves regeneration subsequent to acute injury according to multiple criteria (53).

Factors

The IL-33:St2 axis is as key to the accumulation of Tregs in skeletal muscle as it is to their enrichment in VAT (53). A high proportion of muscle Foxp3$^+$, but not Foxp3$^-$, CD4$^+$ T cells is St2$^+$; this fraction mounts in the first few days after muscle injury and persists at ~30% for weeks thereafter. Mice with Treg-specific ablation of St2 have a substantially smaller population of muscle, but not lymphoid-organ, Tregs after acute injury, along with poorer muscle regeneration. Injection of recombinant IL-33 into old mice at the time of injury boosts the Treg representation almost an order of magnitude and promotes robust regeneration. Analogous to the situation with VAT, these effects likely reflect combined influences of IL-33 on Tregs and ILC2s cooperating within the muscle.

In skeletal muscle, the major cells producing high levels of IL-33 are a subset of FAPs (53). Il33 transcript levels spike within hours of muscle injury, and there is a higher representation of IL-33$^+$ FAPs in damaged muscle; injured muscle of old mice shows a reduction in Il33 transcripts and Il-33$^+$ FAPs. Interestingly, some of the IL-33-producing cells in skeletal muscle are closely associated with neural structures: nerve fiber perineurium, nerve bundles, and muscle spindles, which are stretch-sensitive structures implicated in proprioception (84). This observation begs direct experimental assessment of a role for IL-33 in linking nerve and immunocyte activities within muscle.

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Humans

Foxp3^{+}CD4^{+} T cells have been shown to reside in human skeletal muscle, with an increased representation in muscle lesions of patients with DMD (76). Skewing of the TCR repertoire in patients with DMD has also been reported (85), but it is not known whether Treg TCRs are involved. Normal human muscle also hosts readily detectable IL-33-producing cells, certain of them associated with structures resembling nerve fibers. A higher fraction of IL-33 producers than is typically seen with mice colocalizes with endothelial structures (53).

COLONIC TREGS: OVERSEERS OF INTESTINAL EQUIPOISE

The major functions of the large intestine, or colon, are to extract water and salts from solid wastes before their excretion and to host microbial fermentation of as-yet unabsorbed material, generating vitamins K and B12, thiamine, riboflavin, etc. Indeed, the colon contains by far the largest and most diverse microbiota in the digestive tract. For optimum execution of these tasks, the large intestine is layered into distinct regions (Figure 4): the lumen, where the bulk of colon-associated microbes reside; the mucosa, wherein most local immune responses take place; the submucosa, a dense layer of connective tissue that hosts immunologically active colonic patches and is riddled with parasympathetic nerves; a layer of smooth muscle important for colonic contractions; and the serosa, a thick fibrous covering that segregates the intestine within the peritoneum.

The colonic mucosa can be divided into an epithelial layer; the lamina propria; and a thin muscular layer, the muscularis mucosa (reviewed in 86). The epithelial layer provides an immediate physical and chemical barrier against microbial invasion: Its very rare Paneth cells (most of them reside in the small intestine) secrete antimicrobial peptides; its abundant goblet cells produce a shielding layer of mucus; and most of its cells are decorated with a variety of pattern-recognition receptors, such as Toll-like, NOD, and NOD-like receptors. Both the epithelial layer and the lamina propria host specialized populations of innate and adaptive immune cells: several subsets of mononuclear phagocytes, including distinct types that sample microbes in the lumen and that ferry antigens to colon-draining lymph nodes; ILCs, in particular the IFN-γ secreting ILC1 type; plasma cells, mainly producing the IgA2 isotype; rare CD8αα^{+} intraepithelial lymphocytes; conventional CD8αβ^{+} T cells and CD4^{+} T cells of diverse helper classes (especially Th1 and Th17 cells). It was recently reported that many of these cell types are quite mobile, migrating to and from the colon with measurable frequencies (87). This finding highlights the possibility that mucosal immune responses are transduced systemically, as has been documented (88).

The enormity and diversity of the mucosal immune system reflect its gargantuan task: It is charged with protecting the organism from a constellation of potentially pathogenic microbes and ingested substances while promoting colonization with mutualistic microbes and uptake of beneficial nutrients. Of necessity, the intestinal microbiota and immune system have evolved in concert, each influencing the other's constitution and activities (89). Local Tregs are critical enforcers of colonic balance, which is reflected in their distinct phenotypes, nurturing factors, unique factor dependencies and presence in humans.

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Phenotype

Mice housed in clean, but not germfree, conditions host an abundant population of Foxp3$^+$CD4$^+$ T cells in their colonic lamina propria (90, 91). The precise fraction of the local CD4$^+$ T lymphocyte compartment they represent, ranging from about 15% to 40%, differs according to housing conditions, notably diet and microbial exposure. The colonic Treg population includes two distinct components (Table 1): one becomes a Treg in the thymus (tTregs) and is characterized by expression of the transcription factors Gata3 and Helios; the other differentiates from Foxp3$^-$CD4$^+$ cells in the periphery (pTregs) and expresses the nuclear hormone receptor, RORγt, but little or no Helios (reviewed in 92). Despite their different phenotypes, dependencies, and origins—as detailed below—the two subpopulations have usually been lumped together as a single entity, an unfortunate oversimplification brought to light in a set of recent studies (50, 93–95).

The transcriptomes of RORγt$^+$ and RORγt$^-$ Tregs residing in the large intestine differ by hundreds of transcripts (94). Interestingly, the RORγt imprint on Th17 cells, representing this transcription factor's more traditional function, is only partially recapitulated in colonic RORγt$^+$ Tregs. Although they do transcribe the gene encoding IL-17A, this signature cytokine and its relatives are not actually produced. Within the RORγt$^-$ colonic subpopulation, Gata3 exerts its influence in part by enhancing Foxp3 expression and in part by promoting display of St2 and, thereby, transcription of IL-33-dependent genes such as Areg, Klrg1, and Il1rl1 (encoding St2, itself) (50).

The TCRs expressed by colonic Foxp3$^+$CD4$^+$ T cells show specific reactivity against fecal or cecal bacterial extracts or against individual microbial isolates (96, 97). The extent to which the colonic Treg TCR repertoire overlaps with that of Tregs generated in the thymus or reflects peripheral induction events has been debated (96, 97). Divergent results are likely to reflect aspects of the experimental setups, such as the mouse strains used, their housing conditions, the precise Treg population highlighted, and/or analysis procedures.

Factors

The intestinal microbiota exerts a major influence on the population of Foxp3$^+$CD4$^+$ T cells residing in the large intestine. Colonic Treg TCRs recognize antigens derived from enteric microbes (96, 97), and colonic Treg numbers are reduced in conditions of low bacterial load, e.g., after broad-spectrum antibiotic treatment or under germfree housing conditions (90, 91). It is primarily the Helioslo/$^-$—more specifically, the RORγt$^+$Helioslo/$^-$—subpopulation that responds to intestinal microbes (90, 91, 93–95). Whereas early studies highlighted the importance of a consortium of Clostridium species derived from either the mouse or human intestine (91, 98), it is now clear that many bacterial species can individually induce a robust Helioslo/$^-$ colonic Treg population, as many as one-third of the microbes examined in one report (94, 99). Several groups have proposed a role for short-chain fatty acids (SCFAs) such as butyrate, propionate, or acetate in microbial induction of intestinal Tregs, channeled through changes in the acetylation status of Foxp3 and/or of histones coating the Foxp3 locus (93, 98, 100–102). But different SCFAs were implicated in the different studies, and not all of them could evidence a role for SCFAs in colonic Treg induction by microbes (94). The divergent results likely reflect differences in the baseline microbiotas, diets,

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experimental approaches and/or SCFA assays involved. Other factors implicated in induction of Helioslo/$^{-}$ colonic Tregs have included transforming growth factor (TGF)-β (91, 98); IL-6; IL-23; and the vitamin A derivative retinoic acid (93), although contradictory results concerning IL-23 have been reported (94).

The colonic Treg population does not completely disappear in antibiotic-treated or germfree mice. The Helios^{+}, more specifically the Gata3^{+}Helios^{+}, subpopulation is notably less sensitive to the composition of the intestinal microbiota. Instead, accumulation of these cells is promoted by IL-33 (50). A high percentage (~50–70%) of colonic Tregs display St2, and essentially all of these coexpress Gata3. In vivo injection of IL-33 leads to a more robust Treg population, likely—in analogy to in vitro studies—by inducing serine phosphorylation of Gata3, as well as its recruitment to the Foxp3 promotor and St2 enhancer, followed by binding of RNA polymerase II to the two gene promoters. In chimeric mice with a mixture of St2^{+} and St2^{-} bone marrow–derived hematopoietic cells, there is a dearth of St2^{-} Tregs in the spleen after IL-33 stimulation or in the colon subsequent to Helicobacter infection, arguing for a cell-intrinsic dependence on this cytokine. IL-23 appears to inhibit IL-33 responsiveness in colonic Tregs.

