



## 2009 Research Grant Program Winning Abstract

### Role of Peripheral Conversion in Regulatory T Cell Accumulation in Aging

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Reactivation of persistent infections, inability to combat new infections, impaired responses to vaccination and less effective control of cancer are common consequences of waning immune function in the elderly. A subset of CD4+ T cells, the regulatory T cells (Treg) controls the intensity of immune responses by blocking the activation and function of both effector T cells and antigen-presenting cells. The transcription factor FoxP3 is highly expressed in Treg and is crucial for Treg function. During aging, we and other investigators have shown that the Treg proportion increases in lymphoid tissues. Moreover, we reported that such accumulation of functional Treg is crucial in the spontaneous reactivation of chronic *Leishmania major* infection during aging, underscoring Treg role in the reactivation of chronic infections in aged hosts. Similarly, Treg accumulation plays a critical role in the failure of aged mice to control tumors.

However, despite such emerging evidence of the importance of Treg in age-associated immune suppression, the mechanisms underlying their accumulation in aged hosts remain unclear. Suppressive Treg originate from the thymus or the peripheral conversion of non-Treg. Both subsets exhibit similar phenotype, and contribution of each pathway cannot be determined by a simple phenotyping. Several pathways have been shown to be involved in Treg conversion in young mice and humans, including the signaling of non-Treg by TGF- $\beta$ 1 and T cell receptor (TCR), and the interactions of non-Treg with tolerogenic dendritic cells (DC). The contribution of peripheral conversion in the accumulation of Treg in aging had not yet been studied, and is the principal focus of this application.

The hypothesis underlying this study is that old non-Treg can be converted more readily into Treg than young non-Treg. We will: 1) characterize the conversion of aged murine non-Treg occurring with TGF- $\beta$ 1 in vitro, 2) define the conversion of aged murine non-Treg in presence of different DC subsets in vitro, and 3) determine in vivo the conversion capacity of aged non-Treg.

**Specific aim 1:** Characterize the conversion of aged murine non-Treg into Treg occurring with TGF- $\beta$ 1 in vitro. We will study the ability of TGF- $\beta$ 1, in conjunction with TCR engagement, to convert non-Treg into Treg in vitro, compared to their young counterparts. Conversion of both naïve and memory non-Treg will be evaluated. Indeed, although memory non-Treg cannot be converted in vitro in young animals, it is not known whether memory non-Treg from aged animals exhibit different properties. Using BD magnetic nanoparticles and antibodies for sorting, we will isolate non-Treg, memory and naïve, from FoxP3-GFP knock-in mice (FoxP3-GFP). The conversion into Treg will be determined by flow cytometry using BD reagents, by comparing the percentage of GFP+ cells arising in the culture and their level of proliferation. Expression of markers associated with Treg function, such as CTLA-4, PD-1, will be analyzed by flow cytometry and the functionality of these converted Treg measured by their efficiency to suppress activation of non-Treg and DC. If aged and young non-Treg



were converted in different proportion, or with a different kinetics, we will then study the TGF- $\beta$ 1 signaling pathway.

**Specific aim 2:** Define the conversion of aged murine non-Treg into Treg in presence of different DC subsets. We have shown that DC proportion and maturation status change during aging (Silva Lages et al, submitted). In particular, we found upregulation of the tolerogenic receptor PD-L1, suggesting that these DC could induce more non-Treg conversion than young DC. We will therefore compare the capacity of aged and young DC to convert old non-Treg into Treg. Tolerogenic DC will be purified by a combination of magnetic and flow cytometry sorting using BD antibodies. Memory and naive non-Treg from FoxP3-GFP mice will be purified as described above. The efficiency of conversion and the functionality of converted Treg will be determined as described above.

**Specific aim 3:** Determine the rate of in vivo conversion of aged non-Treg into Treg. We will transfer purified non-Treg into young and old congenic mice, to determine whether old non-Treg can be converted into Treg. We will isolate non-Treg from young and old FoxP3-GFP mice, as described above, and analyze by flow cytometry the emergence over time of Treg in different lymphoid tissues.

This project will allow us to 1) estimate the contribution of non-Treg conversion in the accumulation of functional Treg with aging, 2) clarify the conversion capacity of naive and memory CD4<sup>+</sup> T cells, and 3) evaluate the capacity of different DC subsets to induce conversion of non-Treg. Our proposed experiences will allow us to study the molecular mechanisms involved in Treg peripheral conversion, and to characterize the phenotypic differences between induced Treg and natural Treg. The experiments that we propose will rigorously test whether increased conversion play a significant role in Treg accumulation in aging. Therefore, whatever the outcome of the proposed experiments, they will provide novel insights into Treg biology in aging. Due to the Treg-mediated suppression of critical immune effector functions, such knowledge could help designing novel therapeutic approaches to enhance the control of chronic infections and cancer in aged populations.

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