様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　４ 年　 ５ 月 ２７ 日現在

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研究期間：2018～2021
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研究課題名（和文）The role of the transcription factor Tox2 in Treg and Tfh biology

研究課題名（英文）The role of the transcription factor Tox2 in Treg and Tfh biology

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交付決定額（研究期間全体）：（直接経費）　　3,400,000円

研究成果の概要（和文）：我々は、抗体制御に重要な制御性T細胞（Treg）および濾胞制御性T細胞（Tfr）の生物学上におけるTox2遺伝子の役割を調査した。Treg特異的Tox2ノックアウトシステムを使用し、Treg内のTox2を喪失させると機能欠損が起こり、抗体の産生を助けるT細胞（Tfh）と、高品質の抗体を産生するタイプの胚中心B細胞が増加することがわかった。さらに、Tox2遺伝子を従来のT細胞やTregに過剰発現させたところ、抗体制御に重要な遺伝子を増加させる機能があることが確認された。以上の結果から、Tox2が免疫応答後の制御細胞において重要な役割を担っていることが初めて明らかとなった。

研究成果の学術的意義や社会的意義
This study found new information regarding the control of regulatory T-cells. Since these cells are responsible for the control of vaccine responses and autoimmunity we believe that these results may allow us to control these cells and lead to the production of improved vaccines and medicines.

研究成果の概要（英文）：We explored the role of the Tox2 gene in the biology of regulatory T-cells (Treg) and T-follicular regulatory cells (Tfr), that are critical to control of antibodies. Using a Treg specific Tox2 knockout system we found that loss of Tox2 in Tregs caused a functional defect and an increased number of T-cells that help antibody production (Tfh) and germinal center B-cells, the type of B-cell that produces high quality antibodies. Additionally we found increased levels of IgA antibody that is important to control of immunity at the mucosal sites such as the gut. We also overexpressed the Tox2 gene in conventional T-cells and Tregs which confirmed its function to increase genes important to control antibody regulation. Taken together, our results demonstrate for the first time that Tox2 plays an important role in regulatory cells after immune challenge.

研究分野：Immunology

キーワード：Regulatory T-cells　T-follicular helper　T-follicular regulatory　Antibodies　Germinal center　Mass cytometry (CyTOF)

１．研究開始当初の背景

Our previous research focused on the regulation of T-follicular helper cell (Tfh) cell responses by Tregs and their subset Tfr[1]. Carrying on from that research, I further examined the phenotype of Tfr, being the first to discover that – in contrast to Tregs – mature Tfr in the germinal centers do not express CD25[2]. As part of this earlier work, I carried out RNA-sequencing of Tregs, Tfr and Tfh in both mice and humans, and while analyzing this data it became clear that Tox2 was significantly associated with Tfh, Tfr. At the time of beginning the grant there was no information regarding the function of Tox2 in Tregs. More broadly, very little was known about the function of Tox2 at this time aside from a potential role in the control of Natural killer (NK) cells meaning that the results would be highly novel. We also felt that a further ripple effect may be generated as the information and materials generated in this research, may spur broader interest in Tox2.

1. Wing, J. B., & Sakaguchi, S. (2014). Foxp3+ Treg cells in humoral immunity. International Immunology, 26(2), 61–69.
2. Wing, J. B. et al. (2017). A distinct subpopulation of CD25 − T-follicular regulatory cells localizes in the germinal centers. PNAS, 114(31), E6400–E6409.
3. Vong, QP.　et al. (2014). TOX2 regulates human natural killer cell development by controlling T-BET expression. Blood. 2014 Dec 18;124(26):3905-13.

２．研究の目的

This project focused on the elucidation of the role of the transition factor Tox2 in regulatory T-cells.

３．研究の方法

We used a range of methods in this project. Initially we generated a conditional knock out mouse system in which the Tox2 gene was floxed and then cross bred with Foxp3-cre mice to give Tregs deficient in Tox2 (Tox2-cKO).
As part of this project, we also developed a 45 parameter mass cytometry panel designed to provide fine analysis of murine T and B cells, in particular to allow the simultaneous assessment of Tfh, Tfr, Treg, GC B-cells and plasma cells. Using the cKO mice and analysis by mass cytometry system, we vaccinated Tox2 cKO mice, primarily with NP-Ova and assessed the changes to the murine immune system. We also observed aged mice for any incidence of autoimmunity of disrupted tissue homeostasis. To address the function of Tox2 in human cells we also used viral transfection and overexpression of the Tox2 gene into human T-cells and followed their functional changes with RNA-sequencing.　ATAC sequencing of human T-cells to gain further insight into the epigenetic regulation of Tox2 was also performed.

