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REGULATORY T CELLS IN NON-LYMPHOID TISSUES

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Abstract

Both Foxp3$^+$CD4$^+$ regulatory T cells (T_{reg} cells) and local immune responses within non-lymphoid tissues have long been recognized as important elements of a well-orchestrated immune system, but only recently have these two fields of study begun to intersect. There is growing evidence that T_{reg} cells are present in various non-lymphoid tissues in health and disease, that they have a unique phenotype, and that their functions go beyond the classical modulation of immune responses. Thus, tissular T_{reg} cells might add yet another level to classification of the T_{reg} cell compartment into functional and/or phenotypic subtypes. This review summarizes recent findings in this new field, discussing knowns and unknowns about the origin, phenotype, function and memory of non-lymphoid tissue-resident T_{reg} cells.

Introduction

Regulatory T cells that express the Foxp3 transcription factor, affectionately termed T_{reg} cells, are one of the immune system's main bastions against inappropriate or over-exuberant responses. They control autoimmunity, allergic and inflammatory reactions, and responses to infectious agents and tumors. Over the past decade, innumerable studies have addressed differentiation of the majority of T_{reg} cells in the thymus, generation of a minority in the periphery, homeostasis of the T_{reg} cell compartment, and cellular and molecular mechanisms of T_{reg}-mediated suppression[1]. For the most part, these explorations have taken the average T_{reg} cell residing in the spleen or lymph nodes to be paradigmatic.

However, it eventually became impossible to ignore the considerable heterogeneity of the Foxp3$^+$CD4$^+$ compartment, especially once transcriptome analysis had become a routine tool[2]. Initially, T_{reg} cell subphenotypes were delineated based on expression of activation or memory markers; adhesion molecules, notably CD103; or homing receptors. But a major advance was the discovery of T_{reg} cell functional diversity matched to the type of response being reined in. A subtype of T_{reg} cells was discovered that depends on the transcription factor IRF4 to control T helper (T_H)2 cells, which also critically rely on IRF4 (ref.[3]). In parallel, a discrete CXCR3$^+$ T_{reg} cell subtype was found, dependent on the T-bet transcription factor, that is specialized in regulating the activities of T_H1 cells, which also require T-bet for their differentiation and functions[4]. T_{reg} cells that optimally regulate

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interleukin 17 (IL-17)- or IL-27-dependent responses may be yet different subtypes[5, 6]. The relevance of these various subtypes was serendipitously confirmed in a recent study showing a mutation of Foxp3 to dampen arthritis in an IL-17, IL-4 dependent mouse model, while exacerbating type-1 diabetes in NOD mice, a T_H1-type disorder[7]. Another striking match between the cells that regulate and those that are regulated is found in germinal centers: follicular regulatory (T_{FR}) and helper (T_{FH}) cells both depend on Blimp-1 and Bcl6 for their differentiation/homeostasis and CXCR5 for their localization[8, 9]. The advantage of such a matching is probably that it provokes co-localization to and/or co-survival within discrete locations. Arming regulatory and effector cells with the same capabilities could be dangerous, but safeguards are in place, for example T_H1-type T_{reg} cells poorly up-regulate IL-12Rβ2 upon interferon-γ induction of STAT1, meaning that their differentiation to T_H1 effector cells is aborted[10].

This review focuses on studies that go one step further, highlighting the phenotype and functions – sometimes exquisitely adapted – of T_{reg} cells residing in non-lymphoid tissues. We will survey the populations of tissue-resident T_{reg} cells, focusing on a few particularly interesting examples; consider their origin; discuss potential cellular targets; and weigh the concept of T_{reg} cell memory. Lastly, we will highlight some general principles and knowledge gaps to fill.

Tissue-resident T_{reg} cells: the landscape

The presence of a distinct population of T_{reg} cells has been documented in several non-lymphoid tissues of both mice and humans: skin, intestinal mucosa, lung, liver, adipose tissue, autoimmune target tissues, infected tissues, grafts, placenta, tumors, atherosclerotic plaques and injured muscle are just some examples ([11–21], D.B., C.B. and D.M., manuscript submitted). It is clear from this extensive list that T_{reg} cells can localize in healthy tissues, in tissues with various types and grades of inflammation, and in immunoprivileged sites. In every case where the comparison has been made, tissue-resident T_{reg} cells are distinguishable from classical lymphoid-organ T_{reg} cells in phenotype and function. While they display some features of activated/effector cells[22], certain properties make each tissue-resident T_{reg} cell population unique, such as by the expression of specific transcription factors, chemokine receptors or effector molecules; or by a distinct T cell receptor (TCR) repertoire, migration pattern, mechanism of action or targets.

