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Executive Summary

The COVID-19 pandemic highlights vast clinical differences among SARS-CoV-2 infected individuals, influenced by factors like age, sex, comorbidities, and genetics. However, understanding the degree and drivers of individual and population differences in immune responses to SARS-CoV-2 remains poorly known. Here, we first aimed to define the genes that were differently expressed upon SARS-CoV-2 exposure in human cells ex vivo and quantify individual and population variations in transcriptional responses to this virus. To this end, peripheral blood cells from individuals of different ancestries (Central African, West European, and East Asian) were exposed to SARS-CoV-2 and, as a comparison, influenza A virus (IAV). Using single-cell RNA sequencing, we were able to identify 22 different cell types. We found that exposure to SARS-CoV-2 and IAV induces moderate changes in cell abundance, while lineage identity was the main driver of gene expression variation (~32%), followed by virus exposure (~27%). Both viruses induced strong interferon-stimulated gene (ISG) responses, with SARS-CoV-2 eliciting a specific inflammatory gene network. Interindividual ISG response variability was higher for SARS-CoV-2, predominantly driven by IFN-α. Despite differences in IFN expression between viruses, interindividual ISG response patterns were similar, suggesting a largely shared IFN-driven response between SARS-CoV-2 and IAV.

Abbreviations

FDR	false discovery rate
GWAS	genome-wide association studies
IAV	influenza A virus
ISG	interferon-stimulated gene
IFN	interferon
PBMC	peripheral blood mononuclear cells
pDC	plasmacytoid dendritic cells

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1 LIST OF GENES DIFFERENTIALLY EXPRESSED IN CELL TYPES UPON SARS-COV-2 CHALLENGE EX VIVO

1.1 Background

The COVID-19 pandemic has brought to light considerable clinical variation among individuals infected with SARS-CoV-2, ranging from asymptomatic cases to fatal outcome (1). Factors influencing this variability include advanced age, male sex, comorbidities, and human genetics (2, 3). Specifically, inborn errors of or auto-antibodies against type I interferons (IFNs) have been identified as strong contributors to the severity of COVID-19 pneumonia (4). Additionally, genome-wide association studies (GWAS) involving over 200,000 cases and 3 million controls have pinpointed 51 distinct loci associated with infection susceptibility to SARS-CoV-2 or COVID-19 severity, underscoring the important role of human genetic factors in the pathophysiology of the disease (3, 5).

Despite the unprecedented progress made by immunological and genetic research during the COVID-19 pandemic, several questions remained unanswered. Despite increasing evidence suggesting that human populations can display important differences in their immune responses to pathogen exposure (6), the magnitude and drivers of population variation in immune responses to SARS-CoV-2 have not been thoroughly investigated.

1.2 Results

The purpose of this deliverable was to define a list of genes that are differentially expressed upon SARS-CoV-2 treatment of human cells ex vivo, and thus, to understand not only the nature of the genes and pathways involved in the responses to this virus, but also to quantify the degree of individual and population variation in the response to SARS-CoV-2. To address these questions, we exposed peripheral blood mononuclear cells (PBMCs) from individuals of Central African, West European, and East Asian descent to SARS-CoV-2 and, for comparison purposes, to influenza A virus (IAV) (7). We characterized transcriptional responses to SARS-CoV-2 and IAV by performing single-cell RNA-sequencing (scRNA-seq) on PBMCs from 222 SARS-CoV-2-naïve donors originating from three geographic locations (**Figure 1a**). PBMCs were treated for six hours with a mock-control (non-stimulated), SARS-CoV-2 (ancestral strain, BetaCoV/France/GE1973/2020) or IAV (H1N1/PR/8/1934).

