Editorial

Tregs, Helios and tumor immunity: the sun has not yet risen

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T regulatory cells (Tregs) suppress immune activation and play a critical role in the maintenance of self-tolerance. However, Tregs are enriched in the tumor microenvironment, suppress immune activation and effectively inhibit anti-tumor responses (1). Thus, depletion or blockade of Tregs has been explored as a potential target for cancer immunotherapy. As Tregs were originally defined as CD4+CD25+ T cells, the first attempts to deplete Tregs targeted CD25, the IL2R α-chain. While the results of some mouse studies, with or without vaccination, indicated successful prevention or eradication of tumors, results of human studies have been mixed (1-4). Thus, other approaches to target Tregs are being explored as potential cancer immunotherapies. Tregs differentially express a number of surface receptors and these have been implicated in Treg suppressor function that could serve as targets for immunotherapy (CTLA-4, GITR, OX40, CCR4, FR4 and TIGIT). However, all of these molecules are also up-regulated on activated T cells and it is unclear, in most cases, if antibody therapy depletes Tregs, reverses Treg-mediated suppression, enhances anti-tumor T effector (Teff) cells, or a combination of all three. In fact, the anti-tumor effects of both anti-CTLA-4 mAb and anti-OX40 mAb require their Fc portion to mediate ADCC depletion of Tregs (5-8).

Nakagawa *et al*. (9) recently described a potentially different immunotherapeutic approach, whereby Tregs are not deleted nor is suppressor function inhibited, but rather, Tregs are targeted to promote instability, resulting in the conversion of Tregs to anti-tumor Teff cells. The rationale behind this hypothesis is based on the concept that Tregs (particularly those in the tumor microenvironment) preferentially recognize self-peptide MHC class II complexes including tumor-associated antigens. They propose that targeting signaling pathways that decrease the expression of the transcription factor Helios induces Treg instability and conversion to Teff cells within the tumor.

Helios (Ikzf2) is a member of the Ikaros transcription factor family and is expressed in approximately 70% of Foxp3+ Treg cells. We have postulated that Helios+ Treg may be exclusively thymic-derived (tTreg), while the Helios- subpopulation is enriched in pTreg that are generated extrathymically in peripheral sites (10). We observed a delayed, progressive immune activation in older mice with a Treg conditional deletion of Helios (cKO mice), but did not observe extensive organ-specific autoimmunity (11) or decreased activation of the STAT5 pathway in the steady state (unpublished). We did observe a very large percentage of activated $CD4^+$ and $CD8^+$ T cells that produce IFNγ and significantly elevated T follicular helper (T_{FH}) cells, GC B cells and elevated levels of serum Ig. We postulate that Helios selectively regulates some, but not all, Treg suppressive functions due to the inability of Helios deficient Treg to become activated or remain stable in the activated, effector state. In contrast, Kim *et al*. (12) have reported that mice that lack Helios, either globally or conditionally in Tregs (cKO), develop immune activation, autoantibody production and organ-specific autoimmunity due to an unstable Treg phenotype, presumably secondary to disruption of the STAT5 pathway. While many of the observations of Kim *et al*. were derived from bone marrow chimera experiments, the reasons for the differences in the disease phenotypes observed in the two studies remain unclear.

In their latest study, Nakagawa *et al*. clearly demonstrate that the absence of Helios in Tregs cells results in enhanced tumor immunity (9). When challenged with either B16/F10 melanoma or MC38 adenocarcinoma, Helios cKO mice exhibited decreased tumor size and increased survival. Tumor immunity is enhanced in Helios cKO mice not only as measured by prolonged survival, but also as measured by increased IFNγ and TNFα production by $CD4^+$ and $CD8^+$ T cells. However, the effector T cell pool in these mice, even at a young age, is abnormal (11,12). In the absence of tumor inoculation, $CD4^+$ and $CD8^+$ T cells have an activated phenotype and secrete significantly higher amounts of IFNγ; thus, it remains unclear if, upon tumor inoculation, there is truly an increase in effector cytokine production.

