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研究課題名（和文）Mechanisms of regulatory T-cell　control of humoral immunity

研究課題名（英文）Mechanisms of regulatory T-cell control of humoral immunity

研究代表者
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研究成果の概要（和文）：制御性T細胞(Treg)が抑制性分子であるCTLA4を用いて、濾胞性ヘルパーT細胞（Tfh）の分化を制御し、さらにB細胞胚中心の形成を制御する事を発見した。この現象は、Tfh形成にとって重要な抗原提示B細胞や樹状細胞の細胞表面に発現する共刺激分子であるCD80, CD86の発現が、Tregに発現するCTLA4分子の作用により減少することで引き起こされる。Tfhが高親和性抗体の産生やメモリーB細胞の形成に極めて重要であることから、今回の結果よりTregがCTLA4によってTfh形成を制御する事で、抗体産生を制御し得ることを示す事ができた.

研究成果の概要（英文）：I discovered that Tregs control the formation of Tfh and resulting B-cell germinal center reactions via the suppressive molecule CTLA-4. This is achieved by depletion of the costimulatory molecules CD80 and CD86 from the surface of antigen presenting B-cells and dendritic cells which are critical for the formation of Tfh. Since Tfh are vital for the production of high quality antibody responses and B-cell memory this means that Treg are able to control the production of antibody via CTLA-4.

研究分野：Immunology

キーワード：Regulatory T-cells　T follicular helper　Vaccination　Antibody response

１．研究開始当初の背景

The humoral immune response is critical to protection from pathogens but when incorrectly regulated may cause autoimmunity. B-cells have a dual role as both the source of antibody and also as antigen presenting cells. It has become clear that the role of B-cell antigen presentation and co-stimulatory marker expression is critical to the formation of T-follicular helper cells (Tfh) (Salek-Ardakani et al., 2011).

T-follicular helper cells are a T-helper subset that travel to the B-cell follicle and deliver help to B-cells allowing expansion of antigen specific germinal center B-cells, production of B-cell memory and antibody secreting plasma cells.

Foxp3 expressing regulatory T-cells (Tregs) are critical to the regulation of the immune response, their primary mode of function is via the Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4) dependent control of the co-stimulatory molecules CD80 and CD86 on the surface of antigen presenting cells (Yamaguchi et al., 2011), although it is currently unknown if Treg CTLA-4 is critical to their control of humoral immunity. More recently it has also been discovered that a specialized subset of $Foxp3^+$ Tregs express BCL-6, a transcription factor that allows the localization of Tregs to the B-cell follicle where they become T-follicular Tregs (Tfreg) and control expansion of both germinal center B-cells and Tfh cells by an as yet undefined mechanism (Chung et al., 2011; Linterman et al., 2011).

２．研究の目的

This research project sought to determine the mechanisms by which regulatory T-cells and Tfr control the formation of Tfh and germinal centers. This is critical to understanding the vaccine response.

３．研究の方法

In order to achieve the research objectives I used several different approaches. We measured vaccine responses by both flox cytometry and ELISA. This allowed us to measure both the cellular events such as formation of germinal centers, plasma cells and memory B-cells and also both the quality and quantity of serum antibody levels. In order to assess the effects on both antigen specific B-cells and antigen specific T-cells after vaccination I used the haptan nitrophenol (NP) to detect antigen specific B-cells and MHCII tetramer to allow use to detect antigen specific T-cells. We used a magnetic bead enrichment method to allow collection of rare antigen specific T-cells. To assess the effect of Treg depletion we used a genetically modified mouse strain in which specific depletion of Tregs is possible. To further investigate the mechanisms of Treg function we used a conditional knock out mouse model. In this we used the cre-lox system in which the CTLA-4 gene is flanked by flox excision sites. In most cells this have no effect but by crossing with a Foxp-cre gene in which only Foxp3 expressing cells express cre-recombinase which removes the flox flanked gene we were able to assess the effect of Treg specific loss of CTLA-4 expression. We then confirmed these results using an anti-CTLA-4 blocking antibody system.

４．研究成果

The goals of the project we met. I discovered that Tregs and Tfr control the formation of Tfh and resulting germinal center reactions via the suppressive molecule CTLA-4. This is achieved by depletion of the costimulatory molecules CD80 and CD86 from the surface of antigen presenting B-cells and dendritic cells which are critical for the formation of Tfh. Since Tfh are vital for the production of high quality antibody responses and B-cell memory this means that Treg are able to control the production of antibody via CTLA-4 making understanding of CTLA-4 function critical to vaccination, infectious disease and autoantibody driven autoimmune diseases such as Systemic lupus erythematosus and Type-1 diabetes. The project was published in the journal “Immunity”, novel methodology that we developed in this project was published in “Methods in molecular biology”.

