Comparison between non-pulmonary and pulmonary immune responses in a HIV decedent who succumbed to COVID-19

We read with interest the study by Manuel *et al* showing that chronic immunosuppression could protect against severe COVID-19 in liver transplant patients.[1] Despite increased comorbidities, COVID-19 in liver transplant patients was not more severe than in non-transplant cohorts.[1 2] We present findings from a COVID-19/HIV coinfected decedent who exhibited a significantly longer survival time (46 days) than that of three COVID-19 decedents (average 30 days) (figure 1A). Given the immunosuppressive effects of HIV,[3] the prolonged survival of our COVID-19/HIV patient may reflect protection from severe COVID-19.

To investigate tissue-specific immune responses and viral load, we performed

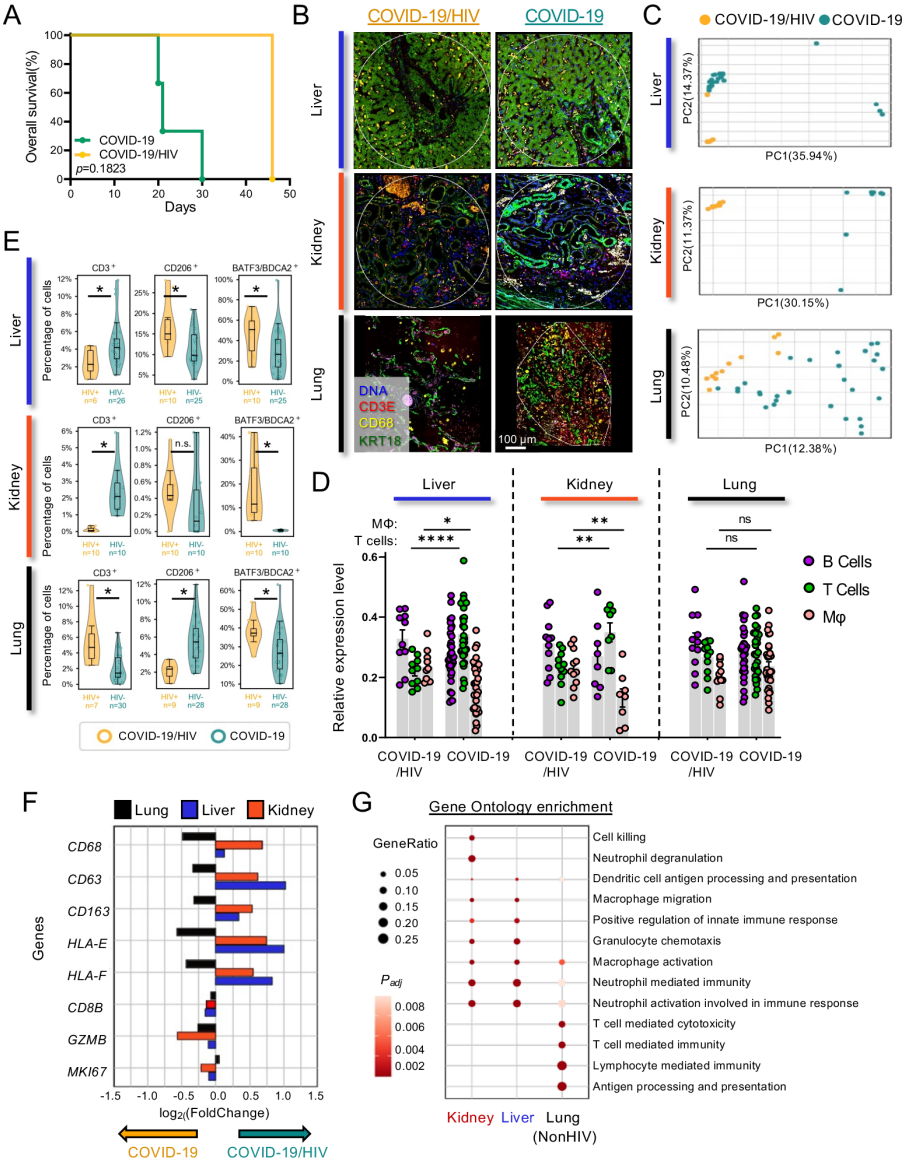

Figure 1 Increased myeloid and reduced T-cell abundance characterises the liver and kidney but not the lungs of a COVID-19/HIV decedent. (A) Kaplan-Meier survival curve of COVID-19/HIV case ($n_{COVID\text{-}19/HIV}$ = 1) and COVID-19 cases ($n_{COVID\text{-}19}$=3). P-value: Log-rank (Mantel-Cox) test. (B) Representative regions of interest (ROI) of the liver, kidney, and lung from the COVID-19/HIV decedent and one COVID-19 decedent. (**C**) Principal component analyses of transcriptional profiles of COVID-19/HIV and COVID-19 decedents from ROIs of the lung ($n_{COVID\text{-}19/HIV}$ = 11, $n_{COVID\text{-}19}$=29), liver ($n_{COVID\text{-}19/HIV}$ = 10, $n_{COVID\text{-}19}$=32), and kidney ($n_{COVID\text{-}19/HIV}$ = 11, $n_{COVID\text{-}19}$=8). (D) Relative estimated levels of immune cells determined by deconvolution of digital spatial profiling ROIs using CIBERSORTx (https://cibersort.stanford.edu/). Grey bars indicate the means. P-values were calculated by a two-tailed *t*-test: *(<0.05), **(<0.01), ***(<0.001), ****(<0.0001), ns (not significant). (E) Comparison of the abundance of various immune cell populations between ROIs of the COVID-19/HIV decedent and COVID-19 decedents by multiplex immunohistochemistry. P-values were calculated by a two-tailed U-test: *(<0.05), ns (not significant). (F) Differential gene expression of selected immune cell phenotypes and function-related genes between the COVID-19/HIV decedent and COVID-19 decedents. (**G**) Gene ontology analysis of pathways enriched in the COVID-19/HIV decedent compared with COVID-19 decedents.

