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Regulatory T cells in nonlymphoid tissues

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Both Foxp3$^+$CD4$^+$ regulatory T cells (T_{reg} cells) and local immune responses in nonlymphoid 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 nonlymphoid tissues in health and disease, that they have a unique phenotype and that their functions go beyond the classical modulation of immune responses. Thus, tissue T_{reg} cells might add yet another level to classification of the T_{reg} cell compartment into functional and/or phenotypic subtypes. In this Review, we summarize recent findings in this new field, discussing knowns and unknowns about the origin, phenotype, function and memory of nonlymphoid tissue-resident T_{reg} cells.

Regulatory T cells that express the Foxp3 transcription factor, termed T_{reg} cells, are one of the immune system's main bastions against inappropriate or overexuberant responses. They control autoimmunity, allergic and inflammatory reactions, and responses to infectious agents and tumors. Over the past decade, many 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} cell–mediated immunosuppression[1]. For the most part, these explorations have taken the average T_{reg} cell residing in the spleen or lymph nodes to be a typical example.

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 integrin 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 2 (T_H2) cells, which also critically rely on IRF4 (ref. 3). In parallel, a discrete chemokine receptor 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 interleukin 17 (IL-17)-dependent 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 an N-terminal insertional mutation of Foxp3 to dampen IL-17–driven and IL-4–driven arthritis in the 'K/BxN' mouse model but to exacerbate type 1 diabetes in nonobese diabetic (NOD) mice, a T_H1 cell–type disorder[7]. Another notable match between the cells that regulate and those that are regulated is found in germinal centers: follicular regulatory (T_{FR}) cells and follicular helper (T_{FH}) cells both depend on transcriptional repressors Blimp-1 and Bcl-6 for their differentiation and homeostasis and on chemokine receptor CXCR5 for their localization[8,9]. The advantage of such a matching is probably that it provokes colocalization to and/or cosurvival in discrete locations. Arming regulatory and effector cells with the same capabilities could be dangerous, but safeguards are in place; for example, T_H1 cell–type T_{reg} cells poorly upregulate IL-12 receptor subunit IL-12Rβ2 upon induction of the transcriptional regulator STAT1 by interferon-γ, meaning that the differentiation of these cells to T_H1 effector cells is aborted[10].

In this Review we focus on studies that go one step further, highlighting the phenotype and functions—sometimes exquisitely adapted—of T_{reg} cells residing in nonlymphoid 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 nonlymphoid 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 (refs. 11–21 and D.B., C.B. and D.M., unpublished data). 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. Although they display some features of activated and/or 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 antigen 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].

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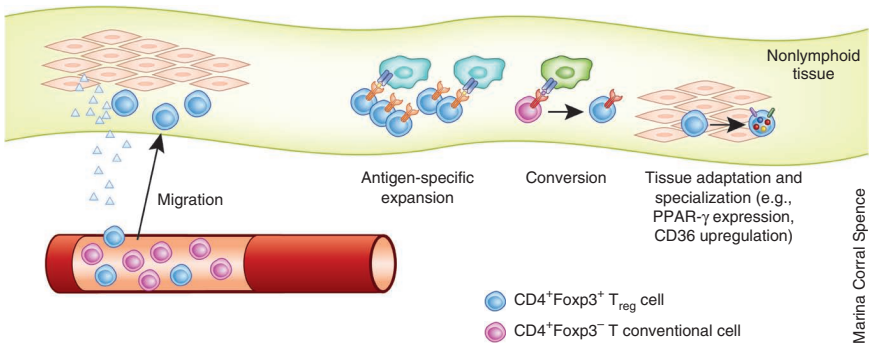

Figure 1 Mechanisms involved in the accumulation of T_{reg} cells in nonlymphoid tissues. Several mechanisms (alone or in combination) have been shown to increase the frequency and numbers of tissue-resident T_{reg} cells: migration and retention of circulating T_{reg} cells, promoted by the expression of specific chemotactic molecules by parenchymal or stromal cells, and the expression of the specific receptors on T_{reg} cells; expression of tissue-specific antigens that induce expansion of particular T_{reg} cell clones; antigen-induced conversion of $CD4^+Foxp3^-$ T conventional cells into peripheral T_{reg} cells (pT_{reg} cells); and acquisition of a tissue-specific phenotype that allows adaptation or specialization in the new environment, promoting survival and enhancing T_{reg} cell functions.

