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Executive Summary

The COVID-19 pandemic has underscored the wide clinical variability among individuals infected with SARS-CoV-2, influenced by factors such as age, sex, comorbidities, and human genetics. However, the extent and drivers of population-level differences in immune responses to SARS-CoV-2—as well as the specific impacts of age and sex—remain poorly understood. Focusing first on population-level variation, we found that 3,389 genes exhibit baseline differential expression across immune lineages, while 898 and 652 genes show differential responses between populations following stimulation with SARS-CoV-2 and IAV, respectively. We show that variation in cellular composition—primarily driven by latent CMV infection—accounts for a substantial portion of these population differences in gene expression, with the strongest effects observed in NK cells. Regarding age and sex, we identified 2,052 genes whose expression changes with age, most prominently in $CD4^+$ naive T cells, and 2,983 genes affected by sex, particularly in NK cells and monocytes. Notably, age-related effects were largely context-dependent, with 71% of age-associated differentially expressed genes manifesting only after viral stimulation. In contrast, sex-related differences were predominantly evident at baseline, with only 35% induced by viral exposure.

Abbreviations

CMV	cytomegalovirus
EMRA	effector-memory re-expressing CD45RA
IAV	influenza A virus
ISGs	interferon-stimulated genes
NK	Natural killer
PBMCs	peripheral blood mononuclear cells
scRNA-seq	single-cell RNA sequencing
UTR	untranslated region

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1 Characterisation of ancestry, age, and sex effects on immune responses to SARS- CoV-2

1.1 Background

The COVID-19 pandemic has highlighted considerable clinical variation among individuals infected with SARS-CoV-2, ranging from asymptomatic cases to fatal outcome (1). Factors contributing to 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 more than 50 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 (2-5), several key questions remained unanswered. First, although growing evidence suggests that human populations can exhibit marked differences in immune responses to pathogen exposure (6), the magnitude and determinants of population-level variation in immune responses to SARS-CoV-2 have not been thoroughly investigated. Second, while age and sex are increasingly recognised as important factors influencing both infectious and non-infectious immunity-related diseases in general (7-10), and COVID-19 severity in particular (1, 2), the extent to which age and sex exert cell type-specific effects on immune responses to SARS-CoV-2 remains largely unknown.

1.2 Results

The purpose of this deliverable was to characterise how ancestry, age and sex can affect transcriptional responses to SARS-CoV-2 ex vivo. Focusing first on ancestry, we exposed PBMCs from 222 SARS-CoV-2-naïve donors of Central African, West European, and East Asian descent to SARS-CoV-2 and, for comparison purposes, to IAV (11) (**Figure 1**) (11). After a 6h-stimulation with a mock-control (non-stimulated), SARS-CoV-2 or IAV, we used scRNA-seq to characterise the transcriptional responses to these respiratory RNA viruses across 21 distinct cell subsets, from 5 major immune lineages. As presented in D1.1, both SARS-CoV-2 and IAV induced robust

transcriptional responses, featuring a strong induction of ISGs across all major lineages. However, myeloid responses markedly differed between the two viruses, with a notable inflammatory signature characterized by the induction of IL1A, IL1B and CXCL8 triggered specifically by SARS-CoV-2.

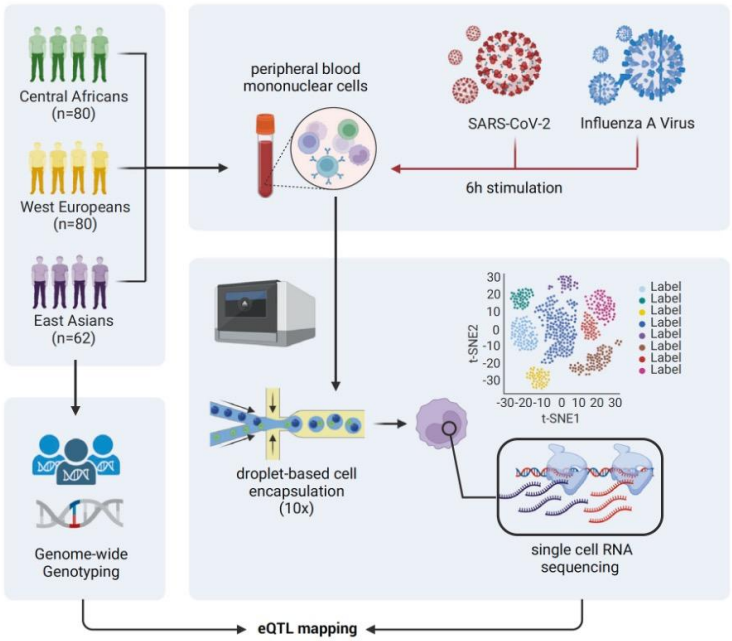

Figure 1. General study design for the study of the impact of ancestry on transcriptional responses of PBMCs to SARS-CoV-2, at single-cell resolution.

