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CORRESPONDENCE

Correspondence: T cells are compromised in tetracycline transactivator transgenic mice

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Tetracycline (Tet)-controlled transcriptional activators allow for the inducible expression of protein-coding genes or shRNAs, and are frequently used to decipher gene function in cultured cells and *in vivo* [1]. The system is based on the bacterial Tet operon that mediates resistance to Tet derivatives, such as doxycycline (Dox). In the Tet-off system, fusion of a Tet repressor protein with the transcriptional activation domain of the herpes simplex virus protein VP16 results in a Tet-controlled transcriptional activator (tTA), which constitutively binds to Tet-responsive elements (TRE), but is inhibited in its transcriptional activity by Dox [1]. In contrast, in the more frequently used Tet-on system, a mutant tTA-version generates a reverse tTA (rtTA) that is only recruited to the TRE upon Dox binding and then mediates transgene expression [1]. Since the Tet-system allows transgene expression in a tissue-specific and temporal manner, it has been extensively used for regulating expression of oncogenes or silencing of tumor suppressor genes [1–3]. Despite its enormous success, however, we report here that, irrespective of the transgene, the Tet-system might compromise activated T-cells, thereby providing a cautionary note for its use in immunological studies.

To investigate T-cell responses, we used transgenic mice carrying an advanced Tet-on transactivator, driving expression of a miR30-based shRNA in frame with a turboGFP reporter. To reduce effects associated with random integration, the Dox-inducible shRNA-reporter transgene was delivered by a recombinase-mediated cassette exchange approach into the type-I collagen (*Col1a1*) locus. During an in-depth analysis of the transgenic mice, we discovered that specific splenic T-cell populations were absent not only in those mice expressing shRNAs targeting particular mRNAs, but surprisingly also in mice carrying a control shRNA for *Renilla* luciferase. These effects were independent of the promoter for rtTA expression, and observed in independent mouse lineages of both ROSA26 and CAG promoter-driven systems. Although total $CD4^+$ T-cells in the spleen were only marginally reduced, antigen-experienced $CD4^+$ T-cells, which we characterized as $CD44^+$ and $CD62L^{low}$, were nearly absent in the GFP-positive fractions (Fig. 1). We confirmed the reduction of activated T-cells in the GFP-positive population by CD25 and CD69 staining (Fig. 1). Importantly, disappearance of antigen-experienced T-cells was not mediated by Dox itself, since Dox-treated mice lacking rtTA expression exhibited normal numbers of activated T-cells (Fig. 1). Already 6–10 days of Dox treatment were sufficient to trigger a profound reduction in $CD25^+/CD44^+/CD4^+$ T-cells, which characterize effector memory or regulatory T-cells. Thus, their rapid disappearance suggests that Dox-activated rtTA/turboGFP expression induces toxicity in these cells (Fig. 1). Interestingly, the frequency of double-negative thymocytes expressing CD25 and CD44 was not altered (Suppl. Fig. S1). However, antigen-experienced GFP-positive $CD8^+$ T-cells, characterized by CD44 expression, were reduced similarly to the $CD4^+$ lineage (Suppl. Fig. S2).

Our findings demonstrate that, in contrast to thymocytes and naïve T-cells, antigen-experienced T-cells are depleted using the rtTA-system. Hundreds of mouse models have been generated with the rtTA-system for the expression of oncogenes or the silencing of tumor suppressor genes. In the latter condition, the blunting effects on T-cell activation might be even advantageous to identifying novel tumor

