

# The Nature of Increased Circulating CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> T Cells in Patients with Systemic Lupus Erythematosus: A Novel Hypothesis

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**Abstract:** The forkhead family transcriptional factor (Foxp3) is an important lineage marker for regulatory T (Treg) cells. Foxp3 expression is primarily restricted to CD4<sup>+</sup>CD25<sup>+</sup> cell population. Recently, an intriguing phenomenon is highlighted that there is a considerable amount of CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> T cells present in the peripheral blood of patients with systemic lupus erythematosus (SLE). Up to now, it is still an open question as to the nature of this cell subset. Following an analyses of the available phenotypic characteristics of CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> T cell subset along with some new findings in research of Treg in human SLE, we propose the hypothesis: the increased circulating CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> T cells in patients with SLE may constitute a peripheral reservoir of CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Treg cells. Under the condition of autoimmune response reactivated, CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> T cells could be recruited to expand the Treg pool upon CD25 regaining, for the effort to try to reverse a homeostatic imbalance shift to more aggressive expansion of autoreactive T cells and B cells. This hypothesis, if confirmed, would provide a new strategy for the treatment of SLE via the generation of therapeutic regulatory T cells.

**Keywords:** Foxp3, regulatory T cells, systemic lupus erythematosus.

## INTRODUCTION

CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> regulatory T (Treg) cells play a crucial role in maintaining peripheral tolerance and provide prevention from autoimmune disease [1]. It is well known that the milestone in Treg research is the discovery of the function of Foxp3. Mutation in the Foxp3 gene has been identified as the disease-causative gene in Scurfy mouse, which spontaneously develops severe autoimmunity, as well as a similar human disease called IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome) [2, 3]. Both of these diseases arise from a lack of functional Treg cells. Foxp3 is the key molecule not only for the development and function of thymus-derived, naturally occurring Treg (nTreg) cells but also for the induced Treg (iTreg) cells which are generated in the periphery. Retroviral transduction of the Foxp3 gene converts naïve CD4<sup>+</sup>CD25<sup>-</sup> T cells to phenotypical and functional Treg cells [4]. Such transduced cells display *in vivo* and *in vitro* suppressive activity. Naïve CD4<sup>+</sup> T cells can differentiate into Foxp3<sup>+</sup> Treg cells in the periphery in the presence of IL-2 and TGF-β [5, 6]. Although transient up-regulation of Foxp3 expression has been observed in human T cells upon activation, conflicting data have also been published concerning the suppressive capacity of T cells with transient Foxp3 expression [7-9].

Foxp3 remains the specific intracellular marker for Treg to date. CD25 retains the conventional surface marker enabling easy isolation of Treg cell subset *ex vivo*. Foxp3 expression is primarily restricted to CD4<sup>+</sup>CD25<sup>+</sup> cell

population. In addition, Foxp3 expression is also detectable at a low level in CD4<sup>+</sup>CD25<sup>-</sup> cells in mice and humans [10, 11]. Partly because of very limited amount, CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> T cell subset attracted little attention in the past years. Very recently, four separate groups have reported one after another that there is a considerable amount of CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> T cells present in the peripheral blood of patients with systemic lupus erythematosus (SLE) [12-16]. The proportion of CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> T cells within CD4<sup>+</sup> lymphocytes is nearly up to 8% [16]. SLE is a disorder of immune regulation characterized by the breakdown of peripheral tolerance to self-antigens and the production of various autoantibodies. Many T-cell and B-cell abnormalities have been described [17], and these include the perturbation of Treg cell subset revealed in recent years [18]. However, the nature of CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> T cell subset and the clinical significance of their increased quantity in patients with SLE have little known.

## PHENOTYPE ANALYSIS OF CD4<sup>+</sup>CD25<sup>-</sup>FOXP3<sup>+</sup> T CELL

By flow-cytometric analysis, several groups reported that CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> T cells in the peripheral blood from lupus patients expressed few level of CD127, another important phenotypic characteristic for Treg [13, 15, 16]. Furthermore, Bonelli and colleagues performed detailed comparative phenotypic analyses of CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> T cells and CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Treg cells among SLE patients and healthy controls. A similar expression pattern was observed for both CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> T cells and CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Treg cells from SLE patients concerning the expression of several surface and intracellular marker molecules that have been described to be associated with a Treg phenotype, such as CD62L, CD95, GITR, CTLA-4 and CD127. Subsequently, they sorted CD4<sup>+</sup>CD25<sup>-</sup>CD127<sup>-</sup>

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cells from SLE patients, substituted for CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> T cells, to evaluate their regulatory function *in vitro*. Unfortunately, these cells were shown to perform partial regulatory activity in that they were only able to suppress effector T cell proliferation but not IFN- $\gamma$  production [16].