in-depth analyses of the patient's immune landscape. Digital spatial profiling (online supplemental materials and methods) on liver, kidney and lung tissues from our study cohort (figure 1B) revealed that the COVID-19/HIV decedent had unique transcriptomic profiles, especially in the liver and kidney (figure 1C). The decedent exhibited a reduction of T-cells and elevation of macrophages only in the non-pulmonary tissues (figure 1D) and no difference in the abundance of natural killer cells and other immune cell types across tissue types (online supplemental figure 1A). Multiplex immunohistochemistry analysis (online supplemental materials and methods) further supported these findings (figure 1E) and additionally revealed increased T-cell and dendritic cell and decreased macrophage cell populations in the lung of the COVID-19/HIV decedent (figure 1E). Hence, at both transcriptomic and protein levels, prolonged survival of the COVID-19/HIV decedent involved differential immunological function in non-pulmonary tissues.

Differential gene expression analysis revealed an upregulation of myeloid cell lineage (*CD68*, *CD63*, *CD163*) and immunoregulatory (*HLA-E*, *HLA-F*) markers only in the non-pulmonary tissues of the COVID-19/HIV decedent while T-cell function-related markers (*CD8*, *GZMB*, *MKI67*) did not differ (figure 1F). Gene ontology enrichment analysis confirmed an enrichment in pathways related to myeloid cell activation exclusively in the non-pulmonary tissues (figure 1G, online supplemental figure 1B). Taken together, these imply that macrophages in the liver and kidney of the COVID-19/HIV decedent were activated and functional.

Liver-associated disease is a leading cause of death in patients with HIV.[4 5] As liver injury has been reported in patients with COVID-19,[6] we investigated the T-cell and myeloid cell responses in the liver. T-cell suppression-associated genes (*HAVCR2*, *LAIR1*, *LILRB1*, *CXCL13*, *LAG3*) were upregulated, and T-cell activation-associated genes (*CD3*, *CD4*, *CD8*, *GZMB*, *GZMK*) were downregulated in CD3-rich regions of the liver (figure 2A). T-cell genes were also broadly down-regulated in the kidney of the COVID-19/HIV decedent (online supplemental figure 2A). In line with a deficient T-cell response due to HIV infection, T-cell abundance was unrelated to SARS-CoV-2 viral load (NP^+) in the COVID-19/HIV decedent but was positively associated with viral load in the COVID-19 decedents (figure 2B, online supplemental figure 2B).

In contrast to the T-cell response, a comparison of CD68-rich regions revealed upregulated myeloid cell activation-associated genes (*CD63*, *CD14*, *CD68*, *IL6*) in the liver (figure 2A) and kidney (online supplemental figure 2A) of the COVID-19/HIV decedent. A greater abundance of activated macrophages was similarly observed (figure 2C, online supplemental figure 2C), together with a significantly higher viral load (NP^+) (figure 2D). Interestingly, macrophage