Currently, one of the best-characterized examples of tissue-resident T_{reg} cells is the population found in visceral adipose tissue (VAT)[12]. T_{reg} cells are enriched in VAT of lean, aged, male mice, constituting more than 50% of the $CD4^+$ T cell compartment at that site, a significantly higher fraction than the 5–15% routinely observed in lymphoid organs, including in aged individuals. Microarray-based gene-expression profiling, confirmed by flow cytometry analyses, revealed VAT T_{reg} cells to have a unique phenotype. While they are *bona fide* T_{reg} cells, as shown by their recapitulation of most of the classical T_{reg} signature and by their functionality in standard *in vitro* suppression assays, they differentially express a panoply of genes in comparison with lymphoid-organ T_{reg} cells. The set of loci overexpressed in VAT T_{reg} cells includes those encoding several chemokine receptors (for example, CC chemokine receptor (CCR)1, CCR2, CCR9),

immunomodulatory cytokines (such as IL-10), and the transcription factor peroxisome proliferator-activated receptor (PPAR)-γ, all of which confer VAT T_{reg} cells with unique functional properties[12, 23]. As flow cytometry studies showed that some of those markers are not expressed by 100% of the fat T_{reg} cells[12, 23], it is not clear how heterogeneous the population is and whether this has functional significance or more prosaically marks cells at different cell-cycle or differentiation stages. Their TCR repertoire is another feature contributing to a distinct phenotype: the TCR sequences they employ have little overlap with those used by lymphoid-organ T_{reg} cells ([12] and Kolodin *et al*., personal communication). In addition, there is a striking expansion of certain T_{reg} clones in the adipose tissue, suggesting that recognition of particular antigens may have an important role in the accumulation of T_{reg} cells in abdominal fat (further discussed below).

The major driver of VAT T_{reg} cell accumulation, phenotype and function is PPAR-γ[23]. A member of the nuclear receptor superfamily characterized as the “master regulator” of adipocyte differentiation and function[24], PPAR-γ interacts with Foxp3 to promote up-regulation of the VAT T_{reg} signature in *in vitro* transduction experiments. Mice lacking PPAR-γ only in T_{reg} cells show a substantial and specific reduction in the frequency and number of T_{reg} cells in VAT vis-à-vis their wild-type littermates, without any changes in lymphoid-organ T_{reg} populations. Moreover, the few VAT T_{reg} cells remaining in PPAR-γ mutant mice show an under-representation of the VAT T_{reg} signature. The fact that PPAR-γ expression in VAT T_{reg} cells is much higher than in anyother T_{reg} population, together with the observation that they can take up lipids by expressing CD36, the scavenger receptor[23], suggests that tissue-resident T_{reg} cells are attuned to local cues, which they can exploit for phenotypic and functional specialization, as well as for preferential survival within the tissue microenvironment (Fig.1).

Another tissue-resident population of great interest are T_{reg} cells that infiltrate injured and regenerating skeletal muscle (D.B., C.B. and D.M., unpublished data). Within days after skeletal muscle injury (of various types), T_{reg} cells start to accumulate locally, their frequency among $CD4^+$ T cells increasing steadily to up to 50–60% and remaining at that frequency for weeks, long after local inflammation has resolved. Microarray-based gene-expression profiling revealed that muscle T_{reg} cells have a unique transcriptome, more closely related to that of VAT T_{reg} cells than to that of lymphoid-organ T_{reg} cells. For example, genes encoding IL-10, amphiregulin (Areg) and T-cell immunoglobulin domain and mucin domain 3 (TIM-3) are highly expressed by T_{reg} cells accumulated in muscle. Muscle T_{reg} cells have a skewed TCR repertoire, which appears not to overlap with that of muscle conventional $CD4^+$ T cells or of splenic T_{reg} cells, and shows clear signs of clonal expansion. Interestingly, T_{reg} cells also accumulate in skeletal muscle of dystrophic mice (such as dystrophin-deficient mdx mice and dysferlin-deficient mice), whose chronic muscle injury has a genetic origin.

Given recent successes with immunomodulatory anti-tumor strategies (see Gajewski et al. reviewed in this issue), there is a growing interest in the more heterogeneous group of tumor-infiltrating T_{reg} cells. In solid tumors of non-lymphoid origin, T_{reg} cells can account for 30–50% of $CD4^+$ T cells, depending on the tumor type[19]. Like other tissue-resident T_{reg} cells, those found in tumors have a distinctive phenotype that differentiates them from T_{reg}

cells found in the circulation or in lymphoid organs. Microarray-based gene-expression profiling of tumor-associated T_{reg} cells is still lacking[25], but $Foxp3^+CD4^+$ T cells from several mouse and human tumors show increased expression of cell-surface markers such as Cytotoxic T-Lymphocyte-Antigen 4 (CTLA-4), Inducible T-cell Costimulator, (ICOS), TIM-3, glucocorticoid-induced TNFR family related gene (GITR); as well as of suppressive cytokines such as IL-10 and transforming growth factor (TGF)-β; and a variety of chemokine receptors[19, 26–29].

Who gets there, when and how?

T_{reg} cells are found in healthy tissues, although usually in very small numbers[11]. Upon challenges of different types (autoimmunity, infection, injury), T_{reg} cell numbers, and often their frequencies, increase considerably. Diverse mechanisms, not mutually exclusive, have been proposed to explain the accumulation of T_{reg} cells at different tissular sites: chemokine-based recruitment of circulating T_{reg} cells, local TCR- or cytokine-driven expansion, conversion of local or circulating conventional $CD4^+$ T cells, or prolonged survival (Fig. 1). In some cases, for example tumor T_{reg} cells, all of these mechanisms have been championed by one investigator or another[19, 25, 26, 30–34].

