Epigenetic orchestration of thymic regulatory T cell development

Marc Beyer[1,2] & Jochen Huehn[3]

[1]Life and Medical Science (LIMES) Institute, University of Bonn, 53115 Bonn, Germany

[2]Single Cell Genomics and Epigenomics Unit at the German Center for Neurodegenerative Diseases and the University of Bonn, 53105 Bonn, Germany

[3]Experimental Immunology, Helmholtz Centre for Infection Research, 38124 Braunschweig, Germany

Correspondence: Jochen Huehn, jochen.huehn@helmholtz-hzi.de; Marc Beyer, marc.beyer@uni-bonn.de

[Summary]

Regulatory T cells develop in the thymus as a distinct lineage of T cells instructed by the lineage-specifying transcription factor Foxp3. Epigenetic imprinting by the genome organizer Satb1 precedes this cell-fate decision during thymocyte development.

[Main Text]

The vast majority of regulatory T cells (T_{reg} cells) is generated within the thymus. At a certain stage of development thymocytes have to make a decision between $CD4^+$ helper T cell-fate and acquisition of T_{reg} cell properties. The T_{reg} cell-lineage-specifying transcription factor Foxp3 is vital for the generation of thymic T_{reg} cells and its loss results in the development of fatal autoimmunity through inadequate suppression of auto-reactive T cells. While the molecular signals required for induction of *Foxp3* expression have been widely studied over the past years, the instructive events governing thymic T_{reg} cell development and allowing *Foxp3* expression in a specific subpopulation of thymocytes has remained largely enigmatic. In the current issue, a study by Kitagawa *et al.*[1] now establishes a concept of inducing a permissive epigenetic landscape that allows for induction of thymic T_{reg} cell programs. This process, which precedes expression of the transcription factor Foxp3, is mediated by the chromatin organizer Satb1.

Published work has substantially increased our knowledge on the molecular events inducing the development of T_{reg} cells in the thymus via pre-committed T_{reg} cell precursor states which can be reached via two separate and distinct ways of development[2,3]. In common for both is the increased signaling via the T cell antigen receptor (TCR) through high-affinity interactions, which combined with additional (costimulatory) signals provided by the interaction with thymic epithelial and dendritic cells is critical for the induction of *Foxp3* expression. Importantly, TCR stimulation was recently found to be not only necessary for the induction of *Foxp3* expression, but also for the Foxp3-independent establishment of a T_{reg} cell-specific epigenetic signature[4]. This T_{reg} cell-specific CpG hypomethylation pattern, particularly at the *Foxp3* locus itself[5], is essentially required for thymocytes to acquire T_{reg} cell-specific gene expression, lineage stability, and full suppressive activity.

Based on these observations, it has become evident that the time point during thymocyte differentiation where T_{reg} cell lineage choice is initialized and what exact steps thymocytes undergo to establish T_{reg} cell fate have not been identified so far. Data from hematopoietic or embryonic stem cells however have instructed us to appreciate epigenetic events as a foundation for subsequent decisions individual cells will make during their differentiation towards mature somatic cells[6], and suggested that the engraving of the T_{reg} cell program in the thymus could also be governed by mechanisms inducing a T_{reg} cell-specific epigenetic landscape.

Following the concept of predetermination of distinct lineage fate by an already established epigenetic landscape in pre-committed precursor cells, Kitagawa *et al.* now introduce a similar concept into thymocyte development and the commitment towards T_{reg} cell differentiation[1]. Using extensive and comprehensive approaches to assess epigenetic and transcriptional regulation in peripheral T_{reg} cells, they linked the activity of a class of enhancers, so-called super-enhancers, suggested to control the expression of the associated lineage-specifying genes in a number of cell types[7], to the expression of several genes defining T_{reg} cell identity, including *Foxp3*, *Ctla4* and *Il2ra*.