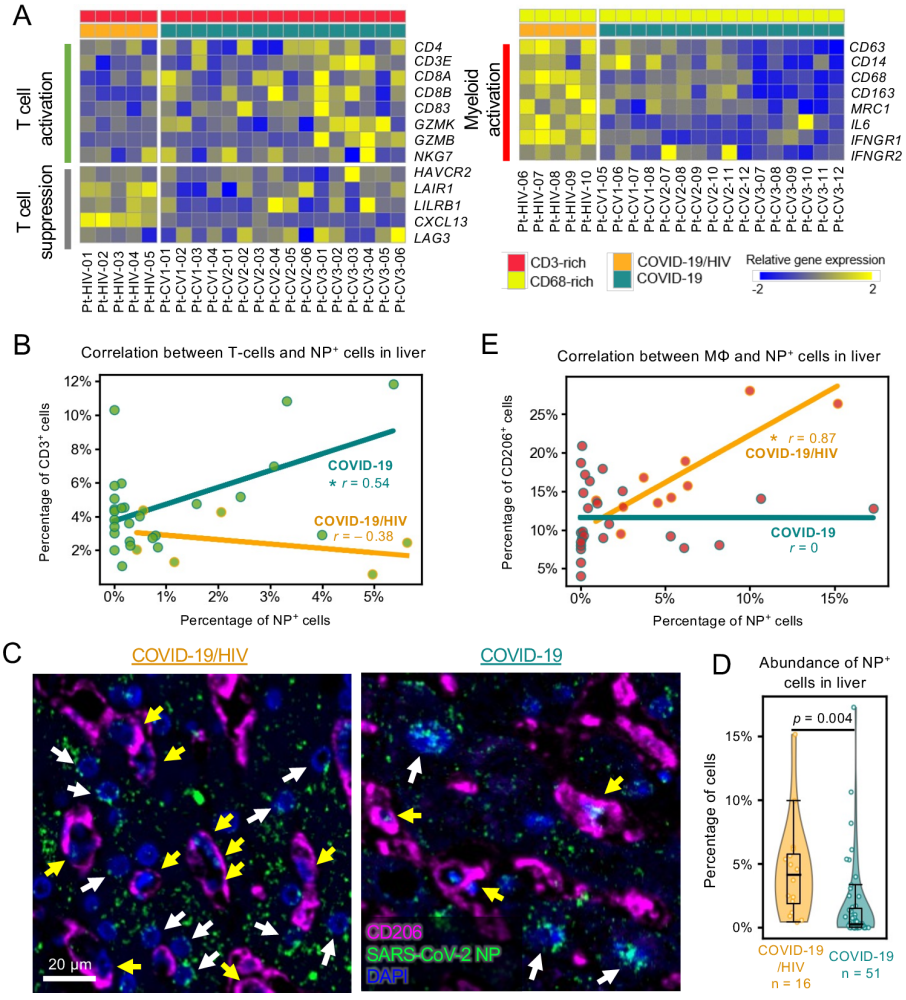

Figure 2 A diminished T-cell response and increased macrophage response in the liver of the COVID-19/HIV decedent is associated with NP$^+$ cell abundance. (**A**) Expression heatmap of genes related to T-cell activation or suppression in CD3-rich regions of interest (ROIs) genes related to myeloid activation in CD68-rich ROIs of the liver. (**B**, **E**) Correlation between the abundance of NP$^+$ cells in the liver ROIs of COVID-19/HIV (red) and COVID-19 (blue) decedents to the abundance of T-cells (B) or macrophages (**E**). P-values were calculated by Wald tests: *(<0.05). (C) Representative liver multiplex immunohistochemistry ROIs of the COVID-19/HIV decedent and COVID-19 decedents (yellow arrows: macrophages; white arrows: NP$^+$ cells). (D) Comparison of abundance of NP$^+$ cells across liver ROIs between the COVID-19/HIV decedent and COVID-19 decedents, as quantified by multiplex immunohistochemistry.

abundance positively correlated with viral load in the liver of the COVID-19/HIV decedent but not in the COVID-19 decedents (figure 2E).

These findings suggest that HIV as a comorbidity promotes virus-induced myeloid cell activation in response to SARS-CoV-2 infection in the absence of a viable T-cell response. Indeed, HIV-infected myeloid cells are long lived and resistant to the cytopathic effect.[7] However, HIV infection renders them functionally impaired.[7 8] Similarly, SARS-CoV-2-infected myeloid cells are also dysregulated[9] and may not partake in the cascade signature of hyperinflammation. Particularly in the liver, where direct SARS-CoV-2 infection and high viral load likely contribute to liver injury,[6 10] HIV-mediated immunosuppression may lower the risk of COVID-19 disease progression. Close monitoring of the immune status of myeloid cells in other organs such as the liver and further research on the COVID-19–HIV relationship is warranted to develop optimal treatment strategies.

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