As for other types of immune-system cells, recruitment of T_{reg} cells to non-lymphoid tissues is governed by specific combinations of chemotactic molecules and their receptors (reviewed in[35]). T_{reg} cells residing in various tissues express distinct patterns of chemokine receptors and adhesion molecules in comparison with their lymphoid-organ counterparts, both at single cell level and in terms of population frequencies[11, 23]. This finding is true even for T_{reg} cells residing in non-inflamed tissues like skin, lung and liver, wherein an enrichment for $CCR4^+CD103$ (integrin $\alpha_E)^+$ T_{reg} cells is observed[11]. *Ccr4*$^{-/-}$ T_{reg} cells show impaired accumulation in healthy non-lymphoid tissues, and this dearth has functional consequences as mice defective in $CCR4^+$ T_{reg} cells develop severe skin inflammation and a less severe lung infiltrate. It seems that antigen-specific activation of T_{reg} cells in subcutaneous lymph nodes under inflammatory conditions induces up-regulation of CCR4, priming T_{reg} cells to migrate to the affected tissue and to suppress immune responses locally. However, the origin of the small fraction of $CCR4^+CD103^+$ T_{reg} cells found in lymphoid tissues in the absence of antigen challenge remains unclear. In this regard, a study showed that, in humans, only few T_{reg} cells express CCR4 at birth but the majority expresses the gut-homing receptor $\alpha_4\beta_7$, while the reverse pattern is true for adults[36]. The switch commences between 1.5 and 3 years of age and correlates with a change from a naïve to memory T_{reg} phenotype, which has been suggested to depend on the recognition of microbial antigens in the gut, although this point has yet to be definitively demonstrated.

In addition to CCR4, an array of chemokine receptors can be involved, usually in a redundant fashion, in the recruitment of T_{reg} cells to non-lymphoid tissues under various inflammatory conditions[37]. In some cases, the expression of a particular receptor by tissue-resident T_{reg} cells is directly associated with the type of tissue, while in others it is related to the type of T cell response (T_H1, T_H2, T_H17) occurring in the tissue and the accompanying T_{reg} cell polarization, for example, the preferential recruitment of $CCR6^+$ and $CXCR3^+$ T_{reg} cells to sites with an ongoing T_H17- or T_H1-driven inflammation, respectively[4, 5].

The pattern and timing of trafficking between lymphoid and non-lymphoid tissues is not well-defined in most cases. Interestingly, in a model of pancreatic islet transplantation, T_{reg} cells first migrate from blood to the inflamed allograft, in a process dependent on CCR2, CCR4, CCR5 and P- and E-selectin ligands[38]. Upon activation in the allograft, they subsequently move to the draining lymph nodes in a CCR2-, CCR5-, and CCR7-dependent fashion. This sequential migration is required for an efficient suppression of alloimmunity, since impairment of either of the steps by abolishing expression of the relevant receptor(s) in T_{reg} cells results in decreased graft survival.

Although not universally accepted, it has been suggested that the T_{reg}-cell TCR repertoire is biased towards the recognition of self-antigens[1], which could facilitate the localization of T_{reg} cells in non-lymphoid tissues to prevent harmful autoimmune attacks, and collateral damage to the tissue resulting from inflammation or injury. Sequencing of the TCR repertoire of VAT T_{reg} cells in either wild-type mice or a mouse line engineered to have only limited TCR diversity yielded several interesting observations ([12], Kolodin *et al*, personal communication). First, the sequences expressed by VAT T_{reg} cells have little overlap with those expressed by conventional $CD4^+$ T cells, either in adipose tissue or in lymphoid organs, arguing against T_{reg} cell conversion in this context. Second, there are striking clonal expansions of T_{reg} cells in VAT even in standard mice. Lastly, in the "limited" line, it is possible to find examples, in the same or different individuals, of a repeated complementary-determining region (CDR)-3 protein sequence specified by different nucleotide sequences. These findings argue that VAT T_{reg} cells are responding to one or more local antigens, whether they be derived from lipids or proteins. By combining an ovalbumin (OVA)-specific TCR transgenic model and the expression of OVA in the skin, a recent study showed that engineered expression of a self-antigen in a peripheral tissue can drive the activation and proliferation of TCR-transgenic T_{reg} cells, which mediate resolution of organ-specific autoimmunity[39]. These antigen-specific T_{reg} cells remain in the target tissue and are primed to attenuate subsequent autoimmune reactions when the antigen is re-expressed (discussed in the next section), suggesting that antigen recognition is important for tissue T_{reg} cell function and memory.

