Immune responses to SARS-CoV-2 in sub-Saharan Africa and western Europe: a retrospective, population-based, cross-sectional study

Mbow, M.; Hoving, D.; Cisse, M.; Diallo, I.; Honkpehedji, Y.J.; Huisman, W.; ... ; BEAT COVID Consortium COVID 19 Leiden Univ Med Ctr Study Team

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Moustapha Mbow, Dennis Hoving*, Marouba Cisse†, Ibrahima Diallo†, Yabo J Honkpehedji†, Wesley Huisman, Cilia R Pothast, Marlieke L M Jongsma, Marion H König, Alicia C de Kroon, Le Thi Kieu Linh, Shohreh Azimi, Tamar Tak, Yvonne C M Kruize, Farid Kurniawan, Yacine Amet Dia, Jaimie L H Zhang, Corine Prins, Anna H E Roukens, Jutte J C de Vries, Linda J Wammes, Hermelijn H Smits, Ayola A Adegnika, Mihaela Zlei, Taco W Kuijpers, Luuk Wieske, Alioune Dieye, Souleymane Mboup, Peter G Kremsner, Filip Eftimov, Thirumalaisamy P Velavan, Ilana Berlin, Mirjam H M Heemskerk, Maria Yazdanbakhsh‡, Simon P Jochems‡, on behalf of BEAT-COVID consortium§, COVID-19 Leiden University Medical Center study team§*

Summary

Background SARS-CoV-2 has been associated with a higher proportion of asymptomatic infections and lower mortality in sub-Saharan Africa than high-income countries. However, there is currently a lack of data on cellular immune responses to SARS-CoV-2 in people living in Africa compared with people in high-income regions of the world. We aimed to assess geographical variation in peripheral and mucosal immune responses.

Methods In this retrospective, population-based, cross-sectional study, we analysed peripheral blood and nasal curettage samples from seven clinical studies involving individuals from Senegal (Senegalese cohort), the Netherlands, and Germany (European cohort). Samples were collected between Nov 1, 2018, and Dec 20, 2021. We included samples from individuals with no, mild, or severe COVID-19. A validation cohort of individuals from Senegal and Gabon (n=64) was used to validate key findings from the main cohort. Matching of individuals between geographical regions by age, sex, viral load, and infection severity and duration was used to address confounding factors. We examined the cellular, humoral, and cytokine immune responses using cytometry by time of flight, spectral flow cytometry, ELISA, and Luminex.

Findings We included 133 individuals (59 from the Senegalese cohort and 74 from the European cohort). In contrast to the European cohort, mild COVID-19 in the Senegalese cohort was not associated with any statistically significant perturbations in blood or nasal immune cell profiles, nor with increased pro-inflammatory cytokines, although SARS-CoV-2-specific adaptive immunity was readily induced, as seen in Europeans. In severe COVID-19, both the Senegalese and European cohorts showed lymphopenia (Senegal: 2·9-times decrease, p=0·0010 *vs* Europe: 1·6-times decrease, p=0·0046) and increased neutrophil frequencies in blood (Senegal: 2·0-times increase, p=0·0044 *vs* Europe: 1·3-times increase, p=0·026) and the nasal mucosa $CD66b^{+}CD16^{low}$ neutrophils (Senegal: 9·9-times increase, p=0·045 *vs* Europe: 392-times increase, p<0·0001). However, in contrast to Europeans, the Senegalese cohort had no significant expansion of immature immune populations, inflammasome activation, or monocyte recruitment to the nasal mucosa.

Interpretation The observed divergent immunological trajectories during SARS-CoV-2 infection offer a potential explanation for the reported attenuated disease course in sub-Saharan Africa and highlight the need to further investigate immune responses to SARS-CoV-2 in understudied populations.

Funding European and Developing Countries Clinical Trials Partnership 2 programme (AIDCO), LUMC Gisela Thier Fellowship, Dutch Research Council (NWO), European Research Council, and Leids Universitair Fonds.

Introduction

Following the emergence of SARS-CoV-2 in 2019, it is estimated that 65% of Africans had been infected with SARS-CoV-2 at least once by December, 2021.[1] Surprisingly, SARS-CoV-2 infection showed a decreased disease severity in sub-Saharan Africa in comparison with high-income regions, with asymptomatic infections estimated to be twice as high in sub-Saharan Africa than the USA or Europe.[1,2,3]

Given the role of excessive immune activation in COVID-19 pathogenesis, geographical variation in immune responses to SARS-CoV-2 could impact the disease course.[4] Geographical variation in immune responses to vaccines, such as the rotavirus and malaria vaccines, is well documented.[5]

Lancet Microbe 2025; 6: 100942

Published Online December 18, 2024 https://doi.org/10.1016/j.lanmic.2024.07.005

*Joint first authors
†Joint second authors, ordered alphabetically
‡Joint senior authors
§Members listed in the appendix (pp 1–2).

