find increased numbers of FOXP3\textsuperscript{pos} cells within the inflammatory infiltrate of patients with AIH. In our study, FOXP3\textsuperscript{pos} cells were detected in most liver biopsies from AIH patients, but they represented a small component of the portal tract inflammatory infiltrate. No comparison was made with normal or pathological control livers. As FOXP3\textsuperscript{pos} cells are reportedly absent or scarce in the normal liver, when tested by immunohistochemistry [6,7], it can be argued that we did find increased numbers of tissue FOXP3\textsuperscript{pos} cells in AIH. In support of their findings, Peiseler et al. refer to Buckner’s review [5], which quotes increased numbers of tissue FOXP3\textsuperscript{pos} cells in human autoimmune disease, such as in the skin in psoriasis and in the gut in inflammatory bowel disease. Interestingly, in the same review [5] Treg function is documented to be decreased in most human autoimmune diseases, including type 1 diabetes, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis and psoriasis, in agreement with what reported by us [4] and others [2,3] in AIH.

Of note, a numerical defect of peripheral Tregs has been reported in primary biliary cirrhosis (PBC), another autoimmune liver disease [6]. In the same paper, the frequency of FOXP3\textsuperscript{pos} cells within the CD3\textsuperscript{pos} liver infiltrating T lymphocytes was approximately 12% in PBC, 18% in AIH, and 28% in chronic hepatitis C [6], though a more recent paper by Oo et al. has reported a frequency of about 12% in both AIH and PBC [7]. As acknowledged by Peiseler et al. [1] and Bukner [5], the use of FOXP3 as a sole marker of Tregs in tissues is inadequate, FOXP3 being also expressed by effector T cells following activation [5]: more accurate information will be obtained when better markers for immunohistochemical detection of Tregs become available.

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.

Reply to: “Regulatory T cell defects in autoimmune hepatitis”

To the Editor:

We read with interest the comment by Ferri et al. to our paper on Tregs in autoimmune hepatitis (AIH) [1], in which they emphasise several discrepancies between their recently published findings [2] and ours. Ferri et al. explain these discrepancies by methodological differences; however, find it difficult to compare the methodologies. We are grateful for the opportunity to clarify our methodology in comparison to that used by Ferri et al.

(1) Ferri et al. report that the frequency of CD4\textsuperscript{+}CD25\textsuperscript{high}FOXP3\textsuperscript{+} cells “is markedly reduced in AIH compared to healthy subjects”, whereas we find that the frequency of CD4\textsuperscript{+}CD25\textsuperscript{[high]}CD127\textsuperscript{[low]}FOXP3\textsuperscript{+} in blood of AIH patients was not reduced compared to healthy subjects. To determine the Treg frequency in peripheral blood, Ferri et al. first purified CD4\textsuperscript{+}CD25\textsuperscript{+} lymphocytes from peripheral blood by a two-step magnetic purification procedure, i.e. depletion of non-CD4 cells followed by enrichment of CD25\textsuperscript{+} cells. Only then, the percentage of FOXP3\textsuperscript{+} cells in the isolated subgroup of blood cells was determined. In contrast, we did not isolate a subfraction of cells from peripheral blood, but instead performed a direct ex vivo analysis of unfractioned peripheral blood cells by staining for CD4, CD25, FOXP3, and CD127. The gating strategy for this analysis is actually shown in Fig. 1A of our paper. We see at least two critical advantages of our approach: first, by direct ex vivo analysis, we avoid the potential selection bias that is inevitable when performing a subfraction analysis after an enrichment procedure; second, by including CD127 as an additional marker, we greatly increase the fidelity of Treg identification. Moreover, we confirmed the findings obtained by this method with another method, i.e. analysis of the FOXP3 TSDR DNA methylation status.

(2) Ferri et al. find a “defective ability of Treg cells to control the proliferation of CD4\textsuperscript{+}CD25\textsuperscript{+} target cells”; while we do not find a reduced suppressor function of Treg from AIH patients. To determine Treg suppressor function, Ferri et al. used the \(^{3}\text{H}\)-thymidine assay; we, in contrast, used

References


Silvia Ferri
Claudine Lalanne
Chiara Masi
Luigi Muratori *
Department of Clinical Medicine, University of Bologna, Bologna, Italy
*Corresponding author.
E-mail address: luigi.muratori@unibo.it
Letters to the Editor

the CFSE assay. The critical advantage of the CFSE assay over the $^{3}$H-thymidine assay is its substantially higher signal to noise-ratio [3–6]. For the full argument on why the CFSE assay is much more meaningful than the $^{3}$H-thymidine assay, we refer to Sakaguchi et al. [3] who summarise “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 dilution of CFSE in effector T cells to determine the percentage and number of proliferating cells”.

(3) We read with great surprise the remark by Ferri et al. that, for performing the CFSE assay, we did not provide data “to assess the efficiency of the purification” or whether the “purified cells are CD127low”. In contrast to Ferri et al., who did not analyse the CD127 status of the purified cells, we did characterise both the purified Treg cells and the purified non-Treg cells for expression of CD127. This was clearly stated in our paper and a representative analysis of FOXP3 and CD127 status of the purified cells is shown in Fig. 2A of our paper.

(4) Ferri et al. criticise the fact that we used a Treg/T effector ratio of 1:1 in our suppressor assay, which is not supposed to reflect the Treg frequency in peripheral blood. However, the Treg frequency in blood is irrelevant for their suppressor function. Treg cells do not suppress on the fly while flowing with the blood stream; they suppress mainly through cell contact between one cell and another within (lymphoid) tissues. Consequently, as others have shown [4–6], the suppressive potential of Treg cells can be reasonably assessed with the CFSE assay at a ratio of 1:1.

(5) Ferri et al. reported the presence of FOXP3+ cells in the livers of 5/7 AIH patients, but did not make a comparison with other inflammatory liver diseases. We find that the numbers of FOXP3+ cells were significantly higher in AIH livers than in NASH livers, and that the number of FOXP3+ cells in the liver correlated with histological disease activity.

In summary, we have reasons to 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

Mid-regional pro-adrenomedullin (MR-proADM): An even better prognostic biomarker than C-reactive protein to predict short-term survival in patients with decompensated cirrhosis at risk of infection?

To the Editor:

We read with great interest the study by Cervoni et al. [1] investigating C-reactive protein (CRP) as a prognostic marker of short-term mortality in patients with advanced cirrhosis. Although persistently elevated CRP levels discriminated patients with poor prognosis due to presumably ongoing systemic inflammation, in their study, baseline CRP was less accurate discriminating bacterial infection and its hemodynamic and renal complications, which substantially contribute to the increased mortality in patients with cirrhosis and might be discriminated using novel biomarkers. A candidate biomarker is the mid-regional fragment of pro-adrenomedullin (MR-proADM), which has been demonstrated to be an excellent predictor of short-term mortality in patients with sepsis [2], lower respiratory tract infections [3] and critically ill patients with new-onset fever [4].

MR-proADM is the non-functional, more stable precursor peptide of adrenomedullin, a vasoactive calcitonin peptide family member that is rapidly cleared from circulation [5]. Circulating adrenomedullin mainly originates from endothelial cells and vascular smooth muscle cells and is upregulated under inflammatory stimuli mediated by pro-inflammatory cytokines or bacterial endotoxin, under hypervolemia and during tissue hypoxia.