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 notably higher fraction than the 5–15% routinely observed in lymphoid organs, including in aged individuals. Microarray-based profiling of gene expression, confirmed by flow cytometry analyses, revealed VAT T_{reg} cells to have a unique phenotype. Although 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, chemokine receptors CCR1, CCR2 and CCR9), immunomodulatory cytokines (such as IL-10) and the transcription factor PPAR-γ, all of which confer unique functional properties to VAT T_{reg} cells[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 importance or more prosaically marks cells at different cell-cycle or differentiation stages. The TCR repertoire is another feature contributing to the distinct phenotype of VAT T_{reg} cells: the TCR sequences they employ have little overlap with those used by lymphoid-organ T_{reg} cells (ref. 12 and D. Kolodin and colleagues, personal communication). In addition, there is a notable population expansion of certain T_{reg} clones in adipose tissue, suggesting that recognition of particular antigens may be important in the accumulation of T_{reg} cells in abdominal fat (see below).

The major driver of VAT T_{reg} cell accumulation, phenotype and function is PPAR-γ (ref. 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 upregulation of the VAT T_{reg} cell 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 compared with their wild-type littermates, without any changes in lymphoid-organ T_{reg} cell populations. Moreover, the few VAT T_{reg} cells remaining in those PPAR-γ–deficient mice exhibit an underrepresentation of the VAT T_{reg} signature. The fact that PPAR-γ expression in VAT T_{reg} cells is much higher than that in any other T_{reg} cell population, together with the observation that these cells 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 in 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, with 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, the growth factor amphiregulin and the regulator of macrophage activation 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. T_{reg} cells also accumulate in skeletal muscle of dystrophic mice (such as mice carrying the mdx mutation in the dystrophin gene and dysferlin-deficient mice), whose chronic muscle injury has a genetic origin.

Given recent successes with immunomodulatory antitumor strategies (see Review by T.F. Gajewski *et al.* in this issue[25]), there is a growing interest in the more heterogeneous group of tumor-infiltrating T_{reg} cells. In solid tumors of nonlymphoid 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 profiling of gene expression in tumor-associated T_{reg} cells is still lacking[26], but $Foxp3^+CD4^+$ T cells from several mouse and human tumors show increased expression of cell-surface markers such as CTLA-4, ICOS, TIM-3 and GITR as well as of suppressive cytokines such as IL-10 and TGF-β, and a variety of chemokine receptors[19,27–30].

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 or 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 tissue sites: chemokine-based recruitment of circulating T_{reg} cells, local TCR-driven or cytokine-driven expansion, conversion of local or circulating conventional $CD4^+$ T cells, or prolonged survival (**Fig. 1**). In some cases, for example, in tumor T_{reg} cells, all of these mechanisms have been championed by one investigator or another[19,26,27,31–35].

As for other types of immune-system cells, recruitment of T_{reg} cells to nonlymphoid tissues is governed by specific combinations of chemotactic molecules and their receptors (reviewed in ref. 36). 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 such as skin, lung and liver, wherein an enrichment for CCR4$^+$CD103 (integrin α_E)$^+$ T_{reg} cells is observed[11]. *Ccr4*$^{-/-}$ T_{reg} cells show impaired accumulation in healthy nonlymphoid 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 upregulation 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 $\alpha_4\beta_7$, and the reverse pattern is true for adults[37]. The switch commences at 1.5–3 years of age and correlates with a change from a naive 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 nonlymphoid tissues under various inflammatory conditions[38]. In some cases, the expression of a particular receptor by tissue-resident T_{reg} cells is directly associated with the type of tissue, but in others it is related to the type of T cell response (T_H1, T_H2 or T_H17) occurring in the tissue and the accompanying T_{reg} cell polarization, for example, the preferential recruitment of CCR6$^+$ T_{reg} cells and CXCR3$^+$ T_{reg} cells to sites with an ongoing T_H17 cell– or T_H1 cell–driven inflammation, respectively[4,5].

The pattern and timing of trafficking between lymphoid and nonlymphoid tissues is not well-defined in most cases. 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[39]. 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 because 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 TCR repertoire of T_{reg} cells is biased toward the recognition of self antigens[1], which could facilitate the localization of T_{reg} cells in nonlymphoid 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 (ref. 12; D. Kolodin and colleagues, 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 notable clonal expansions of T_{reg} cells in VAT even in standard mice. Lastly, in the line with limited TCR diversity, it is possible to find examples, in the same or different individuals, of a repeated complementary-determining region 3 (CDR3) 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-specific TCR transgenic model and the expression of ovalbumin 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[40]. 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 (see below), suggesting that antigen recognition is important for tissue T_{reg} cell function and memory.

A recent report provided the first clear identification of antigen specificity of a natural population of tissue-resident T_{reg} cells[41]. By sequencing the TCR repertoire of prostate tumor–infiltrating T_{reg} cells, the authors identified a TCR $\alpha\beta$ pair recurrently enriched in independent mice (named MJ23), whose sequences they used to generate a MJ23 TCR transgenic line. In tumor-free MJ23 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. Selection of MJ23 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 nonlymphoid tissues, especially in pathological situations such as inflammation or cancer in which an enhanced presentation of self antigen can occur in the periphery.