A recent report provided the first clear identification of the antigen specificity of a natural population of tissue-resident T_{reg} cells[40]. By sequencing the TCR repertoire of prostate-tumor-infiltrating T_{reg} cells, the authors identified a TCR αβ pair recurrently enriched in independent mice, whose sequences they used to generate a TCR transgenic line. In tumor-free TCR-transgenic mice, T_{reg} cells are found selectively in the prostate and its draining lymph nodes, suggesting that the cloned TCR is specific for an antigen expressed in the normal prostate in addition to the malignant tissue. Interestingly, selection of the TCR-transgenic T_{reg} cells is dependent on expression of Aire in the thymus, thereby linking two major mechanisms of immunological tolerance - one central, one peripheral. Although similar demonstrations for other tissue-resident T_{reg} specificities will be necessary before one can definitively generalize, this work supports the hypothesis that tissue-resident T_{reg} cells are generated in the thymus in response to self-antigens, and that this TCR specificity facilitates their localization and amplification in non-lymphoid tissues, especially in

pathological situations such as inflammation or cancer in which an enhanced self-antigen presentation can occur in the periphery.

While the majority of the T_{reg} cells differentiates in the thymus, a small fraction of them can be generated in the periphery, by conversion of Foxp3^{-}CD4^{+} T cells into Foxp3^{+}CD4^{+} Tregs, termed "peripheral T_{reg} cells" (pT_{reg} cells) to distinguish them from their thymic-derived counterparts. There has been a lot of interest in conversion as a mechanism of T_{reg} cell accumulation in non-lymphoid tissues, although few studies have clearly demonstrated that, in certain sites and/or conditions, pT_{reg} cells constitute a substantial proportion of tissular T_{reg} cells. The intestinal lamina propria T_{reg} pool is one of the main populations of non-lymphoid-tissue T_{reg} cells impacted by conversion, and pT_{reg} cells have been shown to contribute substantially to oral tolerance (reviewed in[41]). Exposure to agonist administered orally and specific microbial products strongly induce conversion in the lamina propria, which is reflected in the increased frequency of T_{reg} cells, as well as in the increased proportion of Helios^{-} T_{reg} cells in that tissue[42–45]. The analysis of a mutant mouse bearing a deletion in the CNS1 enhancer region of the *Foxp3* locus necessary for peripheral induction confirmed previous observations on lamina propria T_{reg} cells and suggested that the accumulation and function of pT_{reg} cells might be important at mucosal and feto-maternal interfaces, and perhaps little else[17, 46, 47]. Different populations of tolerogenic antigen-presenting cells promote Foxp3 induction at mucosal surfaces, such as CD103^{+} dendritic cells that produce retinoic acid in the intestinal lamina propria[42, 48], and tissue-resident macrophages in the lung mucosa[49]. Interestingly, a report showed that neurons can elicit conversion of encephalitogenic Foxp3^{-}CD4^{+} T cells into T_{reg} cells *in vitro*, although evidence for *in vivo* conversion was weak[50]. The generation of pT_{reg} cells has also been proposed to play a role in the accumulation of T_{reg} cells in tumors[34, 51], although the exact contribution of this mechanism versus recruitment/expansion of thymic-derived T_{reg} cells remains controversial[25, 41]. In fact, a study using a mouse tumor model showed that both mechanisms can coexist and that the induction of tumoral pT_{reg} cells is intrinsically influenced by the tumor microenvironment and the presence of a tumor-specific antigen[51].

Lastly, the question remains as to how and when the few T_{reg} cells found in healthy, unchallenged tissues are recruited to these sites. It is possible that a combination of specific expression of chemokine receptors and antigen specificity constantly brings a small number of T_{reg} cells to the tissues throughout life. Alternatively, early seeding with T_{reg} cells precursors during fetal life might occur, paralleling the phenomenon observed in tissue macrophages[52], followed by self-renewal of the tissue-resident T_{reg} cells. In theory, seeding during embryonic life could occur after or before thymic development, although extrathymic differentation of TCRαβ T cells remains controversial.

Immunological and non-immunological targets

One of the major functions of T_{reg} cells residing in non-lymphoid tissues is to control local inflammation. Since their initial discovery, Foxp3^{+}CD4^{+} cells were recognized to be potent suppressors of T cell responses, and this activity has been extended to tissular Foxp3^{+} cells. In addition, tissue-resident T_{reg} cells can strongly impact myeloid populations in the

vicinity, inhibiting neutrophils and pro-inflammatory macrophages, and promoting the activities of anti-inflammatory macrophages and monocyte subsets (Fig. 2).

As a particular example, VAT T_{reg} cells may influence a broad spectrum of targets. Their elevated expression of factors like IL-10 and CTLA-4, and their effectiveness in the typical *in vitro* suppression assay[12] suggest that VAT T_{reg} cells are capable of controlling conventional $CD4^+$ and $CD8^+$ T cell populations in the adipose tissue, although *in vivo* experiments to substantiate this point are still lacking. They may also control co-resident myeloid cells, as suggested by an inverse correlation between the frequency of T_{reg} cells and that of pro-inflammatory myeloid populations ($CD11b^+CD11c^+F4/80^+$ macrophages and pro-inflammatory $CD11b^+Ly6c^{hi}$ monocytes) observed in VAT depots[23, 53, 54]. Similarly, muscle T_{reg} cells are important in controlling the pro-inflammatory to anti-inflammatory switch that occurs in the myeloid infiltrate of injured muscle; punctual ablation of T_{reg} cells at the time of muscle injury results in prolonged accumulation of pro-inflammatory $Ly6c^{hi}$ monocytes in the injured tissue (D.B., C.B. and D.M., unpublished data).