Notably, two groups described another phenotype characteristic that CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> T cell subset in SLE patients is CD45RO<sup>+</sup> in the overwhelming majority [15, 16]. On the other hand, this cell subset in healthy donors consists of more CD45RA<sup>+</sup> cells. This may suggest that most of the CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> T cells in the peripheral blood from SLE patients have undergone autoantigen stimulation and been in the memory CD4<sup>+</sup> T cell compartment. In human, it has been suggested that memory CD4<sup>+</sup> T cells may be the peripheral origin of adaptive CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> cells, also called iTregs [19, 20]. Both iTreg cells and nTreg cell constitute the CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Treg pool circulating in the blood. A study by Vukmanovic-Stejic and colleagues revealed that there was extremely close T cell receptor (TCR) clonal homology between human CD4<sup>+</sup>CD25<sup>-</sup>CD45RO<sup>+</sup> T cells and CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Treg cells. These authors proposed that iTreg, emerging at the periphery from the memory T cell compartment, was mainly responsible for the dynamic expansion of the CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Treg pool during an antigen-specific response [20]. Interestingly, there has been accumulating evidence which indicate that the percentages of CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Treg cells in the peripheral blood of SLE patients increase during their disease in the active stage [12, 14, 21, 22].

## HYPOTHESIS

In light of these findings, we propose the hypothesis as follows: The increased CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> T cell subset in the peripheral blood of SLE patients may constitute a peripheral reservoir of CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Treg cell population. Under the condition of autoimmune responses reactivated, CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> T cells could be recruited to expand the Treg pool upon CD25 regaining, for the effort to try to reverse a homeostatic imbalance shift to more aggressive expansion of autoreactive T cells and B cells in SLE.

## DISCUSSION

We propose this hypothesis rather than one which recognizes CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> T cells as conventional Treg cells, based on some new findings in research of Treg in human SLE and several characteristics belonged to this autoimmune disease itself.

There is a wide spectrum in human lupus ranging from solely involvement in skin to systemic disease. Beyond initial studies about Treg in human lupus, emerging data have revealed that the proportion of CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Treg cells as well as CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> T cells in the peripheral blood of lupus patients both increase and positively correlate with disease activity [12-16, 21, 22]. As for Treg function, there is also emerging evidence supporting that a relative deficiency of Treg function, rather than an intrinsic deficiency, is involved in the development of human lupus. This abnormality may be due to the resistant effect on Treg suppression direct from effector T cells, or the blockade effect on Treg suppression indirect from

antigen-presenting cells [18, 21, 22]. In fact, the phenomenon of relative insufficiency of Treg function has been reported in numerous animal models [23]. The same trend is now emerging from human studies, in particular those relating to SLE patients. This scenario just provide a rational explanation for the increased peripheral blood CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Treg quantity in active lupus, which may be the positive feedback response to the resistant / blockade effect on Treg suppression. Subsequently, the following question is: Where do the increased circulating CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Treg cells origin?

It is known that the CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Tregs circulating in the blood consist of nTreg cells and iTreg cells. In SLE patients, nTreg apoptosis is found to be exacerbated due to more sensitive to Fas-mediated apoptosis [24]. The proliferation of limited nTreg seems unlikely sufficient for a bulge in the CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Treg cell pool in the active autoimmune response. What's more, SLE is characterized by a high level of IL-6 [25]. This cytokine can interfere with the function of nTreg [26], and can even convert nTreg cells to IL-17-producing cells [27]. Both IL-2, combined with TGF- $\beta$ , have been suggested to enable the conversion of iTreg from CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>-</sup> precursors in the periphery. However, lymphocyte production of these two cytokines is shown to be decreased in SLE patients [28, 29]. Therefore, the CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> T cell population may serve as the main replenishment for CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> iTreg population that could rapidly be recruited to the Treg pool upon CD25 regaining, in order to combat the more aggressive expansion of autoreactive T cells and B cells during disease flare. Fortunately, Zelenay and his colleagues addressed this question in their mice model and established that Foxp3-expressing cells encompassed in the CD45RB<sup>low</sup> CD25<sup>-</sup> subset were the cells contributing to the pool of CD25<sup>+</sup>Treg during immune activities [30]. This finding may further support our speculation.

## FUTURE PERSPECTIVES

It is still an open question as to the nature of CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> T cell subset and the reason of their increase in patients with SLE. More information must be determined before a definitive conclusion can be made. In particular, whether CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> T cells and CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Treg cells in SLE patients share similar TCR V $\beta$  usage needs to be addressed. The role of CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> T cells and CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Treg cells with respect to TCR clonal homology remains to be clarified. Furthermore, it must be formally established whether the acquisition of surface CD25 by CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> T cells from SLE patients is necessary for their full regulatory capacity, suppressing not only proliferation but also IFN- $\gamma$  production of effector T cells. If this is indeed the case, this would provide another strategy for the generation of therapeutic regulatory T cells for the treatment of SLE.

## STATEMENT OF INTERESTS

Authors' Declaration of Personal Interests

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