Although the majority of T_{reg} cells differentiate in the thymus, a small fraction of them can be generated in the periphery, by conversion of Foxp3$^-$CD4$^+$ T cells into Foxp3$^+$CD4$^+$ T_{reg} cells, 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 nonlymphoid tissues, although few studies have clearly demonstrated that, in certain sites and/or conditions, pT_{reg} cells constitute a substantial proportion of tissue T_{reg} cells. The intestinal lamina propria T_{reg} pool is one of the main populations of nonlymphoid-tissue T_{reg} cells impacted by conversion, and pT_{reg} cells have been shown to contribute substantially to oral tolerance (reviewed in ref. 42). 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[43–46]. The analysis of a mutant mouse bearing a deletion in the conserved noncoding sequence 1 (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[17,47,48]. 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[43,49] and tissue-resident macrophages in the lung mucosa[50]. 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[51]. The generation of pT_{reg} cells has also been proposed to have a role in the accumulation of T_{reg} cells in tumors[35,52], although the exact contribution of this mechanism versus recruitment and/or population expansion of thymically derived T_{reg} cells remains controversial[26,42]. 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[52].

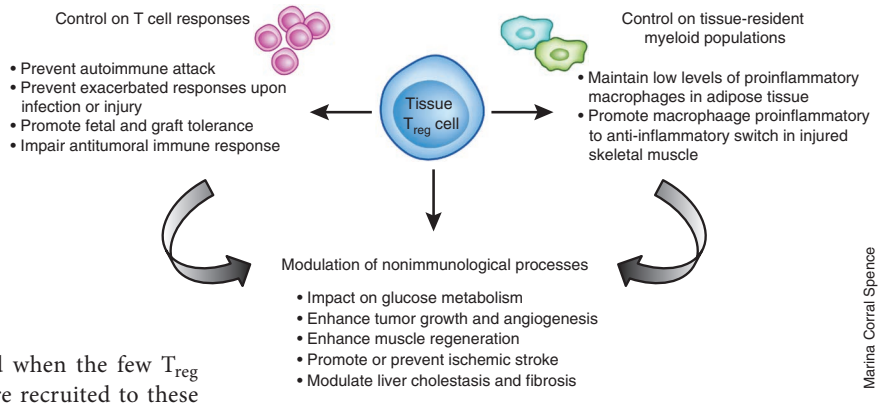

Figure 2 Functions of tissue-resident T_{reg} cells. The functions of tissue-resident T_{reg} cells can be divided in three main groups (representative examples of each group are listed in the figure): control of local T cell responses, control of tissue-resident myeloid populations and modulation of nonimmunological processes. The latter may occur by direct interaction between T_{reg} cells and their nonimmune targets or indirectly through the regulation of other tissue-resident leukocytes, which in turn could affect those targets.

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[53], 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 TCR αβ T cells remains controversial.

Immunological and nonimmunological targets

One of the major functions of T_{reg} cells residing in nonlymphoid 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 tissue $Foxp3^+$ cells. In addition, tissue T_{reg} cells can strongly impact myeloid populations in the vicinity, inhibiting neutrophils and proinflammatory 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 such as IL-10 and CTLA-4, and their effectiveness in the typical *in vitro* suppression assay[12] suggest that VAT T_{reg} cells can control conventional $CD4^+$ T cell 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 proinflammatory myeloid populations ($CD11b^+CD11c^+F4/80^+$ macrophages and proinflammatory $CD11b^+Ly6C^{hi}$ monocytes) observed in VAT depots[23,54,55]. Similarly, muscle T_{reg} cells are important in controlling the proinflammatory 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 proinflammatory $Ly6C^{hi}$ 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[31] or even considerably better[30]; and, in many cases, a high frequency of intratumor T_{reg} cells is correlated with poor prognosis[31,56,57]. 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[58,59]. In line with these observations, in certain tumors, such as colorectal cancers, a high density of T_{reg} cells correlates with a favorable prognosis[60,61]. It has recently been proposed that T_{reg} cell–mediated suppression of proinflammatory T_H17 cell responses to the dense microbiome of the large intestine is key to preventing tumor growth in the intestinal epithelium[61].

