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

平成　２９　年　　５　月　３１　日現在

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研究種目：　若手研究(B)
研究期間：　2015～2016
課題番号：　１５Ｋ１９１２９

研究課題名（和文）Role of Tfh and Tfr in autoimmunity

研究課題名（英文）Role of Tfh and Tfr in autoimmunity

研究代表者
ウィング　ジェイムス（Wing, James）

大阪大学・免疫学フロンティア研究センター・特任助教（常勤）

研究者番号：００６４８６９４

交付決定額（研究期間全体）：（直接経費）　　3,100,000円

研究成果の概要（和文）：濾胞性制御性T細胞（T-follicular regulatory T-cells、以下Tfrと略）は、胚中心B細胞による抗体反応の制御に重要である。本研究では、Tfrが高親和性IL-2受容体であるCD25分子の発現を欠失し、本来IL-2に阻害されるBCL-6転写因子を発現することにより、成熟した胚中心に常在する細胞群に分化することを明らかにした。分化したTfr細胞群は、ヒトとマウスに共通して確認された。これらの結果は、制御性T細胞が、IL-2に依存的・非依存的な細胞群に分類されることを示し、また、この理解は、IL-2を用いた臨床における制御性T細胞の制御法に重要である。

研究成果の概要（英文）：T-follicular regulatory T-cells (Tfr) have a critical role for controlling antibody responses by germinal center B-cells. I have discovered that Tfr can differentiate into a mature germinal center resident subset characterised by a lack of expression of the high affinity IL-2 receptor, CD25. This allows the cells to upregulate the IL-2 inhibited transcription factor BCL-6 and travel to the germinal center to suppress B-cells. These cells can be found in both mice and humans and unlike most regulatory T-cells (Tregs), of which Tfr are a subgroup, are inhibited by the presence of IL-2. This demonstrates a fundamental split in regulatory T-cells into IL-2 dependent Tregs and IL-2 independent Tfr and has important implications for many clinical trials using IL-2 to control Treg function.

研究分野：　Immunology

キーワード：　Regulatory T-cells　Humoral Immunity

１．研究開始当初の背景

The T-dependent antibody response is critical to the defense from infection in a wide range of settings. However, it may also generate autoantibody responses that may prove harmful to the host organism. T-follicular helper cells (Tfh) are critical for this process. Following expression of the lineage defining transcription factor BCL6 Tfh travel to the germinal center and provide T-cell help by the delivery of CD40 signaling, IL-21 and production of other cytokines (Crotty, 2011). This in turn allows the formation of B-cell germinal centers (GCs) that are responsible for the generation of high affinity antibody.

Regulatory T-cells (Tregs) are vital for the maintenance of humoral immune homeostasis (Wing and Sakaguchi, 2014). Tregs also undergo a similar pathway of differentiation to Tfh allowing them to gain expression of the transcription factor BCL6 and become T-follicular regulatory cells (Tfr) (Chung et al., 2011; Linterman et al., 2011) which then regulate Tfh and GC formation. However, due to their recent discovery the biology of Tfr is not well understood.

Citations:

Crotty, S. (2011). Follicular helper CD4 T cells (TFH). Annual Review of Immunology, 29, 621–663.

Chung, Y., et al. (2011). Follicular regulatory T cells expressing Foxp3 and Bcl-6 suppress germinal center reactions. Nat Med 17, 983-988.

Linterman, M.A., et al. (2011). Foxp3+ follicular regulatory T cells control the germinal center response. Nat Med 17, 975-982.

Wing, J. B., & Sakaguchi, S. (2014). Foxp3+ Treg cells in humoral immunity. International Immunology, 26(2), 61–69.

２．研究の目的

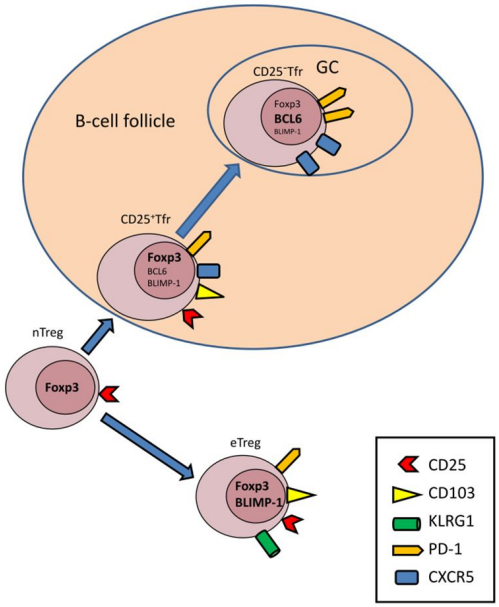
This project aimed to better characterize Tfr and understand the role of Tfh and Tfr in autoimmunity.