Function

The intestinal immune system has the difficult tasks of defending the body against pathogenic microbes while permitting—even facilitating—the installation of a beneficial local microbiota, and of disarming ingested substances that are potentially noxious while allowing uptake of those that are nutritive. Intestinal Tregs regulate these processes, being critical for oral tolerance, for tolerance to microbial mutualists, and for controlling immune responses against enteric pathogens. Given the inverse gradients of digestive enzymes (high→low) and colonizing microbes (low→high) moving proximally to distally along the digestive tract, colonic Tregs are likely to be least involved in oral tolerance.

Locally, colonic Tregs affect the activities of the diverse set of immunocytes in the lamina propria, whether they are long-term residents or just passing through. APCs are one target (e.g., 93); another is likely to be local B or plasma cells and their production of IgAs (103, 104). And, of course, Tregs in the large intestine regulate the activities of CD4^{+} and CD8^{+} effector T cells in the lamina propria. Recent data that Tregs emanating from the distal colon can be found circulating through the peripheral lymphoid system (87) raise the possibility that they can exert extraintestinal effects as well, as has been suggested (91, 105).

Given their clearly distinguishable transcriptomes, the RORγt^{+}Helioslo/$^{-}$, and Gata3^{+}Helios^{+} Treg subpopulations residing in the large intestine may have distinct functions. It has been hypothesized that the former controls local inflammatory responses whereas the latter participates in repair processes (92). RORγt^{+}Helioslo/$^{-}$ colonic Tregs do seem to control inflammatory responses, but there is debate over whether they are focused on type 2 (e.g., proallergic and -fibrotic) reactions (93) or have a more general purview (94, 95). Relatedly, mice lacking pTregs because of a mutation in the conserved CNS1 stretch of the Foxp3 locus spontaneously develop type 2 pathologies at mucosal sites, including the colon (106). However, this large intestinal Treg subpopulation has also been reported to dampen pathology in multiple models of colitis, which depend on diverse immunological

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cells and molecules (e.g., 91, 93, 94, 98, 101). The divergent findings might issue from different microbiotas hosted by mice at the various sites, or they might simply reflect the assays highlighted. Gata3$^+$Helios$^+$ colonic Tregs, on the other hand, appear well equipped to participate in local repair responses, given their induction by the alarmin IL-33 and expression of the tissue-repair factor Areg (50). Tregs lacking Gata3 or St2 have a reduced ability to control enteric inflammation after transfer of Foxp3$^-$CD4$^+$ T cells into lymphopenic mice, but it is not clear at what point inside or outside the colon the defect manifests (50, 107).

Humans

Exploration of these issues in humans is only beginning. Gata3$^+$ Tregs have been observed in human blood (107), and, more relevantly, RORγt$^+$ Tregs can be found in human colonic lamina propria from both healthy controls and patients with Crohn disease (94). It is not clear to what extent these phenotypes parallel the mouse subsets mentioned above.

OTHER TISSUES

While populations of Foxp3$^+$CD4$^+$ T cells located in VAT, skeletal muscle, and colonic lamina propria are the best characterized tissue-Treg communities, several other tissues host Foxp3$^+$CD4$^+$ populations meriting more profound phenotypic and functional exploration. An obvious one is cardiac muscle, which reacts to injury in a manner quite similar to the response of skeletal muscle to damage (108). For example, acute myocardial infarction provokes sterile inflammation entailing recruitment and/or activation of multiple innate and adaptive immune system cell types. As in skeletal muscle, macrophages are important throughout the inflammatory process in the heart, evolving from a pro- to an anti-inflammatory phenotype, a shift that is required for effective tissue repair (109). Tregs begin to accumulate in the infarcted region at the time of this phenotypic shift (109), and systemic depletion or reduction of Foxp3$^+$ cells exacerbates heart inflammation and compromises its repair, leading to a decline in diverse clinical indices (110, 111). Transfer of Tregs inhibited fibrosis in an aortic constriction model of heart injury (112), and coculture of Tregs and cardiac fibroblasts had direct effects on the latter's phenotype and activities, e.g., on expression of extracellular matrix proteins (111). Unfortunately, however, lymphoid-organ rather than cardiac Tregs were used in these two studies, so the relevance of these findings is not clear. Interestingly, biomechanical stress induces cardiomyocytes to produce soluble IL-33, loss of St2 exacerbates the clinical abnormalities provoked by aortic constriction, and injection of IL-33 is cardioprotective (113–115). These results are highly reminiscent of those obtained with skeletal muscle (discussed above).

Another Foxp3$^+$CD4$^+$ population warranting additional attention is that found in the skin (reviewed in 116). This barrier tissue has an unusually high Treg representation at steady state that increases in inflammatory settings (117). Cutaneous Tregs express a distinct constellation of homing receptors, which has been exploited to preferentially deplete them (117, 118). But, beyond that, there exists little information on the phenotypic particularities of Tregs residing in the skin. The development of skin lesions in mice and humans that genetically lack Foxp3$^+$ T cells argues for the functional importance of skin-localized Tregs

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at steady state. They have also been implicated in controlling contact hypersensitivity and atopic dermatitis, as well as in the immunosuppression associated with exposure to ultraviolet light, via the intermediary of epidermal Langerhans cells (116).

Another, fascinating, population of Foxp3$^+$CD4$^+$ T cells is the one that accumulates in the placental decidua of mice bearing embryos that express allogeneic antigens derived from the father (119). This Treg population, which responds to paternal alloantigens, is peripherally induced in a CNS-1-dependent manner. Female mice lacking Tregs (119–123), or, more specifically, missing the CNS-1 stretch of the Foxp3 gene (119), show increased fetal resorption and immune cell infiltration of the placenta when carrying offspring of allogeneic, but not syngeneic, males. Hence, the major function of placental Tregs is to enforce maternal-fetal tolerance (119); a likely trade-off is greater susceptibility to infection by prenatal pathogens (123). While the placental Treg population has some of the features of tissue Tregs, no information has been provided on whether they are truly a distinct population, i.e., whether they have a tissue-specialized transcriptome and/or TCR repertoire.

GENERALITIES

Given the continually growing body of work on distinct populations of Tregs operating within a diversity of tissues, it seems a propitious time to attempt to distill some general principles. To that end, we pose a set of questions—some largely answered, others with partial responses, and still others that have not yet been seriously addressed.

What Is the Provenance of Tissue Tregs?

One element of this question is whether tissue Tregs arise as Foxp3$^+$CD4$^+$ T cells in the thymus (i.e., are tTregs) or are induced in the periphery through conversion of Foxp3$^-$CD4$^+$ T cells (i.e., are pTregs). Different tissue-Treg populations seem to vary in this regard. VAT Tregs are clearly tTregs, as argued by their TCR repertoire (27); their high expression of Helios and Nrp-1 (31) (markers of thymically differentiated Tregs) (124, 125); the lack of any transcriptomic sign of Tconv→Treg cell conversion (31); and the failure of transfer experiments to evidence them (31). According to some of these same criteria, skeletal muscle Tregs are also likely to be tTregs (53, 75). It is generally thought that RORγt$^+$ and Gata3$^+$ colonic Foxp3$^+$CD4$^+$ T cells are pTregs and tTregs, respectively, given their reciprocal expression of Helios (and of Nrp-1) (50, 93, 94), and the former but not the latter subpopulation's absence in mice lacking CNS-1 (106), but this notion has not yet been definitively tested.

Another aspect of the question is to what extent tissue Tregs communicate with the pool of circulating T cells. Here, again, the answer seems to vary according to the tissue queried. For example, there is little contribution of circulating T cells to the VAT Treg population of lean mice, as indicated by data from transfer, parabiosis, and lymph node photoconversion experiments (31), whereas extensive recruitment of Tregs from lymph nodes to injured skeletal muscle can be demonstrated (53). Analogously, there is little exodus of Tregs from VAT into the general circulation, but readily measurable egress from injured muscle. Extensive migration of Tregs into and out of the colon from and to peripheral lymphoid

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organs has been reported (87), although the behaviors of RORγt^{+}Helioslo/$^{-}$ and Gata3^{+}Helios^{+} Tregs were not evaluated separately.