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 nonimmunological processes, including systemic metabolic indices[12,23,55,62], ischemic stroke[63,64], formation of atherosclerotic plaques[65,66], cardiac remodeling after myocardial infarction[67], liver cholestasis and fibrosis[68], and skeletal muscle regeneration (D.B., C.B. and D.M., unpublished data). It is still unclear to what extent such influences reflect direct interaction between T_{reg} cells and their nonimmunological target cells or an indirect effect of T_{reg} suppression of local inflammation (**Fig. 2**). In any case, these nontraditional 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[69,70]. That the frequency of VAT, but not of 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} cell subset might influence local and/or systemic metabolism[12,54]. Loss-of-function experiments, relying on punctual and specific T_{reg} cell ablation in a transgenic mouse expressing the diphtheria toxin receptor under *Foxp3* regulatory elements (*Foxp3*^{DTR} mutant mice), 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 expression of IL-10 in VAT, improved insulin sensitivity and improved glucose tolerance. These results were mirrored in a more recent experiments manipulating PPAR-γ expression or signaling: mice devoid of PPAR-γ only in T_{reg} cells exhibited a strong reduction in VAT T_{reg} cells and degradation of metabolic indices, whereas HFD-fed wild-type mice injected with a PPAR-γ agonist, pioglitazone, had a substantially more robust T_{reg} cell population and metabolic improvements. 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_{reg} cells are important 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 downregulate proinflammatory cytokines and increase glucose uptake.

T_{reg} cells that accumulate in injured skeletal muscle also seem to impact nonimmunological processes, in this case tissue regeneration (D.B., C.B. and D.M., unpublished data). Mice punctually depleted of T_{reg} cells exhibit 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 amphiregulin, a growth factor that induces *in vitro* differentiation of satellite cells, which express amphiregulin receptors (D.B., C.B. and D.M., unpublished data), 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 nonimmune processes regulated by T_{reg} cells. 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 postischemic brain, and that their depletion profoundly enhances brain damage and exacerbates the functional outcome[63]. Production of IL-10 by T_{reg} cells may be their major mechanism of immunomodulation in the brain and of T_{reg} cell–mediated cebroprotection. Unfortunately, these results conflict with another subsequently published study indicating that T_{reg} cells promote acute ischemic stroke in mice by inducing dysfunction of the cerebral microvasculature[64]. 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 versus a *Foxp3*^{DTR} line), by the different stroke models used (permanent versus temporal; different ischemia times) or the timing of readout (late versus acute stages).

A liaison between T_{reg} cells and endothelial cells has also been observed in the tumor microenvironment, in this case reflecting a proangiogenic effect by tumor-infiltrating $Foxp3^+CD4^+$ cells[71]. Tumor hypoxia promotes the recruitment of T_{reg} cells through induction of expression of the chemokine CCL28 in tumor cells. Elevated numbers of T_{reg} cells expressing CCR10 accumulate in $CCL28^+$ tumors, which exhibit increased growth and angiogenesis in comparison with $CCL28^-$ 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 proangiogenic factor themselves[71]. Tumor-infiltrating T_{reg} cells also promote mammary carcinoma metastasis in the lung by the cytokine RANKL[72]. Thus, the impact of tumor-resident T_{reg} cells on tumor growth and patient prognosis reflects both traditional (on immune targets) and nontraditional (on nonimmune targets) T_{reg} cell 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[73,74]. Upon reinfection, these memory T_{reg} cells rapidly expand and effectively control specific antiviral responses by $CD4^+$ and $CD8^+$ T cells. An accelerated accumulation of memory T_{reg} cells is observed in nonlymphoid tissues targeted by the infection; furthermore, memory T_{reg} cells can suppress the collateral tissue damage and inflammation elicited by recall expansion of non–T_{reg} memory $CD4^+$ T cells. A link between T_{reg} memory cells and tissue-resident T_{reg} cells has also been demonstrated in the context of autoimmunity[40]. Using a TCR–neo-self-antigen double-transgenic model (transgenes encoding the DO11.10 TCR combined with transgenes specifying inducible, skin-specific expression of ovalbumin), a recent study showed that upon local induction of ovalbumin expression, ovalbumin-specific T_{reg} cells accumulate in the skin[40]. 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 reexpressed. These memory T_{reg} cells can survive in the nonlymphoid 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[75]. These T_{reg} cells 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 induce their proliferation in a manner dependent on major histocompatibility complex (MHC) class II and IL-15 (ref. 76). It has been proposed that self peptides or the normal skin microbiome might be the source of antigens presented by Langerhans cells. Human skin–resident T_{reg} cells also proliferate in response to pathogens[76] and during the recall response induced by injection of tuberculin purified-protein derivative (PPD) in individuals immunized with bacille Calmette-Guérin[77].

Memory T_{reg} cells also seem to have an important function in preventing fetal rejection in successive pregnancies[78]. 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 reaccumulate with accelerated kinetics during subsequent pregnancies. The precise localization of memory T_{reg} cells in fetal-maternal nonlymphoid 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 specificity to fetal antigen and existing evidence of the presence of T_{reg} cells in placenta and uterus[79–81] 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 nonlymphoid 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

The study of 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. Here we highlight three questions that particularly intrigue us and are interesting realms to explore.

