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Immunity

Letter

Response to Are NKT cells a useful predictor of COVID-19 severity?

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The purpose of our study was to identify immune signatures specific to the immune response against SARS-CoV-2 by subtracting the immune signatures against other pathogens across patients with severe pneumonia. In order to do so, and to specifically interrogate functional features such as cytokine expression and activation/exhaustion states, we generated very broad phenotyping panels that allow the characterization of blood leukocytes. In order to have an unbiased workflow, we did not specifically interrogate certain rare subpopulations of cells such as iNKT cells (defined by CD1d tetramers loaded with α-galactosylceramide; Godfrey et al., 2004). The discovery that the frequency of the FlowSOM-generated $CD3^+$ $CD4^-$ $CD8^-$ $CD56^+$ T cell cluster has a strong predictive value for patient outcomes in COVID-19 was not anticipated.

In our original article, we had used the term "NKT" for T cells that express a natural killer receptor (CD56), and therefore to describe the FlowSOM-generated T cell cluster based on 22 immune cell markers as seen in Kreutmair et al.'s Figure 2B, 7B, S2A, and S2B (Kreutmair et al., 2021). The cluster discriminated from other (both conventional and unconventional) T cells predominantly due to CD56 positivity and CD4 and CD8 negativity, characterizing these cells as $CD3^+$ $CD4^-$ $CD8^-$ $CD56^+$ next to other markers. As pointed out (Koay et al., 2022's Figure S1A), gating on $CD3^+$ $CD56^+$ T cells delivers a heterogeneous subset containing TCRγδ T cells, iNKT, cells and MAIT cells. To be more precise and avoid confusion, we corrected the term "NKT" with "$CD56^+$ T cells."

One reason for discrepancies in frequencies and their predictive value for severe COVID-19 in our study compared to the analysis by Koay et al. may be due to the overall approach in how the unconventional T cell subsets were identified. The FlowSOM algorithm, which we used, is designed to work with high-parametric datasets and takes all included markers into account, while Koay et al. defined the populations based on sequential manual gating of 2-dimensional plots. We measured approximately 5% of $CD56^+$ T cells among T cells in healthy controls (Kreutmair et al., 2021's Figure 5B) compared to approximately 10% detected by Koay et al. (Koay et al., 2022's Figure S1E), making a direct comparison of the predictive values for severe COVID-19 of the identified subsets difficult. Furthermore, their classification of mild and severe COVID-19 patients (patients admitted to a ward versus an ICU) differed from ours, which was based on World Health Organization (WHO)-based grading of COVID-19 severity.

We would like to point out here that the transfer of complex high-parametric algorithm-based population characterizations into daily clinical routines is challenging, where often sequential manual gating using 2-dimensional plots is used to define populations. Therefore, as we had already pointed out in Kreutmair et al., we suggested larger follow-up studies to solidify this measurement as a predictive biomarker for COVID-19 patient outcomes.

Koay et al. and others (Zhang et al., 2020) did not find $CD56^+$ T cell frequency to hold predictive value for COVID-19 patient outcomes. However, several reports demonstrated a predictive value for the $CD3^+$ $CD56^+$ T cell subset as well as MAIT cells in COVID-19 (Flament et al., 2021; Li et al., 2020; Notarbartolo et al., 2021; Odak et al., 2020; Parrot et al., 2020; Zingaropoli et al., 2021). This is in line with our data demonstrating reduced frequencies of the $CD4^-$ $CD8^-$ (Kreutmair et al., 2021's Figure 2C) and the $CD56^+$ T cell cluster (Kreutmair et al., 2021's Figure 5B) in severe COVID-19; both clusters include circulating MAIT cells.

Taken together, regardless of nomenclature and specific research interests, the unbiased algorithm-based analysis suggested that $CD56^+$ T cells have predictive value for COVID-19 patient outcomes. This cell population appears to include TCRγδ T cells, iNKT cells, and MAIT cells. It would be of interest to have a more fine-grained analysis of these populations and, moreover, to understand their functional significance for disease development.

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