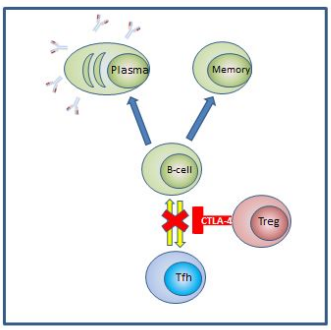

Figure 1: Visual abstract of data. Tregs inhibit Tfh B-cell interaction via CTLA-4.

Citations:
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.
Salek-Ardakani, S., et al. (2011). B cell-specific expression of B7-2 is required for follicular Th cell function in response to vaccinia virus. J Immunol 186, 5294-5303.
Yamaguchi, T., Wing, J.B., and Sakaguchi, S. (2011). Two modes of immune suppression by Foxp3(+) regulatory T cells under inflammatory or non-inflammatory conditions. Semin Immunol 23, 424-430.

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 5 件）

1. Wing JB, Sakaguchi S. In vivo induction of T-follicular helper cells by modulation of regulatory T cell function.
Methods Mol Biol. 2015;1291:77-85.
doi: 10.1007/978-1-4939-2498-1_7.

2. Wing JB, Ise W, Kurosaki T, Sakaguchi S. Regulatory T cells control antigen-specific expansion of Tfh cell number and humoral immune responses via the coreceptor CTLA-4.
Immunity. 2014 Dec 18;41(6):1013-25.
doi: 10.1016/j.immuni.2014.12.006.

3. Maeda Y, Nishikawa H, Sugiyama D, Ha D, Hamaguchi M, Saito T, Nishioka M, Wing JB, Adeegbe D, Katayama I, Sakaguchi S.
Detection of self-reactive $CD8^+$ T cells with an anergic phenotype in healthy individuals.
Science. 2014 Dec 19;346(6216):1536-40.
doi: 10.1126/science.aaa1292.

4. Schubert D, Bode C, Kenefeck R, Hou TZ, Wing JB, (31 other authors), Sakaguchi S, Walker LS, Sansom DM, Grimbacher B.
Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations.
Nat Med. 2014 Dec;20(12):1410-6.
doi: 10.1038/nm.3746. Epub 2014 Oct 20.

5. Wing JB, Sakaguchi S. $Foxp3^+$ T(reg) cells in humoral immunity.
Int Immunol. 2014 Feb;26(2):61-9.
doi: 10.1093/intimm/dxt060.

〔学会発表〕（計 7 件）

1. Wing JB. Regulatory T-cells control humoral immune responses and Tfh formation via CTLA-4. Keystone symposium: T-cells regulation and effector function. Snowbird resort, Utah, USA. 2015/03/30.

2. Wing JB. Regulatory T-cells control humoral immune responses and Tfh formation via CTLA-4. The 43rd annual meeting of the Japanese society for Immunology. Kyoto international conference center Japan. 2014/12/12.

3. Wing JB. Regulatory T-cells control humoral immune responses and Tfh formation via CTLA-4. The fourth international conference on regulatory T-cells and T-helper subsets and clinical application in human diseases. Renaissance hotel, Shanghai, China. 2014/11/4.

4. Wing JB. Regulatory T-cells control humoral immune responses and Tfh formation via CTLA-4. The 13th Awaji international forum on infection and immunity in Nara. Nara prefectural new public hall, Japan. 2014/09/24.

5. Wing JB. Regulatory T-cells modulate T-follicular helpers and germinal center formation via CTLA-4. The 42nd annual meeting of the Japanese society for Immunology. Makuhari Messe, Chiba, Japan. 2013/12/12

6. Wing JB. Regulatory T-cells modulate T-follicular helpers and germinal center formation via CTLA-4. Gordon research conference. T-follicular helper cells: Basic discoveries and clinical applications. The Chinese university of Hong Kong. Hong Kong, China. 2013/07/04.

7. Wing JB. Regulatory T-cells modulate T-follicular helpers and germinal center formation via CTLA-4. Kyoto T-cell conference 2013. Shiran Kaikan, Kyoto, Japan. 2013/06/07.

〔図書〕（計 1 件）

Wing JB, 森田貴義, 坂口志文","制御性 T 細胞による Tfh 細胞および胚中心形成制御機構 (特集 濾胞ヘルパー T (Tfh) 細胞の分化と胚中心形成)",2015,"臨床免疫・アレルギー. 92.

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