Half depletion of Foxp3+ regulatory T cells by diphtheria toxin for long-term study in vivo

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SUMMARY Depletion of regulatory T cells (Tregs) is an appropriate approach to study the function of Tregs in vivo, and most previous studies have focused on complete depletion. The purpose of the current study was to determine an appropriate dose and timing for half depletion of Tregs in vivo. DETREG (DEpletion of REGulatory T cells) mice were produced and injected with different doses of diphtheria toxin (DT) for 7 days and 14 days. The mice were then sacrificed to collect the spleen and mesenteric lymph nodes (MLN) for analysis using flow cytometry. Foxp3+eGFP+ cells were significantly reduced by DT injection. A dose of 5 μg/kg DT led to half depletion and no deaths. A DT dose of 25, 50, or 100 μg/kg led to a progressively higher depletion rate but also a higher mortality rate. In conclusion, a low dose of DT is effective for half depletion of Tregs and long-term study. Half depletion of Tregs may become a new method for the future study of Tregs in vivo.

Keywords half depletion of Foxp3+ regulatory T cells, DETREG mice, Diphtheria toxin (DT)

Researchers have focused on the modulation of regulatory T cells (Tregs) for years because of the important role of Tregs in vivo (1). However, specific in vivo targeting of Tregs is precluded because of the lack of appropriate markers. Now that Foxp3 has been discovered, the development of mouse models of Treg-specific depletion is feasible (2). DETREG (DEpletion of REGulatory T cells) mice carry a diphtheria toxin receptor - enhanced green fluorescent protein (DTR-eGFP) transgene under the control of an additional Foxp3 promoter, thereby allowing specific depletion of Tregs by diphtheria toxin (DT) at any desired point of time during any ongoing immune responses (3). Most previous studies have focused on complete depletion of Tregs, which facilitates the study of Tregs in vivo. However, half depletion of Tregs may be a new method for studying the function of Tregs in vivo.

The aim of the current study was to explore how to use DT to half deplete Tregs in vivo and to determine a proper dose and timing in order to facilitate the long-term study of Tregs.

DT was purchased from Sigma-Aldrich. Flow cytometry antibodies including fluorescein isothiocyanate (FITC)-conjugated anti-CD4, anti-Foxp3-PE, anti-CD25-APC, and their corresponding isotype controls were purchased from eBioscience (San Diego, CA, USA). RT-PCR reagents were purchased from Applied Biosystems (Darmstadt, Germany).

To create a DETREG (Foxp3DTR-EGFP) mouse model, two transgene mouse models were used, pCAG-STOP-DTR-2A-EGFP mice (denoted as DTR-EGFP mice, product number: BCG-TO-0001) were purchased from Beijing Biocytogen Co., Ltd (Beijing, China), and B6.129(cg)-Foxp3tm4(YFP/cre)Ayr/J mice (denoted as Foxp3-Ycre mice) were obtained from the Chinese Academy of Sciences (Dr. Qi-bin Leng’s Lab, Shanghai, China). All mice were bred and reproduced in an animal facility of the Laboratory for Reproductive Immunology, Obstetrics and Gynecology Hospital of Fudan University under specific pathogen-free conditions. This study was approved by the Animal Care and Ethics Committee of the Department of Laboratory Science, Fudan University (approval no. 2020 Obstetrics & Gynecology Hospital JS020). DETREG mice genotyping Primers (5'-3'):

Rosa-GT-F: AGTCGCTCTGAGTTGTTATCAG, Rosa-GT-R: TGAGCATGTCTTTAATCTACCTCGATG;
WPRE-F: GCATCGATACCGTCGACCTC, WPRE-R: GCTGTCCATCTGCACGAGAC;
Ycre-WT-F: GCTGTTCCATCTGCACGAGAC; Ycre-WT-R: TGAGCATGCTTTAATTCACCTCGATG;
WPRE-F: GCTGCTACACCCTGACATTCC; WPRE-R: GCTGTTCCATCTGCACGAGAC.
CCTAGCCCTAGTCCAACC, Ycre-WT-R: AAGGTTCAGTGCTGTTGCT; Ycre-MU-F: AAGATGGAGGGACTACCTGTGA, Ycre-MU-R: TCCTCCTCTGATTCTGGCACATT.

After mice were sacrificed, spleen and mesenteric lymph node (MLN) cells were analyzed using a flow cytometer (Becton Dickinson, Palo Alto, CA, USA).

Continuous variables were expressed as the mean ± SEM. Continuous variables were analyzed using a Student's t-test for two groups and a one-way ANOVA for multiple groups. All analyses were performed using the SPSS 19.0 Statistical Package for the Social Sciences. *P < 0.05 was considered statistically significant.

Genotyping of DETREG mice with RT-PCR is shown in Figure 1. The GFP+ cell and Foxp3+ cell populations in the spleen and MLNs were analyzed using FCM. The number of GFP+ Tregs was much higher in DETREG mice compared to that in wild-type (WT) mice (13.3% vs. 3.01% in the spleen, 16.1% vs. 0.87% in MLNs, *P < 0.05). However, the number of Foxp3+ cells did not differ, suggesting that a DETREG mouse model was successfully created.

In order to achieve half depletion, DETREG and WT mice were injected i.p. with DT (5, 25, 50, or 100 ug/kg) every 48 h. After mice were sacrificed, Foxp3+ and GFP+ cells in the spleen and MLNs were analyzed. As shown in Figure 2, both GFP+ cells in the spleen and MLNs decreased after DT injection. There was a severe drop in Foxp3+ cell as the dose increased, suggesting a higher DT dose led to a higher depletion rate. However, a high dose led to severe weight loss and a high mortality rate in the 50 ug/kg and 100 ug/kg groups (data not shown).

On the day of the eighth DT injection (day 14), the number of Foxp3+ cells did not differ in the DT and WT groups, suggesting a rebound of Tregs in DETREG mice (data not shown).

DT injection allows for a highly specific depletion of Tregs at any desired time point during an ongoing immune response. Most researchers use a large dose of DT to completely deplete Tregs in order to study their function in vivo (4). However, complete depletion of Tregs in vivo causes a fatal autoimmune pathology in mice (5, 6). The aim of the current study was to determine an appropriate dose and timing for half depletion of Tregs in vivo, while previous studies only achieved complete depletion for a short period of time.

In conclusion, previous studies of Tregs using DETREG mice focused on complete depletion for a short period of time. In contrast, the current study achieved a half depletion of Tregs for a prolonged period of time. This approach is potentially a new way to study Tregs in vivo in the future, offering new possibilities for research on Tregs and especially for long-term study of Tregs.

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