

Effector Tregs: middle-men in TGF β activation

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Regulatory T-cells (Tregs) are an inherent suppressive cell of the immune system with an established developmental requirement for the cytokine transforming growth factor β (TGF β). However, the precise mechanisms by which mature Tregs utilize TGF β during disease are unclear. In the May issue of Immunity, we have demonstrated that effector regulatory T-cells are essential activators of latent-TGF β which is crucial to suppress ongoing inflammation.

A failure to regulate effective immunity results in chronic inflammation which can lead to immunopathology and carcinogenesis. One of the key molecules involved in immune cell suppression is the cytokine TGF β , which crucially must be activated from its latent state in order to function [1]. An essential function of TGF β is to drive the development of Tregs, both naturally derived thymic Tregs and the peripherally induced Tregs that are converted from the naïve T-cell population (pTregs) [2]. This critical subset of T-cells with an inherent suppressive role has been a huge focus of research both mechanistically and therapeutically, with current treatments for autoimmunity and transplantation involving the *ex vivo* expansion of patient's Tregs and conversely selective Treg depletion during cancer treatment.

Several *in vivo* studies have clearly shown that, in addition to its role in Treg development, TGF β plays a fundamental role in the suppressive function of Tregs. However, the precise mechanisms by which TGF β mediates Treg biology are unclear, with conflicting reports existing within the literature, most notably in studies utilizing T-cell transfer colitis. Within this colitis model, Tregs are essential for the suppression of disease but are still able to suppress inflammation when they lack the ability to produce TGF β [3, 4]. However, the use of blocking antibodies in the same study demonstrates the complete dependence on TGF β for disease suppression [3]. Furthermore, effector T-cells themselves must respond to TGF β for Treg-mediated prevention of colitis, as T-cells require functional TGF β receptors to be suppressed [3]. Collectively, these data indicate that TGF β is absolutely required for Tregs to suppress effector T-cells, but Tregs themselves do not need to be the source of TGF β .

A particular focus within the Mark Travis lab at the Manchester Collaborative Centre for Inflammation Research, University of Manchester, is the regulation of latent TGF β during intestinal inflammation. The TGF β 1 gene (TGF β 1 being the predominate isoform produced

by the immune system) encodes latency associated peptide (LAP), which after transcription remains non-covalently bound preventing the active TGF β dimer engaging its receptor. This so called LAP "straight-jacket" that surrounds the TGF β dimer contains an RGD motif which can be bound by $\alpha\beta$ integrins allowing either conformational or protease dependent activation of latent TGF β [1]. We have previously shown that tolerogenic CD103 $^{+}$ intestinal dendritic cells, which are key inducers of pTregs, are rich in the integrin $\alpha\beta$ and it is essential for their ability to activate latent TGF β and convert naïve T-cells into pTregs [5]. A lack of this key regulatory molecule on DCs leads to an enhanced ability to fend off intestinal infection [6] but mice succumb to an age-related colitis [7]. We therefore postulated that, rather than produce TGF β , Tregs may be required to activate the latent form to drive suppression.

We now demonstrate high levels of β integrin gene expression within the Treg population and utilising an active TGF β reporter assay show that Tregs do indeed demonstrate an enhanced ability to activate latent TGF β compared to other T-cell subsets. Furthermore, $\alpha\beta$ null Tregs lose their ability to activate latent TGF β , suggesting that Treg cells activate enhanced levels of TGF- β versus other T cell subsets via expression of the integrin $\alpha\beta$.

Interestingly, we identified activated effector Tregs, thought to regulate ongoing inflammation, as the highest expressers of β integrin, indicating that this pathway maybe important in ongoing inflammation rather than homeostasis. As hypothesised, mice lacking β integrin expression specifically in Tregs (via Foxp3-cre), showed no overt autoimmune phenotype even after ageing, and Tregs lacking β integrin were capable of preventing the development of inflammatory T-cells in the intestine when co-transferred with effector T-cells in the transfer colitis model. Collectively, indicating TGF- β activation by Treg-cell-expressed integrin $\alpha\beta$ is not required for Treg-cell-mediated control of T cell tolerance at rest.

In order to examine the role during ongoing inflammation we returned to the T-cell transfer colitis model. In stark contrast to the co-transfer experiments, unlike control Tregs, Tregs that lacked β integrin expression completely lost their ability to cure colitis when transferred after effector T-cells had established ongoing inflammation. Moreover, when we examined the differing Treg and effector T-cell populations for downstream TGF β signalling in the form of Smad2 phosphorylation,

we saw that an increase in TGF β signalling within the colitis-driving effector T-cell population correlated with suppression by Tregs. Importantly this increase was completely absent when the Tregs attempting to rescue colitis lacked β 8 integrin expression, demonstrating that integrin α v β 8-mediated TGF β activation by effector Tregs is essential for suppression of T-cell mediated inflammation. Finally, high expression of β 8 integrin was also seen in human samples upon examination of the equivalent effector Treg populations.

This highlights a new suppressive mechanism by which Tregs control ongoing inflammation and is a pathway that can hopefully be targeted to prevent chronic inflammation, opening up the potential of therapy for a variety of inflammatory and autoimmune diseases via the manipulation of integrin α v β 8.

"Integrin α v β 8-Mediated TGF- β Activation by Effector Regulatory T Cells Is Essential for Suppression of T-Cell-Mediated Inflammation" was recently published in *Immunity*: 2015 May 19;42(5):903-15.

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