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研究課題名（和文）The regulation of the ubiquitin-editing protein A20 in thymic selection

研究課題名（英文）The regulation of the ubiquitin-editing protein A20 in thymic selection

研究代表者
郭　芸（Guo, Yun）

広島大学・医歯薬保健学研究科（医）・助教

研究者番号：５０６０９７６６

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研究成果の概要（和文）：獲得免疫系の司令塔であるT細胞の運命決定は、胸腺という「場」で行われる。しかし、今までの研究で明らかになった部分もあるが、「胸腺でのT細胞の選択」は、以前不明な点が多い。A20(TNFAIP3)はユビキチン修飾酵素であり、多くの疾患との関連が報告されている。今回、我々は、T細胞特異的なA20遺伝子欠損マウスを作成し、胸腺選択におけるA20の機能を検討した。その結果、１）T細胞（Treg, iNKT, nIEL）の選択に重要な役割を果たしている、２）A20欠損によるTreg細胞の減少はTCRシグナル変化と関係する、３）A20欠損により、抗腫瘍活性低下、自己免疫疾患EAEの症状緩和、が観察できた。

研究成果の学術的意義や社会的意義
Knowledge gained from this study will significantly improve our understanding of T cell biology, and will provide important information to advance the design of novel cell-based therapies for human cancer and autoimmune diseases as well as allergenic response.

研究成果の概要（英文）：Thymic selection decides the T cell fate; therefore it decides our body to be healthy or sick. However, the mechanism that underlies the regulation of thymic selection is still poorly understood. A20, also known as TNFAIP3, is a ubiquitin - modifying enzyme and related with multiple autoimmune and inflammatory diseases. In this study, we used A20 T-cell specific deficient mice to examine the role of A20 in thymic selection.
As a result, 1) We found that A20 plays an important role in thymic selection to decide the T cell fate, like increased Treg cells, reduced iNKT and nIEL subsets in A20 deficient mice. 2) The increase of Treg cells in A20 deficient mice was related with the changed TCR signal. 3)We examined the role of A20 deficient T cells in body, and found that loss of A20 in T cell will impair antitumor immunity and reduce the severity of the EAE induction in mice.

研究分野：免疫学

キーワード：A20　thymic selection

１．研究開始当初の背景

As a part of the adaptive immune system, T cells have sophisticated and specific mechanism against pathogens. Thymic selection decides the T cell fate. The successfully passed mature T cells enable no reaction against self-molecules or harmless foreign ones; otherwise they will result in harmful autoimmune or allergic response. However, the molecular mechanisms underlying the regulation of thymic selection is still a mystery.

(1) Thymic selection depends on the affinity of interaction between TCR and self-peptide-MHC complex. Thymocyte expressing a TCR that binds with an appropriate affinity to a self-peptide will receive an optimal signal to survive and differentiate (positive selection). The strong TCR signal can drive clonal deletion (negative selection), which is a major mechanism for self-tolerance. However, some cells seem to escape this fate, they include natural T regulatory cells (nTreg), invariant natural killer T cells (iNKT) and natural CD8aa intraepithelial T cells (nIEL). Some reports showed that these cells have an overall enhanced self-reactivity compared with conventional T cells, their TCR affinity for self peptide- MHCII complex are intermediate between positive selection and negative selection, defined as agonist selection (Fig1). Whether agonist-selected cell development and clonal deletion occur at similar or distinct thresholds, and how the TCR strength and duration regulate the development of these distinct T cell subsets are still unclear.

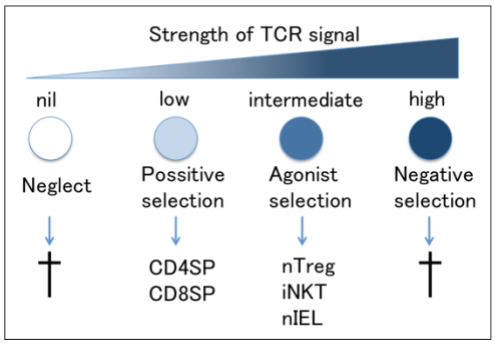

Fig1. Thymic selection and T cell fate in thymus.

(2) Ubiquitination is a key complex mechanism for the regulation of cell signaling and protein modification, and T cells are sensitive to these ubiquitin-mediated regulations. The A20 protein (also known as tumor necrosis factor alpha-induced protein3 or TNFAIP3) has deubiquitinating, E3 ubiquitin ligase and ubiquitin-binding activity (Fig2a). Recent genetic studies also show the association of polymorphisms in the human A20 locus with multiple autoimmune and inflammatory diseases (Fig2b). Thus, the function of A20 in T cells and TEC, especially the role of A20 in thymic selection is an important question to be defined.