Nakagawa *et al*. ascribe the enhanced tumor immunity in the Helios cKO mice as resulting from the conversion of Treg with an unstable phenotype to Teff cells. The only evidence put forth to support this hypothesis is the enhanced production of IFNγ/TNFα by the Treg in the tumors. However, there are many fewer intratumoral Treg in cKO mice and the level of cytokine production from cKO Tregs, although higher than WT Treg, is fairly small, especially in comparison to that of $CD4^+$ and $CD8^+$ Teff. We believe that it is premature to conclude that conversion of Tregs to an effector phenotype significantly contributes to the effector response.

Although studies in the cKO mice suggest that the Tregs are defective, it is necessary to demonstrate that the defect is Treg cell intrinsic. Nakagawa *et al*. transferred wild type (WT) Teff cells with either WT or cKO Treg to $RAG^{-/-}$ recipients. Improved tumor immunity was demonstrated in the presence of cKO Treg compared to WT Treg. However, there are significantly fewer intratumoral cKO Treg that make marginally increased amounts of IFNγ compared to WT Treg, while the Teff cells produce substantially more IFNγ. Thus, it is difficult to determine the contribution of Treg conversion to anti-tumor immunity. Furthermore, tumor growth and Teff cytokine production in the absence of Treg is not shown and it is not possible to determine the true inhibitory effect of WT Treg and/or the purported defect in cKO Treg. Thus, it is never conclusively demonstrated that the Helios deficient Treg alone, producing more cytokines, are solely, or even partially, responsible for increased anti-tumor immunity. A convincing demonstration of conversion of Treg to an anti-tumor Teff cell requires isolation of the "converted" Tregs and a study to prove that they can directly kill tumor cells or inhibit their growth.

We do agree that the experiments of Nakagawa *et al*. convincingly demonstrate that suppression of tumor immunity is one of the Treg functions that is regulated by Helios. The critical question that must be addressed is how to target the function of an intracellular transcription factor in Tregs selectively in the tumor microenvironment without the induction of systemic autoimmunity. As proof of principal, Nakagawa *et al*. select the glucocorticoid-induced TNF receptor (GITR) as a target. In 2005, Ko *et al*. demonstrated that anti-GITR mAb (DTA-1) breaks tolerance and promotes anti-tumor immunity in a mouse model (13). Nakagawa *et al*. observe that intratumoral Treg, but not splenic Treg, from DTA-1 treated mice produce substantially more IFNγ. However, DTA-1 can also activate $CD4^+$ and $CD8^+$ Teff cells and Teff from DTA-1-treated mice make an extremely large amount of IFNγ. Without knowing the ratio and absolute numbers of Teff: Treg, the contribution of the Treg-derived IFNγ to the anti-tumor response is unclear. While Treg from DTA-1 treated animals do show a decrease in Helios expression when transferred to $RAG^{-/-}$ recipients, it is quite clear that Treg are substantially depleted by DTA-1. In fact, DTA-1 has been shown to be a depleting antibody and the anti-tumor effect of anti-GITR mAb requires activating FcγRs to mediate ADCC depletion of Treg (5,14). One explanation for the increased cytokine production observed in Treg from DTA-1-treated mice is that while $Helios^+$ Treg are enriched in tumors, some $Helios^-$ Treg are also present. As $Helios^+$ Tregs also express high levels of the GITR, DTA-1 treatment may preferentially deplete $Helios^+GITR^{hi}$ cells leaving the $Helios^-$ (and thus, $GITR^{lo}$) cells. Helios- Treg are enriched for pTregs and contain a higher frequency of Teff cytokine producing cells. Thus, DTA-1 treatment may produce a relative enrichment of cytokine producing $Helios^-$ Treg, rather than promoting the conversion of Treg to Teff.

While conversion of Treg to Teff cells remains a possible explanation for the enhanced anti-tumor response of Helios cKO mice, the simplest explanation is that the absence of Helios impairs Treg anti-tumor suppressor function. Alternatively, the absence of Helios may impair Treg activation or migration of Treg to the tumor site. While the studies reported by Nakagawa *et al*. do suggest a novel approach to tumor immunotherapy by targeting a transcription factor which is critical for certain aspects of Treg function, we still lack a clear understanding of the function of Helios or its downstream targets in Treg function. Further studies are needed before Helios can be targeted with biologic or pharmacologic agents. Only then, can the sun go down.

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Footnote

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