Because of the ability of T_{reg} cells to dampen immune responses that can eradicate malignancies, their accumulation in tumors is considered to be a tumor 'escape' mechanism. Tumor-infiltrating T_{reg} cells can be as effective as peripheral-blood T_{reg} cells in T cell-suppression assays[30] or even significantly better[29]; and, in many cases, a high frequency of intra-tumoral T_{reg} cells is correlated with poor prognosis[30, 55, 56]. On the other hand, T_{reg} cells can also have anti-tumoral effects by inhibiting inflammation in the tumor environment. A strong link between inflammation and cancer exists in many tissues: inflammation can promote proliferation and survival of malignant cells, angiogenesis and metastasis[57, 58]. In line with these observations, in certain tumors, like colorectal cancers, a high density of T_{reg} cells correlates with a favorable prognosis[59, 60]. It has recently been proposed that T_{reg}-mediated suppression of pro-inflammatory T_H17 responses to the dense microbiome of the large intestine is key to preventing tumor growth in the intestinal epithelium[60].

There is growing evidence that T_{reg} cells (in particular those residing in tissues) not only control T lymphocytes and other immune-system cells, but also regulate certain non-immunological processes, including systemic metabolic indices[12, 23, 54, 61], ischemic stroke[62, 63], formation of atherosclerotic plaques[64, 65], cardiac remodeling after myocardial infarction[66], liver cholestasis and fibrosis[67] and skeletal muscle regeneration (D.B., C.B. and D.M., manuscript submitted). It is still unclear to what extent such influences reflect direct interaction between T_{reg} cells and their non-immunological target cells or an indirect effect of T_{reg} suppression of local inflammation (Fig. 2). In any case, these non-traditional roles can have profound impact on both homeostatic and pathophysiological processes and should be considered an important facet of T_{reg} cell function.

An unusual property of VAT T_{reg} cells is their control of metabolic indices, inhibiting local and systemic insulin resistance and glucose intolerance. VAT of obese individuals exhibits low grade, chronic inflammation which has been directly linked to the appearance of metabolic abnormalities, ultimately type 2 diabetes and the metabolic syndrome[68, 69]. That the frequency of VAT, but not lymphoid-organ T_{reg} cells drops severely upon feeding mice

with a high-fat diet (HFD) or in genetic models of obesity first suggested that this T_{reg} subset might influence local and/or systemic metabolism[12, 53]. Loss-of-function experiments, relying on punctual and specific T_{reg} ablation in a Foxp3-DTR mouse model, demonstrated increases in inflammatory mediators in the visceral fat depot, a decrease in insulin-stimulated insulin receptor tyrosine phosphorylation in VAT and the liver, and insulin resistance upon loss of T_{reg} cells[12]. Conversely, gain-of-function experiments, which entailed treatment of HFD-fed mice with IL-2–anti-IL-2 complexes, resulted in enhanced IL-10 expression in VAT and improved insulin sensitivity and glucose tolerance. These results were mirrored in a more recent report on experiments manipulating PPAR-γ expression or signaling: mice devoid of PPAR-γ only in T_{reg} cells show a strong reduction in VAT T_{reg} cells and degradation of metabolic indices, while HFD-fed wild-type mice injected with a PPAR-γ agonist, pioglitazone, have a substantially more robust T_{reg} cell population and metabolic improvements. Strikingly, and unexpectedly, the restoration of metabolic indices typically induced by treatment of HFD-fed mice with pioglitazone is only very partial in mice lacking PPAR-γ specifically in T_{reg} cells[23], demonstrating not only that the beneficial effects of this drug occur in part by targeting VAT T_{reg} cells, but also that VAT (and not lymphoid) T_{regs} are important players in the control of the metabolic indices downstream of obesity and inflammation of the adipose tissue. At least part of this T_{reg} cell influence may be directly on adipocytes, as one of their major mediators, IL-10, can engage receptors on adipocytes to down-regulate pro-inflammatory cytokines and increase glucose uptake.

T_{reg} cells that accumulate in injured skeletal muscle also seem to impact non-immunological processes, in this case tissue regeneration (D.B., C.B. and D.M., unpublished data). Mice punctually depleted of T_{reg} cells mice show impaired muscle repair according to multiple criteria, notably the functionality of satellite cells, key players in the regeneration of skeletal muscle. Muscle T_{reg} cells overexpress Areg, a growth factor that induces *in vitro* differentiation of satellite cells, which express Areg receptors (D.B., C.B. and D.M., manuscript in preparation), suggesting that local T_{reg} cells could have a direct effect on skeletal muscle precursor cells.

