A new mechanism of regulatory T cell generation to suppress excessive immune responses

—Non-coding DNA sequences indispensable for immune tolerance were discovered—

Keyword：Regulatory T cell (Treg)、Foxp3、Non-coding DNA sequences、Autoimmune disease

❖ Key points

- IFReC team identified non-coding DNA sequences[*1] indispensable for regulatory T cell (Treg) [*2] generation.
- These DNA sequences are distributed into the two separated regions near the Foxp3 gene[*3] locus, the master transcription factor of Tregs. Defect of these elements developed severe autoimmune-diseases[*4].
- Controlling the development and function of Tregs holds promise for the new therapies for immunological diseases.

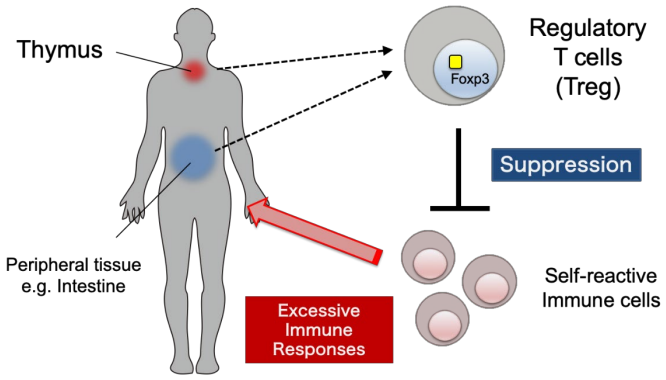
❖ Abstract

IFReC Experimental Immunology research team (Shimon Sakaguchi Lab.) investigated the genomic regions related to Treg development in the thymus. As a result, they found specific DNA elements indispensable for Treg development and the maintenance of immune homeostasis.

❖ Background

The immune system maintains immune tolerance and prevents excessive immune response to tolerable antigens such as self-derived molecules, foods, and symbiotic bacterial antigens. Regulatory T cells (Tregs) are one of the most significant cells in the maintenance of immune tolerance. Tregs work to maintain homeostasis of the body in various situations such as prevention of autoimmune diseases, termination of immune responses and damaging tissue repairing, by braking excessive immune responses.

Tregs are generated from a portion of antigen-stimulated $CD4^+$ T cells. The transcription factor Foxp3 is important for the suppressive function. The detailed mechanisms of Treg generation in the epigenetic and physical three-dimensional chromatin contexts are largely unknown.

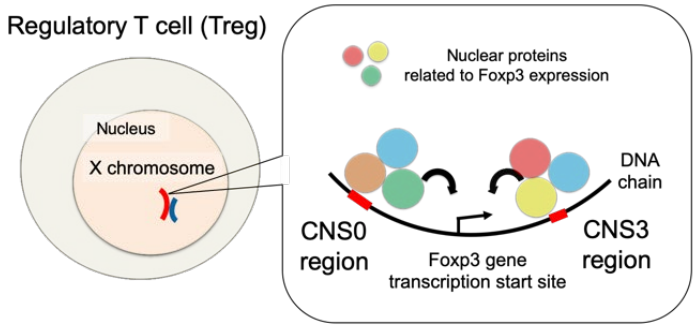


Regulatory T cells (Tregs) derived in the thymus / periphery suppress excessive immune responses.

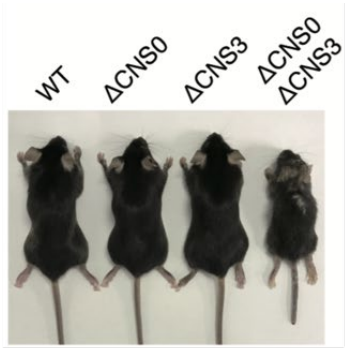
❖ The details of the present study

Treg cells are differentiated from CD4-positive T cells that encounter antigens in the thymus and periphery; e.g. intestinal tissues. Large numbers of Tregs are produced in the thymus during childhood. In order to clarify the developmental mechanism of Tregs, the research group used next-generation DNA sequencing technology[*5] to investigate the DNA regions that are activated during the differentiation of immature cells into Tregs in the mouse thymus.

