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ORIGINAL RESEARCH

Advantages of Foxp3⁺ regulatory T cell depletion using DEREg mice

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Introduction

Regulatory T cells (Tregs) play a non-redundant role in the control of immune responses. The importance of Foxp3 for Treg lineage specification and function in both mice and

Abstract

Several mechanisms enable immunological self-tolerance. Regulatory T cells (Tregs) are a specialized T cell subset that prevents autoimmunity and excessive immune responses, but can also mediate detrimental tolerance to tumors and pathogens in a Foxp3-dependent manner. Genetic tools exploiting the foxp3 locus including bacterial artificial chromosome (BAC)-transgenic DEREg mice have provided essential information on Treg biology and the potential therapeutic modulation of tolerance. In DEREg mice, Foxp3⁺ Tregs selectively express eGFP and diphtheria toxin (DT) receptor, allowing for the specific depletion of Tregs through DT administration. We here provide a detailed overview about important considerations such as DT toxicity, which affects any mouse strain treated with DT, and Treg rebound after depletion. Additionally, we point out the specific advantages of BAC-transgenic DEREg mice including their suitability to study organ-specific autoimmunity such as type I diabetes. Moreover, we discuss recent insights into the role of Tregs in viral infections. In summary, DEREg mice are an important tool to study Treg-mediated tolerance and its therapeutic circumvention.

humans has led several laboratories to the development of genetic tools for the diphtheria toxin (DT)-based depletion of Foxp3⁺ Tregs [1–3]. These mouse models, including bacterial artificial chromosome (BAC)-transgenic DEREg mice [1], have been instrumental in unraveling Foxp3⁺ Treg

biology [1–6]. T cell-restricted Foxp3 expression could be clearly demonstrated previously as a requirement for maintaining tolerance [4, 7–9]. Despite the resolution of these controversies, there is recent confusion about putative disadvantages of DEREK mice. We here provide a detailed technical discussion about DEREK mice and other frequently used and important Foxp3^{DTR} strains.

Results and Discussion

DT Toxicity

DEREG mice express a Foxp3 BAC-driven primate DT-receptor (DTR) fused to eGFP, enabling specific Foxp3⁺ Treg depletion following DT administration [1]. Although the sensitivity of rodent cells to DT is several orders of magnitude lower compared to that of cells expressing the primate/human DTR, it is well known that DT has side effects in mice. For example, DT administration induces weight loss and transient proteinuria [10, 11], and different DT batches can vary in this unspecific toxicity [12]. Additionally, combining DT treatment with adjuvants including pertussis toxin, CFA, or CpG-ODN enhance DT toxicity [6, 10]. It is thus not surprising that viral infection has similar effects [13]. Indeed, we found that DT treatment of WT mice during the peak of sub-lethal influenza A virus infection intensifies lethal inflammation and body weight loss (Fig. 1). Similarly, Schmitz et al. [14] already reported that WT C57BL/6 mice chronically infected with LCMV are much more susceptible to DT toxicity and titrated the DT dose accordingly. Importantly, all these observations are independent of DEREK mice. Unspecific DT effects seen in WT mice should not be confused with effects induced by the depletion of specific cell populations in transgenic mice. Examples for the latter are DC depletion models where the targeting of radioresistant non-hematopoietic cells can cause death, or where neutrophilia can occur following DC

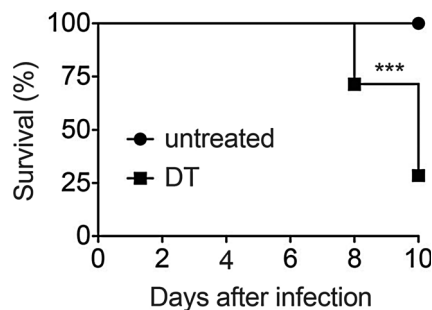


Figure 1. DT induces lethal toxicity in WT mice after influenza infection. WT mice were intranasally infected with a sub-lethal dose of influenza A virus. One group of mice received DT on d4 and d5 after infection, while the other group was left untreated. The graph shows survival of mice in both groups. Data were pooled from two independent experiments ($n = 14$; *** $p < 0.001$, Log-rank and Gehan–Breslow–Wilcoxon tests).

depletion. We conclude that DT has known unspecific effects that are manageable by careful dose titration and by the use of DT-treated WT controls.

Treg Rebound

A rebound of Tregs following the withdrawal of DT treatment is described for several Foxp3^{DTR} strains [1–3]. Thus, the re-appearance of Tregs in short-term depletion protocols [13] is fully expected and has certain advantages (see section “Advantages of Foxp3 BAC Transgenes”). Daily DT treatment of DEREK mice for 5–6 consecutive days reduces Treg outgrowth on d6–7 [1]. However, transient Treg depletion on two consecutive days is often sufficient to demonstrate strong biological effects in comparison to DT-treated controls [12]. Potential anti-DT antibody formation should be considered in long-term depletion regimen depending on the genetic background. Reduced dosing is also favored regarding DT side effects (see section “DT Toxicity”). Of note, Treg rebound can also be influenced by infections (see sections “Advantages of Foxp3 BAC transgenes” and “Tregs in Viral Infections”) and presumably by the commensal microflora.