In searching for differences in immune response across populations, we found that, across immune lineages, 3,389 genes display population differences in baseline expression, and 898 and 652 genes present differential responses between populations after stimulation with SARS-CoV-2 and IAV, respectively (**Figure 2a**). We found that widespread population variation in cellular composition accounted for a substantial portion (up to 50%) of population differences in gene expression, with the most significant impact on NK cells (**Figure 2b**). For example, a subset identified as memory-like NK cells constituted 55.2% of the NK compartment in African-descent individuals, but only 12.2% in Europeans (Wilcoxon's rank-sum test, $P < 1.3 \times 10^{-18}$). Furthermore, we identified latent CMV infection as an important predictor of cellular heterogeneity, with CMV being most prevalent in African-descent individuals (i.e., 99% of African-descent individuals versus 31% of Europeans and 78% of East Asians). CMV serostatus, regardless of the population, was associated with higher proportions of memory-like NK and CD8+ EMRA T cells (**Figure 2c**). Using mediation analysis, we estimated that CMV serostatus accounts for up to 73% of the population differences in the proportion of these cell types, with these differences substantially impacting the transcriptional response to SARS-CoV-2. Together, these results reveal how variation in environmental exposures, like CMV

infection, can contribute to population differences in responses to SARS-CoV-2 through changes in the lymphoid composition.

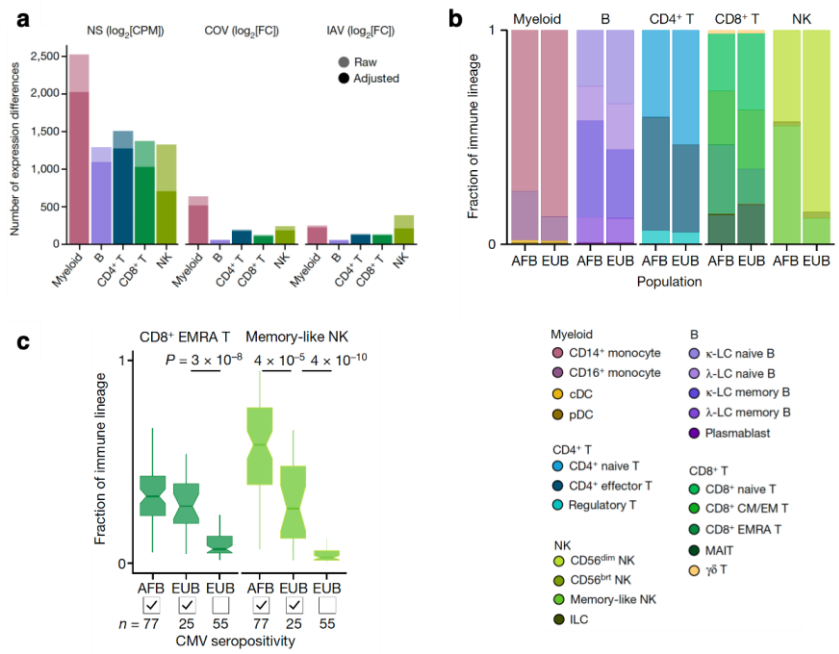

Figure 2. Population differences in responses to SARS-CoV-2. (a) The number of genes differentially expressed between populations at baseline (NS) or in response to SARS-CoV-2 (COV) or IAV, in each immune lineage. Numbers are provided before and after adjustment for cellular composition. (**b**) Cell type proportions within each major immune lineage in Africans (AFB) and Europeans (EUB). (**c**) Distribution of CD8+ EMRA T and memory-like NK cell frequencies in AFB and EUB according to CMV serostatus.

Focusing on the impact of age and sex on immune responses to SARS-CoV-2, we next exposed PBMCs from 415 donors—all of West European ancestry, aged 30 to 80, and with a balanced sex ratio—to SARS-CoV-2 and IAV, followed by scRNA-seq analyses. Examining age-related effects on gene regulation across cell types, we identified 2,052 genes whose expression levels or 3′UTR isoform usage changed with age (**Figure 3a**). The most pronounced changes in gene expression were observed for $CD4^+$ naive T cells, while $CD8^+$ naive T cells showed the greatest shifts in 3′UTR isoform usage. In contrast, sex-related effects were less cell-type-specific: 2,983 genes were differentially expressed (or presented different isoform usage) between males and females in at least one cell type, with the strongest effects observed in innate immune cell types such as NK cells and monocytes (**Figure 3b**).