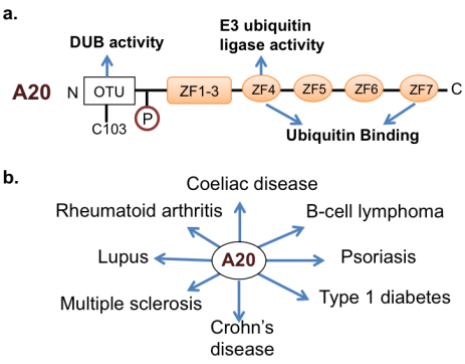

Fig2. a. Doman structure of A20. b. A20 is a susceptibility gene for multiple diseases.

２．研究の目的

Thymic selection decides the T cell fate. Therefore, it decides our body to be healthy or sick. However, the mechanism that underlies the regulation of thymic selection is still poorly understood. A20, as a ubiquitin editor, is related with multiple autoimmune and inflammatory diseases. In this study,

(1) To examine the role of A20 in thymic selection to decide the T cell fate;

(2) To clarify the molecular mechanism that regulates thymic selection by TCR signal etc.;

(3) To examine the role of A20 deficient T cell in cancer and autoimmune disease.

３．研究の方法

To assess the function of A20 in Thymic selection, we mainly use several mice models. To specifically delete the A20 expression, A20 floxed mice were crossed with the Cre-transgenic mice with different promoters

４．研究成果

(1) A20 plays an important role in agonist selection

①In order to elucidate the role of A20 in T cell, especially in thymic selection. We crossed A20 floxed mice with CD4-Cre mice to specificlly delete A20 in T cells from double positive (DP)stage. There was no clear difference appeared in T cell development from DP to single positive (SP) stage. Thymic selection decides the T cell fate such as Treg cell, iNKT and nIEL. We firstly examined the Treg cells in thymus, and found that the number of Treg cells in cKO adult mice was significantly higher than that of control mice. We also found more Treg cells in periphery of A20 deficient mice. To confirm if these increased Treg cell is differentiated from thymus or from blood circulation, we investigated the Treg cell in thymus from baby mice born within 4 days. More Treg cells were showed in A20 deficient thymus (Fig3a). We took embryo thymus on day 16 to perform fetal thymus organ culture (FTOC) in vitro (Fig3b). Similar results were observed in those from baby mice. These results suggest that the deletion of A20 from DP stage will affect the development of Treg cell in thymus.

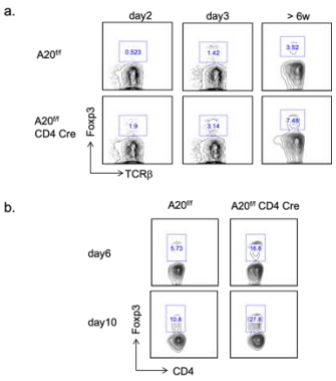

Fig3. a. More Treg cells in A20 cKO mice. b. More Treg cells produced in A20 cKO fetal thymus in FTOC.

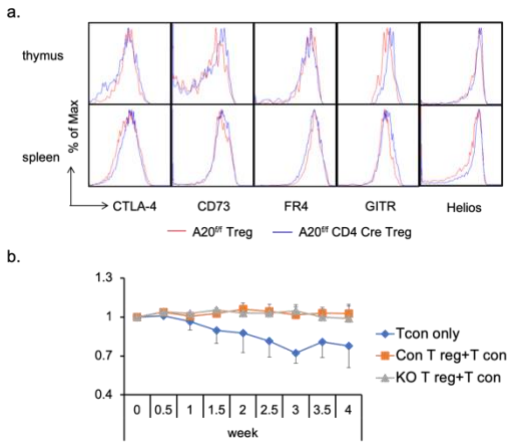

Fig4. a. Normal expression of Treg signature molecules in A20 cKO Treg. b. In vivo suppression assay.

②Next, to investigate whether these increased Treg cell is normal and functional. We examine the expression of several Treg signature molecules, like CTLA-4, CD73, GITR, Helios etc. There were no differences detected between A20 cKO and control Treg cells (Fig4a). To check the function of Treg cells, we performed suppression assay in vitro and in vivo. We confirmed that A20-deficient Treg cells still maintain their suppressive function (Fig4b).