In total, we captured over one million high-quality single-cell transcriptomes. By combining transcriptome-based clusters with cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq), we defined 22 different cell types across myeloid, B, $CD4^+$ T, $CD8^+$ T and natural killer (NK) immune lineages (**Figure 1b**). Following virus exposure, most cell types showed moderate changes in abundance. To assess the extent to which inferred cell states were comparable across stimulation conditions, we conducted differential abundance analyses by contrasting the number of cells assigned to a given cell type or lineage between stimulated (SARS-CoV-2, IAV) and non-stimulated (NS) conditions. We detected significant differences in 46% and 55% of cell types (Bonferroni-adjusted Wilcoxon's signed rank p-value < 0.01) following stimulation with SARS-CoV-2 and IAV, respectively. Yet, for over half of these cell types, the effect of stimulation was very limited (i.e., a change of < 5 cells per sample or < 10% of cells relative to the NS condition). Among the strongest differences, we observed a decrease of up to 62% in the number of CD14+ and CD16+ monocytes in response to both viruses, as well as an IAV-specific transition of myeloid cells towards infected monocytes.

After adjusting for technical factors, we found that lineage identity was the main driver of gene expression variation (~32%), followed by virus exposure (~27%) (**Figure 1b, c**). Both viruses induced a strong transcriptional response, with 2,914 genes upregulated (false discovery rate (FDR) < 0.01, $\log_2$FC > 0.5; out of 12,655 with detectable expression). The list of genes differentially expressed upon SARS-CoV-2 and IAV treatment is presented in **Table 1 (attached as an excel file)**. These responses were highly correlated across immune lineages and featured a strong induction of interferon-stimulated genes (ISGs) (**Figure 2a**). However, myeloid responses were markedly heterogeneous, with SARS-CoV-2 inducing a transcriptional network enriched in inflammatory-response genes (GO:0006954; fold-enrichment (FE) = 3.4, FDR < 4.9×10^{-8}). Importantly, *IL1A*, *IL1B* and *CXCL8* were highly and specifically upregulated in response to SARS-CoV-2 ($\log_2$FC > 2.8, FDR < 2.3×10^{-36}).