A related issue is when during ontogeny tissue Tregs are generated. Results from thymectomy and punctual Treg ablation experiments performed at progressively later ages argue that VAT Tregs are seeded in the first week or two of life, which fits well with the lack of N-region diversity in their TCR-β but not -α chains (31). Although a substantial population of Foxp3^{+}CD4^{+} T cells can already be found in colonic lamina propria at one week of age, the RORγt^{+} subpopulation begins to accumulate only a week later, coincident with changes in the intestinal microbiota that accompany the transition to solid food (94). This issue is of interest because of the recent identification and characterization of a distinct neonatally generated Treg compartment (126). These newly appreciated Tregs overexpress several transcripts reminiscent of tissue Tregs, e.g., Pparg, Il9r, Il1rl1, Icos, and Gpr15.

In short, tissue Tregs appear to have a variety of origins, depending on the particular tissue examined. In most, though not all, cases they are already Foxp3^{+}CD4^{+} T cells when they arrive in the periphery.

Where and How Do Tissue Tregs Take On Their Distinctive Phenotypes?

It is not known to what extent precursor tissue Tregs emerging from the thymus have already acquired their distinctive phenotypes. First, it may be worth emphasizing that the various tissue-localized Treg populations truly have distinct transcriptomes. Unsupervised hierarchical clustering of the totality of differentially expressed transcripts in highly purified Tregs from VAT, skeletal muscle, colonic lamina propria, and the pancreas, compared with control lymphoid-organ Tregs, revealed sets of transcripts shared by all of the tissue-Treg populations, common to Tregs from a few of the tissues, or unique to a single tissue's Treg population (75) (see Figure 5 for differentially expressed transcription factors). These observations counter the notion that tissue-localized and lymphoid-organ Tregs differ simply because the former represents a more activated, effector (127) or memory (128) or reparative (129) state. Single-cell transcriptomics should resolve this issue, addressing the intratissue heterogeneity of tissue-Treg populations, transcriptional module sharing between Treg populations from diverse tissues, and the extent to which tissue Tregs are found in lymphoid organs and vice-versa.

Three scenarios seem possible: that the distinct tissue-Treg phenotypes are imprinted in the thymus as Foxp3^{+}CD4^{+} T cells differentiate; that they appear after the Tregs are installed in their home tissues, in response to local cues; or, in an intermediate version, that a rough phenotype is imparted in the thymus, which is consummated after Treg arrival in the home tissue. The second possibility seems logical and simple, and it coincides with emerging notions concerning the establishment of tissue-specific macrophage phenotypes (130, 131). However, today there exist no convincing data in support of (or against) this option. It has proven possible to turn on expression of certain diagnostic tissue-Treg transcription factors [e.g., PPARγ (34) or Gata-3 (107)] in lymphoid-organ Foxp3^{+}CD4^{+} T cells by cross-linking their TCRs in the presence of particular mixes of cytokines. Unfortunately, the extent to which these cells truly acquire the corresponding tissue-Treg phenotype has not been addressed. Pinpointing where and when tissue Tregs take on their distinctive phenotypes will

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require well-designed lineage-tracing experiments, employing either inducible fluorescent-reporter lines or tissue-Treg TCR transgenic mice.

The molecular mechanism by which tissue Tregs acquire their distinct transcriptomes is a fascinating problem at the crossroads of developmental and molecular biology. Multiple inputs seem to come into play. First, all of the tissue-Treg populations have a readily discernable Treg signature, although it may not be entirely intact (e.g., 27, 75). The significance of less than perfect "Treg-ness" is not obvious given that the classical Treg signature was defined on data derived solely from populations of classical lymphoid-tissue Tregs. Second, there are likely to be developmental imprints reflecting, for example, the thymic versus peripheral origin of the Treg population, or their perinatal versus adult generation (further discussed below). Third, there are critical local cues that drive accumulation of the different tissue-Treg populations, promote their survival, and incite them to arm appropriately to effectively accomplish the specific task(s) required. A cardinal example of this type of input is the dominance of PPARγ in imposing VAT Treg phenotype and functionality—this transcription factor turns on transcriptional programs that enable Tregs to survive in a lipid-laden environment and also to regulate local and systemic metabolic indices. Systems-level analyses of chromatin architecture and transcriptional programs will be required to decipher this complex and variable set of inputs, as has proven so illuminating in the case of tissue-resident macrophages (130, 131).

How Are Tregs Attracted to the Relevant Tissue and Sustained Therein?

Regardless of their provenance, tissue Tregs must arrive at and survive within the appropriate target tissue. Existing data argue for the importance of TCR:MHCII-peptide, chemokine:chemokine receptor and cytokine-cytokine receptor interactions in these processes. As detailed above, several features of the TCR repertoires of the VAT and skeletal muscle Foxp3$^+$CD4$^+$ populations suggest that these cells are responding to local antigens: microclonal expansions, TCR α chains with the same protein sequence specified by different nucleotide sequences, and emergence of one of the TCRs in most mice examined. Presented by tissular APCs, these antigens serve to retain meandering Tregs within the relevant tissue. It will be interesting to see whether the local antigens are akin to those recognized by CD4$^+$ Tconvs at the same site, or whether they represent a new class of tissue-stress signals. The RORγt$^+$Helioslo/$^-$ subpopulation of colonic Foxp3$^+$CD4$^+$ T cells is a special case, as these cells are clearly responding to locally encountered microbial (and likely dietary) antigens. Again, it will be interesting to explore the nature of these antigens—whether they are microbe specific like those that trigger Tconvs in antimicrobe responses or whether they are more general signals of microbial invasion.

In addition, recruitment of Foxp3$^+$CD4$^+$ T cells to parenchymal tissues almost certainly reflects their response to chemokine gradients. Transcripts encoding many chemokine receptors are differentially expressed in tissue Tregs vis-à-vis their lymphoid-organ counterparts. Some—like Ccr8, Ccr5, CCrl2, and Ccr2—are upregulated in all tissues; the induction of others, e.g., Ccr9, is more specific. Tissue-specific homing receptors also come into play, one example being GPR15, which was found to direct Tregs to the colonic lamina propria (132).

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There are certainly examples of cytokine dependencies being limited to Treg populations localized in particular parenchymal tissues. For example, VAT Tregs produce large amounts of IL-10, and this cytokine has a strong in situ impact on their profile of gene expression (27), whereas colonic RORγt$^+$Helioslo/$^-$ and pancreas Tregs display little if any IL-10 receptor (75). Different tissue-Treg populations also show variable reliance on IL-23 and IL-1 (discussed above). Potentially more interesting is the case of IL-33 [and perhaps IL-18 (129)]. For all tissue-localized tTreg populations examined to date (VAT, skeletal muscle, colon), IL-33 seems to be a critical factor for local expansion and stability. This cytokine has markedly less of an effect on classical lymphoid-organ Treg populations, largely reflecting these cells' low-level display of St2. This differential reliance makes sense given IL-33's alarmin function and the potent tissue-repair programs downstream of St2 engagement. Investigators should keep their eyes open for other pairings of tissular alarmins with particular Treg effector programs. It is also important to explore how these tissue-localized programs are extinguished once the inciting challenge has been resolved.

What Processes Are Tregs Targeting Within Parenchymal Tissues?

Certainly, some of the activities of tissue Tregs are directed at controlling inflammatory cells in the vicinity. Indeed, the accumulation of Foxp3$^+$CD4$^+$ T cells in both VAT and skeletal muscle accompanies a sterile inflammatory response, whereas their counterparts in the colonic lamina propria are confronted with incipient immune reactions to a myriad of microbial and dietary antigens. It may not be a coincidence that VAT and muscle Tregs increase in representation just as anti-inflammatory macrophages do—perhaps they foster M1 to M2 phenotypic shifts. Tissue Tregs seem well equipped to perform such anti-inflammatory activities: They are at least as effective as lymphoid-organ Tregs in standard in vitro suppression assays (e.g., 27); and they have transcriptomes exhibiting many of the elements characteristic of mature, effector Tregs (127).

But tissue Tregs also have the capacity to directly influence surrounding parenchymal cells: VAT Tregs make high levels of IL-10; adipocytes display the IL-10 receptor; and this cytokine blocks the synthesis of inflammatory mediators by cultured adipocytes (27 and references therein). Skeletal muscle Tregs produce abundant Areg; muscle satellite cells express this mediator's receptor, Egfr; and Areg can improve satellite cell function in culture and after injection in vivo (75). The Gata3$^+$Helios$^+$ subpopulation of colonic Tregs also makes substantial amounts of Areg (50), a growth factor recently proposed to be an important element of a Treg mucosal barrier repair program, one employed by lung Tregs as well (129). Thus, it seems that a component of the distinct transcriptomes of tissue Tregs is dedicated to controlling potentially pathological processes unleashed within nonimmunological cells in the home tissue. This notion was recently extended by the demonstration of inflammatory (and anti-inflammatory) cross talk between VAT and the intestine, encompassing local Tregs (133).

