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Commentary

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The unwavering commitment of regulatory T cells in the suppression of autoimmune encephalomyelitis: Another aspect of immune privilege in the CNS

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FoxP3$^+$ regulatory T (Treg) cells accumulate in the central nervous system (CNS) during experimental autoimmune encephalomyelitis and have been shown to limit the extent of neuroinflammation and to facilitate clinical recovery. The recent demonstration that Treg cells lose FoxP3 expression and assume effector cell characteristics upon stimulation with proinflammatory cytokines has raised questions about their stability in the inflamed CNS. In this issue of the European Journal of Immunology, O'Connor et al. [Eur. J. Immunol. 2012. 42: 1164–1173] show that CNS-infiltrating Treg cells maintain their suppressor phenotype by downregulating the IL-6 receptor. This commentary discusses the finding particularly with relevance to therapy of multiple sclerosis.

Keywords: Autoimmunity • Experimental autoimmune encephalomyelitis • Interleukin-6 • Neuroimmunology • Regulatory T cells

See accompanying article by O'Connor et al.

The central nervous system (CNS) microenvironment is not conducive to the generation of immune responses, a characteristic that is likely the consequence of evolutionary adaptation. The brain and spinal cord are especially vulnerable to collateral damage from inflammatory insults due to the poor regenerative capacities of neurons and axons. This is illustrated by the pathological landmarks of multiple sclerosis (MS), in which progressive neurodegeneration is driven by perivascular inflammatory infiltrates [1, 2]. The immune privileged status of the CNS is based, in large part, on the lack of an "infrastructure" to support lymphocyte recruitment and activation. Hence, it is one of the few tissues bereft of lymphatic vessels. Cerebrovascular endothelial cells express relatively low levels of adhesion molecules and resident myeloid cells express low levels of MHC class II and costimulatory molecules during homeostasis [3]. In the current issue of the *European Journal of Immunology*, O'Connor et al. [4] demonstrate that the CNS microenvironment also dampens inflammation by bolstering the stability of infiltrating FoxP3$^+$ CD4$^+$ regulatory T (Treg) cells. Specifically, they found that Treg cells isolated from the CNS, but not from peripheral lymphoid tissues, of mice with experimental autoimmune encephalomyelitis (EAE), retain their suppressor phenotype and resist conversion to IL-17-producing cells in response to the proinflammatory cytokine IL-6.

FoxP3$^+$CD25$^+$CD4$^+$ Treg cells accumulate in the brain and spinal cord during EAE, reaching their highest frequency coincident with clinical remission [5]. Several groups have found that Treg cells isolated from the inflamed CNS are more potent suppressors of conventional T-cell proliferation than Treg cells isolated from peripheral lymphoid tissues [6, 7]. The role of Treg cells in modulating encephalitogenic T cells in vivo was first suggested by the observation that mice expressing a transgenic myelin protein-reactive T-cell receptor succumbed to spontaneous EAE when backcrossed to a T-cell deficient background [7]. It was subsequently shown that resistance of these mice to autoimmune demyelination is restored by reconstitution with CD25$^+$CD4$^+$ Treg cells [8, 9]. Consistent with these findings, the adoptive transfer of Treg cells (either isolated from the CNS of mice that had recovered

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from EAE, or generated in vitro by the stimulation of naïve $CD25^-$ $CD4^+$ T cells with IL-2 and TGF-β), reduces the incidence and severity of EAE in wild-type mice [5, 10]. Conversely, depletion of Treg cells enhances disease severity and impedes clinical recovery [5, 11]. Treg cells from individuals with MS have been found to be relatively inefficient in in vitro suppression assays [12, 13]. Together, these data demonstrate that $FoxP3^+CD25^+CD4^+$ Treg cells can limit the extent and duration of neuroinflammation. However, their mechanism and site of action in that capacity are poorly understood.

Based on the preferential accumulation of adoptively transferred $CD4^+CD25^+$ donor T cells in the lymphoid tissues of mice with EAE shown in their study, Kohm et al. [14] speculated that Treg cells exert their protective functions in the periphery. In support of this notion, recent insights regarding the plasticity of Treg cells suggest that their suppressive functions could be undermined in the inflamed CNS. Hence, multiple laboratories have independently demonstrated that Treg cells downregulate FoxP3 and upregulate IL-17 (as well as other Th17 cell-associated molecules, including IL-17F, RORγt, and IL-23 receptor) following activation in the presence of proinflammatory cytokines that are expressed in the CNS during EAE, such as IL-6, IL-1β, and IL-23 [15–1815-18]. Among these factors, IL-6 has been most prominently implicated [17, 18].

