Comment on "Stability of human rapamycin-expanded CD4$^+$CD25$^+$ T regulatory cells" Haematologica 2011;96(9):1357-65.

We recently read with great interest the article by Tresoldi *et al.*[1] in the September 2011 issue of *Haematologica*. Their study examined the stability of human CD4$^+$ regulatory T (Treg) cells exposed to rapamycin *in vitro* and *in vivo*, and concluded that rapamycin-expanded Treg cells maintained a stable phenotype. We believe that the data from this investigation are interesting but that they do not, however, provide adequate support for their conclusion.

In this study, it was shown that the frequency of FOXP3$^+$ cells within CD4$^+$CD25$^+$ T cells in the non-rapamycin group of patients declined more markedly than that in the rapamycin group after *in vitro* expansion. Accordingly, the rapamycin-expanded CD4$^+$CD25$^+$ T cells displayed a stronger suppressive activity and contained a smaller fraction of pro-inflammatory cytokine-producing T cells compared to expanded CD4$^+$CD25$^+$ T cells in the absence of rapamycin. These results may be due to the distinct effects of rapamycin on FOXP3$^+$ Treg cells and effector T cells, because rapamycin was shown to be able to selectively promote *in vitro* expansion of Treg cells while inhibiting proliferation of effector T cells.[2,3] In this study, the initial population (CD4$^+$CD25$^+$ T cells) was contaminated with more than 30% effector T cells. Thus, we could not draw any conclusion about whether rapamycin has an intrinsic role in the stability of Treg cells. Furthermore, recent studies demonstrated that natural Treg cells were instable during *in vitro* culture and could convert to so-called exFOXP3 cells that produced pro-inflammatory cytokines.[4-6] Therefore, the pro-inflammatory cytokines detected in the expanded T cells in this study can be from either preferentially expanded effector T cells or exFOXP3 cells converted from FOXP3+ Treg cells.

Additionally, the stability of Treg cells *in vivo* remains controversial. Several recent studies have reported that a fraction of FOXP3+ Treg cells can lose FOXP3 expression after *in vivo* transfer,[7] especially in lymphopenic conditions.[6,8,9] However, the study by Rubtsov *et al.*[10] reported that FOXP3$^+$ Treg cells are notably stable under physiological and inflammatory conditions. In this study, Tresoldi and colleagues also tested the stability of Treg cells *in vivo*. However, the article reports that they only displayed the frequency of FOXP3$^+$ Treg cells after and not before *in vivo* transfer. Furthermore, it was unfortunate that they did not obtain data concerning the number of non-rapamycin expanded FOXP3$^+$ Treg cells recovered from the injected mice. It is, therefore, difficult to compare the stability of FOXP3$^+$ Treg cells before and after *in vivo* transfer in the rapamycin group, as well as those cultured with and without rapamycin. Therefore, we think that the data reported in the article cannot offer substantial support for the conclusion that rapamycin fixed the Treg cell phenotype.

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