A Fox of a different color: FoxA1 programs a new regulatory T cell subset

Greg M Delgoffe & Dario A A Vignali

Interferon-β (IFN-β) is widely used to treat multiple sclerosis (MS), but its mechanism of protection remains obscure. A new study shows that IFN-β induces FoxA1$^+$ regulatory T cells, a new regulatory T cell population, which suppress conventional T cells via programmed cell death 1 ligand 1. This cell subset limits disease in a mouse model of MS and was found in patients with MS who responded to IFN-β therapy (pages 272–282).

The power of a highly specific adaptive immune system is that pathogens can be eliminated efficiently from the body. However, when left unchecked, the immune response can wreak havoc on host tissues, resulting in severe autoimmune pathologies. Regulatory T (T_{reg}) cells are a specialized population of suppressive T cells that have crucial roles in maintaining immune homeostasis by suppressing autoreactive cells and limiting immunopathology. However, they have also been shown to be dysregulated in autoimmune diseases, including MS. Whereas most of the attention has been focused on CD4$^+$CD25$^+$Foxp3$^+$ thymus-derived T_{reg} cells—the predominant suppressive T cell population—it has become clear hat additional Foxp3$^-$ suppressive T cell populations exist, such as interleukin-10 (IL-10)-producing T regulatory 1 cells[1], induced IL-35–producing T_{reg} (iT_R35) cells[2] and CD8$^+$ T_{reg} cells[3].

In this issue of *Nature Medicine*, Liu *et al.*[4] describe a new T_{reg} cell population called FoxA1$^+$ T_{reg} cells, which express the forkhead family transcription factor FoxA1 and the inhibitory cell surface ligand programmed cell death 1 ligand 1 (PD-L1) and suppress and kill autoreactive T cells in the central nervous system (CNS) during relapsing-remitting experimental autoimmune encephalomyelitis (RR-EAE), a mouse model of MS. Samples from patients with relapsing-remitting MS who were responsive to IFN-β therapy showed increased numbers of FoxA1$^+$ T_{reg} cells in the peripheral blood, suggesting this new T_{reg} cell subset may mediate the therapeutic effect of IFN- β (**Fig. 1**).

Previous studies have shown that mice lacking *Ifnb* (encoding IFN-β) and *Ifnar1* (encoding the IFN receptor) have an increased severity of EAE compared to wild-type mice with EAE[5], which is consistent with the observed efficacy of IFN-β treatment for MS over the last two decades[6]. Although the authors had previously reported that the cytokine milieu and the numbers of T helper and T_{reg} cells remained unchanged in EAE in these mutant mice[5,7], the authors speculated that some new tissue-resident T_{reg} cells might expand in the presence of IFN-β. The number and phenotype of the Foxp3$^+$ T_{reg} cells did not change; however, a population of CD4^{hi}PD-L1^{hi} T cells was enriched in the inflamed CNS of wild-type but not IFN-β–deficient mice with EAE[4]. Using an *ex vivo* system with an encephalitogenic T cell clone that specifically reacts against myelin basic protein (MBP), Liu *et al.*[4] showed that the generation of a PD-L1^{hi} subset was not dependent on strong or repetitive activation; instead, it depended on co-culture with neurons.

Gene expression profiling revealed that PD-L1^{hi} T cells were a distinct hypoproliferative cell type expressing the transcription factor FoxA1, as well as the integrin-associated cell surface protein CD47 and the early activation cell surface glycoprotein CD69. Referred to as FoxA1$^+$ T_{reg} cells, this population seems to possess a transcriptome distinct from Foxp3$^+$ T_{reg} cells. What was not assessed, but would be interesting to determine, is whether FoxA1$^+$ T_{reg} cells bear any similarities to other cytokine-induced CD4$^+$ T_{reg} populations, such as T_R1 cells[1] and iT_R35 cells[2].

