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: Marinos Kallikourdis

Reviewer’s report:

In this manuscript Majowicz and coworkers elegantly show that CD4+CD25- T cells activated in vitro via PMA/ionomycin express Treg-like markers and are able to reduce the disease severity of experimental colitis.

The experimental methods are well-described and the experiments follow a logical sequence and are thoroughly executed and reported. The in vivo protective effect is studied from a variety of parameters and is convincing.

There is one issue with the interpretation of the results, which I outline below.

Discretionary Revisions:

The manuscript does not present data examining the in vitro suppressive capacity of these cells. Whilst this could be considered not necessary, given the convincing in vivo data, presenting in vitro suppression data could substantially strengthen the conclusions drawn by the in vivo studies.

Minor Essential Revisions:

The number of CD45RBlow cells injected in “negative control” animals should be stated.

Major Compulsory Revisions:

Experimental colitis, as performed by the authors and by many of the previous reports cited, is induced by administration of 4*10^5 CD45RBhi T cells into a lymphopenic recipient. Administration of an equal number of CD45RBLow cells can protect from colitis, as indeed the authors and others have shown. However, as shown in Barthlott, Kassiotis and Stockinger, J Exp Med (2003), injection of increased numbers (6*10^6) of CD45RBhi cells, that have no suppressive capacity, is sufficient to show a reduction in the severity of colitis, due to “competition with neighboring T cells for limited resources” that limits the activation of the T cells in the lymphopenic host.

The authors used 1.2*10^6 TregPMA cells, which is appreciably less than the number used by Barthlott and colleagues, though still 3 times more than the number of CD45RBhi that cause the colitis. As a consequence, it is not possible to exclude that at least part of the protective effect observed in vivo is not due to the increased number of administered cells.

This caveat should be made clear in the discussion.
Dr M Kallikourdis

**Level of interest:** An article whose findings are important to those with closely related research interests

**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