Published in final edited form as:

Curr Opin Organ Transplant. 2015 August ; 20(4): 376–384. doi:10.1097/MOT.0000000000000212.

New insights into the mechanisms of Treg function

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Abstract

Purpose of review—CD4+Foxp3+ regulatory T cells (Tregs) are crucial in controlling immunity and self-tolerance. Consequently, in transplantation, Tregs play a central role in inhibiting acute rejection and promoting allograft tolerance. A more complete understanding of Treg biology may lead to novel therapeutic approaches to enhance Treg numbers and function.

Recent findings—The maintenance of self-tolerance in non-lymphoid tissues requires the differentiation of Tregs in secondary lymphoid organs from naïve-like central Tregs into effector Tregs. Antigen and environmental cues guide this Treg differentiation, which parallels the types of adaptive immune responses taking place, allowing them to enter and function within specific non-lymphoid tissues. In addition to controlling inflammation, tissue-infiltrating Tregs unexpectedly regulate non-immune processes, including metabolic homeostasis and tissue repair. Finally, Tregs can be directly and specifically targeted in vivo to augment their numbers or enhance their function in both secondary lymphoid organs and non-lymphoid tissues.

Summary—Tregs exhibit a previously unrecognized breadth of function, which includes tissue-specific specialization and the regulation of both immune and non-immune processes. This is of particular importance in transplantation since allo-reactive memory T cells can act directly within the allograft. Thus, therapeutic approaches may need to promote Treg function in transplanted tissue as well as in secondary lymphoid organs. Such therapy would not only prevent inflammation and acute rejection, but may also promote non-immune processes within the allograft such as tissue homeostasis and repair.

Keywords

CD4+ Regulatory T cells; specialization of function; tissue homeostasis; tissue repair; inflammation

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Conflicts of interest

The authors have no conflict of interest to disclose.

Introduction

Regulatory CD4⁺ T cells expressing the transcription factor Foxp3 (Tregs) play a crucial role in the balance between immunity and tolerance. Dysregulation of Treg ontogeny or function leads to uncontrolled immune responsiveness, tissue damage and autoimmunity [1,2]. In animal models, Tregs are central in promoting and maintaining allograft tolerance [3–5].

While initial studies focused on the role of Tregs in inhibiting effector T cell (Teff) priming in secondary lymphoid organs (SLO), it is now apparent that in response to environmental cues, Tregs responses adapt to the type of immune response (i.e. Th1, Th2, Th17, and Tfh). This allows Tregs to both gain access to inflamed peripheral tissues and to limit the immune response at hand. New data demonstrate further specialization of Treg function within peripheral tissues where they contribute to tissue homeostasis and repair. Thus, the complexity of Treg function is greater than previously envisioned, and extends to the control of non-immunological processes in non-lymphoid tissues. Accordingly, in transplantation, a rethinking of Treg-associated therapies should concentrate on promoting allo-immunity type-specific that not only act in SLO, but also in the allograft where they prevent ongoing immune attack by both Teff and memory T cells and promote healing and organ homeostasis.

This review provides an overview of the recent findings pertaining to Treg diversity and specialization of function in SLO and in non-lymphoid tissues. In addition, we describe recent therapeutic approaches that modulate Treg function in vivo. The pertinence of these findings to transplantation will be highlighted. Given the central role of thymic-derived Tregs (tTregs) in controlling immunity in SLO and in non-lymphoid tissues (other than in gut and placenta) [6,7], and given the important advances pertaining to tTreg, this review will entirely focus on this regulatory subset.

Treg diversity in secondary lymphoid organs

Tregs populate primary lymphoid organs and SLO, as well as non-lymphoid tissues [8,9]. It is now clear that the presence and function of Tregs in various non-lymphoid tissues is required for protection against immune damage at those sites [8]. Moreover, Tregs are heterogeneous and can be divided into two major subsets. While *central Tregs* are predominantly found in SLO, *effector Tregs* populate non-lymphoid tissues, as well as SLO. These can be distinguished using cell surface markers. In mice, central Tregs are CD44^{low} and express CCR7⁺ CD62L^{hi} (allowing them to migrate within T cell zones in SLO). In contrast, effector Tregs phenotypically resemble conventional CD4⁺ Teff cells (CCR7[−] CD62L^{low} CD44^{hi}) [9–11]. The effector Treg subset differentiates from central Tregs after antigen exposure [9,10,12]. Consequently, effector Tregs also partially upregulate markers found on the surface of recently activated T cells (e.g. CD103, KLRG1, CxCR3, and CD69). In human peripheral blood, similar subsets have been identified using different markers. Central Tregs (termed “resting” in this report) are FOXP3^{low} CD45RA^{hi} CD25^{low} and effector Tregs express FOXP3^{hi} CD45RA^{low} CD25^{hi} [13].