The authors hypothesized that FoxA1$^+$ T_{reg} cells may contribute to suppression of CNS inflammation *in vivo*[4]. FoxA1$^+$ T_{reg} cells generated *in vitro* in the presence of neurons suppressed T cell proliferation and induced T cell death *in vitro*, inhibited delayed-type hypersensitivity responses in mouse models of tissue inflammation and ameliorated disease severity in mice with EAE lacking *Ifnb* after adoptive transfer[4]. Mouse and human FoxA1$^+$ T_{reg} cells could also be generated *in vitro* in the presence of IFN-β alone, suggesting that IFN-β is the molecular mediator of FoxA1$^+$ T_{reg} cell differentiation in the CNS. Notably, FoxA1$^+$ T_{reg} cells were induced in the CNS in mice with EAE in an IFN-β–dependent manner, and systemic treatment of *Ifnb*-deficient mice with EAE with recombinant IFN-β restored the development of FoxA1$^+$ T_{reg} cells, which limited disease severity. Interestingly, FoxA1$^+$ T_{reg} cells seemed to be stable *in vivo*, as they retained FoxA1 expression for 40 d after adoptive transfer, although their suppressive capacity was not assessed. Collectively, these data suggest that FoxA1$^+$ T_{reg} cells are a bona fide suppressive T_{reg} cell population that is induced in an IFN-β–dependent manner *in vivo* during autoimmune disease in the CNS.

Liu *et al.*[4] then sought to determine the contribution of the transcription factor FoxA1 to FoxA1$^+$ T_{reg} cell function. RNAi-mediated knockdown of FoxA1 expression in T cells prevented IFN-β–induced PD-L1 upregulation and resulted in loss of immune suppressive capacity *in vitro* and in mice with EAE. Furthermore, ectopic overexpression of FoxA1 in conventional T cells was sufficient to endow suppressive capacity. Conversely, RNAi-mediated knockdown of PD-L1 expression or monoclonal antibody-mediated PD-L1 blockade limited the suppressive capacity of FoxA1$^+$ T_{reg} cells and exacerbated EAE. The authors found that PD-L1 was required for suppression of T cell proliferation and the induction of their cell death, which were mediated by inhibition of Akt and p38 signaling and induction of active caspase-3, respectively. FoxA1 may directly induce PD-L1 expression, as it binds to the *Cd274* (PD-L1) promoter and can drive promoter activity. Thus, IFN-β–mediated induction of FoxA1, which seems to act as a key transcriptional regulator for this T_{reg} cell subset, drives PD-L1 expression, which in turn mediates the suppressive activity of T_{RFoxA1} cells.

Finally, the authors assessed whether the presence of FoxA1$^+$ T_{reg} cells correlated with responsiveness of patients with RR-MS to IFN-β treatment. The percentage of CD4$^+$CD47$^+$PD-L1$^+$ T cells was higher in patients that responded well to IFN-β, and purified PD-L1^{hi} cells from these patients were able to suppress T cell proliferation and induce T cell death *in vitro*. Curiously, not all CD4$^+$CD47$^+$PD-L1$^+$ T cells expressed FoxA1, suggesting a heterogeneity in patients with RR-MS that warrants further investigation. Although it remains to be determined whether

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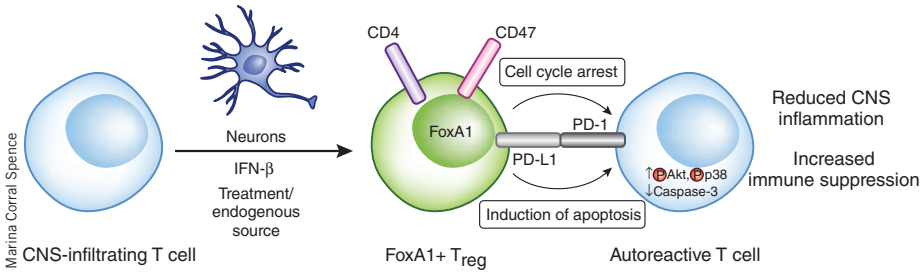

Figure 1 IFN-β can generate FoxA1$^+$ T_{reg} cells in the CNS, which inhibit autoreactive T cells. CNS-infiltrating T cells, upon activation in the presence of neurons and/or IFN-β treatment, upregulate the transcription factor FoxA1, which promotes the expression of PD-L1 on the cell surface. CD4 and CD47 serve as additional cell surface markers. FoxA1$^+$ T_{reg} cells suppress autoreactive T cells in the inflammatory milieu via PD-L1–PD-1 interaction, which limits proliferation by inhibiting Akt and p38 phosphorylation or promotes cell death by inducing active caspase-3. The induction and function of FoxA1$^+$ T_{reg} cells results in ameliorated disease in mouse EAE.

these cells directly limit disease in patients, it is possible that FoxA1$^+$ T_{reg} cell counts could be used to monitor the clinical benefit of IFN-β therapy in patients with RR-MS.

