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HIGHLIGHTS

Basic

REVIEW

Foxp3$^+$ regulatory T cells in the central nervous system and other nonlymphoid tissues

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Foxp3$^+$ regulatory T (Treg) cells are indispensable for the maintenance of immunologic self-tolerance as well as for the confinement of autoimmune inflammation after the breach of self-tolerance. In order to fulfill these tasks, Treg cells operate in secondary lymphoid tissues and nonlymphoid tissues. The conditions for Treg cell stability and for their modes of action are different according to their compartment of residence. In addition, Treg cells initiate residency programs to inhabit niches in nonlympoid tissues (NLT) in steady state and after re-establishment of previously deflected homeostasis for extended periods of time. These NLT Treg cells are different from lymphoid tissue residing Treg cells and are functionally specialized to subserve not only immune functions but support intrinsic functions of their tissue of residence. This review will highlight current ideas about the functional specialization of NLT Treg cells in particular in the central nervous system (CNS) and discuss challenges that we are facing in an effort to exploit the power of NLT Treg cells for maintenance of tissue homeostasis and perhaps also tissue regeneration.

Keywords: central nervous system · Foxp3$^+$ Treg cell · heterogeneity · residency · stability

Introduction

Foxp3$^+$ regulatory T (Treg) cells are an integral constituent of any adaptive immune response. Whenever a T-cell response is started, conventional T cells provide IL-2 to transiently facilitate their own expansion but also to concomitantly expand and harness the function of Treg cells to control the conventional T-cell response [1]. A pethora of excellent reviews on the transcriptional make-up and function of Treg cells has been published since the discovery of their master transcription factor Foxp3 and its fundamental downstream targets in Treg cells including IL-2Rα and CTLA-4 [2, 3]. Due to the skewing of the TCR repertoire of Treg cells toward autoantigens [4], the functional deficiency of Foxp3 (e.g., due to mutations) results in multiorgan autoimmunity in mice (*scurfy* phenotype) and men (Immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) syndrome) [5–7]. Moreover, depletion of Treg cells in adult individuals recapitulates the *scurfy* phenotype and results in the death of the animals within 3 weeks [8]. While *scurfy* mice suffer from massive lymphoproliferation and multiorgan autoimmunity, the CNS is not primarily affected by the systemic inflammatory response—most likely due to the fact that neuroantigen reactive precursors in the conventional T-cell repertoire are exceedingly rare. When this precursor frequency is genetically increased, Treg cell depletion almost immediately results in spontaneous encephalomyelitis [9]. Treg cells have a fundamental role in controlling neuroantigen-specific conventional T cells both in the systemic immune compartment but also within the CNS even though a strong inflammatory response in the target tissue is able to mask their suppressive capacity [10].

Treg cells are also believed to be crucial for maintaining T-cell tolerance against neuroantigens in humans. Patients with MS

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have functional deficits in their Treg cell compartment. Moreover, an antibody to IL-2Rα that had been given to MS patients with the aim to expand regulatory NK cells also deprived patients of Foxp3$^+$ Treg cells, resulting in detrimental cases of encephalitis and other autoimmune disorders [11, 12]. These events led to the immediate retraction of this compound from the market, teaching us the lesson that it is never a great idea to neglect fundamental principles of vertebrate immune regulation when selecting potential drug targets for clinical use in humans.

During the last decade, it has become clear that Treg cells are not a monolithic population of regulators. It was shown that effector T cells committed to a certain Th cell subset might require a specifically biased subset of Foxp3$^+$ Treg cells to control them. An appealing concept suggests that the transcriptional make-up of Treg cells needs to mirror in part the key transcriptional modules of the effector T-cell response. For instance, in order to be appropriately controlled, Th1 responses might require Tbet$^+$ Treg cells, Th2 response might need Treg cells expressing Irf4, and Th17 cells Treg cells with a prominent activation of the STAT3 pathway [13–15].

Heterogeneity of Treg cells might even be more profound as far as Treg cells that reside in nonlymphoid tissue (NLT) niches are concernced [16]. Here, Treg cell heterogeneity likely reflects a functional specialization of Treg cells in distinct microenvironments and might be associated with noncanonical tasks of NLT-residing Treg cells [17]. In this review, I will discuss features of tissue imprinting of Treg cells as a determinant of their heterogenity and functional specialization with a particular focus on Treg cells in the CNS.