③Then to continue to determine the function of A20 in T cell fate, we examined iNKT by the detection of CD1d-Tetromer with flow cytometry in thymus, as well as spleen. We found that the reduced iNKT in thymus and spleen in A20 deficient

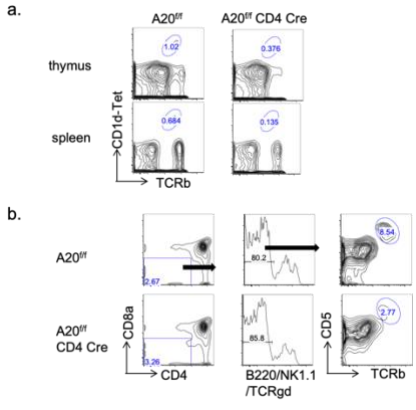

Fig5. a. less iNKT in A20 cKO mice. b. Less progenitor of IEL in A20 cKO thymus.

mice compared with that of control mice (Fig5a). Agonist selection also guides the development of the progenitor of TCRαβ CD8αα intestinal intraepithelial lymphocytes (IEL). We identified the progenitor IEL cells as CD4-CD8-NK1.1-B220-TCRγδ-TCRβ+CD5+, and found that there were reduced percentage of progenitor of IEL in A20 cKO mice (Fig5b).

(2) Changed TCR signal in A20 cKO mice.

TCR signals induce thymic selection, strong and week signaling will give rise to different cell fate. To clarify the mechanism that regulates thymic selection in A20 deficient mice, we checked the TCR signal by the detection of the phosphorylation of Zap70, ERK1/2 and AKT. The reduced phosphorylation of ERK1/2 and AKT was appeared in the A20 deficient cell after TCR stimulation (Fig6). On the other hand, calcium influx is an important signal event on the TCR stimulation. We detected the calcium influx in thymocyte after CD3e antibody stimulation, and found that A20 deficient CD4SP cells reduced Ca2+ influx. We confirmed the influence of the TCR signal in A20 deficient Treg cells by in vitro cell culture.

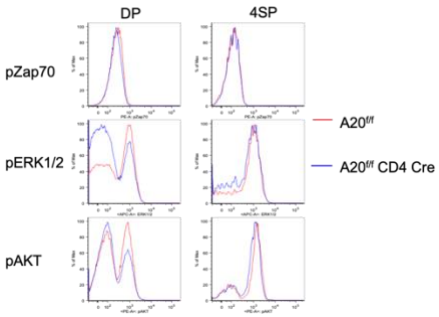

Fig6.The phosphorylation of Zap70, ERK1/2 and AKT in thymocyte after stimulation.

(3) The role of A20 deficient T cell in cancer and autoimmune disease.
A20 is susceptibility gene for multiple diseases. More Treg cells in body are propitious to control autoimmune and inflammatory diseases, but also will impair the anticancer ability. To further investigate the effect of A20 deficient T cell in disease, we used two mice models. One is the tumor formation assay. We found that the size of tumor grew on A20 cKO mice was significantly bigger than that of on the control mice, and more Treg cells appeared in the tumor (Fig7a). So, loss of A20 in T cell will impair antitumor immunity in mice. The other model is experimental autoimmune encephalomyelitis (EAE) induction. We found that T cell-specific A20 deletion could reduce the severity of the EAE induction in mice (Fig7b).

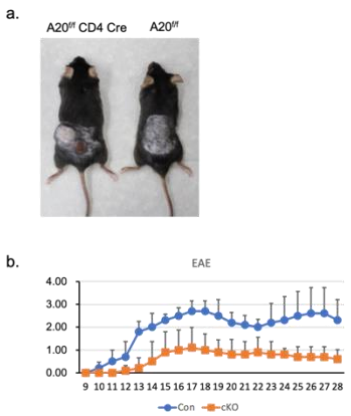

Fig7. a. Bigger tumor grew on A20 cKO mice. b. EAE induction in mice.

In this study, we found that the deletion of A20 in T cells will cause more Treg cells, fewer iNKT and nIEL cell in mice. In addition, loss of A20 will lead to impaired antitumor capability and rescue the severity of EAE in mice. Thus, A20 plays an important role in thymic selection, especial in agonist selection, to decide the T cell fate. Therefore, it affects cancer and autoimmune diseases in mice.

５．主な発表論文等

〔雑誌論文〕（計　0　件）

〔学会発表〕（計　1　件）

Yun Guo, Ubiquitin-modifying enzyme A20 plays a role in regulatory T cell, The 46th Annual Meeting of The Japanese Society for Immunology, 2017

〔図書〕（計　0　件）

〔産業財産権〕
○出願状況（計　0　件）

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発明者：
権利者：
種類：
番号：
出願年：
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：
国内外の別：

〔その他〕
ホームページ等

６．研究組織

(1)研究分担者
研究分担者氏名：
ローマ字氏名：
所属研究機関名：
部局名：
職名：
研究者番号（8 桁）：

(2)研究協力者
研究協力者氏名：
ローマ字氏名：