Based on this identification of a group of T_{reg} cell-specific super-enhancers in peripheral T_{reg} cells, Kitagawa *et al.* postulated that these super-enhancers could be the aforementioned instructive upstream event necessary for the commitment of thymic T_{reg} cell precursors towards T_{reg} cell lineage fate[1]. Applying a broad set of genome-wide approaches to determine the epigenetic and transcriptional landscape within thymic populations of T_{reg} cells and their precursors, the initial hypothesis was confirmed that permissive remodeling of the epigenetic landscapes at this set of super-enhancers is required for the commitment towards the T_{reg} cell lineage, the induction of *Foxp3* expression and the fixation of the unique T_{reg} cell-specific phenotype by subsequent hypomethylation of T_{reg} cell-specific epigenetic signature genes[1].

But how are these T_{reg} cell-specific super-enhancers activated during thymic T_{reg} cell development? To address this question, Kitagawa *et al.*[1] again turned to analogies from lineage commitment in other cell types, e.g. myeloid cells, where transcription factors like PU.1 have been described as pioneering factors required for alterations in the epigenetic landscape allowing for subsequent expression of myeloid lineage-specific genes[8]. Global organizers of chromatin being highly expressed in the respective cell type mainly exert this function. Special AT-rich binding protein 1 (Satb1) is highly expressed during thymocyte development and had been introduced as a key event necessary for the generation of functional single-positive thymocytes[9], while its suppression in peripheral T_{reg} cells is key to prevent effector differentiation of mature T_{reg} cells[10]. Thus far, its function has been described as a global genome organizer able to induce both transcriptional and epigenetic regulation via formation of long-range chromatin loops from base-unpairing regions thereby bringing distal genes in close proximity[11]. In addition, Satb1 can recruit chromatin-remodeling and histone-modifying enzymes as well as transcription factors to Satb1-bound gene loci. Making use of the comprehensive dataset of thymic T_{reg} cell precursor populations, Satb1 is presented as a possible upstream molecule of the T_{reg} cell-specific super-enhancer landscape. From elegant knockout studies comparing deletion of *Satb1* at different stages of thymocyte and peripheral T_{reg} cell development, Kitagawa *et al.* concluded that the action of Satb1 is already necessary during early stages of thymic T_{reg} cell development[1]. At this point, Satb1 can even bind to super-enhancers within closed chromatin, where ATAC-seq signals measuring chromatin accessibility were low. This finding led to the conclusion that Satb1 might act as a pioneering factor for T_{reg} cell commitment through selective priming of T_{reg} cell-specific super-enhancers.

Could this also alter our current understanding of the induction of the lineage-specifying transcription factor Foxp3? To address this question, Kitagawa *et al.* fine-mapped a super-enhancer containing the three pre-described conserved non-coding sequence elements (CNS1-3) at the *Foxp3* locus associated with enhancer activity important for thymic and peripheral T_{reg} cell differentiation as well as stability of *Foxp3* expression[12], and could identify a novel conserved element they termed CNS0, which is bound by Satb1 already in a closed chromatin confirmation prior to commitment of thymocytes to the T_{reg} cell lineage[1]. They propose that this element through binding of Satb1 and subsequent alterations in chromatin accessibility or histone modifications acts as a pioneering element required for the subsequent activities of the other CNS elements, leading to the initiation of *Foxp3* expression.

The current study adds another piece to the fascinating events taking place within the thymus and during specialization of thymocytes to the T_{reg} cell lineage. Still, important questions remain open, including the intermediate factor or reaction translating Satb1 binding into final T_{reg} cell lineage commitment, as Satb1 binding alone is not sufficient to activate T_{reg} cell-specific super-enhancers. Here, TCR signaling as suggested by recent evidence[4] could be the next step further promoting T_{reg} cell differentiation, however also additional events including unique signals provided by thymic antigen-presenting cells (own unpublished data) might be required to fully commit cells towards the T_{reg} cell lineage. Furthermore, it is only incompletely understood how the activity and specificity of TET enzymes enforcing CpG hypomethylation in Satb1-bound regions or stretches of DNA marked by not yet identified intermediates is controlled on a molecular level, leading to the final epigenetic fixation of T_{reg} cell lineage fate.

