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Comment on Adamzik et al: an increased alveolar CD4 + CD25 + Foxp3 + T-regulatory cell ratio in acute respiratory distress syndrome is associated with increased 30-day mortality

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Dear Editor

Adamzik et al [1] reported that an elevated ratio of $CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs) to overall $CD4^+$ cells in bronchoalveolar lavage (BAL) fluid portends a poor outcome for ARDS patients. A cellular biomarker able to provide prognostic information would be useful for ICU decision-making. However, we wish to highlight that the functional significance of alveolar Tregs remains undefined in ARDS.

Our group has shown in mouse models that Tregs—a lymphocyte subset that attenuates immune system activation and promotes repair in damaged tissues—resolve experimental lung injury via pro-repair effects on macrophage function and neutrophil efferocytosis [2], epithelial regeneration [3], and fibroproliferation [4]. It is unclear from the authors' data, obtained at a single time point, whether Tregs were actually pathogenic or if they accumulated as a pro-repair response to parenchymal damage. We hypothesize that severe lung inflammation, rather than the Tregs themselves, mediated the observed increase in mortality. A few findings call into question the study's conclusions:

1. Validated illness severity markers such as the SAPS II and lung injury scores did not predict mortality in their cohort. Additionally, in the authors' quantification of Treg-to-$CD4^+$ cell ratio there was one extreme outlier in the non-survivor group. Would the authors' conclusions hold if this data point were excluded in a sensitivity analysis?
2. Study group characteristics likely affected the results. All ARDS subjects and an unknown proportion of non-ARDS controls received glucocorticoids, which probably altered lymphocyte population features. The fact that control subjects had active malignancies may also have skewed the findings, as Tregs are known to play a major role in cancer biology.
3. Phenotypically, $CD4^+CD25^+Foxp3^+$ status is insufficient to identify human Tregs and may include newly-activated effector T cells. Other markers including CD3,

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CD127, and CD45 isoforms are usually advocated to increase specificity when analyzing human T lymphocyte populations [5]. A more specific Treg definition could have changed the conclusions of the study.

4. Finally, in addition to the reported ratio value, was the absolute Treg number per unit volume of BAL fluid different between the groups?

Many questions remain regarding the impact of alveolar Tregs in ARDS. Foremost, if these potent immunoregulatory cells are indeed lung inflammation markers, what are the mechanisms that limit their pro-repair activity following severe lung injury? Further characterization of alveolar Treg phenotype and function during resolution of human lung injury will help determine their usefulness as a biomarker and potential therapeutic target for ARDS.

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