様式第１９号

学位論文審査の結果の要旨

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論文題目

TRAF6 signaling in dendritic cells plays protective role against infectious colitis by limiting C. rodentium infection through the induction of Th1 and Th17 responses
（樹状細胞の TRAF6 シグナルは Th1 および Th17 応答の誘導を介して C. rodentium 感染を抑制し、感染性大腸炎に対する防御的役割を果たす）

論文掲載雑誌名

Biochemical and Biophysical Research Communications

論文要旨

Authors generated intestinal epithelial cells (IECs)-specific TRAF6-deficient mice (TRAF6ΔIEC) and dendritic cell (DC)-specific TRAF6-deficient (TRAF6ΔDC) mice to assess the protective role of TRAF6 during the infectious colitis caused by C. rodentium infection.

WT and TRAF6ΔIEC mice maintained their body weight, while TRAF6ΔDC mice lost their body weight. TRAF6ΔDC mice displayed a significant reduction in colon length compared to WT and TRAF6ΔIEC mice. In TRAF6ΔDC but not WT and TRAF6ΔIEC mice, marked crypt damage, immune cell infiltration into the lamina propria, and altered epithelial and mucosal structures were observed. Before infection, the frequencies of DCs but not neutrophils and macrophages in TRAF6ΔDC mice were significantly lower compared to WT mice. The frequencies of neutrophils, macrophages, and DCs were significantly enhanced by C. rodentium infection in TRAF6ΔDC mice compared to WT mice. Western blotting using BMDCs derived from TRAF6ΔDC mice showed TRAF6 deficiency. TRAF6ΔDC mice showed reduction in the frequency of CD4+CD8α−DCs in the spleen. The frequencies of Th1 cells and Th17 cells were significantly reduced in the colon of TRAF6□DC mice compared with those of WT mice. The upregulations of p40, IL-12, IL-23, and TNF-α in response to C. rodentium infection were significantly attenuated in TRAF6-deficient BMDCs compared to WT BMDCs. The frequencies of Th1 and Th17 cells were reduced under the cocultured condition with TRAF6-deficient BMDCs.Their study demonstrated that the pathology of infectious colitis was exacerbated with higher pathogen burdens during infection in TRAF6ΔDC mice but not in TRAF6ΔIEC mice. Notably, the frequencies of IFN-γ producing Th1 cells and IL-17A producing Th17 cells in the colonic lamina propria were significantly reduced in TRAF6ΔDC mice compared to control WT mice. Moreover, BMDCs from TRAF6ΔDC mice failed to produce both IL-12 and IL-23 in response to C. rodentium stimulation and to induce Th1 and Th17 cells in vitro. Thus, TRAF6 signaling in DCs, but not in IECs, protects against colitis induced by C. rodentium infection by producing IL-12 and IL-23 that induce Th1 and Th17 responses in the gut.

本研究は，樹状細胞の TRAF6 シグナルは Th1 および Th17 応答の誘導を介して C. rodentium 感染を抑制し、感染性大腸炎に対する防御的役割を果たすことを証明した。

このため，審査員の合議により本論文は学位論文に値するものと判定した。

様式第２０号

最終試験
の結果の要旨
~~学力の確認~~

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学位申請者は本論文の公開発表を行い，各審査委員から研究の目的，方法，結果，考察について以下の質問を受けた。

1. You mentioned impaired Th1 Th17 differentiation capacity T6 deficient BM. These immune cells transport to intestine? Is this evidence is proved?
2. Do you think TRAF6 can applied to clinical situation in human?
3. The authors used conditional knock-out model of TRAF6, please explain the phenotype of TRAF6 knockout or heterozygous knockout mouse.
4. Are there any disease or conditions caused by somatic/germline alteration in TRAF6 or MyD88 gene?
5. In the CD11c-Cre/TRAF6 floxed mice, the authors showed complete deletion of TRAF6 in the dendritic cells. However, there was also a small decrease in TRAF6 protein level in B cells. Did the authors check whether there were "leakages" of Cre expression in other cell types or not?
6. What would happen if the authors continue observation of TRAF6△DC mice beyond 14 days? How normal mice eradicate C. rodentium infection?
7. Previous reports showed the impairment of Th1 and Th17 was related with Citrobacter colitis (Ref 25 and 26). Could you please explain what kinds of experiments were conducted in these reports?
8. You showed that the colon length became short by Citrobacter infection in TRAF6DC knock-down mice. Was the decrease of colon length correlated with pathological scoring?
9. Without the infection of Citrobacter, the number of dendritic cells in colon was significantly lower in TRAF6DC knock-out mice than in WT mice. Could you explain the reason?
10. Could you explain why TRAF6IEC knock-out mice did not have colitis, but MyD88IEC knock-out mice did?