IL-6 is expressed in acute and chronic active plaques in the brains of patients with MS and animals with EAE, where it is associated with resident glial cells [19, 20]. Treg cells have been shown to catalyze their own transformation into IL-17 producers, as well as to promote the Th17-cell differentiation of uncommitted "bystander" T cells, by acting as a source of TGF-β in an IL-6 rich environment [18]. Treg cells that have transitioned into IL-17 producers (so called "exTreg" cells) are compromised in their ability to inhibit the activation of conventional T cells in vitro and to suppress inflammatory events in vivo [15, 17]. These observations beg the question as to whether autoreactive exTreg cells can mediate destructive inflammation within a target organ, commensurate with their Th effector counterparts. Indeed, exTreg cells specific for a pancreatic islet autoantigen induce diabetes following transfer in lymphopenic hosts [16, 21]. However, a paradoxical disease-inciting role of Treg cells in EAE is belied by the fact that in vivo depletion of $CD25^+$ T cells or a genetic deficiency of Treg cells exacerbates, rather than suppresses, clinical signs [5, 7, 9, 11]. This suggests that endogenous Treg cells are actively stabilized in the CNS during EAE, in spite of the presence of proinflammatory cytokines. O'Connor et al. [4] now report that such Treg-cell stabilization is achieved via downregulation of both chains of the IL-6 receptor (IL-6R), CD126 and gp130. It is interesting that FoxP3 negative T cells, as well as Treg cells, were found to be IL-6R negative in the CNS of mice with EAE. The downregulation of IL-6R on uncommitted conventional T cells could provide an additional layer of protection against EAE relapse or progression, by preempting the emergence of Th17 effectors reactive against secondary myelin epitopes in the context of epitope spreading [22].

The plasticity of Treg cells is determined, in part, by their ontogeny. Most recently, it was reported that the majority of ex-FoxP3 cells are derived from a minor population of conventional T cells that transiently upregulate FoxP3 but that never fully commit to a regulatory lineage (as reflected by a lack of suppressor function and the presence of a methylated Treg cell-specific demethylation region, or TSDR) [23]. In the current article, bisulfite sequence analysis of CNS-infiltrating $FoxP3^+$ cells revealed complete demethylation of the TSDR, making it unlikely that they represent conventional T cells with promiscuous FoxP3 expression. In fact, the demethylated status of the TSDR in CNS Treg cells is most reminiscent of the thymically derived "natural" Treg (nTreg) cell subset. It has been claimed that nTreg cells are more susceptible to IL-6-mediated Th17 deviation than peripherally derived, "inducible" Treg (iTreg) cells [16, 17]. Collectively, these data suggest that the insensitivity of CNS nTreg cells to IL-6 is imperative for their stability during EAE. Since IL-12, IL-23, and IFNγ can stimulate Treg cells to acquire effector cell characteristics [15, 16, 24], it would be instructive to determine whether expression of each of their receptors is also reduced on CNS Treg cells.

A number of important questions have yet to be answered. For example, are Treg cells triggered to downregulate IL-6R after they enter the brain and spinal cord? If so, what are the factors in the brain and spinal cord that suppress IL-6R expression? IL-10 merits consideration as a candidate Treg-cell stabilizing factor because it is upregulated in the CNS during EAE remissions and because IL-10 deficiency or neutralization delays clinical recovery [5, 25]. Alternatively, are IL-6R expressing Treg cells selectively recruited to the CNS from the periphery or do they have a survival/proliferative advantage over other Treg-cell subsets within the CNS microenvironment? The majority of Treg cells isolated from the CNS of mice with EAE express CXCR3, a chemokine receptor that has been implicated in CNS homing of effector T cells and in Treg cell migration to sites of inflammation [24]. $CXCR3^+$ Treg cells isolated from the spleen of the same mice tended to be IL-6R positive. Hence, peripheral Treg cells that migrate to the brain during the course of EAE are likely enriched with IL-6R expressing cells. In experimental models of gliomas, the CCR4/CCL22-CCL2 axis drives Treg cell recruitment to the brain [26]. The importance of CCR4 expression for Treg cell trafficking in mice with EAE, and the relationship between CCR4 and IL-6R expression on Treg, remain to be elucidated.

Irrespective of the answers to the above questions, the findings of O'Conner et al. [4] hold important implications for the treatment of MS. As the authors themselves discuss, concerns over the therapeutic administration of Treg cells (and, in particular, autoreactive Treg cells) based on their potential for transformation into pathogenic effectors, might be defused if it were established that the CNS provides a stabilizing environment. That statement is made with the caveats that: (i) the behavior of endogenous Treg cells with a demethylated TSDR might not reflect the behavior of Treg cells that are induced from $FoxP3^-$ precursors and/or that are artificially expanded in vitro for therapeutic purposes, and (ii) if donor Treg cells are administered systemically, Th17-cell deviation could occur in the more permissive lymphoid tissues. The current study [4] highlights the potential efficacy of IL-6

neutralization or IL-6 receptor blockade as therapeutic interventions in MS. In addition to thwarting the development of encephalitogenic Th17 cells [19], IL-6/IL-6R targeting agents could augment the immunosuppressive activities of Treg cells at the very site of neuroinflammation. An issue of wide-ranging therapeutic significance concerns the universality of the phenomena reported by O'Connor et al. [4]. Hence, are pathways that reinforce Treg cell properties unique to the immune-privileged CNS, or do they also exist in other body compartments? If the latter is the case, then stabilization of Treg cells via downregulation of IL-6R could contribute to the restraint and resolution of a spectrum of organ-specific autoimmune diseases.

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Abbreviations: **nTreg**: natural regulatory T cell · **IL-6R**: interleukin 6 receptor

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