

Research Article

DNA Methyltransferase Inhibition Promotes Th1 Polarization in Human CD4⁺CD25^{high} FOXP3⁺ Regulatory T Cells but Does Not Affect Their Suppressive Capacity

Sija Landman ¹, Marjan Cruijsen,² Paulo C. M. Urbano,¹ Gerwin Huls,^{2,3} Piet E. J. van Erp,⁴ Esther van Rijssen,¹ Irma Joosten,¹ and Hans J. P. M. Koenen ¹

¹Department of Laboratory Medicine-Medical Immunology, Radboud University Medical Center (Radboudumc), Nijmegen, Netherlands

²Department of Hematology, Radboud University Medical Center (Radboudumc), Nijmegen, Netherlands

³Department of Hematology, University Medical Center Groningen, Groningen, Netherlands

⁴Department of Dermatology, Radboud University Medical Center (Radboudumc), Nijmegen, Netherlands

Correspondence should be addressed to Hans J. P. M. Koenen; hans.koenen@radboudumc.nl

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Regulatory T cells (Treg) can show plasticity whereby FOXP3 expression, the master transcription factor for Treg suppressor function, is lost and proinflammatory cytokines are produced. Optimal FOXP3 expression strongly depends on hypomethylation of the *FOXP3* gene. 5-Azacytidine (Aza) and its derivative 5-aza-2'-deoxycytidine (DAC) are DNA methyltransferase inhibitors (DNMTi) that are therapeutically used in hematological malignancies, which might be an attractive strategy to promote Treg stability. Previous *in vitro* research primarily focused on Treg induction by DAC from naïve conventional CD4⁺ T cells (Tconv). Here, we examined the *in vitro* effect of DAC on the stability and function of FACS-sorted human naturally occurring CD4⁺CD25^{high} FOXP3⁺ Treg. We found that *in vitro* activation of Treg in the presence of DAC led to a significant inhibition of Treg proliferation, but not of Tconv. Although Treg activation in the presence of DAC led to increased IFN γ expression and induction of a Thelper-1 phenotype, the Treg maintained their suppressive capacity. DAC also induced a trend towards increased IL-10 expression. *In vivo* studies in patients with hematological malignancies that were treated with 5-azacytidine (Vidaza) supported the *in vitro* findings. In conclusion, despite its potential to increase IFN γ expression, DAC does preserve the suppressor phenotype of naturally occurring Treg.

1. Introduction

Regulatory T cells (Treg) are important for homeostasis of the immune system [1]. Immune regulation by Treg depends on the stability of these cells [1, 2], which in turn is controlled by stable expression of the transcription factor FOXP3 [3]. In the past, we have shown that Treg reveal plasticity as indicated by loss of FOXP3 expression and gain of proinflammatory cytokine (IL-17a, IFN γ) production [4]. Stable FOXP3 expression requires hypomethylation of CpG-rich regions of the *FOXP3* gene, which is known as Treg-specific demethylated region (TSDR) [5–8]. Treg instability and plasticity have been demonstrated in a number

of immune-related pathologies and are thought to promote chronic inflammation [9–12]. Demethylating agents, such as the DNA methyltransferase inhibitor (DNMTi) 5-azacytidine (Vidaza, Aza) and its derivative 5-aza 2'-deoxycytidine (decitabine, DAC), are used in the treatment of hematological malignancies and seem an attractive therapeutic strategy to promote Treg stability. Aza and DAC have related mechanisms of action, including depletion of DNMTs and hypomethylation of DNA [13, 14]. Aza/DAC shows immunomodulatory potential *in vitro* and *in vivo* and have been shown to induce demethylation of the *FOXP3* gene [15, 16]. Administration of DAC in experimental mouse models of inflammation (lung inflammation [17–19], diabetes