Advantages of Foxp3 BAC Transgenes

We have previously described that eGFP⁻Foxp3⁺ Tregs can be selected by long-term DT treatment or by certain infections combined with DT treatment of DEREK mice [12, 15, 16]. Similar observations were made with Foxp3. LucidTR mice undergoing prolonged DT treatment [3]. The existence of DT-resistant Tregs has provided important insights into Treg homeostasis and self-tolerance [3, 17]. Additionally, adult DEREK mice are protected from lethal autoimmunity as opposed to Foxp3^{DTR} knock-in mice due to DT-resistant Tregs [1, 2, 17]. This important advantage allows studying the specific effects of transient Treg depletion. For example, the DEREK transgene is particularly suited to study organ-specific autoimmunity in mice on genetically susceptible backgrounds. We have crossed DEREK mice on the NOD background as a model of human type I diabetes. Interestingly, 70% of non-diabetic NOD.DEREK mice develop overt diabetes after Treg depletion, whereas DT-treated NOD controls remain non-diabetic (Fig. 2). Thus, Foxp3⁺ Tregs maintain self-tolerance to insulin-producing beta cells in the absence of TCR transgenes. Independent of Treg depletion, the Foxp3 BAC-encoded eGFP in DEREK mice has been instrumental in Treg isolation and functional analyses [18]. This is particularly important to mention because Foxp3 modifications in knock-in strains can alter Treg functions [17]. In contrast, Foxp3 maintains its native state in DEREK mice.

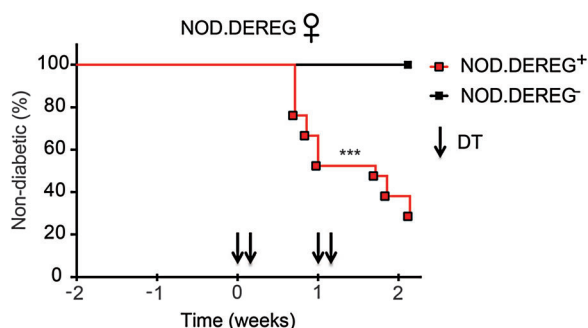


Figure 2. Rapid diabetes onset upon acute Foxp3⁺ Treg cell ablation in NOD.DEREG mice. Normoglycemic NOD.DEREG⁺ females ($n = 21$; $N = 3$) were injected with DT (arrowheads). Age-matched NOD.DEREG⁻ females were included as controls ($n = 6$; $N = 1$). Pooled percentages of normoglycemic mice are shown over time (** $p < 0.001$, Log-rank and Gehan–Breslow–Wilcoxon tests).

Tregs in Viral Infections

DT toxicity must be especially controlled in viral infections (see section “DT toxicity”). After careful DT dose titration, Schmitz *et al.* [14] demonstrated enhanced pathology after Treg depletion during LCMV infection. Treatment of DEREG mice with low dose DT effectively depleted all transgenic GFP⁺Foxp3⁺ cells but spared a considerable proportion of expanded GFP^{neg}Foxp3⁺ T cells. An overall reduction of 50–60% Foxp3⁺ T cells in DEREG compared to C57BL/6 mice was sufficient to reduce T cell exhaustion by increasing anti-viral CD8⁺ T cell effector function. Inhibition of T cell exhaustion is known to drive immunopathology. Consequently, partial depletion of Foxp3⁺ T cells in DEREG mice resulted in 100% mortality [14]. In contrast to LCMV clone 13, Friend Virus (FV) induces a life-long chronic infection and does not induce severe immunopathology since this retrovirus mainly replicates and induces effector T cell responses in lymphatic organs. Hence, Treg depletion is associated with improved control of FV infection rather than enhanced pathology [19–21]. Thus, if immunopathology is not a critical factor in an infection model, Treg depletion has no detrimental effects given that the right source and dose of DT is administered. This underlines the power of the method despite the circumstance that Foxp3⁺ T cells are depleted only partially in some settings [13]. Our data suggest that Treg manipulation might be an interesting new therapeutic approach in certain persistent viral infections. In patients, such a treatment could only be performed for a short period of time to avoid the onset of severe autoimmune diseases [16, 17]. In this regard, the DEREG mouse is an ideal model to study the effect of a transient Treg depletion on a chronic viral infection. Interestingly, such a transient Treg depletion resulted in a sustained reduction in chronic retroviral set points in the FV model [21, 22].

In conclusion, Foxp3 BAC transgenic mice have known limitations, as every model, while DEREG mice also

have several important advantages that underline their broad use.

Material and Methods

Mice

WT (C57BL/6JRj) mice and DEREG mice [1] were maintained at the animal facilities of Twincore (Hanover, Germany) and the Helmholtz-Center for Infection Research (Braunschweig, Germany) under SPF conditions. DEREG mice were crossed to the NOD background for 12 generations and maintained at the DFG-Center for Regenerative Therapies (Dresden, Germany) under SPF conditions. All animal experiments were in accordance with institutional and state guidelines.

Influenza infection

10–14 weeks old female WT mice were intranasally infected with a sub-lethal dose of influenza A virus (mouse-adapted H1N1 PR8, 0.1 LD₅₀). One group of mice received an intraperitoneal injection with 1 µg DT (Calbiochem) diluted in 100 µl PBS on days 4 and 5 after infection, while the other group was left untreated. Health status was followed every second day until day 10. Mice showing very restricted activity for two consecutive days or losing more than 20% of body weight within two days were euthanized, with infection being considered lethal.

Diabetes

15–18 week-old normoglycemic NOD.DEREG⁺ or control NOD.DEREG⁻ females were injected intraperitoneally with 0.5 µg DT (Calbiochem) on days 0, 1, 7 and 8. Blood glucose levels were determined every other day. Mice were considered diabetic at blood glucose levels above 150 mg/dl. 71.4% (15 out of 21) of initially normoglycemic NOD.DEREG⁺ mice developed hyperglycemia within 2 weeks after the first DT injection.

Statistical analysis

Survival curves were assessed by Log-rank and Gehan–Breslow–Wilcoxon tests using Prism. p values < 0.05 are considered significant.

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Conflict of Interest

None declared.

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