Clearly, then, tissue Tregs and their impact on organismal homeostasis are a new world for immunologists to explore. We might expect an onslaught of studies in this realm, likely yielding new immunological principles.

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References

1. Kotas ME, Medzhitov R. Homeostasis, inflammation, and disease susceptibility. Cell. 2015; 160:816–27. [PubMed: 25723161]
2. Chovatiya R, Medzhitov R. Stress, inflammation, and defense of homeostasis. Mol Cell. 2014; 54:281–88. [PubMed: 24766892]
3. Varol C, Mildner A, Jung S. Macrophages: development and tissue specialization. Annu Rev Immunol. 2015; 33:643–75. [Abstract] [Web of Science ®]. [PubMed: 25861979]
4. Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. Annu Rev Immunol. 2012; 30:531–64. [PubMed: 22224781]
5. Rosen ED, Spiegelman BM. What we talk about when we talk about fat. Cell. 2014; 156:20–44. [PubMed: 24439368]
6. Osborn O, Olefsky JM. The cellular and signaling networks linking the immune system and metabolism in disease. Nat Med. 2012; 18:363–74. [PubMed: 22395709]
7. 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. [PubMed: 7678183]
8. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA. 2001; 286:327–34. [PubMed: 11466099]
9. Wen H, Gris D, Lei Y, Jha S, Zhang L, et al. Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling. Nat Immunol. 2011; 12:408–15. [PubMed: 21478880]
10. Xu H, Barnes GT, Yang Q, Tan G, Yang D, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Investig. 2003; 112:1821–30. [PubMed: 14679177]
11. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Investig. 2003; 112:1796–808. [PubMed: 14679176]
12. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Investig. 2007; 117:175–84. [PubMed: 17200717]
13. 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. [PubMed: 17192460]
14. Nguyen MT, Favelyukis S, Nguyen AK, Reichart D, Scott PA, 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. [PubMed: 17916553]
15. Patsouris D, Li PP, Thapar D, Chapman J, Olefsky JM, Neels JG. Ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin resistant animals. Cell Metab. 2008; 8:301–9. [PubMed: 18840360]
16. Weisberg SP, Hunter D, Huber R, Lemieux J, Slaymaker S, et al. CCR2 modulates inflammatory and metabolic effects of high-fat feeding. J Clin Investig. 2006; 116:115–24. [PubMed: 16341265]
17. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, et al. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. J Clin Investig. 2006; 116:1494–505. [PubMed: 16691291]
18. Kamei N, Tobe K, Suzuki R, Ohsugi M, Watanabe T, et al. Overexpression of monocyte chemoattractant protein-1 in adipose tissues causes macrophage recruitment and insulin resistance. J Biol Chem. 2006; 281:26602–14. [PubMed: 16809344]

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19. Chawla A, Nguyen KD, Goh YP. Macrophage-mediated inflammation in metabolic disease. Nat Rev Immunol. 2011; 11:738–49. [PubMed: 21984069]
20. McNelis JC, Olefsky JM. Macrophages, immunity, and metabolic disease. Immunity. 2014; 41:36–48. [PubMed: 25035952]
21. Mathis D, Shoelson SE. Immunometabolism: an emerging frontier. Nat Rev Immunol. 2011; 11:81. [PubMed: 21469396]
22. Mathis D. Immunological goings-on in visceral adipose tissue. Cell Metab. 2013; 17:851–59. [PubMed: 23747244]
23. Lynch L, Michelet X, Zhang S, Brennan PJ, Moseman A, et al. Regulatory iNKT cells lack expression of the transcription factor PLZF and control the homeostasis of Treg cells and macrophages in adipose tissue. Nat Immunol. 2015; 16:85–95. [PubMed: 25436972]
24. Nishimura S, Manabe I, Takaki S, Nagasaki M, Otsu M, et al. Adipose natural regulatory B cells negatively control adipose tissue inflammation. Cell Metab. 2013; 18:759–66. [CrossRef] [Web of Science ®].
25. Wensveen FM, Jelencic V, Valentic S, Sestan M, Wensveen TT, et al. NK cells link obesity-induced adipose stress to inflammation and insulin resistance. Nat Immunol. 2015; 16:376–85. [PubMed: 25729921]
26. Moraes-Vieira PM, Yore MM, Dwyer PM, Syed I, Aryal P, Kahn BB. RBP4 activates antigen-presenting cells, leading to adipose tissue inflammation and systemic insulin resistance. Cell Metab. 2014; 19:512–26. [PubMed: 24606904]
27. Feuerer M, Herrero L, Cipolletta D, Naaz A, Wong J, et al. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat Med. 2009; 15:930–39. The first molecular, cellular, and functional characterization of a parenchymal-tissue Treg population, residing in visceral adipose tissue, is reported in this paper. [PubMed: 19633656]
28. Cipolletta D, Cohen P, Spiegelman BM, Benoist C, Mathis D. Appearance and disappearance of the mRNA signature characteristic of Treg cells in visceral adipose tissue: age, diet, and PPARγ effects. PNAS. 2015; 112:482–87. [PubMed: 25550516]
29. Pettersson US, Walden TB, Carlsson PO, Jansson L, Phillipson M. Female mice are protected against high-fat diet induced metabolic syndrome and increase the regulatory T cell population in adipose tissue. PLOS ONE. 2012; 7:e46057. [PubMed: 23049932]
30. Tran TT, Yamamoto Y, Gesta S, Kahn CR. Beneficial effects of subcutaneous fat transplantation on metabolism. Cell Metab. 2008; 7:410–20. [PubMed: 18460332]
31. Kolodin D, van PN, Li C, Magnuson AM, Cipolletta D, et al. Antigen- and cytokine-driven accumulation of regulatory T cells in visceral adipose tissue of lean mice. Cell Metab. 2015; 21:543–57. [PubMed: 25863247]
32. Tontonoz P, Spiegelman BM. Fat and beyond: the diverse biology of PPARγ. Annu Rev Biochem. 2008; 77:289–312. [PubMed: 18518822]
33. Cipolletta D, Feuerer M, Li A, Kamei N, Lee J, et al. PPAR-γ is a major driver of the accumulation and phenotype of adipose tissue Treg cells. Nature. 2012; 486:549–53. Reports the intriguing observation that tissue Tregs can co-opt the archetypal transcriptional program of surrounding parenchymal cells. [PubMed: 22722857]
34. Vasanthakumar A, Moro K, Xin A, Liao Y, Gloury R, et al. The transcriptional regulators IRF4, BATF and IL-33 orchestrate development and maintenance of adipose tissue-resident regulatory T cells. Nat Immunol. 2015; 16:276–85. Uncovers an important role for the IL-33:St2 axis in VAT Treg accumulation and identifies upstream transcription factors. [PubMed: 25599561]
35. Medrikova D, Sijmonsma TP, Sowodniok K, Richards DM, Delacher M, et al. Brown adipose tissue harbors a distinct sub-population of regulatory T cells. PLOS ONE. 2015; 10:e0118534. [PubMed: 25714366]
36. Deiuliis J, Shah Z, Shah N, Needleman B, Mikami D, et al. Visceral adipose inflammation in obesity is associated with critical alterations in T regulatory cell numbers. PLOS ONE. 2011; 6:e16376. [PubMed: 21298111]
37. Eller K, Kirsch A, Wolf AM, Sopper S, Tagwerker A, et al. Potential role of regulatory T cells in reversing obesity-linked insulin resistance and diabetic nephropathy. Diabetes. 2011; 60:2954–62. [PubMed: 21911743]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

38. Winer S, Chan Y, Paltser G, Truong D, Tsui H, et al. Normalization of obesity-associated insulin resistance through immunotherapy. Nat Med. 2009; 15:921–29. [PubMed: 19633657]

39. Ilan Y, Maron R, Tukpah AM, Maioli TU, Murugaiyan G, et al. Induction of regulatory T cells decreases adipose inflammation and alleviates insulin resistance in ob/ob mice. PNAS. 2010; 107:9765–70. [PubMed: 20445103]

40. Cho KW, Morris DL, Delproposto JL, Geletka L, Zamarron B, et al. An MHC II-dependent activation loop between adipose tissue macrophages and $CD4^+$ T cells controls obesity-induced inflammation. Cell Rep. 2014; 9:605–17. [PubMed: 25310975]