４．研究成果

We explored the role of the Tox2 gene in the biology of regulatory T-cells (Treg) and T-follicular regulatory cells (Tfr), that are critical to control of antibodies. Using a Treg specific Tox2 knockout system we found that loss of Tox2 in Tregs caused a functional defect and an increased number of T-cells that help antibody production (Tfh) and germinal center B-cells, the type of B-cell that produces high quality antibodies. Additional disruption to PD1 expressing T-cells was also seen suggesting that Tox2 deficient mice have some further disruption of exhausted T-cells.
Additionally, we found increased levels of IgA antibody that is important to control of immunity at the mucosal sites such as the gut.
In humans we found that Tox2 is widely expressed in Tfh and Tfr in the tonsils but that we could not see a clear difference in its expression in autoimmune disease such as Systemic lupus erythematosus (SLE). We also overexpressed the Tox2 gene in conventional T-cells and Tregs which confirmed its function to increase genes important

to the control of antibody regulation such as ASCL-2, BCL6, CXCR5 and PD1. Additionally, downregulation of the chemokine receptors CCR2 and CCR5 was seen further indicating tha tTox2 controls the localization and trafficking of Treg cells. ATAC-seq analysis also indicated that activated Tfr and Tfh cells have increased open chromatin regions of the Tox2 gene promotor indicating epigenetic control of Tox2 expression. We did not find any evidence that mice lacking Tox2 in Tregs developed any spontaneous autoimmunity or organ disfunction after aging. As a result, it seems that the function of Tox2 in Tregs lies primarily in the control of Tfr function. Taken together, our results demonstrate for the first time that Tox2 plays an important role in regulatory cells after immune challenge.

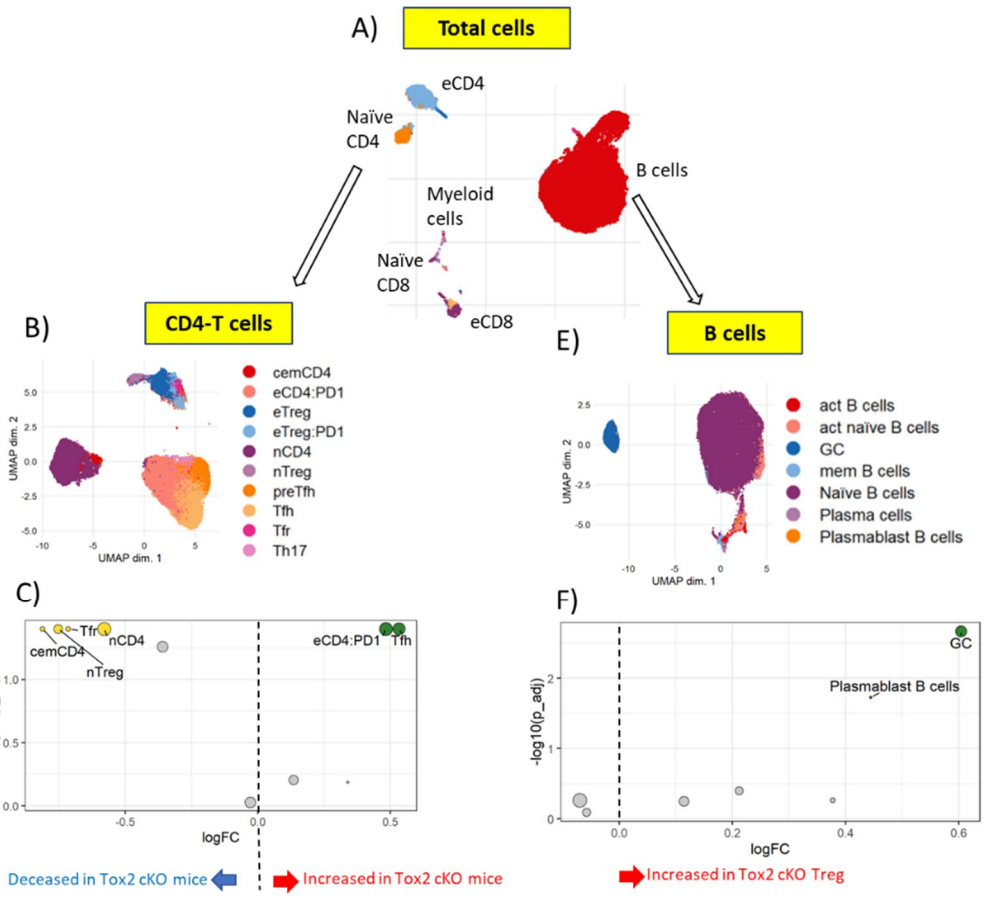

Figure 1: Effect of Treg specific Tox2 cKO. Mice were vaccinated with Np-Ova and 7 days later payer's patch cells isolated and assessed by mass cytometry analysis. A) Overall mass cytometry analysis of immune cells. B) Dimensionality reduction and population identification of CD4 T-cells. C) Volcano plot showing significant changes in cellular phenotypes in Tox2 cKO mice. E) Dimensionality reduction and population identification of B-cells. F) Volcano plot showing significant changes in B-cell cellular phenotypes in Tox2 cKO mice.