Importantly, these Treg subsets differ not only in their anatomical location, but also in their biology and function. Tregs are generally believed to express a TCR repertoire that is skewed towards self-reactivity [14,15]. In addition, Treg homeostasis requires antigen presentation by dendritic cells (DCs) [16] and signaling through CD28 [17,18]. In fact, recent data demonstrated that Tregs constantly receive TCR signals [10], which are essential for the differentiation from central to effector Tregs, and for Treg suppressor function [12,19]. Indeed, inducible ablation of TCR signaling in Tregs in adult mice led to a rapid fall in the number of effector Tregs in SLO and non-lymphoid tissues and induced systemic autoimmunity. This occurred despite initial maintenance of normal numbers of Foxp3⁺ central Tregs [12]. However, the number of central Tregs lacking TCR expression diminished by half on day 46 [19]. Also, recent thymic central Treg émigrés failed to differentiate into effector Tregs in absence of TCR expression [12]. Thus, constant antigen recognition is required for the differentiation and maintenance of effector Tregs and for Treg suppressor function.

Additional characteristics differentiate central and effector Tregs. First, their distribution within the spleen differs: central Tregs are predominantly found within T cell zones, whereas effector Tregs localize to the marginal zone, red pulp and B cell follicle [10,20]. Second, distinct signals are required for their homeostasis and survival: central Tregs depend on IL-2R signaling while effector Tregs require ligation through the co-stimulatory molecule ICOS [10]. This correlates with the expression of each of these molecules on the cell surface. Central Tregs express high levels of CD25 (IL-2Ra) and effector Tregs express high levels of ICOS. Although effector Tregs respond normally to IL-2R signaling upon ex vivo exposure to IL-2, when analyzed directly in vivo, a significantly higher proportion of central Tregs demonstrate constant IL-2R signaling [10]. Thus, the localization of central Tregs within T cell zones, likely due to their expression of CCR7, provides access to IL-2 produced by conventional CD4⁺ Foxp3⁺-T cells (Tconv).

Tregs have been shown to exhibit a higher rate of homeostatic proliferation than Tconv [21]. Further characterization now demonstrates that this heightened rate of proliferation occurs within the effector Treg subset, while central Tregs are quiescent [10]. This is consistent with the requirement of effector Treg for constant TCR signaling [12,19]. However, a compensatory mechanism prevents the over-accumulation of these cells. Effector Tregs express low levels of the anti-apoptotic molecule Bcl-2, and are prone to cell death [10].

Thus, there appears to be a division of labor between central and effector Treg subsets. Central Tregs arise in the thymus and serve as a longer-lived pool of recirculating Tregs in SLO. Upon activation, they become effector Tregs, which is required for the maintenance of self-tolerance in non-lymphoid tissues, as well as in SLO (Figure 1). The role of these respective Treg subsets in transplantation tolerance has not been demonstrated. However, because allograft tolerance promotes the expansion of donor-specific Tregs, it is tempting to speculate that the differentiation step from central to effector Tregs is essential for long-term allograft survival in tolerant animals. In addition, only effector Tregs accumulate in non-lymphoid tissues, and this is likely to be essential for the protection of allografts from immune attack. It is clear that exposure to infections and environmental antigens generates effector/memory T cells that cross-react with alloantigens. These effector/memory T cells

make up a significant part of the alloimmune response and are relatively resistant to immunosuppressive and tolerogenic regimens [22,23]. Importantly, unlike naïve T cells, effector/memory T cells can directly migrate to the allograft to mount rejection, without prior activation in SLO [24,25]. Allografts, a source of persistent antigen, are under the constant threat of rejection by these cells. Thus, graft-infiltrating Tregs are likely to be essential to prevent allograft damage. While it is known that Tregs infiltrate allografts and that Tregs isolated from allografts are potent suppressors *ex vivo* [26,27], the specific role of graft-infiltrating Tregs remains to be demonstrated.