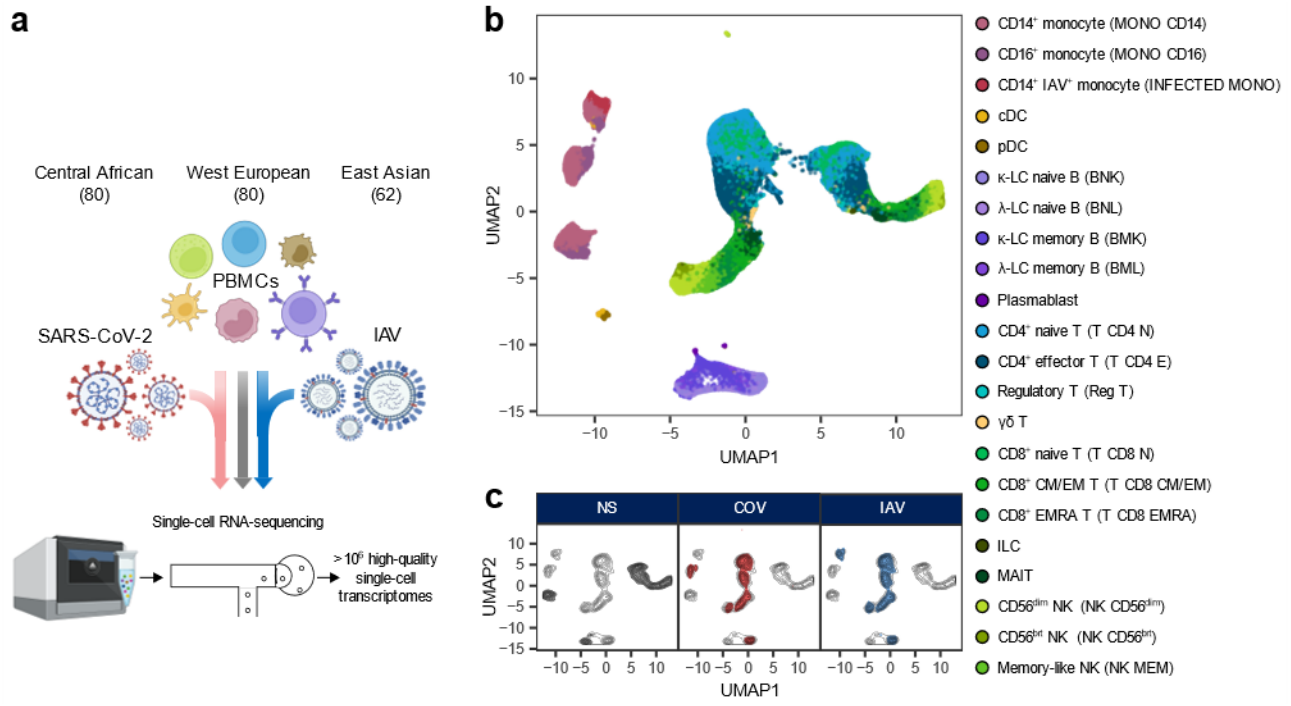

Figure 1. Population single-cell responses to SARS-CoV-2 and IAV. **a**, Study design. **b and c**, Uniform manifold approximation and projection (UMAP) of 1,047,824 peripheral blood mononuclear cells: resting (non-stimulated; NS), stimulated with SARS-CoV-2 (COV), or influenza A virus (IAV) for six hours. **b**, The colours indicate the 22 cell types inferred. **c**, Distribution of cells in NS, COV and IAV conditions on UMAP coordinates. Contour plot indicates the overall density of cells, and coloured areas delineate regions of high cell density in each condition (gray: NS, red: COV, blue: IAV).

To assess interindividual variability in the response to viruses, we summarized each individual's response as a function of their mean ISG expression. SARS-CoV-2 induced more variable ISG activity than IAV across lineages, with myeloid cells displaying the strongest differences (Levene test p-value < 6.2×10^{-6}, **Figure 2b**). We determined the contributions of the various interferons (IFNs) to variation of ISG activity, using single-molecule arrays (SIMOA) to quantify the levels of secreted IFN-α, β and γ. In the SARS-CoV-2 condition, IFN-α accounted for up to 57% of ISG variability (**Figure 3a, b**), consistent with its determinant role in COVID-19 pathogenesis. *IFNA1-21* transcripts were mostly produced by infected $CD14^+$ monocytes and plasmacytoid dendritic cells (pDCs) after IAV stimulation, while pDCs were the only important source of *IFNA1-21* after SARS-CoV-2 stimulation (i.e., producing 88% of transcripts; **Figure 3c**). *IFNA1-21* expression by pDCs was weaker after stimulation with SARS-CoV-2 ($\log_2$FC = 6.4 *vs*. 12.5 for IAV, Wilcoxon's rank-sum p-value = 1.2×10^{-16}). Nevertheless, patterns of interindividual variability for ISG activity were remarkably similar after virus treatment ($r = 0.60$, Pearson's p-value < 1.2×10^{-22}, **Figure 3d**), indicating that the IFN-driven response is largely shared between SARS-CoV-2 and IAV.

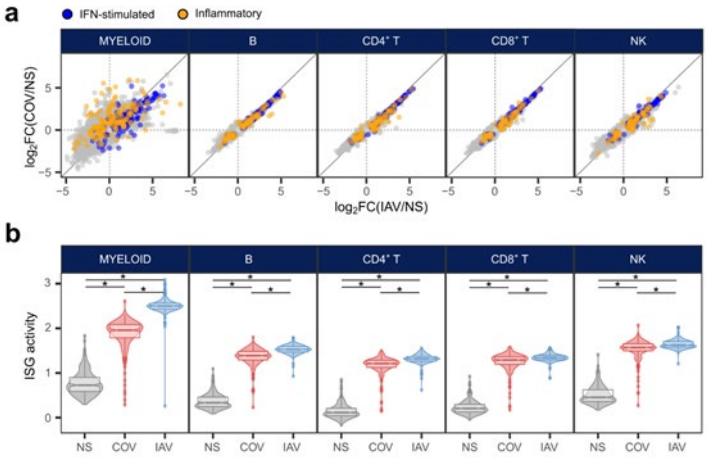

Figure 2. Transcriptional responses to SARS-CoV-2 and IAV stimulation. **a**, Comparison of transcriptional responses to SARS-CoV-2 and IAV across major immune lineages. **b**, Distribution of ISG activity in the non-stimulated state and in response to SARS-CoV-2 (COV) and IAV across the five major immune lineages. *: Wilcoxon's two-sided signed-rank p-value < 2.2×10^{-16}.