First, how widespread is the phenomenon? Given the distinct T_{reg} cell compartments already found to be associated with adipose

tissue, muscle, the colon and other tissues, it is theoretically possible that there is a specialized type of T_{reg} cells dedicated to maintaining homeostasis in 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 analyses, should yield critical information bearing on this question. It may also prove informative to compare T_{reg} cell 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 subphenotype, is retained in a particular tissue, likely because its TCR reacts to an antigen therein and, in response to tissue-specific cues, takes on a tissue-specific subphenotype. But it is difficult to rule out the possibility that small numbers of precursors precommitted to a particular T_{reg} cell subphenotype 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[53]. 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' coopting of PPAR-γ provides these cells with properties (such as expression of the lipid transporter, CD36) that promote survival in adipose tissue. Similarly, high-level expression of amphiregulin by T_{reg} cells in regenerating tissues (for example, muscle or intestine) arms them with the capacity to favorably impact their local environment. How common it is for tissue-resident T_{reg} cells to coopt transcriptional programs characteristic of tissue cells (like VAT T_{reg} cells' use of PPAR-γ, the 'master-regulator' of adipocytes) and whether there might be something special about the chromatin organization or epigenetic makeup of T_{reg} cells that favors such adaptability remains an open question.

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COMPETING FINANCIAL INTERESTS

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1. Josefowicz, S.Z., Lu, L.F., Rudensky, A.Y. & Regulatory, T. Cells: mechanisms of differentiation and function. *Annu. Rev. Immunol.* **30**, 531–564 (2012).
2. Feuerer, M., Hill, J.A., Mathis, D. & Benoist, C. Foxp3+ regulatory T cells: differentiation, specification, subphenotypes. *Nat. Immunol.* **10**, 689–695 (2009).
3. Zheng, Y. *et al.* Regulatory T-cell suppressor program co-opts transcription factor IRF4 to control T(H)2 responses. *Nature* **458**, 351–356 (2009).
4. Koch, M.A. *et al.* The transcription factor T-bet controls regulatory T cell homeostasis and function during type 1 inflammation. *Nat. Immunol.* **10**, 595–602 (2009).
In references 3 and 4, the concept is introduced that T_{reg} cells adapt their phenotype to match the type of immune response they are controlling, sometimes sharing key segments of the transcriptional program with co-resiging T effector cells.
5. Chaudhry, A. *et al.* CD4+ regulatory T cells control TH17 responses in a Stat3-dependent manner. *Science* **326**, 986–991 (2009).
6. Hall, A.O. *et al.* The cytokines interleukin 27 and interferon-gamma promote distinct Treg cell populations required to limit infection-induced pathology. *Immunity* **37**, 511–523 (2012).
7. Darce, J. *et al.* An N-terminal mutation of the Foxp3 transcription factor alleviates arthritis but exacerbates diabetes. *Immunity* **36**, 731–741 (2012).
8. Chung, Y. *et al.* Follicular regulatory T cells expressing Foxp3 and Bcl-6 suppress germinal center reactions. *Nat. Med.* **17**, 983–988 (2011).
9. Linterman, M.A. *et al.* Foxp3+ follicular regulatory T cells control the germinal center response. *Nat. Med.* **17**, 975–982 (2011).
10. Koch, M.A. *et al.* T-bet(+) Treg cells undergo abortive Th1 cell differentiation due to impaired expression of IL-12 receptor beta2. *Immunity* **37**, 501–510 (2012).
11. Sather, B.D. *et al.* Altering the distribution of Foxp3(+) regulatory T cells results in tissue-specific inflammatory disease. *J. Exp. Med.* **204**, 1335–1347 (2007).