Tissue residing Treg cells

Some Treg cells reside in NLT environments for extended periods of time even though a certain turn-over with the systemic immune compartment might still occur [18]. Based on analyses of visceral adipose tissue, muscle, and lung tissue, an NLT Treg cell signature has been defined, comprising a set of specific sensors like the insulin receptor, IL-33R, effectors like amphiregulin, and transcription factors including PPAR-γ, Batf (also known as Batf1), Prdm1 (which encodes Blimp1), and Gata3 [19–22]. It has been a fundamental question in the field whether tissue Treg cells constitute a universal Treg cell subset that would be similar irrespective of the specific niche or organ system in which it resides, or whether each NLT would host their private tissue Treg cells. This question has neither been resolved as to the TCR repertoire of Treg cells in various tissues nor to the transcriptome and functional phenotype of NLT Treg cells: Even though a substantial overlap has been postulated, the TCR repertoire of Treg cells is already different depending on whether they reside in axillary, inguinal, cervical, or mesenteric lymph nodes [23] and may facilitate a preferential recruitment of antigen specific Treg cells into certain NLT niches [24]. Similarly, an NLT Treg cell "core" transcriptome has been proposed [21] while at the same time, niche-specific features of NLT Treg cells have been described [25–27].

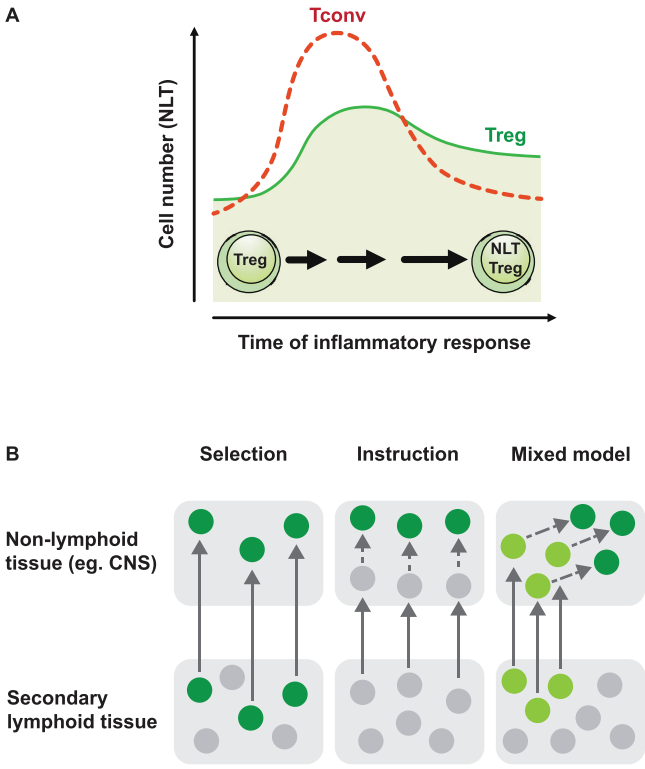

Figure 1. Dynamics of Treg cells in the CNS. In steady state, the CNS harbors only few Treg cells. After an inflammatory perturbation, the Treg cell compartment in the CNS increases (**A**) and Treg cells persist in the the CNS longer than conventional T cells. A subset of these Treg cells may become tissue resident (NLT Treg cells). (**B**) In principle, the NLT Treg cell pool (in any tissue) may be built by differential recruitment of precursors from the systemic compartment (selection), in situ imprinting of specific properties in generic Treg cell recruits (instruction), or a mixture of these possibilities.

A second key question concerning NLT Treg cells refers to their development and dynamics (Fig. 1). Perhaps except for mucosa associated Treg cells (in the lamina propria and mucosa associated secondary lymphoid tissue), NLT Treg cells are thymus-derived Treg cells. However, it is not clear whether their tissue specific profile is the result of a differential recruitment of pre-existing NLT precursor Treg cells from the secondary lymphoid tissue [28] (e.g., via CCR2 [26]) or whether it is due to local imprinting processes in situ. A two-step model of pre-committed Nfil3$^+$ Treg cell precursors prone to migrate to NLTs where they then differentiate into Nfil3$^+$Klrg1$^+$ NLT Treg cells would integrate these two ideas [21, 29]. The Treg cell residency programs in various tissues may be largely overlapping. However, distinct features of NLT Treg cells might still exist depending on the respective tissue niche.