Transcriptional control of Treg specialization of function to immunity

The differentiation from central into effector Tregs involves the differential expression of several hundred genes [12]. However, the transcription factor interferon regulatory factor 4 (IRF4) appears to be a key regulator in effector Treg differentiation. In its absence, mice develop autoimmunity [12,28,29]. In addition, IRF4 promote the expression of Blimp-1 in effector Tregs, which is required for their function [29]. Furthermore, the differentiation of Tregs exhibits additional complexity. During the course of an immune response, specific transcription factors direct the differentiation of Tconv into different Th (effector) subsets (e.g. Th1, Th2, Th17, and Tfh). It was recently shown that Tregs parallel this differentiation paradigm, and expression of the corresponding transcription factors (in conjunction with the Foxp3 transcriptome) is necessary for optimal regulation of each of these conventional Th responses. For example, expression of T-bet, GATA3, STAT3 and BCL-6 in Tregs is necessary to control Th1, Th2, Th17, and Tfh responses, respectively [30–33]. Similarly to the polarization of naïve Tconv into Th subsets, environmental signals direct the expression of these transcription factors in Tregs. For example, exposure to IL-27 or IFN- γ promotes T-bet expression in effector Tregs [30,34,35], while exposure to IL-4 drives GATA3 expression in those cells [36]. Notably, T-bet, STAT3, or BCL-6 in Tregs promotes the expression of specific chemokine receptors (CXCR3, CCR6 or CXCR5, respectively) that allow their recruitment to sites of Th1, Th17 or Tfh cell responses [30,32,33,37]. Thus, in some instances, this specialization in Treg function permits their migration to non-lymphoid sites of inflammation or to specific sub-compartments in SLO (Figure 1). Interestingly, these effector Treg subsets are unlikely terminally differentiated since the expression of some of the Th-specific transcription factors can be dynamic [36]. Because allogeneic exposure predominantly generates Th1 (T-bet) immune responses, effective regulation of these responses in SLO and in allografts would necessitate T-bet-expressing effector Tregs. However, this has yet to be demonstrated.

Non-immunological Treg function in non-lymphoid tissues

Tregs populate non-lymphoid tissues in steady state, where they maintain self-tolerance [38]. Recent evidence demonstrates that these Tregs are phenotypically and functionally distinct from the overall Treg population in SLO [39–41]. Indeed, Tregs isolated from visceral adipose tissue (VAT) or from injured skeletal muscles express a distinct transcriptome, not only in comparison to Tregs isolated from SLOs, but also when compared to one another [39,40]. In particular, Tregs infiltrating VAT express high levels of the nuclear receptor peroxisome proliferator-activated receptor (PPAR)- γ , a central regulator of

adipocyte differentiation. Tregs lacking PPAR- γ expression failed to accumulate in VAT and lack expression of GATA3 and CD103 [40,42]. A recent study also demonstrated that, unlike Tregs in SLO or in other non-lymphoid tissues, nearly all VAT Tregs express high levels of the receptor for the alarmin IL-33 (ST2), which is necessary for their accumulation in VAT [36]. More interestingly, the absence of PPAR- γ in Tregs prevented the therapeutic normalization of glucose and systemic insulin metabolism in mice fed a high-fat diet. Paralleling these observations, injured skeletal muscle-infiltrating Tregs express high levels of the growth factor amphiregulin, which can directly act on satellite muscle cells to promote muscle fiber formation in vitro and muscle repair in vivo [39]. Treg depletion impairs muscle repair, while promoting Treg accumulation in these tissues has the opposite effect. However, whether amphiregulin expression by Tregs is essential for their muscle repair capacity has not been directly addressed. In support of this, amphiregulin is not specific to muscle Tregs and is also highly expressed by VAT-infiltrating Tregs [39].

Additional data further support the idea that distinct Tregs accumulate in different non-lymphoid tissues. A recent principal component analysis of the transcriptomes of Tregs found in various non-lymphoid tissues as well as in SLO, demonstrates tissue-dependent cluster segregation of Tregs. For example, all non-lymphoid tissue Tregs cluster separately from SLO Tregs. In addition, VAT and skeletal muscle Tregs had similar gene expression and clustered close to one another, but were separate from Tregs extracted from liver, kidney and skin (which clustered together). Additionally, analysis of CDR3 TCR sequences of VAT, and muscle Tregs reveals tissue-specific enrichment of Treg clones that are neither found within the SLO Treg population, nor within Tconv in these respective tissues [39]. Thus, Tregs infiltrating tissues may recognize tissue-specific antigens, which promotes their accumulation and retention in those sites. This is in line with the essential requirement for TCR signaling in the homeostasis of Tregs (discussed above; [12]). In addition, because TCR sequences from Tregs and Tconv differ in muscle and in VAT, it suggests that these Tregs are of thymic origin (tTreg) and not from conversion from Tconv (pTreg). In support of this, a recent report demonstrated that in mice, tissue-protective Tregs that are crucial for prevention of autoimmunity, are generated in the thymus perinatally (within 10 days), though they persist through adulthood [43]. These Tregs are distinct from Tregs generated subsequently, in that their generation requires AIRE expression and their TCRs appear to recognize peripheral tissue antigens with a high affinity.