This report described the homing receptor expression and tissue localization of T_{reg} cells in the steady state, and showed that impairing T_{reg} cell migration to nonlymphoid tissues results in the development of tissue-specific inflammatory disease.
12. Feuerer, M. *et al.* Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nat. Med.* **15**, 930–939 (2009).
This study reported for the first time the presence of a unique population of T_{reg} cells in male visceral adipose tissue and their role in controlling metabolic parameters.
13. Herman, A.E., Freeman, G.J., Mathis, D. & Benoist, C. CD4+CD25+ T regulatory cells dependent on ICOS promote regulation of effector cells in the prediabetic lesion. *J. Exp. Med.* **199**, 1479–1489 (2004).
14. Nguyen, L.T., Jacobs, J., Mathis, D. & Benoist, C. Where FoxP3-dependent regulatory T cells impinge on the development of inflammatory arthritis. *Arthritis Rheum.* **56**, 509–520 (2007).
15. Suffia, I.J. *et al.* Infected site-restricted Foxp3+ natural regulatory T cells are specific for microbial antigens. *J. Exp. Med.* **203**, 777–788 (2006).
16. Lee, I. *et al.* Recruitment of Foxp3+ T regulatory cells mediating allograft tolerance depends on the CCR4 chemokine receptor. *J. Exp. Med.* **201**, 1037–1044 (2005).
17. Samstein, R.M. *et al.* Extrathymic generation of regulatory T cells in placental mammals mitigates maternal-fetal conflict. *Cell* **150**, 29–38 (2012).
This work demonstrated that peripheral T_{reg} cells specific for paternal antigens accumulate in the placenta and prevent fetal resorption, and suggested that extrathymic differentiation of T_{reg} cells emerged in placental animals to enforce maternal-fetal tolerance.
18. Tilburgs, T. *et al.* Evidence for a selective migration of fetus-specific $CD4^{+}CD25^{bright}$ regulatory T cells from the peripheral blood to the decidua in human pregnancy. *J. Immunol.* **180**, 5737–5745 (2008).
19. Tanchot, C. *et al.* Tumor-infiltrating regulatory T cells: phenotype, role, mechanism of expansion *in situ* and clinical significance. *Cancer Microenviron.* **6**, 147–157 (2013).
20. de Boer, O.J. *et al.* Low numbers of FOXP3 positive regulatory T cells are present in all developmental stages of human atherosclerotic lesions. *PLoS ONE* **2**, e779 (2007).
21. Meng, X. *et al.* Statins induce the accumulation of regulatory T cells in atherosclerotic plaque. *Mol. Med.* **18**, 598–605 (2012).
22. Cretney, E., Kallies, A. & Nutt, S.L. Differentiation and function of Foxp3(+) effector regulatory T cells. *Trends Immunol.* **34**, 74–80 (2013).
23. Cipolletta, D. *et al.* PPAR-gamma is a major driver of the accumulation and phenotype of adipose tissue T_{reg} cells. *Nature* **486**, 549–553 (2012).
This report identified PPAR-γ as a crucial molecular orchestrator of the accumulation, phenotype and function of T_{reg} cells in male visceral adipose tissue, demonstrating that a specific transcription factor can drive the unique characteristics of a particular tissue T_{reg} population.
24. Tontonoz, P. & Spiegelman, B.M. Fat and beyond: the diverse biology of PPARgamma. *Annu. Rev. Biochem.* **77**, 289–312 (2008).
25. Gajewski, T.F., Schreiber, H. & Fu, Y.-X. Innate and adaptive immune cells in the tumor microenvironment. *Nat. Immunol.* (18 September 2013) doi:10.1038/ni.2703.
26. Savage, P.A., Malchow, S. & Leventhal, D.S. Basic principles of tumor-associated regulatory T cell biology. *Trends Immunol.* **34**, 33–40 (2013).
27. Gobert, M. *et al.* Regulatory T cells recruited through CCL22/CCR4 are selectively activated in lymphoid infiltrates surrounding primary breast tumors and lead to an adverse clinical outcome. *Cancer Res.* **69**, 2000–2009 (2009).
28. Gao, X. *et al.* TIM-3 expression characterizes regulatory T cells in tumor tissues and is associated with lung cancer progression. *PLoS ONE* **7**, e30676 (2012).

