CNS T_{reg} cells - new functions, conventional fuel

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Ectopic expression of IL-2 in the central nervous system (CNS) is sufficient to create an organ-restricted niche for tissue-resident regulatory T cells that support re-establishment of homoestasis after multiple types of tissue injury in the CNS.

Foxp3$^+$ regulatory T (T_{reg}) cells are prone to apoptosis unless they reside in a milieu rich in interleukin 2 (IL-2)[1]. In the systemic immune compartment, conventional T (T_{conv}) cells are the major providers of IL-2, which T_{reg} cells cannot produce themselves. However, T_{reg} cells are also found to reside in non-lymphoid tissues where they control adaptive immune responses in a low-grade inflammatory environment but might also subserve "homeostatic" functions for tissue integrity and regeneration [2, 3]. Since T_{conv} cells may not be available as sources of IL-2 in that scenario, it has been a key question which factors shape functional niches for the maintenance of T_{reg} cells in non-lymphoid tissues such as the CNS. In the current study, Liston and colleagues show that IL-2 is sufficient to increase the local pool of T_{reg} cells in the CNS [4]. In a series of either genetically encoded systems or by adeno-associated viral (AAV)-vector mediated expression systems, CNS-intrinsic cells (e.g. neurons or astrocytes) were inducibly forced to locally produce IL-2 ("behind the blood-brain-barrier"), which led to an enlargement of the T_{reg} cell population in the brain (but not the systemic compartment) with beneficial effects in traumatic brain injury, stroke, and autoimmunity [4]. Notably, microglia expressed higher levels of major histocompatibility complex (MHC) class II molecules and PD-L1, suggesting that a positive-feedback loop between microglia and T_{reg} cells involving cognate and co-stimulatory signals might eventually result in the creation of an expanded CNS T_{reg} niche as long as IL-2 is locally expressed in the brain.

The significance of tissue-resident immune cells is increasingly recognized not only in terms of the immune competence of non-lymphoid tissues but also as to their structural integrity and homeostatic function. A tissue-specific functional specialization has been described for T_{reg} cells in particular in adipose tissue and muscle - but perhaps also in the CNS [5]. Even though the number of T_{reg} cells in the naive CNS is very low, CNS-resident T_{reg} cells form a sizeable population under infection-, autoimmune-, or damage-induced inflammatory conditions. As has been illustrated for visceral adipose tissue, CNS T_{reg} cells exhibit a CNS-specific transcriptional profile that is distinct from the transcriptome of lymphoid system-residing T_{reg} cells and T_{reg} cells in other anatomical sites [6]. Yet, some key features of T_{reg} cells, such as high expression of CD25, are also present in CNS T_{reg} cells. While the prerequisites for the maintenance of T_{reg} cells in lymphoid tissues have been worked out to some detail, it is unclear which are the growth and maintenance factors and their cellular sources in non-lymphoid tissues. In fact, it has been questioned whether T_{reg} cells in the CNS depend upon IL-2 since the concentration of IL-2 in the CNS is low and that the CNS is usually devoid of T_{conv} cells as the prime producers of this cytokine.

Since in their present study [4] Liston and colleagues do not introduce a CNS-conditional loss-of-function system for IL-2, they cannot make the claim that IL-2 is necessary for the maintenance of CNS T_{reg} cells. Yet, they provide clear evidence that synthetic IL-2 is sufficient to maintain and increase the pool of T_{reg} cells in the CNS.

Tissue-resident T_{reg} cells are diverse and adapted to their niches. In ongoing analyses, tissue specific signatures of T_{reg} cells are being identified. Presently, a "core signature" of tissue T_{reg} cells comprises the phenotypic markers KLRG1, ST2, CD103 and the transcription factor Blimp-

1 [7]. This signature is overlapping with a signature of effector T_{reg} cells that has previously been proposed [8]. A commonality between the effector T_{reg} and tissue-resident T_{reg} cell signatures is a strong signalling bias towards the NF-κB pathway, and it has been hypothesized that this signaling bias is linked to the maintenance of T_{reg} cells in non-lymphoid tissues [9]. From these findings, two key areas of investigation have evolved in order to understand the biology of tissue T_{reg} cells and perhaps exploit their functions for interventional strategies in disease. First, the field has wondered whether the pool of tissue-resident T_{reg} cells in a specific organ is formed by selective recruitment of precursor T_{reg} cells that can already be found in the systemic immune compartment or whether a distinct functional phenotype is instructed in local T_{reg} cells by the specific milieu in a given anatomical niche [10]. Second (and related to previous problem), the tissue-specific signals that shape and support T_{reg} cells in non-lymphoid tissues need to be defined - and this refers to both the TCR specificity of tissue-resident Treg cells, i.e. the cognate signals that they receive, and the differentiation and growth factors that they might sense in a distinct organ. We are far from having the answers to these fundamental questions. However, the present study provides compelling evidence that IL-2 can be used to expand T_{reg} cells not only in secondary lymphoid tissue but also in non-lymphoid tissue, including the CNS.