これらの質疑に対して，申請者は概ね適切に回答した。よって審査委員の合議の結果，申請者は学位取得有資格者と認定した。

（注）不要の文字は２本線で抹消すること。

様式第８号 No. 1

学　位　論　文　要　旨

氏名 Thanyakorn Chalalai

論　文　題　目

TRAF6 signaling in dendritic cells plays protective role against infectious colitis by limiting C. rodentium infection through the induction of Th1 and Th17 responses

(樹状細胞の TRAF6 シグナルは Th1 および Th17 応答の誘導を介して C. rodentium 感染を抑制し、感染性大腸炎に対する防御的役割を果たす)

要　　旨

Background:

Tumor necrosis factor receptor-associated factor 6 (TRAF6) plays a pivotal role in the induction of inflammatory responses not only in innate immune cells but also in non-immune cells, leading to the activation of adaptive immunity. The critical role of the TRAF6 signaling pathway in regulating intestinal homeostasis in different cell types has been documented in multiple studies using animal diseases model, such as dextran sulfate sodium (DSS)-induced colitis. *Citrobacter rodentium (C. rodentium)* has been used as a mouse infection model for studying human enterohaemorrhagic and enteropathogenic Escherichia coli (EHEC and EPEC). However, it remains unclear whether the TRAF6 signaling pathway is essential for maintaining mucosal homeostasis during *C. rodentium* infection. We generated intestinal epithelial cells (IECs)-specific TRAF6-deficient mice (TRAF6ΔIEC) and dendritic cell (DC)-specific TRAF6-deficient (TRAF6ΔDC) mice to assess the protective role of TRAF6 during the infectious colitis caused by *C. rodentium* infection.

Method:

Wild-type (WT), TRAF6ΔDC, and TRAF6ΔIEC mice were infected orally with 2×10^9 CFU *C. rodentium.*

1) The survival rate and body weight changes were monitored.

2) Analysis of bacterial load in feces, tissues, and cecal contents was performed.

3) Colon length and the mRNA levels of IL-1β and IL-6 in the colons were measured.

4) Histological changes were evaluated using hematoxylin and eosin staining of colonic tissues.

5) Frequencies of neutrophils, macrophages, and dendritic cell in colon were analyzed using flow cytometry.

6) Expression levels of TRAF6 protein in bone marrow derived dendritic cells (BMDCs) were evaluated by Western blotting.

7) Expression pattern of cell surface protein on splenic DCs were analyzed using flow cytometry.

8) Th1 and Th17 populations in the colon were quantified by flow cytometry.

9) BMDCs were infected with *C. rodentium*. The expression levels of inflammatory cytokines were measured by quantitative RT-PCR and ELISA.

10) $CD4^+$ T cells of OT-II Tg mice were co-cultured with OVA peptide-pulsed BMDCs stimulated with heat-killed *C. rodentium* under Th0 or Th17 polarizing conditions, and quantified Th1 or Th17 populations by flow cytometric analysis.

Result:

1) WT and TRAF6ΔIEC mice maintained their body weight, while TRAF6ΔDC mice lost their body weight. Eventually, all WT and TRAF6ΔIEC mice survived, whereas 40% of TRAF6ΔDC mice died.

2) The bacterial loads in the feces, liver, spleen, colon, and cecal contents in TRAF6ΔDC mice were higher than those in WT and TRAF6ΔIEC mice.

3) TRAF6ΔDC mice displayed a significant reduction in colon length compared to WT and TRAF6ΔIEC mice. The expression levels of IL-1β and IL-6 were upregulated by *C. rodentium* infection in WT mice, which were further enhanced in TRAF6ΔDC mice.

4) In TRAF6ΔDC but not WT and TRAF6ΔIEC mice, marked crypt damage, immune cell infiltration into the lamina propria, and altered epithelial and mucosal structures were observed.

5) Before infection, the frequencies of DCs but not neutrophils and macrophages in TRAF6ΔDC mice were significantly lower compared to WT mice. The frequencies of neutrophils, macrophages, and DCs were significantly enhanced by *C. rodentium* infection in TRAF6ΔDC mice compared to WT mice.

6) Western blotting using BMDCs derived from TRAF6ΔDC mice showed TRAF6 deficiency.

7) TRAF6ΔDC mice showed reduction in the frequency of $CD4^{+}CD8\alpha^{-}$DCs in the spleen.

8) The frequencies of Th1 cells and Th17 cells were significantly reduced in the colon of TRAF6ΔDC mice compared with those of WT mice.

9) The upregulations of p40, IL-12, IL-23, and TNF-α in response to *C. rodentium* infection were significantly attenuated in TRAF6-deficient BMDCs compared to WT BMDCs.

10) The frequencies of Th1 and Th17 cells were reduced under the cocultured condition with TRAF6-deficient BMDCs.

Conclusion:

Our study demonstrated that the pathology of infectious colitis was exacerbated with higher pathogen burdens during infection in TRAF6ΔDC mice but not in TRAF6ΔIEC mice. Notably, the frequencies of IFN-γ producing Th1 cells and IL-17A producing Th17 cells in the colonic lamina propria were significantly reduced in TRAF6ΔDC mice compared to control WT mice. Moreover, BMDCs from TRAF6ΔDC mice failed to produce both IL-12 and IL-23 in response to *C. rodentium* stimulation and to induce Th1 and Th17 cells *in vitro*. Thus, TRAF6 signaling in DCs, but not in IECs, protects against colitis induced by *C. rodentium* infection by producing IL-12 and IL-23 that induce Th1 and Th17 responses in the gut.