In conclusion, we would urge the authors to be more cautious in the interpretation of their and published observations and we agree with the editorial comment that improved methodologies will help better understand the role of T-regs in disease.

Conflict of interest

The authors declare that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Financial support

M.S.L. is supported by a Clinician Scientist Fellowship from the Medical Research Council, UK.

References


Maria Serena Longhi
Yun Ma
Giorgina Mieli-Vergani
Diego Vergani*
Institute of Liver Studies, King's College London School of Medicine at King's College Hospital, Denmark Hill, London SE5 9RS, UK
*Corresponding author. Tel.: +44 203 2993305; fax: +44 203 2994224
E-mail address: diego.vergani@kcl.ac.uk

Reply to: “Regulatory T cells in autoimmune hepatitis”

To the Editor:

We read with interest the comment by Longhi et al. on our paper on Tregs in autoimmune hepatitis (AIH) [1] and the accompanying editorial by Oo and Adams [2]. The discrepant results by Longhi et al. and us on Treg function in autoimmune hepatitis (AIH) may originate from the use of different methodologies to purify and assess the suppressor activity of Tregs [3,4]. Therefore, we will reply to the criticism by comparing the methodologies used by the Kings College group and by us, and explain why we chose the methodology as we did.

To determine Treg function, Longhi et al. used the 3H-thymidine assay. First, Treg were purified with magnetic beads to enrich CD25+CD4+ T cells; indeed, >95% of these cells were CD25+. These CD25+ cells were then co-cultured together with CD25−CD4+ T cells at a ratio of 1:8 for several days, after which 3H-thymidine was added to determine proliferation of the cell mixture. We also enriched CD25+CD4+ T cells with magnetic beads, and found that >95% of these cells expressed CD25. However, in humans, CD25 alone cannot be used as a marker for Treg cells, since, in human blood, up to 30% of the CD4+ T cells can express CD25, but only 1–2% of these are functional suppressors [4]. In contrast to Longhi et al., we therefore further characterised the CD25+ cells by staining Foxp3 and CD127 (Fig. 2A of our manuscript), demonstrating that the majority of these cells (80–90%) were indeed Treg. More importantly, we also characterised the CD25−effector population by showing that more than 90% of cells were non-Tregs (Fig. 2A). We then determined Treg function with the CFSE dilution assay, in which only CD25−CD4+ effector T cells were labelled with CFSE and subsequently co-cultured together with the unlabelled CD25+ Treg fraction. The great advantage of the CFSE assay over the 3H-thymidine assay is that it is able to specifically evaluate the proliferation of the CD25−CD4+ responder T cell population [5–7]. The 3H-thymidine assay, in contrast, cannot distinguish which cells in the co-culture have incorporated 3H-thymidine, which can result in false estimates of Treg suppression [7]. In addition, unlike the CFSE assay, 3H-thymidine assays can only give a snapshot of proliferation for the period of time during which 3H-thymidine was present in the culture [7]. Moreover, unlike murine Treg cells, human Treg cells have a tendency to proliferate after activation [4], making the distinction of those cells that actually proliferated in the culture even more problematic. We thus would like to refer to Sakaguchi et al. [4] who state that ‘this indicates that thymidine uptake by cultured Treg and effector cells is not appropriate to assess suppressive activity of human Treg cells’, and instead ‘propose that it is more accurate to assess dilation of CFSE in effector T cells to determine the percentage and number of proliferating cells’.

Another point addressed by Longhi et al. is that we cultured Treg cells and effector cells at a ratio of 1:1, instead of 1:8 as they did, arguing that this ratio is ‘utterly non-physiological’. We do agree that this assay is non-physiological, as basically any in vitro assay. However, the aim of the assay was not to determine the Treg physiology, but to assess their suppressive potential, which, as others have shown [5–7], can be reasonably performed with the CFSE assay using ratios in a range between 2:1 and 1:4.

Open access under CC BY-NC-ND license.
Letters to the Editor

Longhi et al. further criticise our gating strategy and claim that the Treg frequencies in peripheral blood reported by us were vastly lower than those reported in the literature [8]; however, in the paper they refer to, we find a gating strategy to determine CD25(high) cells (see Fig. 1 in that paper) that is very similar to that used by us (see our Fig. 1A). Moreover, it is actually stated in this paper that only ‘the top 1% of CD25-staining CD4 T cells’ contain Treg cells [8], a number not vastly different from the frequencies reported by us.

Longhi et al. also refer to a conference abstract, in which they have reported that Treg cell numbers are similar in AIH patients and healthy subjects; we apologise for having cited only previous papers of this group, which had reported impaired Treg frequencies in AIH.

Longhi et al. quote us having attributed the discrepancies between previous findings and our report to the use of ‘improved methodology’ to define ‘true Treg’. We would like to emphasise that we did not use the quoted terms in our paper. Nevertheless, we believe that the methodology used by us was appropriate and that the conclusions drawn from these experiments are valid.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

References


Moritz Peiseler
Marcial Sebode
Christoph Schramm*
Johannes Herkel*
Department of Medicine I,
University Medical Centre Hamburg-Eppendorf,
20246 Hamburg,
Germany

* Corresponding authors.

E-mail addresses: cschramm@uke.de, jherkel@uke.de