41. Meijer K, de Vries M, Al-Lahham S, Bruinenberg M, Weening D, et al. Human primary adipocytes exhibit immune cell function: Adipocytes prime inflammation independent of macrophages. PLOS ONE. 2011; 6:e17154. [PubMed: 21448265]

42. Deng T, Lyon CJ, Minze LJ, Lin J, Zou J, et al. Class II major histocompatibility complex plays an essential role in obesity-induced adipose inflammation. Cell Metab. 2013; 17:411–22. [PubMed: 23473035]

43. Choi JH, Banks AS, Estall JL, Kajimura S, Bostrom P, et al. Anti-diabetic drugs inhibit obesity-linked phosphorylation of PPARγ by Cdk5. Nature. 2010; 466:451–56. [PubMed: 20651683]

44. Choi JH, Banks AS, Kamenecka TM, Busby SA, Chalmers MJ, et al. Antidiabetic actions of a non-agonist PPARγ ligand blocking Cdk5-mediated phosphorylation. Nature. 2011; 477:477–81. [PubMed: 21892191]

45. Banks AS, McAllister FE, Camporez JP, Zushin PJ, Jurczak MJ, et al. An ERK/Cdk5 axis controls the diabetogenic actions of PPARγ. Nature. 2014; 517:391–95. [PubMed: 25409143]

46. Molofsky AB, Van GF, Liang HE, Van Dyken SJ, Nussbaum JC, et al. Interleukin-33 and interferon-gamma counter-regulate group 2 innate lymphoid cell activation during immune perturbation. Immunity. 2015; 43:161–74. [PubMed: 26092469]

47. Han JM, Wu D, Denroche HC, Yao Y, Verchere CB, Levings MK. IL-33 reverses an obesity-induced deficit in visceral adipose tissue $ST2^+$ T regulatory cells and ameliorates adipose tissue inflammation and insulin resistance. J Immunol. 2015; 194:4777–83. [PubMed: 25870243]

48. Molofsky AB, Savage AK, Locksley RM. Interleukin-33 in tissue homeostasis, injury, and inflammation. Immunity. 2015; 42:1005–19. [PubMed: 26084021]

49. Turnquist HR, Zhao Z, Rosborough BR, Liu Q, Castellaneta A, et al. IL-33 expands suppressive $CD11b^+$ Gr-1int and regulatory T cells, including $ST2L^+Foxp3^+$ cells, and mediates regulatory T cell-dependent promotion of cardiac allograft survival. J Immunol. 2011; 187:4598–610. [PubMed: 21949025]

50. Schiering C, Krausgruber T, Chomka A, Frohlich A, Adelmann K, et al. The alarmin IL-33 promotes regulatory T-cell function in the intestine. Nature. 2014; 513:564–68. Uncovers the IL-33 dependence of colonic Tregs, of the $Gata3^+Helios^+$ subpopulation in particular. [PubMed: 25043027]

51. Miller AM, Asquith DL, Hueber AJ, Anderson LA, Holmes WM, et al. Interleukin-33 induces protective effects in adipose tissue inflammation during obesity in mice. Circ Res. 2010; 107:650–58. [PubMed: 20634488]

52. Pichery M, Mirey E, Mercier P, Lefrancais E, Dujardin A, et al. Endogenous IL-33 is highly expressed in mouse epithelial barrier tissues, lymphoid organs, brain, embryos, and inflamed tissues: in situ analysis using a novel Il-33-LacZ gene trap reporter strain. J Immunol. 2012; 188:3488–95. [PubMed: 22371395]

53. Kuswanto W, Burzyn D, Panduro M, Wang KK, Jang YC, et al. Poor repair of skeletal muscle in aging mice reflects a defect in local, interleukin-33-dependent accumulation of regulatory T cells. Immunity. 2016; 44:355–67. [PubMed: 26872699]

54. Morita H, Arae K, Unno H, Miyauchi K, Toyama S, et al. An interleukin-33-mast cell-interleukin-2 axis suppresses papain-induced allergic inflammation by promoting regulatory T cell numbers. Immunity. 2015; 43:175–86. [PubMed: 26200013]

55. Fabrizi M, Marchetti V, Mavilio M, Marino A, Casagrande V, et al. IL-21 is a major negative regulator of IRF4-dependent lipolysis affecting Tregs in adipose tissue and systemic insulin sensitivity. Diabetes. 2014; 63:2086–96. [PubMed: 24430438]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

56. Priceman SJ, Kujawski M, Shen S, Cherryholmes GA, Lee H, et al. Regulation of adipose tissue T cell subsets by Stat3 is crucial for diet-induced obesity and insulin resistance. PNAS. 2013; 110:13079–84. [PubMed: 23878227]
57. Bassaganya-Riera J, Dominguez-Bello MG, Kronsteiner B, Carbo A, Lu P, et al. Helicobacter pylori colonization ameliorates glucose homeostasis in mice through a PPAR gamma-dependent mechanism. PLOS ONE. 2012; 7:e50069. [PubMed: 23166823]
58. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. Nat Rev Immunol. 2011; 11:98–107. [PubMed: 21233852]
59. Zeyda M, Wernly B, Demyanets S, Kaun C, Hammerle M, et al. Severe obesity increases adipose tissue expression of interleukin-33 and its receptor ST2, both predominantly detectable in endothelial cells of human adipose tissue. Int J Obes. 2013; 37:658–65.
60. Jang YC, Sinha M, Cerletti M, Dall'Osso C, Wagers AJ. Skeletal muscle stem cells: effects of aging and metabolism on muscle regenerative function. Cold Spring Harb Symp Quant Biol. 2011; 76:101–11. [PubMed: 21960527]
61. Rudnicki MA, Le Grand F, McKinnell I, Kuang S. The molecular regulation of muscle stem cell function. Cold Spring Harb Symp Quant Biol. 2008; 73:323–31. [PubMed: 19329572]
62. Tabebordbar M, Wang ET, Wagers AJ. Skeletal muscle degenerative diseases and strategies for therapeutic muscle repair. Annu Rev Pathol. 2013; 8:441–75. [PubMed: 23121053]
63. Tidball JG, Villalta SA. Regulatory interactions between muscle and the immune system during muscle regeneration. Am J Physiol Regul Integr Comp Physiol. 2010; 298:R1173–87. [PubMed: 20219869]
64. Bosurgi L, Manfredi AA, Rovere-Querini P. Macrophages in injured skeletal muscle: a perpetuum mobile causing and limiting fibrosis, prompting or restricting resolution and regeneration. Front Immunol. 2011; 2:62. [PubMed: 22566851]
65. Arnold L, Henry A, Poron F, Baba-Amer Y, van Rooijen N, et al. Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. J Exp Med. 2007; 204:1057–69. [PubMed: 17485518]
66. Ruffell D, Mourkioti F, Gambardella A, Kirstetter P, Lopez RG, et al. A CREB-C/EBPβ cascade induces M2 macrophage-specific gene expression and promotes muscle injury repair. PNAS. 2009; 106:17475–80. [PubMed: 19805133]
67. Mounier R, Theret M, Arnold L, Cuvellier S, Bultot L, et al. AMPKα1 regulates macrophage skewing at the time of resolution of inflammation during skeletal muscle regeneration. Cell Metab. 2013; 18:251–64. [PubMed: 23931756]
68. Rosenberg AS, Puig M, Nagaraju K, Hoffman EP, Villalta SA, et al. Immune-mediated pathology in Duchenne muscular dystrophy. Sci Transl Med. 2015; 7:299rv4.
69. Villalta SA, Rinaldi C, Deng B, Liu G, Fedor B, Tidball JG. Interleukin-10 reduces the pathology of mdx muscular dystrophy by deactivating M1 macrophages and modulating macrophage phenotype. Hum Mol Genet. 2011; 20:790. [PubMed: 21118895]
70. Villalta SA, Nguyen HX, Deng B, Gotoh T, Tidball JG. Shifts in macrophage phenotypes and macrophage competition for arginine metabolism affect the severity of muscle pathology in muscular dystrophy. Hum Mol Genet. 2009; 18:482–96. [PubMed: 18996917]
71. Farini A, Meregalli M, Belicchi M, Battistelli M, Parolini D, et al. T and B lymphocyte depletion has a marked effect on the fibrosis of dystrophic skeletal muscles in the scid/mdx mouse. J Pathol. 2007; 213:229–38. [PubMed: 17668421]
72. Iannitti T, Capone S, Feder D, Palmieri B. Clinical use of immunosuppressants in Duchenne muscular dystrophy. J Clin Neuromuscul Dis. 2010; 12:1–21. [PubMed: 20808159]
73. Eghtesad S, Jhunjhunwala S, Little SR, Clemens PR. Rapamycin ameliorates dystrophic phenotype in mdx mouse skeletal muscle. Mol Med. 2011; 17:917–24. [PubMed: 21607286]
74. Morrison J, Partridge T, Bou-Gharios G. Nude mutation influences limb skeletal muscle development. Matrix Biol. 2005; 23:535–42. [PubMed: 15694130]
75. Burzyn D, Kuswanto W, Kolodin D, Shadrach JL, Cerletti M, et al. A special population of regulatory T cells potentiates muscle repair. Cell. 2013; 155:1282–95. Describes skeletal muscle Tregs for the first time and demonstrates their importance for muscle repair after acute or chronic injury. [PubMed: 24315098]

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76. Villalta SA, Rosenthal W, Martinez L, Kaur A, Sparwasser T, et al. Regulatory T cells suppress muscle inflammation and injury in muscular dystrophy. Sci Transl Med. 2014; 6:258ra142.