It has long been known that IFN-β can have immunosuppressive properties, especially in the CNS, and it has been used as an effective and safe treatment for MS for many years. The intriguing observations of Liu *et al.*[4] provide a possible cellular mechanism for IFN-β treatment, which may work, in part, by inducing suppressive FoxA1$^+$ T_{reg} cells. Given the apparent *in vivo* stability of FoxA1$^+$ T_{reg} cells, it is possible they could also be used in cell-based therapies. It will be interesting to determine whether they are generated in, and affect, other autoimmune or inflammatory diseases, especially those with high IFN-β activity, such as some viral infections. It is not clear whether FoxA1$^+$ T_{reg} cells are induced only by IFN-β or whether other factors can enhance or contribute to their induction and whether they are generated in the thymus, given the high levels of IFN-β suggested to be produced by AIRE$^+$ thymic epithelial cells[8].

It is also intriguing that PD-L1 seems to be the sole effector molecule of this regulatory population, and one of only a few immunologic molecules upregulated in the FoxA1$^+$ T_{reg} cell transcriptome, albeit these are characteristics shared by other induced T_{reg} cell populations. It remains to be determined whether FoxA1$^+$ T_{reg} cells use additional regulatory mechanisms, although the PD-1–PD-L1 axis alone has been shown to mediate potent T cell exhaustion in tumors and chronic viral infections[9]. Given that PD-L1–targeted therapies to treat cancer have entered the clinic[10], it is possible that inadvertent inhibition of FoxA1$^+$ T_{reg} cells might underlie some of the inflammatory toxicities observed in some patients.

The identification of FoxA1$^+$ T_{reg} cells adds another member to the expanding panoply of induced T_{reg} cell populations that seem to be generated in tissue- and/or disease-restricted microenvironments and may serve unique roles in mediating suppression in these niches. One wonders whether additional induced T_{reg} cell populations remain to be discovered.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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Malarial liver parasites awaken in culture

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The cure and elimination of malaria caused by *Plasmodium vivax* is hindered by the threat of relapse infections from undetectable dormant forms of the parasite in the liver. In a new breakthrough, using a related parasite, *Plasmodium cynomolgi*, it has been shown that the small nongrowing forms of the parasite, termed hypnozoites, can be reactivated in primary simian hepatocytes that have been infected and maintained in culture for 40 days, providing a system to study this parasite form with the development of potential new antihypnozoite drugs in mind (pages 307–312).

When injected into the host by mosquitoes, the infecting sporozoites of certain malaria parasite species, such as *P. vivax* or *P. cynomolgi*, invade liver cells. There, some sporozoites typically transform into actively growing parasites that repeatedly divide, producing within a week's time thousands of merozoites, which invade red blood cells, whereas others remain as small ~4-nm bodies known as hypnozoites and become metabolically quiescent (**Fig. 1**). Hypnozoites lurk undetected inside hepatocytes, awaiting some undefined internal or external signal weeks, months or years later to cause activation, growth and multiplication[1]. One infectious mosquito bite can result in multiple bouts of relapsing blood-stage illness and hypnozoites are undetectable in infected people with no other signs of disease, posing a challenge to the elimination of the parasite[2].

Whereas several available antimalarial drugs can kill growing parasites in the liver, only one drug will eliminate hypnozoites. This drug, primaquine, has a long dosing schedule (up to 14 days), which makes it challenging to effectively treat people carrying the hypnozoite reservoir. Primaquine can also cause severe hemolysis in people with glucose-6-phosphate dehydrogenase deficiency, a common condition in areas where malaria is endemic, further limiting the

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