Related to these observations, in a model where the expression of a surrogate self-antigen is specifically induced in skin, antigen-specific Tregs accumulated in skin and reduced the severity of autoimmunity. Moreover, Tregs that were maintained in skin provided enhanced protection against autoimmunity upon antigen re-expression [44]. These skin-resident Tregs were termed “memory Tregs” and a recent report by the same group shows that Tregs infiltrating human skin display a CD45RO memory phenotype [45].

Taken together, these studies show that non-lymphoid tissues recruit and retain unique Treg subpopulations that restrain local immune responses, and act to regulate tissue homeostasis and metabolism. Thus, in addition to their central role in controlling alloimmune responses in SLO and allografts, these data raise the intriguing possibility that Tregs may also contribute towards tissue repair and homeostasis. However, it remains unknown which Treg

subpopulations or antigen specificities are required for induction and maintenance of allograft tolerance. For example, the initial induction of tolerance and inhibition of naïve T cell priming in SLO and control of effector T cells in both SLO and allograft may be controlled by alloreactive Tregs, while the control of chronic rejection and the promotion of tissue repair could require the recruitment of self-reactive tissue-specific Tregs within the allograft. Answering these questions would generate strategies to improve Treg therapy.

Treg control of immunity in non-lymphoid tissues

Tregs utilize various mechanisms to suppress immunity in vitro and in vivo [46,47]. These include secretion or generation of inhibitory soluble factors (e.g. TGF β , IL-10, IL-35 and adenosine), engagement by inhibitory receptors (CTLA-4), direct killing of targets (through Granzyme A/B), or deprivation of IL-2 or the amino acid Tryptophan (through high IL-2R expression or induction of indoleamine 2,3-dioxygenase in DCs, respectively). These different mechanisms may be necessary to control various immune effector cell types and inflammatory settings, or may be a reflection of the specialization of Treg function in different anatomical sites. Given that inflammatory signals can affect both the stability of Foxp3 expression in Treg and possibly their function [1,48], identifying which mechanisms are essential for Treg suppressor function in different inflamed tissues is key to specifically targeting enhanced Treg function.

Tregs have been shown to directly suppress Teff in vitro. However, the use of intravital microscopy revealed that in vivo, Tregs in SLO inhibit effector T cells indirectly, through the modulation of DC function [49–51]. Specifically, after interacting with Tregs, DCs were unable to form stable interactions with or present antigen to naïve T cells. Parallel findings have recently been seen in tumors where infiltrating Tregs formed antigen-dependent short-tethering interactions with DCs, leading to a reduction in DC function and exhausted tumor-infiltrating cytotoxic T cells [52]. In addition, Treg function within tumors required antigen presentation by tumor-infiltrating DCs, demonstrating a central role for DCs in non-lymphoid tissues in promoting both immunity and tolerance. Similarly, the presence of Tregs in VAT and skeletal muscles correlates with a switch from pro-inflammatory to anti-inflammatory innate cells [39,40]. However, the nature of the inhibition of DCs by Tregs remains unclear. Moreover, whether Tregs have the same effect in more inflammatory settings, such as an allograft, is unclear.

A recent study attempted to address Treg function in islet allografts using intravital microscopy to examine transplanted islets in the anterior chamber of the eye [53]. In this setting, graft-infiltrating Tregs and Teff were mostly immobile and appeared to contact one another directly. However, it remains unclear whether these Tregs and Teff were also contacting the same DC, since both expressed the same transgenic TCR, and could be competing for the same MHC-II-antigen complexes on antigen-presenting cells (APCs). In this regard, both Teff and Tregs exhibited prolonged interactions with DCs detected in the periphery of the islets, and a large fraction of Treg and Teff contacted the same DC. Moreover, in our hands, intravital microscopy of allogeneic islets transplanted under the kidney capsule reveals that polyclonal Tregs and Teff are both highly motile and predominantly exhibit short-lived interactions with DCs, rather than with one another (G.C.

unpublished observations). The discrepancy in cellular dynamics and behavior between these studies may be explained by differences in the transplantation site (eye anterior chamber vs. kidney capsule), or by differences in TCR affinity and antigen-competition, between TCR-transgenic and polyclonal T cells.