This study found new information regarding the control of regulatory T-cells and their subset Tfr by the chromatin remodeler Tox2. Since these cells are responsible for the control of vaccine responses and autoimmunity, we believe that these results may allow us to control these cells and lead to the production of improved vaccines and medicines. The primary conclusions of this work are now being prepared for publication. Several ripple effects were also seen since the mass cytometry panel development that we conducted as part of this study has also been applied by us in several collaborative projects for the analysis of murine Tfh, Tfr and germinal center biology. Additionally, several groups have contacted us to obtain the Tox2 flox mouse line for use in their own research.

５．主な発表論文等

〔雑誌論文〕　計12件（うち査読付論文　8件／うち国際共著　10件／うちオープンアクセス　2件）

１．著者名 Y Yang, X Li, Z Ma, C Wang, Q Yang, M Byrne-Steele, R Hong, Q Min, G Zhou, Y Cheng, G Qin, JV. Youngyunpipatkul, JB. Wing, S Sakaguchi, C Toonstra, L Wang, JG. Vilches-Moure, D Wang, MP. Snyder, J Wang, J Han, L Herzenberg	４．巻 12
２．論文標題 CTLA-4 expression by B-1a B cells is essential for immune tolerance	５．発行年 2021年
３．雑誌名 Nature Communications	６．最初と最後の頁 521
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41467-020-20874-x	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Wing James B.Lim Ee Lyn、Sakaguchi Shimon	４．巻 296
２．論文標題 Control of foreign Ag‐specific Ab responses by Treg and Tfr	５．発行年 2020年
３．雑誌名 Immunological Reviews	６．最初と最後の頁 104～119
掲載論文のDOI（デジタルオブジェクト識別子） 10.1111/imr.12888	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Shigeta Naoya、Kumasawa Keiichi、Tanaka Atsushi、Badger Wing James、Nakamura Hitomi、Sakaguchi Shimon、Kimura Tadashi	４．巻 140
２．論文標題 Dynamics of effector and naive Regulatory T cells throughout pregnancy	５．発行年 2020年
３．雑誌名 Journal of Reproductive Immunology	６．最初と最後の頁 103135～103135
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.jri.2020.103135	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Sakaguchi Shimon、Mikami Norihisa、Wing James B.、Tanaka Atsushi、Ichiyama Kenji、Ohkura Naganari	４．巻 38
２．論文標題 Regulatory T Cells and Human Disease	５．発行年 2020年
３．雑誌名 Annual Review of Immunology	６．最初と最後の頁 541～566
掲載論文のDOI（デジタルオブジェクト識別子） 10.1146/annurev-immunol-042718-041717	査読の有無 有
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1．著者名 Wing James Badger、Tay Christopher、Sakaguchi Shimon	4．巻 1189
2．論文標題 Control of Regulatory T Cells by Co-signal Molecules	5．発行年 2019年
3．雑誌名 Co-signal Molecules in T Cell Activation	6．最初と最後の頁 179～210
掲載論文のDOI（デジタルオブジェクト識別子） 10.1007/978-981-32-9717-3_7	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

1．著者名 Cossarizza A,…150 authors…,Wing, JB….16 authors… Zychlinsky A	4．巻 49
2．論文標題 Guidelines for the use of flow cytometry and cell sorting in immunological studies (second edition)	5．発行年 2019年
3．雑誌名 European Journal of Immunology	6．最初と最後の頁 1457～1973
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/eji.201970107	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

1．著者名 Wing James B.、Tanaka Atsushi、Sakaguchi Shimon	4．巻 50
2．論文標題 Human FOXP3+ Regulatory T Cell Heterogeneity and Function in Autoimmunity and Cancer	5．発行年 2019年
3．雑誌名 Immunity	6．最初と最後の頁 302～316
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.immuni.2019.01.020	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