Tissular immune functions of Treg cells

Immune regulation by Treg cells is not limited to secondary lymphoid tissues. In fact, Treg cells are abundantly found in inflamed NLTs (including the CNS) as well as in tumor microenvironments

[30, 31]. Due to the lack of appropriate models to delete Treg cells only from NLT without touching the systemic compartment, it has not been formally proven that Treg cells exert a crucial immune function in NLTs. However, since the ratio of Treg cells versus conventional T cells in NLT increases toward the resolution of an inflammatory episode [10] and manipulation of NLT Treg-cell-specific transcriptional programs (that are not active in systemic Treg cells) unleashes uncontrolled inflammation in the target tissue [32], NLT Treg cells likely exert a crucial immune modulatory function in situ. In line with this concept, destabilization of the Treg cell functional phenotype specifically in the tumor bed (but not systemically) facilitates anti-tumor responses [33].

Since the signature of NLT Treg cells has been defined for some tissues, loss of function perturbations were performed to interrogate NLT-specific molecules expressed in Treg cells for their functional relevance as to the control of immunopathology during inflammatory episodes. Klrg1 is expressed in NLT Treg cells but not in secondary lymphoid tissue Treg cells. Since Klrg1 is a target of the transcriptional regulator Blimp1, Blimp1 was hypothesized to support the tissue residency program in Treg cells [22], as had been suggested for other resident immune cells as well [34]. When Blimp1 is ablated in Treg cells, NLT Treg cells are still generated but are unable to secrete IL-10, gradually lose expression of Foxp3, and initiate a proinflammatory program with the production of IFN-γ and IL-17 [32]. Moreover, Blimp1 is an efficient suppressor of IL-23R [35]. Therefore, Blimp1 is an essential transcription factor to maintain the functional phenotype of NLT Treg cells in an inflammatory environment. Due to the loss of their suppressive capacity, autoimmune inflammation cannot be controlled in experimental animals with Treg cell conditional Blimp1 deficiency. While some of the disregulated genes in Blimp1-deficient Treg cells are directly trans-activated (or repressed) by Blimp1, others (including Foxp3) are only indirectly regulated by Blimp1 [32, 36].

The immune function of NLT Treg cells is beneficial in autoimmunity and detrimental in the case of malignant tumors. In infectious disease settings, the presence of Treg cells in the CNS inhibits short lived effector cells responses, which may slow down sterile immunity but prevent overshooting immunopathology, and at the same time promote the development of tissue resident memory T cells [37]. In addition, Treg cell responses in the context of viral infections in the CNS likely suppress co-evolving autoimmune responses in a bystander fashion [38].

In summary, NLT Treg cells exert immune functions in situ under inflammatory conditions. Their transcriptional outfit is suited to preserve their functional phentype and allows for efficient local immune regulation with beneficial effects in chronic inflammation and perhaps infection but with rather detrimental outcomes in tumor immunity.

Tissular nonimmune functions of Treg cells

It is an emerging concept that NLT Treg cells form a "functional unit" with their host niche. Here, NLT Treg cells control autonomous functions of certain tissues. For instance, Treg cells are responsible for the maintenance of insulin susceptibility in visceral adipose tissue, they facilitate tissue regeneration in lung and muscle, as well as the regeneration of hair follicles in the skin [20, 25, 39, 40]. In the CNS, the number of Treg cells is very low in steady state. In steady state, the median dwell time of $CD69^+$ conventional T cells as well as of $CD69^+$ Treg cells is in the range of eight weeks [18]. However, steady-state Treg cells acquire a tissue resident phenotype faster than their conventional counterparts, and in contrast to conventional T cells, they show signs of TCR engagement in the CNS [18] also under homeostatic conditions. Conversely, steady-state conventional T cells need to be activated in the systemic compartment to enter the CNS but then are largely ignorant for CNS antigens in situ. It has been suggested that interaction with $CD4^+$ T cells is required for the maturation of microglial cells to their adult phenotype. It will be important to understand whether Treg cells (but perhaps not conventional T cells) need to cognately interact with microglia in order to support the developmental trajectory of microglia in newborns.

Under conditions of "perturbed homeostasis," the Treg cell population in the CNS increases dramatically. Even after re-establishment of homeostasis, a substantial fraction of Treg cells persists in the CNS for at least several weeks. Depending on the nature of the perturbation, CNS Treg cells were shown to fulfill nonimmune functions in the CNS during re-establishment of homeostasis, for example, prevention of astrogliosis after stroke by amphiregulin-mediated inhibition of IL-6/STAT3 signaling into astrocytes [27] or remyelination after toxic demyelination by secretion of the growth regulatory protein CCN3 [41]. Conversely, in a model of Alzheimer's disease, Treg cells might impair appropriate β-amyloid plaque clearance by blocking the choroid plexus as an entry gateway of immune cells [42] (Fig. 2).