Therapeutic manipulation of Tregs

Given the obvious role of Tregs in the induction and maintenance of transplantation tolerance, there is a great interest in therapeutic manipulation of Tregs. Here, we will concentrate on the recent developments in therapeutic manipulation of Tregs either through exogenous Treg therapy (i.e. ex vivo expansion and infusion of Tregs) or using therapeutic agents that directly promote Tregs in vivo.

Exogenous Treg therapy

The efficacy of Treg therapy in pre-clinical animal studies has been clearly demonstrated [54,55]. This has led to four published Phase I/II clinical trials in graft-vs-host disease and early onset of type-1 diabetes. These studies showed that Tregs therapy was safe. However, only minimal benefit was achieved [56–59]. This might be explained by the poor survival of the transferred Tregs in these patients. Indeed, elevated Treg fractions in peripheral blood were reported after Tregs transfer early on, but this was not sustained, and Treg proportions returned to baseline after 2 weeks [59]. Similarly, a recent study of Treg therapy in non-human primates demonstrated that infused Tregs were rapidly decreased within 5 days and almost undetectable in peripheral blood or in bone marrow by day 16. It is possible that constant TCR and IL-2R signaling required for Treg survival are not readily available to the majority of infused Tregs. In addition, the plasticity of adoptively transferred Tregs remains a potential issue, and this study reports a dramatic loss of Foxp3 expression in the transferred cells [60]. However, recent work in humans by investigators at UCSF using deuterium-labeled ex vivo expanded Tregs indicate that 320 million infused Tregs peak at ~5% of the overall Treg population in peripheral blood. These transferred Tregs retain Foxp3 expression and could be detected for at least 30 days, demonstrating that prolonged maintenance of stable ex-vivo expanded Tregs is achievable (Tang Q, personal communication).

Two other Phase I/II trials using exogenous Treg therapy registered in www.clinicaltrials.gov (NCT02244801 and NCT02188719) in liver and in kidney transplantation, respectively, distinguish themselves due to the use of donor-alloantigen-reactive Tregs (as opposed to polyclonal amplification of Tregs). The ex vivo expansion of large numbers of donor-alloantigen-reactive Tregs can be achieved by direct alloantigen recognition provided by donor B cells [61]. Although this group demonstrated that infusion of directly alloantigen-reactive Tregs can prevent allograft rejection of islets in mice [62], previous reports suggest that Treg therapy with a mixture of directly and indirectly alloantigen-reactive Tregs is more effective [54,63]. Directly alloantigen-reactive Tregs may encounter donor-derived APCs early on, however, these APCs are rapidly lost [64–67] and can no longer drive the alloimmune response or provide the requisite TCR-signals to sustain Tregs. Thus, after infusion of directly alloantigen-reactive Tregs in mice, the majority of Tregs infiltrating allografts on days 4–6 are of exogenous origin. However, by day 14, these

exogenous Tregs are nearly absent and are replaced by endogenous Tregs [62]. Taken together, this suggests that direct alloantigen-reactive Tregs may contribute to control of early rejection, but that ongoing control of the immune response is provided by Tregs recognizing indirect allo- or self-antigens.

Augmenting Treg number and function in vivo

Enhancing endogenous Treg numbers and function through the use of therapeutic agents promises logistical advantages over exogenous Treg therapy in terms of time, cost and effort. In mice, various tolerance-inducing reagents ultimately result in development of Tregs that are important for maintaining tolerance. However, in many instances such tolerogenic agents appear to control the acute effector response (through potent inhibition or depletion of Teff), and provide the time necessary for allo-responsive Tregs to ultimately expand due to their relative sparing and/or faster homeostatic expansion [68]. On the other hand, development of agents that specifically and directly augment Treg numbers or function in vivo has been a challenge.