The influence of T_{reg} cells on the pathophysiology of ischemic stroke provides yet another example of non-immune processes regulated by T_{regs}. However, in this case, opposite effects have been reported by different investigators, so whether T_{reg} cells are beneficial or detrimental to the outcome of ischemic stroke remains controversial. One study showed that T_{reg} cells can be found in the post-ischemic brain; and that their depletion profoundly enhances brain damage and exacerbates the functional outcome[62]. IL-10 production by T_{reg} cells may be their major mechanism of immunomodulation in the brain and of T_{reg} -mediated cebroprotection. Unfortunately, these results conflict with another study published subsequently that indicated that indicating that T_{reg} cells promote acute ischemic stroke in mice by inducing dysfunction of the cerebral microvasculature[63]. Notably, this negative impact of T_{reg} cells on recovery from stroke is unrelated to their classical immunoregulatory functions; in fact, the $CD4^+$ T cell and macrophage populations infiltrating the ischemic tissue are not affected by the depletion of T_{reg} cells. Rather, the negative influence seems to result from an interaction between T_{reg} cells and activated cerebral endothelial cells and

platelets. These contradictory findings on the role of T_{reg} cells in stroke issue from different models of T_{reg} depletion (anti-CD25-mediated depletion vs a Foxp3-DTR line), by the different stroke models used (permanent vs temporal; different ischemia times), or the timing of read-out (late vs acute stages).

A liaison between T_{reg} cells and endothelial cells has also been observed within the tumor microenvironment, in this case reflecting a pro-angiogenic effect by tumor-infiltrating $Foxp3^+CD4^+$ cells[70]. Tumor hypoxia promotes the recruitment of T_{reg} cells through induction of expression of the chemokine, CC-chemokine ligand 28 (CCL28), in tumor cells. Elevated numbers of T_{reg} cells expressing CCR10 accumulate in CCL28-positive tumors, which exhibit increased growth and angiogenesis in comparison with CCL28-negative tumors. Angiogenesis is dependent on the presence of tumor-infiltrating T_{reg} cells, which not only enhance expression of vascular endothelial growth factor A (VEGFA) in tumor cells, but also directly contribute to the tumor VEGFA pool by producing the pro-angiogenic factor themselves[70]. Tumor-infiltrating T_{reg} cells also promote mammary carcinoma metastasis in the lung by producing receptor activator of nuclear factor kappa-B ligand (RANKL)[71]. Thus, the impact of tumor-resident T_{reg} cells on tumor growth and patient prognosis reflects both traditional (on immune targets) and non-traditional (on non-immune targets) T_{reg} functions. Dissection of the individual pathways regulated by tissue T_{reg} cells will be important for the design of effective and specific therapies aimed at modulating T_{reg} cells and their products in cancer and other diseases.

T_{reg} cell memory in tissues

The generation of memory is one of the hallmarks of adaptive immunity, and it is becoming clear that T_{reg} cells are no exception to the rule. During viral infections, the expansion of antigen-specific T_{reg} cells is followed by a contraction phase and the formation of a memory pool[72, 73]. Upon re-infection, these memory T_{reg} cells rapidly expand and effectively control specific anti-viral responses by $CD4^+$ and $CD8^+$ T cells. An accelerated accumulation of memory T_{reg} cells is observed in the non-lymphoid tissues targeted by the infection; furthermore, memory T_{reg} cells are capable of suppressing the collateral tissue damage and inflammation elicited by recall expansion of non-T_{reg} memory $CD4^+$ T cells. A link between T_{reg} memory and tissue-resident T_{reg} cells has also been demonstrated in the context of autoimmunity[39]. Using a TCR–neo-self-antigen (OVA) double-transgenic model, a recent study showed that upon local induction of ovalbumin expression, ovalbumin-specific T_{reg} cells accumulate in the skin. After resolution of the inflammatory response, activated T_{reg} cells remain in the target tissue in high frequencies and are primed to attenuate subsequent autoimmune responses when the antigen is re-expressed. These memory T_{reg} cells can survive in the non-lymphoid tissues in the absence of overt expression of antigen, and show enhanced functional activity.

In human skin, resident T_{reg} cells comprise 5–10% of total skin T cells[74]. These T_{regs} express the memory T cell marker CD45RO, and are thus typically referred to as ‘effector-memory T_{reg} cells’, although their true memory nature has not been formally demonstrated. They reside preferentially in the epidermis, where Langerhans cells (LCs) induce their proliferation in an MHC-class-II- and IL-15-dependent manner[75]. It has been proposed that

self-peptides or the normal skin microbiome might be the source of antigens presented by LCs. Human skin-resident T_{reg} cells also proliferate in response to pathogens[75] and during the recall response induced by injection of tuberculin purified-protein derivative (PPD) in individuals immunized with bacille Calmette-Guérin (BCG)[76].

Memory T_{reg} cells seem also to have an important function in preventing fetal rejection in successive pregnancies[77]. Fetal-antigen-specific T_{reg} cells, generated by conversion of $Foxp3^{-}CD4^{+}$ T cells, expand >100 fold through parturition, remain at enriched numbers after delivery, and re-accumulate with accelerated kinetics during subsequent pregnancies. The precise localization of memory T_{reg} cells in feto-maternal non–lymphoid tissues remains unknown. Although in the case of pregnancy the target tissue is present only transiently, and memory T_{reg} cells seem to be maintained in lymphoid organs, their fetal-antigen specificity and existing evidence of the presence of T_{reg} cells in placenta and utero[78–80] suggest that memory T_{reg} cells might migrate to these tissues in recurrent pregnancies to exert their protective role.

