Reviewer’s report

Title: Murine CD4+CD25- cells activated in vitro with PMA/ionomycin and anti-CD3 acquire regulatory function and ameliorate experimental colitis in vivo

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Reviewer: Franco Scaldaferrri

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Potentially interesting paper, assessing the T regulatory capacity of subset of T cells manipulated in vitro in the experimental murine CD45RB transfer model of colitis.

Well written, clear however it displays several major limitations.

Authors state that murine cd4+cd25- cells activated in vitro with pma/ionomycin and anti-cd3 acquire regulatory function.

However, even if they are associated to a “clinical amelioration” of colitis in mice, the causality could not be related so strictly to those “supposed” transferred regulatory T cells.

Those cells, in fact, have not been fully characterized in vitro, in simplified system. Do they display regulatory properties in vitro? are they able to suppress proliferation of activated T cells in a dose dependent manner? Do they inhibit T cells cytokines production upon activation? Do they stay alive in mice following the transfer?

Furthermore, authors state that it is the treatment with pma/ionomycin, by modulating ion channel function, that enhances the expression of cd25 on murine cd4+cd25- cells. A control group of mice receiving cd4+cd25- cells activated only with anti-CD3 and then cultured in IL2 supplemented medium should also be included in the paper.

Finally introduction should contain more information about stimulation of T cells with (PMA)/ionomycin and anti-CD3.

Aim of the study should be stated instead of anticipating, already in the introduction, results of the study and analysis.

In the discussion session more room should be dedicated to compare pma/ionomycin and anti-cd3-activated murine cd4+cd25- cells to other in vitro generated regulatory T cells. Limits of the paper should also be better presented.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:

I declare that I have no competing interests.