As a result, two DNA elements were found as activated regions from the early stage of T cell differentiation scattered around the Foxp3 gene region, which is a transcription factor important for the differentiation and function of regulatory T cells. These two regions are located on the X chromosome and are small DNA regions of 400 base pairs and 200 base pairs, respectively, called the CNS0 and CNS3 regions. Importantly, CNS0 and CNS3 are non-coding DNA regions that do not have any sequence information for protein translation. However, the research team hypothesized that CNS0 and CNS3 may be involved in the development of regulatory T cells due to significant conservation of these sequences was found in mammalian genomes including humans.



CNS0, CNS3 regions on Chromosome X control the expression of Foxp3 gene.



CNS0 and CNS3 double-deficient mice cause severe auto-immune disease due to the defect of Treg development. (WT; Wild Type, Δ;CNS-deficient)

In this study, mice lacking either CNS0 or CNS3, or both, were generated using a genome-editing technology called the CRISPR/Cas9 system[*6], and the effects on the differentiation and function of regulatory T cells were examined. Mice lacking only one of CNS0 or CNS3 showed only a slight decrease in regulatory T cells, but the individuals developed normally. On the other hand, mice lacking both of the two sites simultaneously failed to differentiate into normal regulatory T cells in the thymus and developed serious autoimmune diseases in various organs of the body. These experiments demonstrated that the regulation of Foxp3 expression through non-coding DNA regions, plays an important role in the development of Treg cells and the establishment of immune tolerance.

CNS0 and CNS3 regions each harbored DNA sequences that were recognized by various nuclear proteins involved in regulatory T cell differentiation and function. However, these protein species did not necessarily overlap, suggesting that they are not functionally redundant. Furthermore, these regions are independently activated during T cell differentiation and form steric interactions between DNA regions around the Foxp3 gene. These results collectively suggest that CNS0 and CNS3 regions physically interact with the promoter region of the Foxp3 gene during the differentiation of regulatory T cells thereby control Foxp3 gene expression.

❖ Significance of this study

In many cases, there is no universal prevention or treatment regimens for diseases that damage the body by hyper-activation of the immune system, such as autoimmune diseases and new coronavirus-related diseases that are currently prevalent worldwide. This study revealed the significance of DNA elements for the function and development of Treg cells, which is a major step forward in understanding the mechanism of their development. This will enable us to develop methods to convert over-active T cells into Treg cells or create an environment in the body that facilitates Treg generation, which leads to the development of fundamental prevention and treatment methods for various immune diseases.

These results were published in an Elsevier's journal Immunity in collaboration with the Institute for Frontier Life and Medical Sciences, Kyoto University and Stanford University.

❖ Terminology

＊1：**Non-coding sequence**
Genomic DNA regions that are not used as information for protein translation. They occupy most of the total eukaryotic genome although the majority of their functions are unclear.

＊2：**Regulatory T cell; Treg**
A subset of CD4-positive helper T cell that specializes in immunosuppression.

＊3：**Foxp3 gene**
A transcription factor that is important for the function of regulatory T cells. It controls the expression of various genes and provides T cells with immunosuppressive properties.

＊4：**Autoimmune disease**
A term for various diseases that occur when the immune system misrecognizes to attack self.

＊5：**Next-generation DNA sequencing technology**
Recently advanced high-throughput DNA sequencing technologies that enable to rapid and comprehensively obtain information on the complete nucleotide sequence of a particular species' genome and gene expression.

＊6：**Genome-editing**
A technology that targets and modifies specific DNA sequences in the genome.

❖ Publication

Title: “Distinct Foxp3 enhancer elements coordinate development, maintenance and function of regulatory T cells”

Authors: Ryoji Kawakami*, Yohko Kitagawa*, Kelvin Y. Chen, Masaya Arai, Daiya Ohara, Yamami Nakamura, Keiko Yasuda, Motonao Osaki, Norihisa Mikami, Caleb A. Lareau, Hitomi Watanabe, Gen Kondoh, Keiji Hirota, Naganari Ohkura, and Shimon Sakaguchi**
(*; contributed equally, **; corresponding)

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