Thus, evidence suggests that there is a close association between tissue-resident T_{reg} cells and memory T_{reg} cells. The generation of regulatory memory is emerging as an important factor to control the accelerated and enhanced recall responses to secondary exposure to antigens. The localization of memory T_{reg} cells in the target non-lymphoid tissues seems to be key for their function and, in some cases, for the maintenance of the memory T_{reg} cell pool during the remission period.

Perspectives

Tissue-resident T_{reg} cells is a relatively new area of T_{reg} cell biology. It is not surprising, then, that some key questions remain unanswered, or even unaddressed. We would like to highlight three questions that particularly intrigue us:

First, how widespread is the phenomenon? Given the distinct T_{reg} cell compartments already found to be associated with adipose tissue, muscle, the colon, etc, it is theoretically possible that there is a specialized type of T_{reg} cell dedicated to maintaining homeostasis within each tissue. Or it might be that evolution has provided dedicated T_{reg} cell types only for those tissues particularly prone to insult (for example, the skin and muscle) or in especially close communication with the environment (such as the skin and the gut). An alternative possibility is that there are a limited number of T_{reg} cell types and each tissue T_{reg} compartment represents a different optimum mix of the various subsets – that is what we are seeing are really only unique “averages.” A broader survey of tissue T_{reg} cells, especially in single-cell-analysis mode, should yield critical information bearing on this question. It may also prove informative to compare T_{reg} compartments from tumors and their matched normal tissues.

Second, where do tissue-resident T_{reg} cells come from? So far, it seems clear that, except for the case of the gut and placental compartment, tissue T_{reg} cells do not emanate from conversion of conventional $Foxp3^{-}CD4^{+}$ T cells. It is perhaps simplest to envisage that a precursor cell, already $Foxp3^{+}$ but not committed to a particular T_{reg} cell sub-phenotype, gets retained within a particular tissue, likely because its TCR reacts to an antigen therein

and, in response to tissue-specific cues, takes on a tissue-specific sub-phenotype. But it is difficult to rule out the possibility that small numbers of precursors precommitted to a particular T_{reg} cell sub-phenotype are generated in the thymus and monitor their corresponding peripheral tissues, either as residents or as members of the circulating T cell pool. Such a scenario is reminiscent of the recent finding that populations of tissue-resident macrophages are generated before birth and are important elements in enforcing tissue homeostasis[52]. Perhaps these macrophages need T_{reg} cell monitors to keep them in line. Identifying the origin of tissue-resident T_{reg} cells would be aided by generating appropriate inducible lineage-tracer mice, for example in PPAR-γ reporters for VAT T_{reg} cells.

Third, what is the advantage of having unique populations of tissue-resident T_{reg} cells? It makes perfect evolutionary sense to optimize T_{reg} compartments for both effective survival and appropriate effector activities. This is especially true for T_{reg} cells that will reside in a given tissue long-term, either as tissue-resident sentries or in response to chronic insult. For example, adipose tissue is an environment not generally hospitable for lymphocytes – VAT T_{reg} cells co-opting of PPAR-γ provides these cells with properties (such as expression of the lipid transport, CD36) that promote survival in adipose tissue. Similarly, high-level expression of Areg by T_{reg} cells in regenerating tissues (for example muscle or intestine) arms them with the capacity to favorably impact their local environment. We wonder how common it is for tissue-resident T_{reg} cells to co-opt transcriptional programs characteristic of tissular cells (like VAT T_{reg} use of PPAR-γ, the “master-regulator” of adipocytes), and whether there might be something special about the chromatin organization or epigenetic make-up of T_{reg} cells that favors such adaptability.

Interesting realms to explore!

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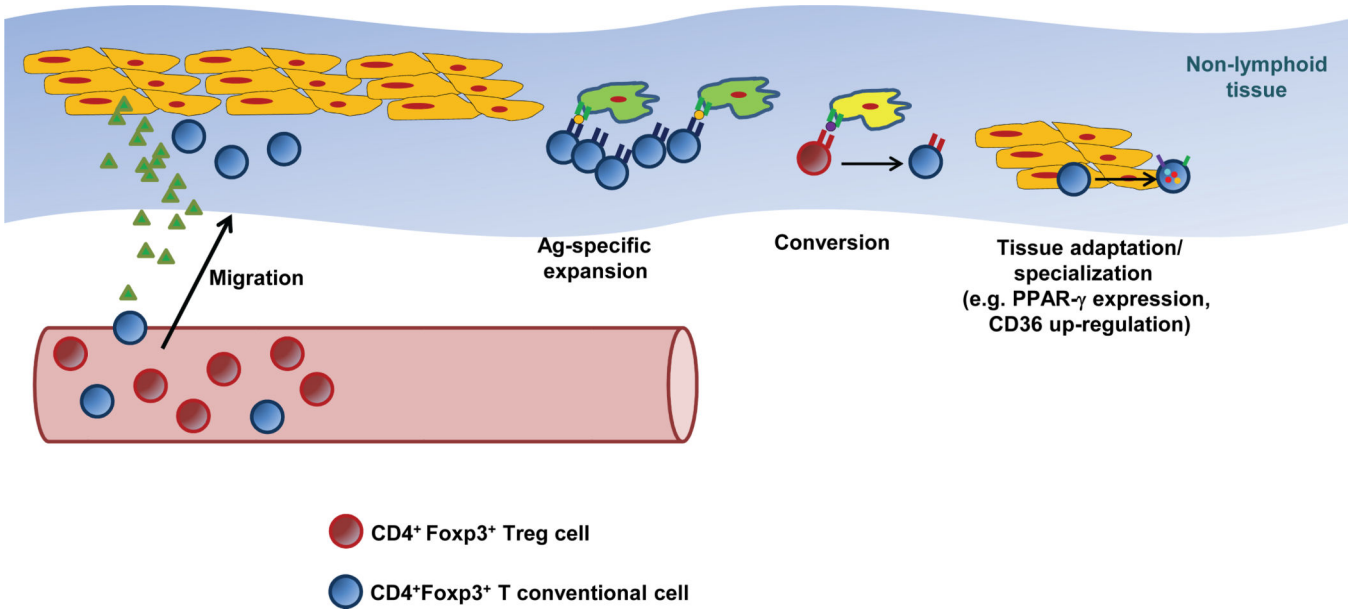