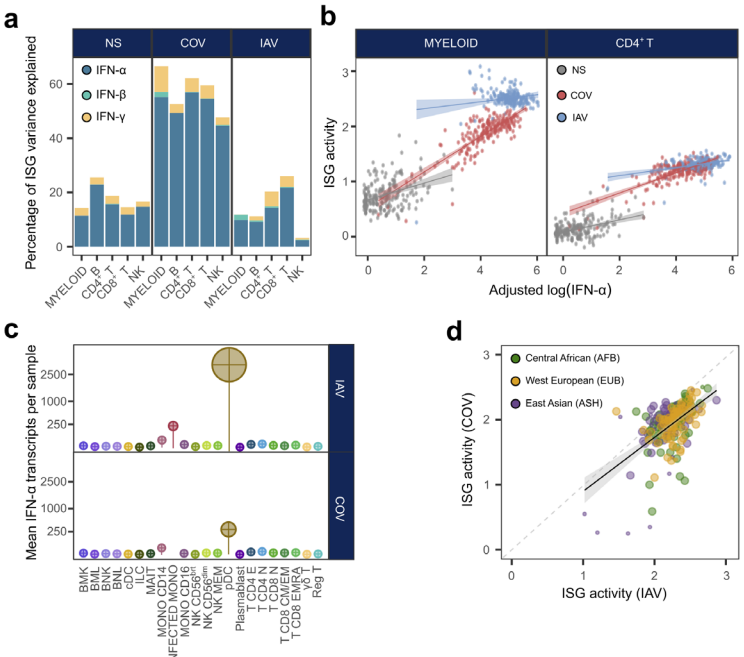

Figure 3. Drivers of population variation in expression of interferon-stimulated genes. a, Proportion of the variance of ISG activity explained by IFN-α, IFN-β and IFN-*γ* in the non-stimulated condition and in response to SARS-CoV-2 and IAV, across the five major immune lineages. **b,** Correlation between levels of IFN-α in the supernatants and ISG activity in myeloid and CD4⁺ T cells, adjusted for cellular mortality. **c**, Relative expression of IFN-α-encoding transcripts by each immune cell type in response to SARS-CoV-2 and IAV. Bar lengths indicate the mean number of IFN-α transcripts contributed by each cell type to the overall pool. Dot area is proportional to the mean level of IFN-α transcripts in each cell type (counts per million). **d**, Correlation of ISG activity scores between SARS-CoV-2 and IAV-stimulated samples. Each dot corresponds to a single individual and its colour indicates the self-reported ancestries of the individual concerned. Shaded error band shows the 95% confidence interval (mean ± 2 SEM) of the expected ISG activity in COV-stimulated sample, given ISG activity in IAV-stimulated samples. Samples with a cellular viability below the 10th percentile are indicated by smaller dots.

2 Conclusion

Using single-cell RNA sequencing technologies, we showed that both SARS-CoV-2 and IAV elicit robust transcriptional responses, characterized by a strong induction of ISGs across all major immune lineages. However, myeloid responses markedly differed between the two viruses, with a notable inflammatory signature, characterized by the induction of IL1A, IL1B, and CXCL8, specifically triggered by SARS-CoV-2. Additionally, besides this unique pro-inflammatory signature in myeloid cells, our study reveals that SARS-CoV-2 induces a weaker but more heterogeneous interferon-stimulated gene activity compared to IAV. Overall, our results underscore the importance of single-cell approaches in capturing the full diversity of peripheral immune responses to respiratory RNA viruses, particularly SARS-CoV-2.

3 References

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