The report by Liston and colleagues [4] is technically very strong and provides a series of ideas to translate the concept of tissue-restricted IL-2 expression into a clinical setting with the aim to increase the niche size for tissue-resident T_{reg} cells at defined anatomical sites. This idea builds on the understanding that it might be a better strategy to expand established (functionally specialized) tissue-resident T_{reg} cell populations with their complex properties, rather than to adoptively transfer "generic" T_{reg} cells with all the issues of stability and tissue adaptation

including the potential of aberrant adaptation. In addition, in recent years we have learnt quite a bit about interfering with the IL-2 system in therapeutic interventions. IL-2 is already used as a therapeutic agent in metastatic melanoma. Here, systemic application of IL-2 is meant to harness effector T cell responses against tumor cells. However, the systemic application of high-dose IL-2 in cancer therapy has been problematic due to immediate toxicities (mostly related to vascular leakage syndrome) but also due to systemic expansion of $ICOS^{high}$ T_{reg} cells [11], which is an unwanted event in tumor therapy. Conversely, a blocking monoclonal antibody to the IL-2Rα (daclizumab) that was applied in Multiple Sclerosis (MS) was associated with loss of T_{reg} cell number and function [12]. In some cases, the application of daclizumab resulted in hyperinflammatory syndromes including severe encephalitis [13] that led to the withdrawal of this drug for MS. Together these data suggest that targeting IL-2 in humans unfolds some of its key effects through modulation of $Foxp3^+$ T_{reg} cells. In order to refine IL-2 directed interventional approaches and to exploit them for organ-restricted inflammatory and degenerative diseases, the present study will be instrumental because it proves that locally and temporally restricted administration of IL-2 that has a short half-life and only acts over short distances is sufficient to support a preexisting pool of tissue-resident T_{reg} cells. Therefore, the concept of expanding an already established tissue-adapted T_{reg} cell population via the IL-2 system without modulating the systemic T_{reg} cell pool is promising and bears a realistic translational potential (**Figure 1**).

T_{reg} cells are a rare cell population in the naive CNS, but are established in the CNS for extended periods of time after trauma, vasular tissue damage, or an inflammatory assault. While these CNS T_{reg} cells might constitute a cellular checkpoint of tissue recovery in the CNS, more homeostatic functions of CNS T_{reg} cells have been proposed. Since the molecular unraveling of

these functions has only started, an alternative strategy to exploit this T_{reg} cell potential for next generation immune interventions builds on the idea to appropriately size the niche of these tissue adapted T_{reg} cells by interfering with crucial tissue-specific growth or maintenance factors. It has been proposed that tumor necrosis factor (TNF) might be a factor that specifically supports the functional phenotype of CNS T_{reg} cells [14]. However, the best known growth factor for T_{reg} cells (IL-2) might also do the job in controling the size of tissue T_{reg} cell populations. Nevertheless, we need to explore alternative fuels of tissue-resident T_{reg} cells (perhaps TNF or IL-33), and novel technologies that allow for single-cell analysis in the spatial context of distinct anatomical niches may help to identify cellular interaction partners of tissue-resident T_{reg} cells and inform us on new and perhaps short-ranging growth and maintenance factors of T_{reg} cells in non-lymphoid tissues. However, in the meantime, tissue conditioning to enhance resilience to recurrent injury and perhaps to promote regeneration of the CNS by T_{reg} cells might as well be facilitated by providing their conventional fuel.

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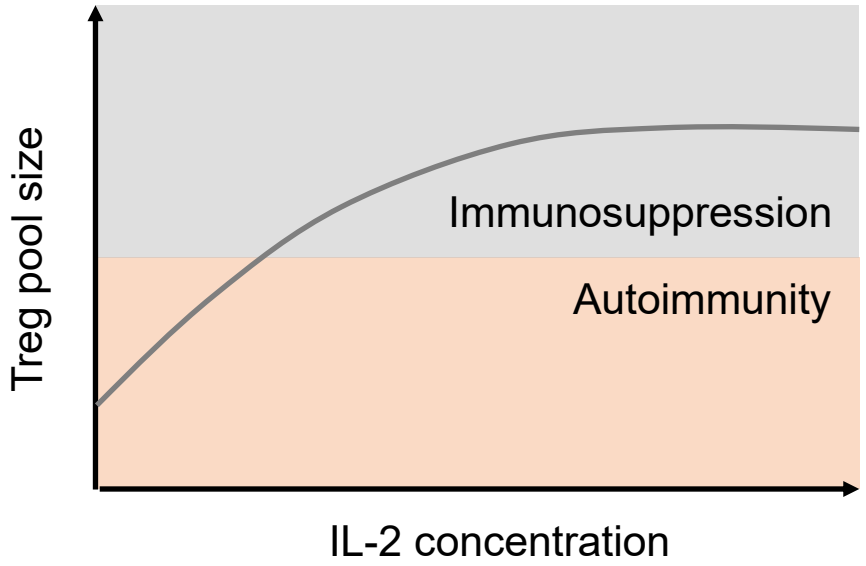
Systemic lymphoid tissue



Non-lymphoid tissue niche