77. Blau HM, Cosgrove BD, Ho AT. The central role of muscle stem cells in regenerative failure with aging. Nat Med. 2015; 21:854–62. [PubMed: 26248268]

78. Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. Nature. 2005; 433:760–64. [PubMed: 15716955]

79. Brack AS, Conboy MJ, Roy S, Lee M, Kuo CJ, et al. Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. Science. 2007; 317:807–10. [PubMed: 17690295]

80. Ruckh JM, Zhao J-W, Shadrach JL, van Wijngarden P, Rao TN, et al. Rejuvenation of regeneration in the aging central nervous system. Cell Stem Cell. 2012; 10:96–103. [PubMed: 22226359]

81. Sinha M, Jang YC, Oh J, Khong D, Wu EY, et al. Restoring systemic GDF11 levels reverses age-related dysfunction in mouse skeletal muscle. Science. 2014; 344:649–52. [PubMed: 24797481]

82. Lages CS, Suffia I, Velilla PA, Huang B, Warshaw G, et al. Functional regulatory T cells accumulate in aged hosts and promote chronic infectious disease reactivation. J Immunol. 2008; 181:1835–48. [PubMed: 18641321]

83. Castiglioni A, Corna G, Rigamonti E, Basso V, Vezzoli M, et al. FOXP3^{+} T cells recruited to sites of sterile skeletal muscle injury regulate the fate of satellite cells and guide effective tissue regeneration. PLOS ONE. 2015; 10:e0128094. [PubMed: 26039259]

84. Takeoka A, Vollenweider I, Courtine G, Arber S. Muscle spindle feedback directs locomotor recovery and circuit reorganization after spinal cord injury. Cell. 2014; 159:1626–39. [PubMed: 25525880]

85. Gussoni E, Pavlath GK, Miller RG, Panzara MA, Powell M, et al. Specific T cell receptor gene rearrangements at the site of muscle degeneration in Duchenne muscular dystrophy. J Immunol. 1994; 153:4798–805. [PubMed: 7963545]

86. Mowat AM, Agace WW. Regional specialization within the intestinal immune system. Nat Rev Immunol. 2014; 14:667–85. [PubMed: 25234148]

87. Morton AM, Sefik E, Upadhyay R, Weissleder R, Benoist C, Mathis D. Endoscopic photoconversion reveals unexpectedly broad leukocyte trafficking to and from the gut. PNAS. 2014; 111:6696–701. [PubMed: 24753589]

88. Wu HJ, Ivanov II, Darce J, Hattori K, Shima T, et al. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. Immunity. 2010; 32:815–27. [PubMed: 20620945]

89. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. Science. 2012; 336:1268–73. [PubMed: 22674334]

90. Geuking MB, Cahenzli J, Lawson MA, Ng DC, Slack E, et al. Intestinal bacterial colonization induces mutualistic regulatory T cell responses. Immunity. 2011; 34:794–806. [PubMed: 21596591]

91. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, et al. Induction of colonic regulatory T cells by indigenous Clostridium species. Science. 2011; 331:337–41. [PubMed: 21205640]

92. Hegazy AN, Powrie F. Microbiota RORgulates intestinal suppressor T cells. Science. 2015; 349:929–30. [PubMed: 26315421]

93. Ohnmacht C, Park JH, Cording S, Wing JB, Atarashi K, et al. The microbiota regulates type 2 immunity through RORγ^{+} T cells. Science. 2015; 349:989–93. 93 and 94. Report on the phenotype and function of the microbiota-dependent RORγt^{+}Helios1o/$^{-}$ subpopulation of Tregs in the colonic lamina propria. [PubMed: 26160380]

94. Sefik E, Geva-Zatorsky N, Oh S, Konnikova L, Zemmour D, et al. Individual intestinal symbionts induce a distinct population of RORγ^{+} regulatory T cells. Science. 2015; 349:993–7. 93 and 94. Report on the phenotype and function of the microbiota-dependent RORγt^{+}Helios1o/$^{-}$ subpopulation of Tregs in the colonic lamina propria. [PubMed: 26272906]

95. Yang BH, Hagemann S, Mamareli P, Lauer U, Hoffmann U, et al. Foxp3^{+} T cells expressing RORγt represent a stable regulatory T-cell effector lineage with enhanced suppressive capacity during intestinal inflammation. Mucosal Immunol. 2016; 9:444–57. [PubMed: 26307665]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

96. Lathrop SK, Bloom SM, Rao SM, Nutsch K, Lio CW, et al. Peripheral education of the immune system by colonic commensal microbiota. Nature. 2011; 478:250–54. [PubMed: 21937990]
97. Cebula A, Seweryn M, Rempala GA, Pabla SS, McIndoe RA, et al. Thymus-derived regulatory T cells contribute to tolerance to commensal microbiota. Nature. 2013; 497:258–62. [PubMed: 23624374]
98. Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, et al. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature. 2013; 500:232–36. [PubMed: 23842501]
99. Faith JJ, Ahern PP, Ridaura VK, Cheng J, Gordon JI. Identifying gut microbe-host phenotype relationships using combinatorial communities in gnotobiotic mice. Sci Transl Med. 2014; 6:220ra11.
100. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science. 2013; 341:569–73. [PubMed: 23828891]
101. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature. 2013; 504:446–50. [PubMed: 24226770]
102. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature. 2013; 504:451–55. [PubMed: 24226773]
103. Cong Y, Feng T, Fujihashi K, Schoeb TR, Elson CO. A dominant, coordinated T regulatory cell-IgA response to the intestinal microbiota. PNAS. 2009; 106:19256–61. [PubMed: 19889972]
104. Kawamoto S, Maruya M, Kato LM, Suda W, Atarashi K, et al. Foxp3^{+} T cells regulate immunoglobulin A selection and facilitate diversification of bacterial species responsible for immune homeostasis. Immunity. 2014; 41:152–65. [PubMed: 25017466]
105. Sun J, Furio L, Mecheri R, van der Does AM, Lundeberg E, et al. Pancreatic beta-cells limit autoimmune diabetes via an immunoregulatory antimicrobial peptide expressed under the influence of the gut microbiota. Immunity. 2015; 43:304–17. [PubMed: 26253786]
106. Josefowicz SZ, Niec RE, Kim HY, Treuting P, Chinen T, et al. Extrathymically generated regulatory T cells control mucosal TH2 inflammation. Nature. 2012; 482:395–99. [PubMed: 22318520]
107. Wohlfert EA, Grainger JR, Bouladoux N, Konkel JE, Oldenhove G, et al. GATA3 controls Foxp3^{+} regulatory T cell fate during inflammation in mice. J Clin Investig. 2011; 121:4503–15. [PubMed: 21965331]
108. Frangogiannis NG. The inflammatory response in myocardial injury, repair, and remodelling. Nat Rev Cardiol. 2014; 11:255–65. [PubMed: 24663091]
109. Yan X, Anzai A, Katsumata Y, Matsuhashi T, Ito K, et al. Temporal dynamics of cardiac immune cell accumulation following acute myocardial infarction. J Mol Cell Cardiol. 2013; 62:24–35. [PubMed: 23644221]
110. Weirather J, Hofmann UD, Beyersdorf N, Ramos GC, Vogel B, et al. Foxp3^{+} CD4^{+} T cells improve healing after myocardial infarction by modulating monocyte/macrophage differentiation. Circ Res. 2014; 115:55–67. [PubMed: 24786398]
111. Saxena A, Dobaczewski M, Rai V, Haque Z, Chen W, et al. Regulatory T cells are recruited in the infarcted mouse myocardium and may modulate fibroblast phenotype and function. Am J Physiol Heart Circ Physiol. 2014; 307:H1233–42. [PubMed: 25128167]
112. Kanellakis P, Dinh TN, Agrotis A, Bobik A. CD4^{+}CD25^{+}Foxp3^{+} regulatory T cells suppress cardiac fibrosis in the hypertensive heart. J Hypertens. 2011; 29:1820–28. [PubMed: 21785365]
113. Sanada S, Hakuno D, Higgins LJ, Schreiter ER, McKenzie AN, Lee RT. IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system. J Clin Investig. 2007; 117:1538–49. [PubMed: 17492053]
114. Kakkar R, Hei H, Dobner S, Lee RT. Interleukin 33 as a mechanically responsive cytokine secreted by living cells. J Biol Chem. 2012; 287:6941–48. [PubMed: 22215666]
115. Chen WY, Hong J, Gannon J, Kakkar R, Lee RT. Myocardial pressure overload induces systemic inflammation through endothelial cell IL-33. PNAS. 2015; 112:7249–54. [PubMed: 25941360]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