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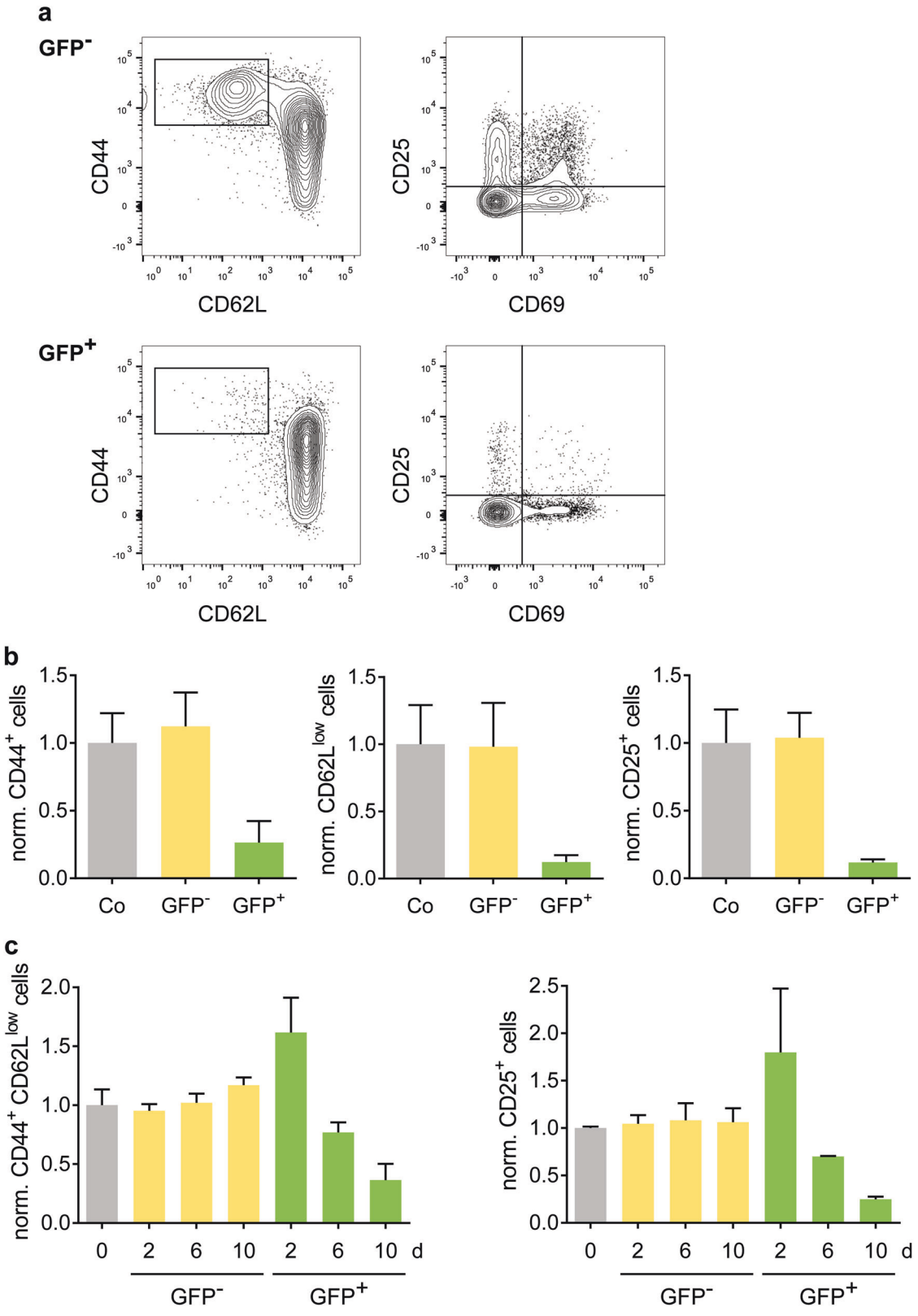
suppressors. More recently, Tet-transactivators proved very useful for modulating gene activities by CRISPR/Cas9 and Cre/LoxP technologies [4, 5]. Adverse effects of the rtTA-protein might not be restricted to activated T-cells, but could also affect other cell populations [6, 7]. Importantly, a recent study provided compelling evidence that not only



◀ **Fig. 1** Quantification of antigen-experienced splenic T-cells in ROSA26-rtTA TRE-turboGFP-shRenilla mice. **a** Flow cytometric analysis of total splenocytes isolated from mice receiving 2 mg/ml doxycycline (Dox) for 4 weeks. Splenocytes were gated for $CD4^+$ cells and segregated by their GFP intensity in two populations (GFP^- and GFP^+). Antigen-experienced $CD4^+$ T-cells were quantified by the combination of $CD44^+$ and $CD62L^{low}$ or by the cell surface expression of the activation markers CD25 and CD69. **b** The frequency of $CD44^+$, $CD62L^{low}$, and $CD25^+$ splenic $CD4^+$ T-cells from ROSA26-rtTA TRE-turboGFP-shRenilla mice was normalized to control mice lacking rtTA expression. Following Dox treatment for four weeks, at least six mice were analyzed per group. **c** Quantification of antigen-experienced splenic $CD4^+$ T-cells during short-term Dox treatment. After 2, 6, and 10 days, the frequency of splenic $CD4^+$ T-cells expressing $CD44^+$, $CD62L^{low}$, and $CD25^+$ was determined in the GFP^+ and GFP^- population and normalized to untreated ROSA26-rtTA TRE-turboGFP-shRenilla mice

activated T-cells, but also B-cells are diminished in tTA-transgenic mice [8]. This study further showed an inhibition of lymphocyte depletion by Bcl-2, suggesting that tTA expression triggers an apoptotic response. Thus, in addition to the ability of Dox to interfere with mitochondrial functions [9], these results provide a cautionary note for using proper controls to reveal potential side effects of Tet transactivator expression alone.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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