In summary, besides their immunoregulatory function, Treg cells switch on residency programs to reside in the CNS for extended periods of time after perturbations and build a niche, in which they might exert noncanonical functions that are connected to re-establishment of tissue structure and function. That has been shown for a variety of peripheral tissues but might also be the case for the CNS. It will be important to determine how "deeply" Treg cells become integrated into NLTs. In fact, tissue residency of Treg cells might be different for various tissues, for example, while visceral adipose tissue harbors Treg cells with almost no exchange with the systemic compartment, this might be different for the CNS.

Determinants of NLT Treg cell stability

From a systems point of view, it has been a long standing question in the field whether the stability of NLT Treg cells is maintained by feedforward or feedback loops and what molecular pathways constitute these loops. A feedforward concept would posit that molecules that are used as effector molecules of Treg cells would also be required to induce and maintain their regulatory phenotype. For instance, by sensing IL-10, Treg cells would be licensed

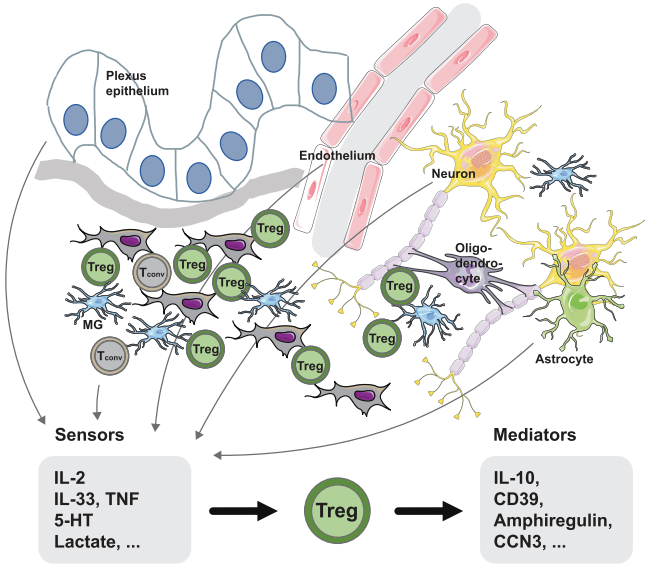

Figure 2. Integration of Treg cells into distinct CNS niches. In the CNS, resident Treg cells (e.g., after resolution of an inflammatory episode) may reside in distinct anatomical niches. Here, they might sense cues (and metabolites) provided by other immune cells (conventional T cells, microglial cells, MG) but also by CNS intrinsic cells including neurons, astrocytes, or oligodendrocytes to shape their functional phenotype. In contrast to Treg cells in the systemic immune compartment, CNS Treg cells might exhibit specialized functions in terms of the re-establishment and maintenance of the structural and functional integrity of CNS intrinsic cells.

to produce more IL-10 to then re-inforce the production of more downregulatory molecules by their target cells [43]. In contrast, in a feedback system, Treg cells would have to sense molecules whose production they inhibit. For example, IFN-γ produced by conventional T cells might promote Treg cells to efficiently produce IL-10, which in turn would suppress the production of IFN-γ by the target cells of the Treg cell response. Here, STAT1-deficient Treg cells fail to express sufficient levels of Blimp1, which is a major determinant of Treg cell stability in the CNS [32]. Apparently, only a feedback system would place Treg cells in the position of key regulators of a perturbed environment while under conditions of homeostasis, a feedforward system might be useful to increase the resilience to perturbations. While a plethora of pathways (TCR trigger, cytokine cues, and metabolic fluxes) were shown to be associated with a stable Treg cell phenotype as assessed by high expression of Foxp3 [16], a fundamental decision as to whether feedforward or feedback loops are essential for maintaining Treg cell stability in NLT has not been made and is perhaps not possible since varying degrees of deflection from homeostasis of a given niche might afford different regulatory loops, very similar to what has been proposed in terms of different modes of suppression by Treg cells under noninflammatory and inflammatory conditions [44].

Treg cells need a TCR trigger for their maintenance in lymphoid tissue [45, 46], and a variety of observations support the idea that the TCR of Treg cells is also triggered in NLT both in steady state and under inflammatory conditions [10, 18, 47, 48]. Moreover, synthetic (ectopic) production of IL-2 in the CNS is able to expand CNS Treg cells [49]. However, the major natural source of IL-2, that is, conventional T cells, are not available in sufficient numbers in the CNS in steady state or after re-establishment of homeostasis when the conventional T-cell population has contracted while niches of CNS resident Treg cells still exist.