116. Honda T, Miyachi Y, Kabashima K. Regulatory T cells in cutaneous immune responses. J Dermatol Sci. 2011; 63:75–82. [Medline] [Web of Science ®]. [PubMed: 21708454]

117. Sather BD, Treuting P, Perdue N, Miazgowicz M, Fontenot JD, et al. Altering the distribution of Foxp3$^+$ regulatory T cells results in tissue-specific inflammatory disease. J Exp Med. 2007; 204:1335–47. [PubMed: 17548521]

118. Dudda JC, Perdue N, Bachtanian E, Campbell DJ. Foxp3$^+$ regulatory T cells maintain immune homeostasis in the skin. J Exp Med. 2008; 205:1559–65. [PubMed: 18573908]

119. Samstein RM, Josefowicz SZ, Arvey A, Treuting PM, Rudensky AY. Extrathymic generation of regulatory T cells in placental mammals mitigates maternal-fetal conflict. Cell. 2012; 150:29–38. Identifies a placental decidua Treg population that is critical for preventing maternal rejection of a fetus expressing paternally derived alloantigens. [PubMed: 22770213]

120. Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. Nat Immunol. 2004; 5:266–71. [PubMed: 14758358]

121. Shima T, Sasaki Y, Itoh M, Nakashima A, Ishii N, et al. Regulatory T cells are necessary for implantation and maintenance of early pregnancy but not late pregnancy in allogeneic mice. J Reprod Immunol. 2010; 85:121–29. [PubMed: 20439117]

122. Rowe JH, Ertelt JM, Xin L, Way SS. Pregnancy imprints regulatory memory that sustains anergy to fetal antigen. Nature. 2012; 490:102–6. [PubMed: 23023128]

123. Rowe JH, Ertelt JM, Aguilera MN, Farrar MA, Way SS. Foxp3$^+$ regulatory T cell expansion required for sustaining pregnancy compromises host defense against prenatal bacterial pathogens. Cell Host Microbe. 2011; 10:54–64. [PubMed: 21767812]

124. Thornton AM, Korty PE, Tran DQ, Wohlfert EA, Murray PE, et al. Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3$^+$ T regulatory cells. J Immunol. 2010; 184:3433–41. [PubMed: 20181882]

125. Weiss JM, Bilate AM, Gobert M, Ding Y, Curotto de Lafaille MA, et al. Neuropilin 1 is expressed on thymus-derived natural regulatory T cells, but not mucosa-generated induced Foxp3$^+$ T reg cells. J Exp Med. 2012; 209:1723–42. [PubMed: 22966001]

126. Yang S, Fujikado N, Kolodin D, Benoist C, Mathis D. Immune tolerance: Regulatory T cells generated early in life play a distinct role in maintaining self-tolerance. Science. 2015; 348:589–94. [PubMed: 25791085]

127. Cretney E, Kallies A, Nutt SL. Differentiation and function of Foxp3$^+$ effector regulatory T cells. Trends Immunol. 2013; 34:74–80. [PubMed: 23219401]

128. Rosenblum MD, Gratz IK, Paw JS, Lee K, Marshak-Rothstein A, Abbas AK. Response to self antigen imprints regulatory memory in tissues. Nature. 2011; 480:538–42. [PubMed: 22121024]

129. Arpaia N, Green JA, Moltedo B, Arvey A, Hemmers S, et al. A distinct function of regulatory T cells in tissue protection. Cell. 2015; 162:1078–89. Describes a TCR-independent repair program in lung Tregs and highlights the important role of amphiregulin. [PubMed: 26317471]

130. Lavin Y, Winter D, Blecher-Gonen R, David E, Keren-Shaul H, et al. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. Cell. 2014; 159:1312–26. Uses a systems-level analysis to explore how tissue-resident macrophages acquire unique phenotypes, a potential model for tissue Tregs. [PubMed: 25480296]

131. Gosselin D, Link VM, Romanoski CE, Fonseca GJ, Eichenfield DZ, et al. Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. Cell. 2014; 159:1327–40. [PubMed: 25480297]

132. Kim SV, Xiang WV, Kwak C, Yang Y, Lin XW, et al. GPR15-mediated homing controls immune homeostasis in the large intestine mucosa. Science. 2013; 340:1456–59. [PubMed: 23661644]

133. Luck H, Tsai S, Chung J, Clemente-Casares X, Ghazarian M, et al. Regulation of obesity-related insulin resistance with gut anti-inflammatory agents. Cell Metab. 2015; 21:527–42. [PubMed: 25863246]

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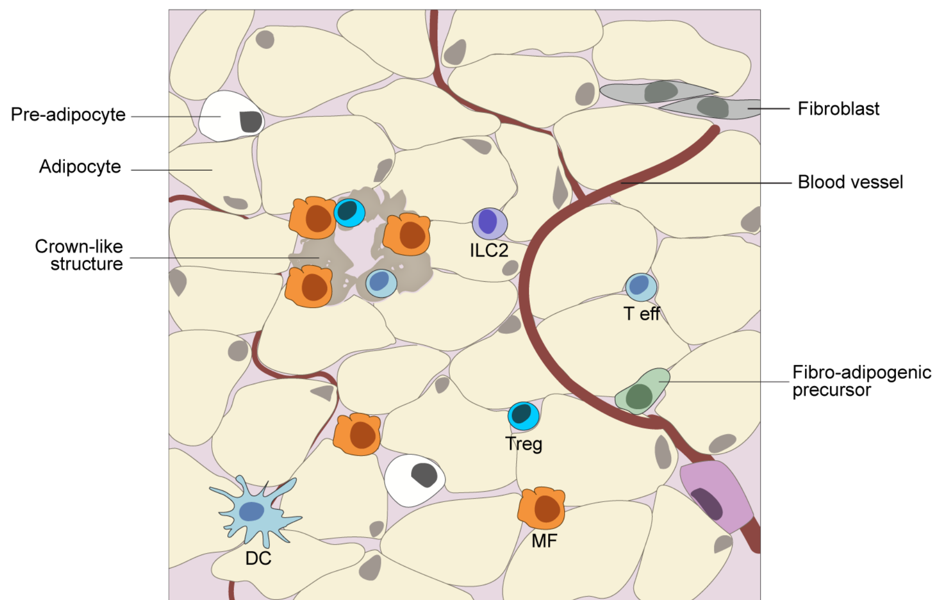

Figure 1.

Visceral adipose tissue. Schematic representation of a transverse section of epididymal fat from a lean adult B6 mouse. Abbreviations: DC, dendritic cell; ILC2, type 2 innate lymphoid cell; MF, macrophage; Teff, effector T cell (either $Foxp3^{-}CD4^{+}$ or $CD8^{+}$); Treg, regulatory T cell.

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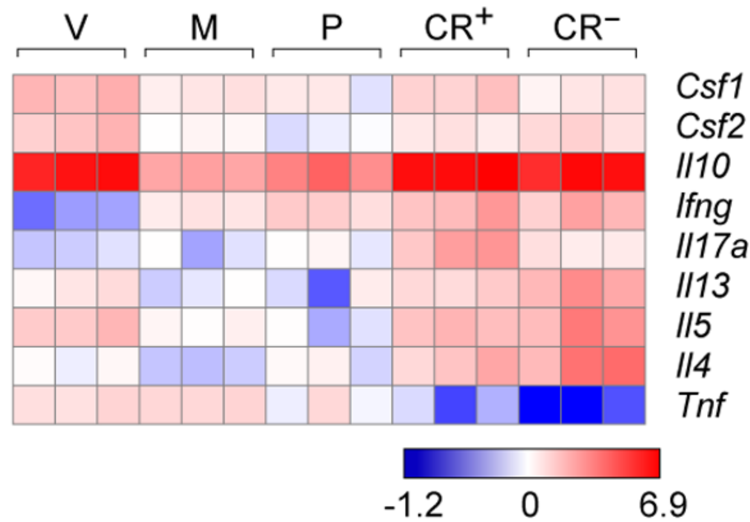

Figure 2.