IL-2 has been shown to expand Tregs in vivo. Pre-incubation of IL-2 with certain anti-IL-2 mAbs (forming IL-2/anti-IL-2 complexes; IL-2c) can prolong serum half-life and binding to the high-affinity IL-2R [69,70]. IL-2c treatment of mice only results in transient expansion of endogenous Tregs in SLO. On the other hand, IL-2c therapy leads to a sustained increase of Tregs in non-lymphoid tissues, and promotes their function at those sites [39,41,69,70]. Such Tregs demonstrated a phenotype similar to effector Tregs, except that they expressed high levels of CD25 (IL-2Ra)[69]. Moreover, treatment of mice with IL-2c prior to islet cell transplantation induced long-term survival. Interestingly, IL-2c treatment appears to enhance Treg suppressor function within the allograft, rather than in SLO [69]. Thus, IL-2c may promote the accumulation of tissue-resident Tregs. On the other hand, the efficacy of IL-2c therapy during (rather than before) an acute immune response is unclear, because Teff upregulate CD25 and will also respond to the cytokine [71].

The mechanisms underlying tolerance induction by another potent therapeutic agent, anti-CD45RB, have been recently reported by our group. Tolerance induced by anti-CD45RB treatment of mice is donor-specific and depends on the presence of Tregs [72–74]. We showed that anti-CD45RB treatment acutely increases Treg numbers in SLO by augmenting integrin-dependent Treg-DC interactions, which leads to an amplification of antigen-dependent Treg proliferation [75]. While anti-CD45RB expands Tregs to exogenous antigen, it also promotes homeostatic expansion of Tregs (to self, or possibly gut-derived antigens). In contrast, anti-CD45RB has no effect on the proliferation or the interactions of Tconv with DCs. This suggests that anti-CD45RB capitalizes on differences in TCR-mediated stop-signaling between Tregs and Tconv during their initial interactions with DCs. Such differences have been noted in response to CTLA-4 ligation [76]. The biochemical mechanisms of anti-CD45RB-mediated regulation of LFA-adhesiveness in Tregs remain to be elucidated.

Taken together, these studies demonstrate that Tregs can be directly and specifically targeted in vivo for the expansion of antigen-specific Tregs in SLO (through CD45 ligation) or accumulation in non-lymphoid tissues (through IL-2c). These reagents could be combined

with other therapeutics that specifically block effector/memory T cell responses, or with exogenous Treg therapy to promote their survival and expansion. The degree and duration of Treg expansion in all Treg-based therapies will need to be tailored to avoid infection and malignancy. Further understanding of the regulation of Treg numbers in the tissues and SLO will provide new insight into maintaining optimal immune balance.

Conclusions

Tregs respond to environmental cues during immune responses resulting in their differentiation into subsets exhibiting additional functional capacity and tissue localization. Recent studies demonstrate a crucial role for Tregs within non-lymphoid tissues regulating both immune and non-immune processes. The former may be key in the allograft setting, where heterologous immunity gives rise to memory T cells that can act directly within the transplanted tissue. Moreover, Tregs within the allograft may also promote tissue homeostasis and repair. Improved understanding in Treg biology should aid the development of new therapeutic approaches that significantly promote allograft survival by augmenting Treg function in both SLO and within the allograft itself.

Acknowledgments

We apologize to our colleagues for the many articles that could not be quoted because of space limitations. We thank our lab members for constructive discussions on this topic.

Financial support and sponsorship

This work is supported by a NIH grant (AI097361) to D.M.R.

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Key Points

- Treg adaption to various antigens and environmental cues leads to specialization of Treg function
- Specialization of Treg function is accomplished by distinct Treg subsets
- Treg function includes the control of immune and non-immune processes, such as tissue homeostasis and tissue repair
- Therapeutic approaches can specifically and directly target Treg function in vivo

Figure 1. Diversity of Treg subsets and specialization of function

Thymic-derived central Tregs recirculate through secondary lymphoid organs. Upon TCR and CD28 signaling, and expression of the transcription factors IRF4 and Blimp-1, central Tregs differentiate into effector Tregs. In response to inflammatory signals, effector Tregs adopt additional transcription factors that provide the necessary means to suppress specific subsets of effector helper T cells (red lines). This also allows effector Tregs to gain expression of specific chemokine receptors that promote their migration to the targeted tissues. In non-lymphoid tissues, effector and tissue-resident Tregs suppress inflammation and immunity, and promote tissues homeostasis and repair. The origin of tissue-resident Tregs is uncertain (indicated by dashed lines and question marks), but display an effector Treg phenotype.