29. Park, H.J. *et al.* Tumor-infiltrating regulatory T cells delineated by upregulation of PD-1 and inhibitory receptors. *Cell. Immunol.* **278**, 76–83 (2012).
30. Strauss, L. *et al.* A unique subset of CD4+CD25^{high}Foxp3+ T cells secreting interleukin-10 and transforming growth factor-beta1 mediates suppression in the tumor microenvironment. *Clin. Cancer Res.* **13**, 4345–4354 (2007).
31. Curiel, T.J. *et al.* Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat. Med.* **10**, 942–949 (2004).
This study demostrated that T_{reg} cells have an immunopathological role in human cancer by showing that they accumulate in ovarian tumors, recruited by CCL22, and that they suppress antitumoral T cell responses.
32. Tan, M.C. *et al.* Disruption of CCR5-dependent homing of regulatory T cells inhibits tumor growth in a murine model of pancreatic cancer. *J. Immunol.* **182**, 1746–1755 (2009).
33. Quezada, S.A. *et al.* Limited tumor infiltration by activated T effector cells restricts the therapeutic activity of regulatory T cell depletion against established melanoma. *J. Exp. Med.* **205**, 2125–2138 (2008).
34. Kuczma, M. *et al.* Intratumoral convergence of the TCR repertoires of effector and Foxp3+ CD4+ T cells. *PLoS ONE* **5**, e13623 (2010).
35. Liu, V.C. *et al.* Tumor evasion of the immune system by converting CD4+. *J. Immunol.* **178**, 2883–2892 (2007).
36. Ding, Y., Xu, J. & Bromberg, J.S. Regulatory T cell migration during an immune response. *Trends Immunol.* **33**, 174–180 (2012).
37. Grindebacke, H. *et al.* Dynamic development of homing receptor expression and memory cell differentiation of infant CD4+CD25^{high} regulatory T cells. *J. Immunol.* **183**, 4360–4370 (2009).
38. Campbell, D.J. & Koch, M.A. Phenotypical and functional specialization of FOXP3+ regulatory T cells. *Nat. Rev. Immunol.* **11**, 119–130 (2011).
39. Zhang, N. *et al.* Regulatory T cells sequentially migrate from inflamed tissues to draining lymph nodes to suppress the alloimmune response. *Immunity* **30**, 458–469 (2009).
This report described the migration pattern of T_{reg} cells in a model of islet transplantation and proposed that T_{reg} cells, to efficiently control an alloimmune response, need to be educated first in the target tissue before entering the draining lymph node.
40. Rosenblum, M.D. *et al.* Response to self antigen imprints regulatory memory in tissues. *Nature* **480**, 538–542 (2011).
41. Malchow, S. *et al.* Aire-dependent thymic development of tumor-associated regulatory T cells. *Science* **339**, 1219–1224 (2013).
This work identified an endogenous population of thymus-derived T_{reg} cells that infiltrates mouse prostate tumors and is specific for a normal prostate antigen, and demonstrated that Aire-mediated expression of peripheral-tissue antigens can drive the generation of tissue-specific T_{reg} cell subsets.
42. Bilate, A.M. & Lafaille, J.J. Induced CD4+Foxp3+ regulatory T cells in immune tolerance. *Annu. Rev. Immunol.* **30**, 733–758 (2012).
43. Sun, C.M. *et al.* Small intestine lamina propria dendritic cells promote *de novo* generation of Foxp3 T reg cells via retinoic acid. *J. Exp. Med.* **204**, 1775–1785 (2007).
44. Haribhai, D. *et al.* A central role for induced regulatory T cells in tolerance induction in experimental colitis. *J. Immunol.* **182**, 3461–3468 (2009).
45. Atarashi, K. *et al.* Induction of colonic regulatory T cells by indigenous Clostridium species. *Science* **331**, 337–341 (2011).
This study demonstrated that a cocktail of *Clostridia* species, a component of the mammalian colonic microbiota, promote anti-inflammatory immune responses by expanding and activating T_{reg} cells in the colonic lamina propria.
46. Thornton, A.M. *et al.* Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3+ T regulatory cells. *J. Immunol.* **184**, 3433–3441 (2010).
47. Zheng, Y. *et al.* Role of conserved non-coding DNA elements in the *Foxp3* gene in regulatory T-cell fate. *Nature* **463**, 808–812 (2010).
48. Josefowicz, S.Z. *et al.* Extrathymically generated regulatory T cells control mucosal TH2 inflammation. *Nature* **482**, 395–399 (2012).
This paper showed that mice deficient in peripheral T_{reg} cells spontaneously develop pronounced T_H2 cell–type pathologies at mucosal sites and have altered gut microbial communities, demostrating the functional specialization of peripheral T_{reg} cells and confirming that thymus-derived T_{reg} cells are the major controllers of systemic and tissue-specific autoimmunity.
49. Coombes, J.L. *et al.* A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J. Exp. Med.* **204**, 1757–1764 (2007).
50. Soroosh, P. *et al.* Lung-resident tissue macrophages generate Foxp3+ regulatory T cells and promote airway tolerance. *J. Exp. Med.* **210**, 775–788 (2013).
51. Liu, Y., Teige, I., Birnir, B. & Issazadeh-Navikas, S. Neuron-mediated generation of regulatory T cells from encephalitogenic T cells suppresses EAE. *Nat. Med.* **12**, 518–525 (2006).
52. Zhou, G. & Levitsky, H.I. Natural regulatory T cells and de novo–induced regulatory T cells contribute independently to tumor-specific tolerance. *J. Immunol.* **178**, 2155–2162 (2007).