NLT Treg cells express IL-33R (ST2), which together with IL-1RAP forms the functional IL-33 receptor and is linked to the MyD88/NFκB signaling pathway. It has been speculated that NLT Treg cells need to sense IL-33 in order to expand and maintain their functional phenotype in NLTs including the CNS [27, 47, 50-53]. However, more recently it was suggested that the residency of Treg cells in distinct NLTs appeared to be differentially dependent on IL-33 signaling, that is, more dependent in the lung than in the skin [54]. Together, these observations support the idea that NLT Treg cells may no longer (exclusively) rely on IL-2 as the classic Treg cell "fuel" but use other maintenance factors as compared to their lymphoid tissue counterparts. In fact, NFκB (RelA)-associated signaling is essential for NLT Treg cells [55]. Consistent with this idea, TNF signaling is important to promote the immune function of Treg cells in NLT: Loss of TNFR2 signaling into Treg cells results in exacerbated EAE [56, 57], and loss of TNFR2 signaling directly reduces Blimp1 expression in CNS Treg cells but not LN residing Treg cells [57].

Metabolically, the CNS might be a favorable niche for Treg cells since astrocytes are a sink for glucose and in turn provide lactate to nurture neurons [58]. The limited availability of glucose may actually be stabilizing Treg cells since enforced glycolysis is inhibitory to Treg cell function [59], and conversely, Foxp3 promotes a metabolic outfit of Treg cells to benefit from high lactate concentrations [60]. After uptake into the cell, Treg cells oxidize lactate to pyruvate which can be channeled into the tricarboxylic acid cycle to fuel oxidative phosphorylation. Whether the ability of Treg cells (but not effector T cells) to take up short chain fatty acids [61] also confers a "metabolic" advantage to Treg cells in the CNS has not been determined.

NLT Treg cell metabolic signaling is characterized by low activity of the Akt pathway, that is, Foxo1 needs to be present in the nucleus and transactivate Foxp3. Loss of Foxo1 results in a "toxic" gain of function of Treg cells, which then start producing IFN-γ [62]. In general, the Akt-mTOR pathway is considered as a negative regulator of Treg cell generation [63]. On the other hand, the mTOR complex 1 (mTORC1), which is also downstream of Akt needs to be active (at low to intermediate levels) for appropriate Treg cell function since Treg cell conditional ablation of Raptor, associated with mTORC1, leads to loss of de novo Treg cell cholesterol synthesis, Treg cell function, and generalized autoimmunity [64].

Although it is likely that metabolic pathways contribute to the stability of CNS Treg cells, in particular in a postlesional situation (after stroke or inflammation), few of the known metabolic controllers of Treg cells (e.g., the Akt-mTOR pathway) have specifically been investigated in CNS Treg cells.

Conclusion and outlook: specific targeting of NLT Treg cells

After the molecular definition of Treg cells at the beginning of the new century, the therapeutic exploitation of these cells faced a number of difficulties: Ex vivo expansion, stability, and specificity of Treg cells have been a major focus in these efforts. The motivation to overcome these issues was driven by the idea to generate a large number of highly efficient (i.e., suppressive) Treg cells that would be available "off the shelf" for universal application in autoimmunity or organ transplantation [65, 66]. However, the functional diversity of Treg cells according to the tissue niche and organ system they reside in has only recently been increasingly appreciated. For example, the integration of Treg cells into tissues results in the establishment of functional units, in which parenchymal cells and Treg cells cannot be conceived as disconnected constituents. Even developmental programs in parenchymal cells and resident immune cells (such as microglia in the CNS) require the interaction with T cells and Treg cells.

In particular, regeneration and re-establishment of appropriate function in a distinct tissue might crucially rely on the presence of specialized Treg cells. In order to specifically target them, we need to understand how these specialized Treg cells develop, how they turn over or self-renew and how they talk to their niche. Targeted approaches to manipulate NLT Treg cells without touching the systemic Treg cell compartment are possible in experimental settings [49]. The refinement of these interventions, their safety, and scalability are major challenges that we need to tackle in order to exploit the power of NLT Treg cells for regenerative and sustainable medicine. While most of these new insights are being obtained in mouse models, the fundamental biology of mouse and human Treg cells appears to be largely conserved [67]. Nevertheless, it will be important to confirm key functional features of murine NLT Treg cells in humans before considering to exploit their therapeutic potential.

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Abbreviation: **NLT:** nonlympoid tissues

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