1．著者名 Ha Danbee、Tanaka Atsushi、Kibayashi Tatsuya、Tanemura Atsushi、Sugiyama Daisuke、Wing James Badger、Lim Ee Lyn、Teng Karen Wei Weng、Adeegbe Dennis、Newell Evan W.、Katayama Ichiro、Nishikawa Hiroyoshi、Sakaguchi Shimon	4．巻 116
2．論文標題 Differential control of human Treg and effector T cells in tumor immunity by Fc-engineered anti-CTLA-4 antibody	5．発行年 2018年
3．雑誌名 Proceedings of the National Academy of Sciences	6．最初と最後の頁 609～618
掲載論文のDOI（デジタルオブジェクト識別子） 10.1073/pnas.1812186116	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

1．著者名 Wing James B.、Tekguc Murat、Sakaguchi Shimon	4．巻 9
2．論文標題 Control of Germinal Center Responses by T-Follicular Regulatory Cells	5．発行年 2018年
3．雑誌名 Frontiers in Immunology	6．最初と最後の頁 1910-1917
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fimmu.2018.01910	査読の有無 有
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1．著者名 木本　富子, Wing James B.	4．巻 268
2．論文標題 濾胞性制御性T細胞	5．発行年 2019年
3．雑誌名 Journal of Clinical and Experimental Medicine (Igaku No Ayumi)	6．最初と最後の頁 1106-1110
掲載論文のDOI（デジタルオブジェクト識別子） なし	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

1．著者名 Wing James B.、Sakaguchi Shimon	4．巻 T-Follicular Helper Cells
2．論文標題 Using Mass Cytometry to Address Tfh and Tfr Heterogeneity	5．発行年 2021年
3．雑誌名 Methods in molecular biology	6．最初と最後の頁 47～57
掲載論文のDOI（デジタルオブジェクト識別子） 10.1007/978-1-0716-1736-6_5	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

1．著者名 Tulyeu Janyerkye、Sondergaard Jonas N.、Sakaguchi Shimon、Wing James B.	4．巻 1
2．論文標題 Isolation and Characterization of Both Human and Mouse Tfh/Tfr Cells	5．発行年 2021年
3．雑誌名 Current Protocols	6．最初と最後の頁 e283
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/cpz1.283	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計10件（うち招待講演　7件／うち国際学会　2件）

1．発表者名
James Wing

2．発表標題
High dimensional analysis of T-follicular regulatory cells

3．学会等名
IMSUT young investigators symposium “Rising stars in cutting edge Immunology research”（招待講演）

4．発表年
2020年

1．発表者名
James Wing

2．発表標題
Exploring regulatory T cell function and phenotype with mass cytometry

3．学会等名
2019 Japanese society for Immunology, lunch time technical seminar（招待講演）

4．発表年
2019年

1．発表者名
James Wing

2．発表標題
High dimensional analysis of T-follicular regulatory cells

3．学会等名
2019 Japanese society for Immunology

4．発表年
2019年

1．発表者名
James Wing

2．発表標題
Exploring regulatory T cell function and phenotype with mass cytometry

3．学会等名
8th Annual Fluidigm Mass Cytometry Summit（招待講演）

4．発表年
2019年

１．発表者名
Wing James B.

２．発表標題
T-follicular regulatory cells in mice and humans

３．学会等名
Invited lecture at National cancer center, Chiba.（招待講演）

４．発表年
2018年

１．発表者名
Wing James B.

２．発表標題
T-follicular regulatory cells in mice and humans

３．学会等名
Invited lecture at Nagoya city university, Nagoya.（招待講演）

４．発表年
2018年

１．発表者名
Wing James B.

２．発表標題
Exploring regulatory T-cell phenotype and function in mice and humans with CyTOF

３．学会等名
第1回 Japan CyTOF User meeting, Tokyo.（招待講演）

４．発表年
2018年

１．発表者名
Wing James B.

２．発表標題
T-follicular regulatory cells in human blood

３．学会等名
47th Annual meeting of the Japanese society for Immunology（国際学会）

４．発表年
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Wing James B.

２．発表標題
High dimensional analysis of T-follicular regulatory cells

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B Cell-T cell interactions, Keystone Symposium, Colorado, USA（国際学会）

４．発表年
2019年

１．発表者名
Wing James B.

２．発表標題
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３．学会等名
25th annual meeting of the Japanese society for vaccinology.（招待講演）

４．発表年
2021年

〔図書〕　計1件

１．著者名	４．発行年
Wing, James Badger. Shimon Sakaguchi.	2018年
２．出版社	５．総ページ数
Elsevier	1319
３．書名	
Clinical Immunology: Principles and practice. Fifth Edition. Chapter: Regulatory Immune cells.	

〔産業財産権〕

〔その他〕

６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関