３．研究の方法

Several different approaches were used to achieve the research results. Flow cytometry and RNA-sequencing was used to characterize and phenotype the new Tfr populations. Assessment of the epigenetic status of the cells by measuring DNA demethylation was used to confirm that the CD25-Tfr were truly Tregs. In vitro assays of suppressive function were used to define the suppressive capability of the cells. In vivo murine experiments in which IL-2 was either added or blocked by anti-IL-2 antibodies were used to better define the regulation of the cells by IL-2. Human blood and tonsils were used to identify the presence of the CD25-Tfr in humans.

４．研究成果

This project has led to an important finding in Tfr biology. I have discovered that Tfr can differentiate into a mature germinal center resident subset characterised by a lack of expression of the high affinity IL-2 receptor, CD25. This allows the cells to upregulate the IL-2 inhibited transcription factor BCL-6 and travel to the germinal center to suppress B-cells. These cells are found in both mice and humans and unlike most regulatory T-cells (Tregs), of which Tfr are a subgroup, are inhibited by the presence of IL-2. The CD25 negative Tfr display an overall gene-expression pattern equidistant between Tfh and effector Tregs demonstrating that this is a significant shift in their identity. However despite this they retain expression of key Treg suppressive molecules such as CTLA-4 that we have previously demonstrated to be critical for Tfr control of Tfh responses (Wing et al. 2014). Further to this the CD25-Tfr retain demethylation of Foxp3 CNS2 demonstrating that they are epigenetically stable Tregs and we also demonstrate that these cells are formed from thymically derived naïve $CD25^{+}$Tregs but not converted from Tfh or other Foxp3 negative cells. These findings demonstrate a fundamental split in regulatory T-cells into IL-2 dependent effector Tregs, characterise by expression of BLIMP-1, KLRG1 and CD103 and IL-2 independent CD25-Tfr and has important implications for many clinical trials using IL-2 to control Treg function.



Graphical abstract

Graphical abstract: Naïve Tregs (nTregs) may differentiate into CD25+ effector Tregs or travel into the B-cell follicle where they become at first CD25+Tfr before losing CD25 and moving into the germinal Center. This work has recently been accepted for publication at PNAS (Wing et al 2017).

Citations:

Wing, J. B., Ise, W., Kurosaki, T., & Sakaguchi, S. (2014). Regulatory T cells control antigen-specific expansion of Tfh cell number and humoral immune responses via the co-receptor CTLA-4. Immunity, 41(6), 1013–1025.

Wing JB, Kitagawa Y, Locci M, Hume H, Tay C, Morita T, Kidani Y, Matsuda K, Inoue T, Kurosaki T, Crotty S, Coban C, Ohkura N, Sakaguchi S.
A distinct subpopulation of CD25 negative T-follicular regulatory cells localizes in the germinal centers. Accepted for publication in PNAS, 2017.

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　3　件）

Wing JB, Kitagawa Y, Locci M, Hume H, Tay C, Morita T, Kidani Y, Matsuda K, Inoue T, Kurosaki T, Crotty S, Coban C, Ohkura N, Sakaguchi S.
A distinct subpopulation of CD25 negative T-follicular regulatory cells localizes in the germinal centers. Accepted for publication in PNAS, 2017.

Glatman A, Konradt C, Dépis F, Wing JB, Goenka R, Atria DG, Silver JS, Cho S, Wolf AI, Quinn WJ, Engiles JB, Brown DC, Beiting D, Erikson J, Allman D, Cancro MP, Sakaguchi S, Lu LF, Benoist CO, Hunter CA.
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Science. 2015 Aug 28;349(6251):989-93.

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2. Wing JB. A germinal center resident subset of T-follicular regulatory cells. Keystone symposium: T Follicular Helper cells and Germinal Centers. Hyatt Regency Montery, Montery, California, USA. 2016/2/28.

3. Wing JB. T-follicular regulatory cells. Invited lecture at National Cancer Center, Kashiwa, Chiba, Japan. 2015/11/24.

〔図書〕（計　3　件）

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Wing JB, Sakaguchi S. Encyclopedia of Immunobiology: Treg cells. Elsevier. 2016, 5 pages.

Kitagawa Y, Wing JB, Sakaguchi S. Progress in Molecular Biology and Translational Science: Transcriptional and epigenetic control of Regulatory T-cell development. Elsevier. 2015, 33 pages.

〔産業財産権〕

○出願状況（計　0　件）

名称：
発明者：
権利者：
種類：
番号：
出願年月日：
国内外の別：

○取得状況（計　0　件）

名称：
発明者：
権利者：
種類：
番号：
取得年月日：
国内外の別：

〔その他〕
ホームページ等

６．研究組織
(1)研究代表者
James Badger Wing　(James Badger Wing)
大阪大学・免疫学フロンティア研究センター・特任助教
研究者番号：２０５７２３６６

研究者番号：
(　　)

(2)研究分担者
(　　)

研究者番号：

(3)連携研究者
(　　)

研究者番号：

(4)研究協力者
(　　)