53. Yona, S. *et al.* Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* **38**, 79–91 (2013).
54. Deiuliis, J. *et al.* Visceral adipose inflammation in obesity is associated with critical alterations in T regulatory cell numbers. *PLoS ONE* **6**, e16376 (2011).
55. Ilan, Y. *et al.* Induction of regulatory T cells decreases adipose inflammation and alleviates insulin resistance in ob/ob mice. *Proc. Natl. Acad. Sci. USA* **107**, 9765–9770 (2010).
56. Bates, G.J. *et al.* Quantification of regulatory T cells enables the identification of high-risk breast cancer patients and those at risk of late relapse. *J. Clin. Oncol.* **24**, 5373–5380 (2006).
57. Perrone, G. *et al.* Intratumoural FOXP3-positive regulatory T cells are associated with adverse prognosis in radically resected gastric cancer. *Eur. J. Cancer* **44**, 1875–1882 (2008).
58. Colotta, F. *et al.* Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis* **30**, 1073–1081 (2009).
59. Grivennikov, S.I., Greten, F.R. & Karin, M. Immunity, inflammation, and cancer. *Cell* **140**, 883–899 (2010).
60. deLeeuw, R.J., Kost, S.E., Kakal, J.A. & Nelson, B.H. The prognostic value of FoxP3+ tumor-infiltrating lymphocytes in cancer: a critical review of the literature. *Clin. Cancer Res.* **18**, 3022–3029 (2012).
61. Ladoire, S., Martin, F. & Ghiringhelli, F. Prognostic role of FOXP3+ regulatory T cells infiltrating human carcinomas: the paradox of colorectal cancer. *Cancer Immunol. Immunother.* **60**, 909–918 (2011).
62. Eller, K. *et al.* Potential role of regulatory T cells in reversing obesity-linked insulin resistance and diabetic nephropathy. *Diabetes* **60**, 2954–2962 (2011).
63. Liesz, A. *et al.* Regulatory T cells are key cerebroprotective immunomodulators in acute experimental stroke. *Nat. Med.* **15**, 192–199 (2009).
64. Kleinschnitz, C. *et al.* Regulatory T cells are strong promoters of acute ischemic stroke in mice by inducing dysfunction of the cerebral microvasculature. *Blood* **121**, 679–691 (2013).
65. Ait-Oufella, H. *et al.* Natural regulatory T cells control the development of atherosclerosis in mice. *Nat. Med.* **12**, 178–180 (2006).
66. Klingenberg, R. *et al.* Depletion of FOXP3+ regulatory T cells promotes hypercholesterolemia and atherosclerosis. *J. Clin. Invest.* **123**, 1323–1334 (2013).
67. Tang, T.T. *et al.* Regulatory T cells ameliorate cardiac remodeling after myocardial infarction. *Basic Res. Cardiol.* **107**, 232 (2012).
68. Katz, S.C. *et al.* Obstructive jaundice expands intrahepatic regulatory T cells, which impair liver T lymphocyte function but modulate liver cholestasis and fibrosis. *J. Immunol.* **187**, 1150–1156 (2011).
69. Hotamisligil, G.S., Shargill, N.S. & Spiegelman, B.M. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* **259**, 87–91 (1993).
70. Hotamisligil, G.S. Inflammation and metabolic disorders. *Nature* **444**, 860–867 (2006).
71. Facciabene, A. *et al.* Tumour hypoxia promotes tolerance and angiogenesis via CCL28 and T(reg) cells. *Nature* **475**, 226–230 (2011).
72. Tan, W. *et al.* Tumour-infiltrating regulatory T cells stimulate mammary cancer metastasis through RANKL-RANK signalling. *Nature* **470**, 548–553 (2011).
73. Sanchez, A.M., Zhu, J., Huang, X. & Yang, Y. The development and function of memory regulatory T cells after acute viral infections. *J. Immunol.* **189**, 2805–2814 (2012).
74. Brincks, E.L. *et al.* Antigen-specific memory regulatory CD4+Foxp3+ T cells control memory responses to influenza virus infection. *J. Immunol.* **190**, 3438–3446 (2013).
75. Clark, R.A. & Kupper, T.S. IL-15 and dermal fibroblasts induce proliferation of natural regulatory T cells isolated from human skin. *Blood* **109**, 194–202 (2007).
76. Seneschal, J. *et al.* Human epidermal Langerhans cells maintain immune homeostasis in skin by activating skin resident regulatory T cells. *Immunity* **36**, 873–884 (2012).
77. Vukmanovic-Stejic, M. *et al.* The kinetics of CD4+Foxp3+ T cell accumulation during a human cutaneous antigen-specific memory response *in vivo*. *J. Clin. Invest.* **118**, 3639–3650 (2008).
78. Rowe, J.H., Ertelt, J.M., Xin, L. & Way, S.S. Pregnancy imprints regulatory memory that sustains anergy to fetal antigen. *Nature* **490**, 102–106 (2012).
This study showed that pregnancy selectively stimulates the accumulation of maternal T_{reg} cells with fetal specificity, which, after delivery, persist at elevated levels, maintain tolerance to preexisting fetal antigen and rapidly reaccumulate during subsequent pregnancy, demonstrating the importance of T_{reg} cells for sustaining protective regulatory memory to fetal antigen.
79. Erlebacher, A. Mechanisms of T cell tolerance towards the allogeneic fetus. *Nat. Rev. Immunol.* **13**, 23–33 (2013).
80. Kallikourdis, M., Andersen, K.G., Welch, K.A. & Betz, A.G. Alloantigen-enhanced accumulation of CCR5+ 'effector' regulatory T cells in the gravid uterus. *Proc. Natl. Acad. Sci. USA* **104**, 594–599 (2007).
81. Perez Leiros, C. & Ramhorst, R. Tolerance induction at the early maternal-placental interface through selective cell recruitment and targeting by immune polypeptides. *Am. J. Reprod. Immunol.* **69**, 359–368 (2013).