Most notably, our analysis revealed that the effect of age on gene expression is largely context-dependent, with 71% of age-related differentially expressed genes manifesting only after viral stimulation (**Figure 3a**). Conversely, sex differences in gene expression were predominantly

observed at baseline, with only 35% of these differences induced by stimulation (**Figure 3b**). These effects translated into functional differences between sexes; for example, females exhibited stronger antiviral responses in CD14+ monocytes mediated by type I IFN signalling (GO:0034340) and inflammatory regulation (GO:0050729). This prompted us to investigate whether sex-specific effects could arise from age-associated mosaic loss of the Y chromosome (mLoY). We found that mLoY is lineage- and cell-type-specific, primarily affecting NK and myeloid cells. Furthermore, genes overexpressed in mLoY cells after viral stimulation, compared to non-mLoY cells from the same individuals, were enriched in innate immune pathways (GO:0045087). These preliminary findings suggest that Y chromosome loss may contribute to inter-individual differences in antiviral responses through enhanced innate immunity.

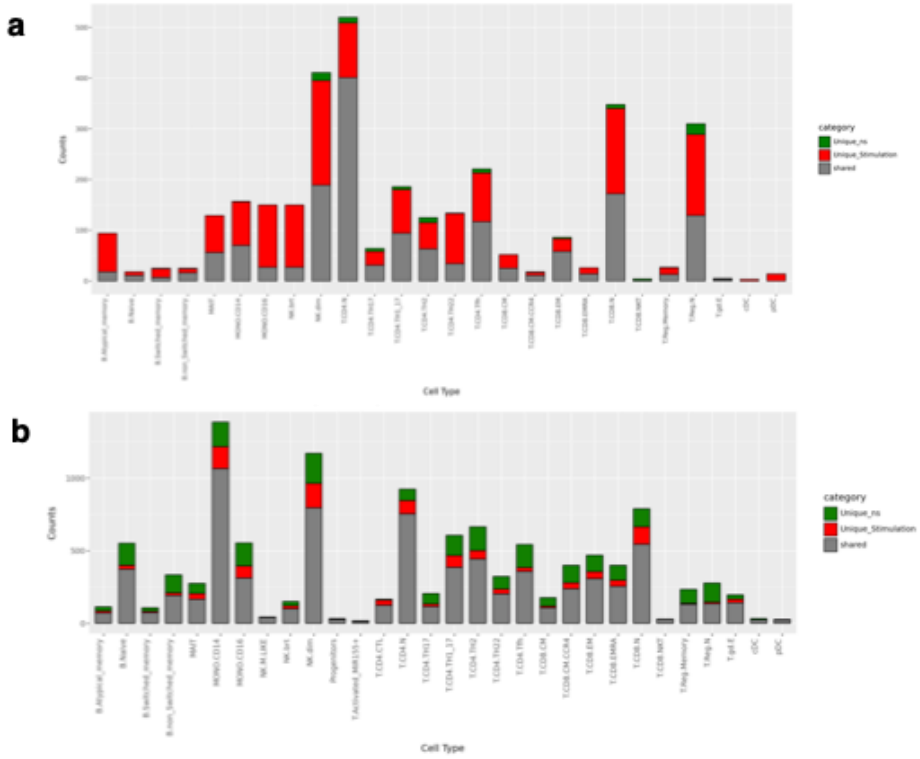

Figure 3. Impact of age and sex on immune responses to SARS-CoV-2. Total number of genes whose expression levels vary with (**a**) age and (**b**) sex. Green areas indicate genes differentially expressed either by age or sex only at the baseline (unstimulated) conditions, red areas represent genes affected only upon viral stimulation, and grey areas denote genes influenced in both conditions. Notably, the impact of age is predominantly observed after viral stimulation (red areas), in contrast with the effect of sex (**b**), which shows a more limited influence of virus-specific responses.

2 Conclusion

Using single-cell RNA sequencing technologies, we showed that populations with different ancestry exhibit marked differences in immune responses to SARS-CoV-2 exposure. A large fraction of these differences is accounted by baseline differences in immune cell composition, which were largely attributed to population differences in environmental exposure, notably CMV infection. We also found that age and sex both have a substantial impact on cell-type transcriptional profiles, with age showing a much stronger, specific impact upon viral stimulation than sex, the main impact of which is already present at baseline. The extent to which ancestry-, age- and sex-specific effects on leukocyte responses to SARS-CoV-2 also result from, or depend on, variation in human genetics is currently under investigation.

3 References

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