In addition, the current study also raises another set of questions that focus on the possible upstream mechanism inducing Satb1 expression. How is Satb1 expression translated into binding at specific super-enhancers? Is this a pre-committed event e.g. dependent on the three-dimensional configuration of chromatin or close association within topologically associating domains or is this alternatively a rather stochastic event induced by probing certain differentiation programs within the developing thymocyte? It will be fascinating to follow how these key questions will be addressed in future studies and which of these possibilities will crystalize as the preferred pathway of T_{reg} cell lineage commitment.

One very practical implication of this study could be the translation of its findings into novel approaches to improve the therapeutic usage of T_{reg} cells for clinical applications. If T_{reg} cell stability and commitment is induced by fixation through epigenetic modifications at T_{reg} cell-specific super-enhancers dependent on Satb1 than this could provide a vital lesson for the generation and quality control of T_{reg} cell products used for transfusion into patients suffering from autoimmune diseases, graft-versus-host disease after stem cell transplantation or chronic graft rejection after solid organ transplantation to prevent loss of T_{reg} cell identity after transfer.

In summary, the study by Kitagawa *et al.* has altered our current view of T_{reg} cell lineage commitment within the thymus, extending it to an early, Stab1-dependent epigenetic imprinting of lineage identity at T_{reg} cell-specific super-enhancers, which allows T_{reg} cells to stably exert their unique immunosuppressive properties in a long-lasting manner.

Competing financial interest

The authors declare no competing financial interests.

Acknowledgements

MB is a member of the Excellence Cluster ImmunoSensation. This work was supported by the German Research Foundation (CRC738 to JH and BE 4427/3-1).

Figure: Thymic T_{reg} cell development is governed by Satb1-dependent super-enhancer establishment. Satb1 expression in double-positive (DP) or immature CD4 single-positive (imCD4SP) thymocytes precedes induction of precursor T_{reg} cells (pre-tT_{reg}) within the thymus. Satb1 acts as a pioneering factor of T_{reg} cell development by establishing T_{reg} cell-specific super-enhancers, as exemplified here for the *Foxp3* locus. Satb1 recruitment to a novel conserved non-coding sequence element (CNS0) within the *Foxp3* locus alters the epigenetic state of this T_{reg} cell-specific super-enhancer, e.g. relaxed chromatin, nucleosome positioning, and increased permissive histone marks, finally allowing the initiation of *Foxp3* expression through CNS3.

References

1. Kitagawa, Y. *et al*. *Nat. Immunol.* **18**, XXX–XXX (2017).
2. Lio, C.W. & Hsieh, C.S.. *Immunity* **28**, 100–111 (2008).
3. Tai, X. *et al. Immunity* **38**, 1116–1128 (2013).
4. Ohkura, N. *et al. Immunity* **37**, 785–799 (2012).
5. Toker, A. *et al. J. Immunol.* **190**, 3180–3188 (2013).
6. Hemberger, M., Dean, W. & Reik, W. *Nat. Rev. Mol. Cell Biol.* **10**, 526–537 (2009).
7. Whyte, W.A. *et al. Cell* **153**, 307–319 (2013).
8. Heinz, S. *et al. Mol. Cell* **38**, 576–589 (2010).
9. Alvarez, J.D. *et al. Genes Dev.***14**, 521–535 (2000).
10. Beyer, M. *et al. Nat. Immunol.* **12**, 898–907 (2011).
11. Cai, S., Han, H.J. & Kohwi-Shigematsu, T. *Nat. Genet.* **34**, 42–51 (2003).
12. Zheng, Y. *et al. Nature* **463**, 808–812 (2010).