Cytokines differentially expressed in tissue Tregs. K-means clustering (K = 4) of cytokine transcripts more than twofold upregulated in Tregs from at least one of the indicated parenchymal tissues vis-à-vis their lymphoid-organ Treg counterparts. Abbreviations: V, VAT (epididymal fat) from 20- to 25-week-old B6 mice; M, skeletal muscle 2 weeks after cardiotoxin-induced injury of young adult B6 mice; CR^+ and CR^-, RORγt^+ and RORγt^- Treg subpopulations from colonic lamina propria of young adult B6 mice; P, pancreas from 10-week-old NOD mice. Bar refers to fold-change differences on a log2 scale.

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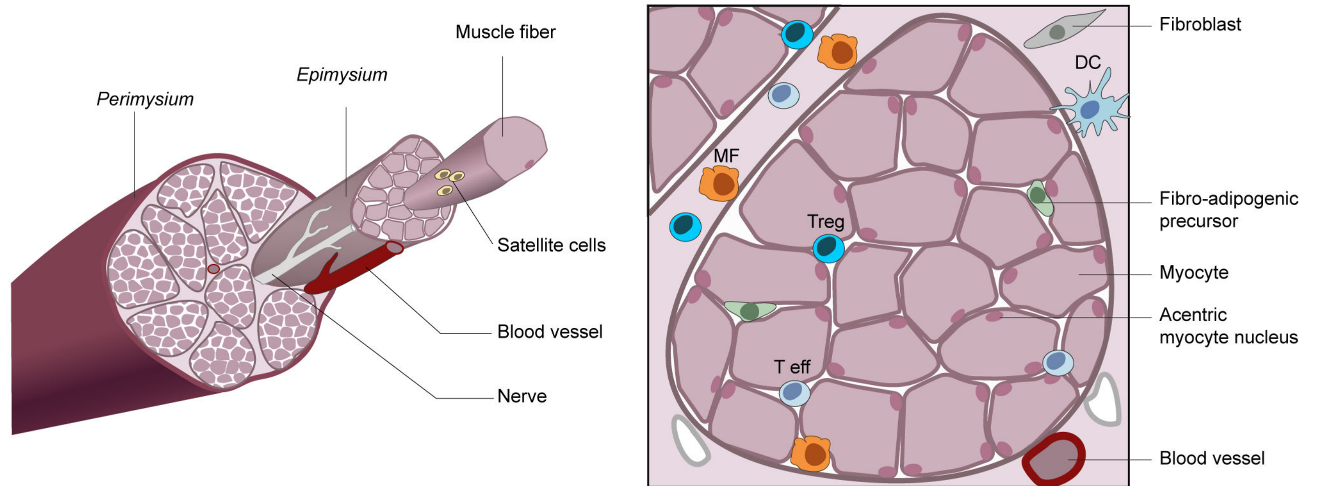

Figure 3.

Schematic representation of bundles of skeletal muscle fibers (a) and of a transverse section of one bundle (b). Representative of uninjured gastrocnemius muscle from a young adult mouse. Abbreviations: DC, dendritic cell; MF, macrophage; Teff, effector T cell (either Foxp3^{-}CD4^{+} or CD8^{+}); Treg, regulatory T cell.

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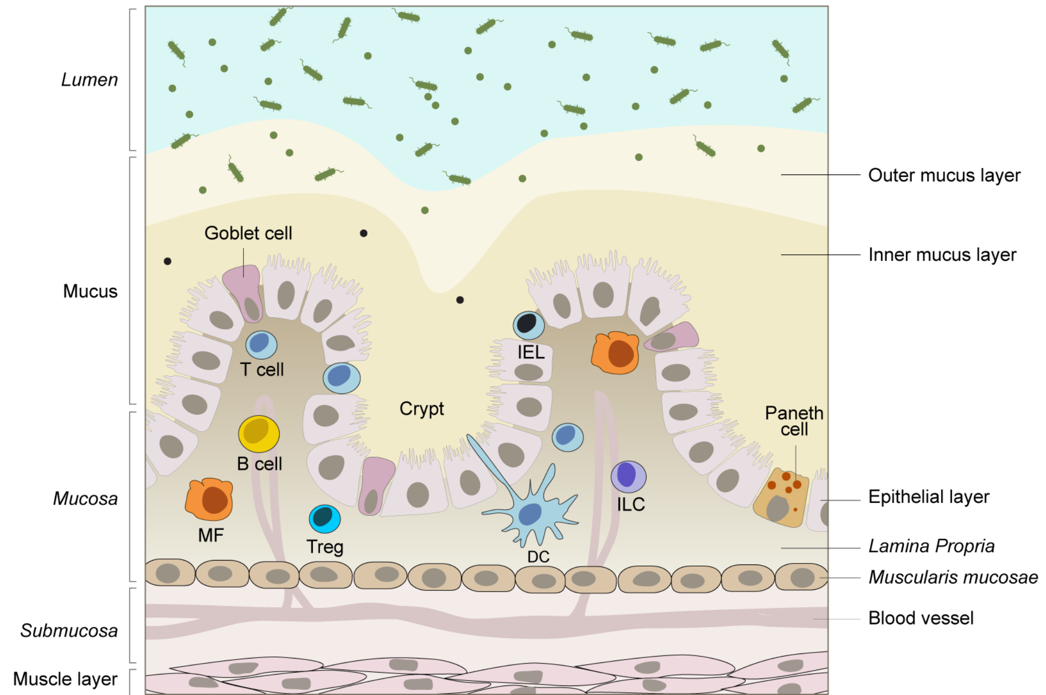

Figure 4.

Schematic representation of a transverse section of the colon of a young adult mouse. The DC projection is sampling microbes. Abbreviations: DC, dendritic cell; IEL, intraepithelial lymphocyte; ILC, innate lymphoid cell; MF, macrophage; Teff, effector T cell (either Foxp3^{-}CD4^{+} or CD8^{+}); Treg, regulatory T cell.

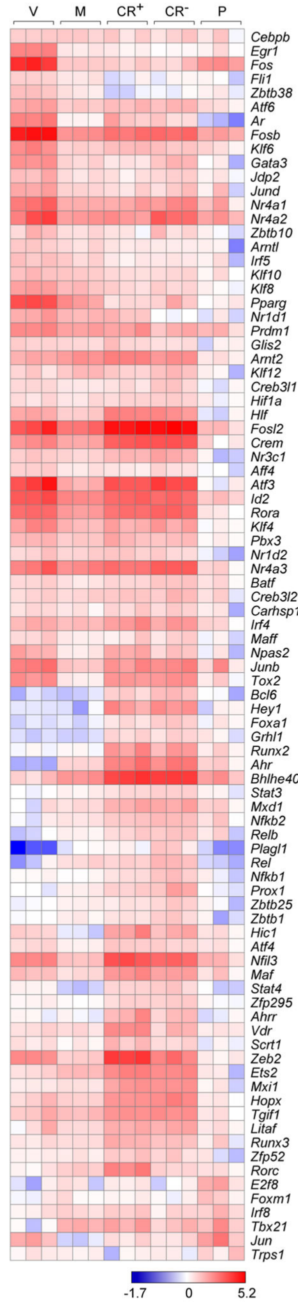

Figure 5.

Transcription factors differentially expressed in tissue Tregs. K-means unsupervised clustering (K = 5) of transcription factor gene transcripts more than twofold upregulated in Tregs from at least one of the indicated parenchymal tissues vis-à-vis their lymphoid-organ Treg counterparts. Abbreviations: V, VAT (epididymal fat) from 20- to 25-week-old B6 mice; M, skeletal muscle 2 weeks after cardiotoxin-induced injury of young adult B6 mice; CR^{+} and CR^{-}, RORγt^{+} and RORγt^{-} Treg subpopulations from colonic lamina propria of

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young adult B6 mice; P, pancreas from 10-week-old NOD mice. Bar refers to fold-change differences on a log2 scale.

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TABLE 1

TWO COLONIC Treg SUBPOPULATIONS

	RORγt+	**Gata3+**
Became a Treg in the:	periphery	thymus
Microbe dependence	++	+/−
IL-33 dependence	+/−	++
Repair program	−	+