Figure 1. Mechanisms involved in the accumulation of T_{reg} cells in non-lymphoid tissues

Several mechanisms (alone or in combination) have been shown to increase the frequency and numbers of tissue-resident T_{reg} cells: 1) Migration and retention of circulating T_{regs}, promoted by the expression of specific chemotactic molecules by parenchymal or stromal cells, and the expression of the specific receptors on T_{reg} cells; 2) Expression of tissue-specific antigens that induce expansion of particular T_{reg} clones; 3) Antigen-induced conversion of $CD4^{+}Foxp3^{-}$ T conventional cells into pT_{regs}; and 4) Acquisition of a tissue-specific phenotype that allows adaptation or specialization in the new environment, promoting survival and enhancing T_{reg} functions.

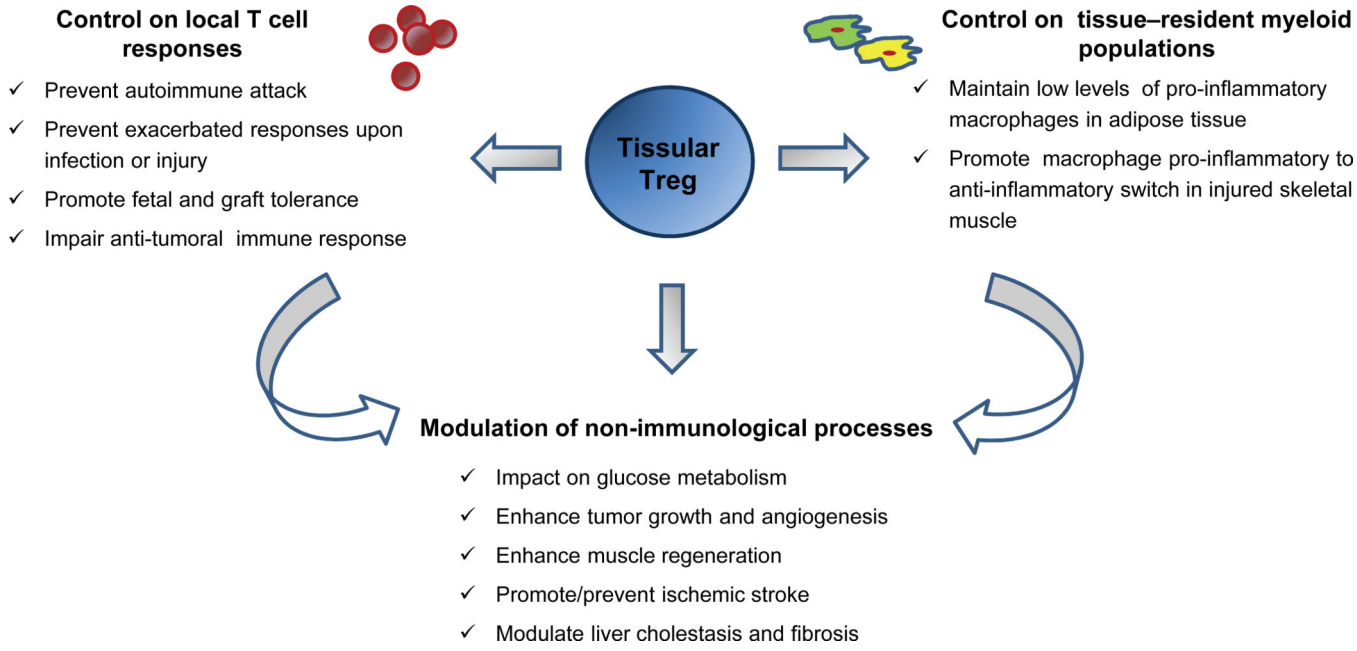

Figure 2. Functions of tissue-resident T_{reg} cells

The functions of tissue resident T_{reg} cells can be divided in three main groups (representative examples of each group are listed in the figure): control on local T-cell responses, control on tissue-resident myeloid populations and modulation of non-immunological processes. The latter may occur by direct interaction between T_{reg} cells and their non-immune targets, or indirectly through the regulation of other tissue-resident leukocytes which in turn could affect those targets.