03.11.2014

Goettingen, Germany

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Dear Sirs,

We are grateful for the comments and suggestions for how to improve our manuscript.

Reviewer 00038879:

This is a good short study. My comments:

1. In the Abstract and Discussion the authors conclude that the 2 tested assays are not equivalent to measure nTregs and iTregs. Are the authors implying that one method is testing one subtypte rather than the other? they should clarify this point.

We have reworded the Abstract and Discussion to try to clarify that we were not suggesting that one method measures nTregs while the other measures iTregs: rather that the two Treg subtypes are differentially detected by the two methods.

2. As the authors mention in the Discussion it is possible that one explanation for their results is that Foxp3 is present in non-Tregs cells. Indeed, it has clearly been shown in the recent past that Tregs, Th1 and Th17 are indeed the same cell - expressing one gene or the other depending on the microenvironment. The authors should expand this aspect.

We have reworded the Conclusion to more clearly state that one explanation for the differences is that non Tregs could have been expressing Foxp3. However, if this were the explanation then the FACS assay should have produced lower percentages of Tregs which was in fact not found.

3. The results of the 2 methods are compared in the table - however I don't see calculated p values. Why?

We agree that the p-values should have been presented. The correlation coefficients and respective p-values were calculated according to Spearman Rho and are now included in Table 1.

Reviewer 00074323:

The authors clearly show that there is poor correlation between two different methods for measuring Tregs in peripheral blood. Thus, studies on Tregs in various inflammatory disorders should be read with great caution. Sample and methods are adequate to support results. It is true to state that the two methods evaluated may reflect the identification of different types of Tregs (natural or induced), but also conventional activated T cells can share many phenotypic features with Tregs. For example, in IPEX syndrome, activated FOXP3high cells can be found in blood, which likely represent activated T cells.

We have reworded the Discussion to clarify that one possible explanation of the results is that the Foxp3 gene could be expressed in activated, but non T_{reg} cells (see answer to other reviewer) and a comment about using caution in interpreting studies has been included.

Again, we appreciate all of the critical comments and considerations.

Sincerely,

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