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研究課題名（和文）Identification of Genes Controlling Treg Development by Combining the CRISPR-Cas9 System and Bone Marrow Chimeric Mouse Model

研究課題名（英文）Identification of Genes Controlling Treg Development by Combining the CRISPR-Cas9 System and Bone Marrow Chimeric Mouse Model

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研究成果の概要（和文）：制御性 T 細胞(Treg)は免疫応答の抑制的制御に枢要な細胞群である。その発生、機能の分子基盤の解明により、自己免疫疾患の発症機構のさらなる理解が予想されます。筆者らは Tregの分化早期段階の Mbd3 欠損により、Treg 前駆細胞およびTreg が減少、それに従って、 マウスの多臓器における自己免疫疾患の発症を確認した。そこで、Mbd3がTregの分化制御により、自己寛容の樹立に関する肝要な役割を果たしていることを明らかにした。今回の研究により、自己免疫疾患発症の分子また細胞基盤につきまして、新たな知見を得られた。

研究成果の概要（英文）：Regulatory T cells (Tregs) play an indispensable role in maintaining immune homeostasis. Understanding the molecular basis governing their development will help to understand the pathogenesis of autoimmune diseases and establish new strategies for the treatment and prevention of immunological diseases. We found that Mbd3 plays an essential role in thymic Treg development, and thus establishment of immune homeostasis. Mice with T cell-specific Mbd3 deletion have impaired Treg development, development multiple autoimmune diseases and die prematurely. Development of autoimmune diseases could be prevented by adoptive transfer of wild type Tregs. The requirement of Mbd3 for Tregs is restricted at developmental stage, since mice with Treg-specific deletion of Mbd3 could largely maintain their immune homeostasis. Our findings expand the notion that the defects in genetic and epigenetic control of thymic Tregs development could be potentially the cause of a wide range of autoimmune diseases.

研究分野：　免疫学　制御性T細胞　エピジェネティクス

キーワード：　免疫寛容　自己免疫疾患　エピジェネティクス

１．研究開始当初の背景

Regulatory T cells (Tregs) play an indispensable role in maintaining immune homeostasis. The molecular basis of their functions has been intensively investigated, while the molecular mechanisms controlling their development is relatively untouched. Its understanding will help to understand the pathogenesis of autoimmune diseases and establish new strategies for the treatment and prevention of immunological diseases.

２．研究の目的

The purpose of this study is to identify critical regulators of thymic Treg (tTreg) development, explore their potential roles in the establishment of self-tolerance and investigate the underlying molecular mechanisms by which they control tTreg development.

３．研究の方法

Mice

T cell-specific *Mbd3* conditional knockout (CKO) mice were generated by crossing *Mbd3*$^{fl/fl}$ mice with *Cd4*-Cre^{+}mice. Treg specific *Mbd3* conditional knockout mice (FIC CKO) were generated by crossing *Mbd3*$^{fl/fl}$ mice with *Foxp3*-Cre^{+}mice. Both T cell-specific and Treg-specific *Mbd3* CKO mice were backcrossed onto BALB/c mice for 8 generations (C576L/6 →BALB/c N=8). In order to facilitate the detection and isolation of Tregs, some *Mbd3*$^{fl/fl}$*Cd4*-Cre^{+} were further crossed with *Foxp3-eGFP* fusion knock-in mice to generate *Mbd3*$^{fl/fl}$*Cd4*-Cre^{+} eFOX mice. *Rag2* KO mice (BALB/c background); CD45.1 wild type (WT) mice (BALB/c background); *CTLA-4* KO mice (BALB/c background).

Main methods

Flow cytometry analysis; histological analysis; serological analysis (ELISA); adoptive transfer of lymphocytes; generation of mixed bone marrow chimeric mice; next-generation sequencing (NGS) related methods (RNA-seq, ChIP-seq, ATAC-seq).

４．研究成果

1) Identification of Mbd3 as a key regulator of tTreg development.

By generation and analysis of CKO mice, we observed decreases of tTregs and their precursors as well as reductions of Tregs in lymph nodes and the spleen, revealing a critical role of Mbd3 in regulating tTreg development. Consistent with the established crucial role of Tregs in immune homeostasis, comparing to WT mice, CKO mice have a significantly shorter lifespan (80% mice died within 200 days).

2) Identification of a critical role of Mbd3 in the establishment of self-tolerance.

Based on the initial analysis, it was clear that in the absence of Mbd3, tTreg development was impaired and CKO mice died prematurely. It is conceivable that CKO mice failed to establish self-tolerance due to Treg deficiency and consequently developed lethal autoimmune diseases. To confirm this

possibility, we systemically characterized CKO mice. CKO mice showed increased cell numbers in lymph nodes and the spleen. Regarding the lymphocytes, more class switched B cells as well as plasma cells were observed in CKO mice; a higher proportion of T cells in CKO mice was revealed to be activated, and was identified as inflammatory cytokine-secreting effector cells. In addition, pathological changes involving multi-organs were identified by histological analysis. Notably, gastromegaly as well as thickening of gastric wall was grossly evident in CKO mice. Microscopic examination of the gastric tissue revealed mucosal hyperplasia and infiltration of mononuclear cells within the lamina propria. The titer of anti-parietal cell antibodies in the serum of CKO mice was detected to be several orders of magnitude higher than that in the serum of their WT counterparts. Furthermore, when T cells from CKO mice transferred into *RAG2* KO mice, they induced gastritis in the recipient mice with a high frequency. Taken together, mice with T cell-specific deletion of Mbd3 develop autoimmune diseases.

3) Demonstration of a causal role of defect of Tregs in the development of autoimmune diseases in CKO mice.

It is reasonable to assume that the onset of autoimmune diseases is due to the defect of tTreg development. However, another major possibility could be conceived. Namely, Mbd3 functions as a cell-intrinsic "check-point" within conventional T cells (Tconvs), the loss of which leads to aberrant activation of Tconvs and consequently breakdown of self-tolerance. Role for Mbd3 in tTreg development does not necessarily exclude its cell-intrinsic role within Tconvs per se. To exclude the latter possibility, we performed two additional experiments. First, bone marrow cells from CKO mice were mixed with that from WT counterparts in a 1:1 ratio, and were transferred into *RAG2* KO recipients. The resulting chimera mice never developed severe autoimmune gastritis, indicating that Mbd3 does not contribute to the establishment of self-tolerance by keeping Tconvs in check in a cell-intrinsic manner. Second, when co-transferred with splenocytes from CKO mice, WT Tregs prevented the onset of autoimmune gastritis which would develop when splenocytes from CKO mice transferred alone. Based on these results, we concluded that the onset of autoimmune diseases in CKO mice was due to the defect of Treg development.

4) Elucidation of the underlying molecular mechanisms by which Mbd3 control Tregs development.

Having established a critical role of Mbd3 in the induction of self-tolerance via controlling tTreg development, we next seek to understand the underlying molecular mechanisms. We carried out the profiling of transcriptomes, histone modifications and chromatin accessibility of cells at different stages during tTreg development. The analysis

revealed that in the absence of Mbd3, the chromatin accessibility was barely affected during tTreg development. Rather, Mbd3 contributes to the establishment of tTreg transcriptome via shaping lineage specific histone modifications during the developmental process.

In conclusion, our findings identify a key role of Mbd3 in the establishment of self-tolerance via controlling thymic Treg development and expand the notion that the defects in genetic and epigenetic control of thymic Treg development could be the cause of a wide range of autoimmune diseases.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計　0　件）

〔学会発表〕（計　1　件）

Jun Huang, Yohko Kitagawa, Atsushi Tanaka, Naganari Ohkura, Shimon Sakaguchi. Mbd3, a component of the NuRD complex, shapes the T-cell receptor repertoire of regulatory t cells. 3rd Cold Spring Harbor Laboratory meeting on Epigenetics & Chromatin. Cold Spring Harbor Laboratory, NY, USA. 2016

〔図書〕（計　0　件）

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〔その他〕
ホームページ等

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