Department of Immunology, Faculty of Medicine, Pharmacy, and Odontology, Cheikh Anta Diop University of Dakar, Dakar, Senegal (Prof M Mbow PhD, M Cisse PharmD, I Diallo PharmD, Prof A Dieye PhD); **Institute of Health Research, Epidemiological Surveillance and Training, Dakar, Senegal** (Prof M Mbow, Y A Dia PharmD, Prof S Mboup PharmD); **Leiden University Center for Infectious Diseases** (D Hoving PhD, M Cisse, I Diallo, Y J Honkpehedji MD, W Huisman PhD, Marion H König BSc, A C de Kroon BSc, S Azimi PhD, T Tak PhD, Y C M Kruize BSc, F Kurniawan MD, Prof H H Smits PhD, Prof A A Adegnika PhD, Prof M Yazdanbakhsh PhD, S P Jochems PhD, J L H Zhang MD, C Prins BSc, A H E Roukens MD, J J C de Vries MD, L J Wammes MD), **Department of Hematology** (C R Pothast MSc, Prof M H M Heemskerk PhD), **Department of Cell and Chemical Biology, ONCODE Institute** (M L M Jongsma PhD, I Berlin PhD), **and Department of Immunology** (M Zlei PhD), **Leiden University Medical Centre, Leiden, Netherlands; Centre de Recherches Médicales de Lambaréné, Lambaréné, Gabon** (Y J Honkpehedji, Prof A A Adegnika, Prof P G Kremsner FRCP);

Institut für Tropenmedizin, Universitätsklinikum Tübingen, Tübingen, Germany (Prof A A Adegnika, Prof P G Kremsner, Prof T P Velavan PhD, L T K Linh); **The German Center for Infection Research (DZIF), Tübingen, Germany** (Prof A A Adegnika, Prof P G Kremsner, Prof T P Velavan, L T K Linh); **Vietnamese-German Center for Medical Research, VG-CARE, Hanoi, Viet Nam** (Prof T P Velavan, L T K Linh); **Division of Endocrinology, Metabolism, and Diabetes, Department of Internal Medicine, Dr. Cipto Mangunkusumo National General Hospital, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia** (F Kurniawan); **Department of Flow Cytometry, Regional Institute of Oncology, Iasi, Romania** (M Zlei); **Department of Pediatric Immunology, Rheumatology and Infectious Disease** (Prof T W Kuijpers MD), **and Department of Neurology and Neurophysiology** (L Wieske MD, F Eftimov MD), **Amsterdam UMC, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands; Faculty of Medicine, Duy Tan University, Da Nang, Viet Nam** (Prof T P Velavan)

Correspondence to: Dr Simon P Jochems, Leiden University Center for Infectious Diseases, Leiden University Medical Centre, 2333 ZA Leiden, Netherlands. **s.p.jochems@lumc.nl**

See Online for appendix

Research in context

Evidence before this study

SARS-CoV-2 has been associated with more asymptomatic infections and reduced mortality in sub-Saharan Africa compared with high-income countries. However, the underlying immune mechanisms remain speculative. Geographical differences in the immune response have been linked to vaccine hyporesponsiveness and a reduced incidence of inflammatory- and allergic diseases in low-income countries, including countries in sub-Saharan Africa. We conducted a PubMed search from database inception to Jan 12, 2024, using the following terms: (SARS-CoV-2[Title/Abstract] OR COVID-19[Title/Abstract]) AND Africa[Title/Abstract]) AND immune[Title/Abstract]. We found no studies that compared immune responses during infection with SARS-CoV-2 in individuals from sub-Saharan Africa with high-income regions.

Added value of this study

This study is, to our knowledge, the first to compare immune responses during SARS-CoV-2 infection in individuals from sub-Saharan African countries (Senegal, with validation from a Gabonese dataset) with high-income countries (the Netherlands and Germany). By using standardised protocols for sample collection and storage, and batched analysis in a discovery and validation cohort, comparable immunological data from blood and nasal mucosa were generated. Matching of individual characteristics such as age, sex as well as infection severity and duration was used to address confounding factors. Our study showed that individuals with mild COVID-19 in Europe showed inflammation and typical antiviral cellular immune responses, particularly in the nasal mucosa; however, these were absent in sub-Saharan Africans (Senegalese and Gabonese) with mild COVID-19. Inflammation was clearly observed in hospitalised patients from Senegal, but with a different trajectory compared with European individuals; for example, IL-18 was not increased and there was no evidence of inflammasome activation. Moreover, hospitalised Senegalese patients did not show the emergence of myeloid derived suppressor cells or immature neutrophils, which are considered hallmark features of severe COVID-19 based on studies in high-income regions.

Implications of all the available evidence

This study highlights clear differences in immune responses to SARS-CoV-2 infection in individuals from different geographical regions, with individuals from Senegal and Gabon having a dampened and altered inflammatory response. Together with the previously reported differences in disease course, this study highlights the need to study the immune response in understudied populations to understand the pathophysiology of, and immune responses to, infectious diseases.

However, little is known on how geographical variation affects immune responses to infections.

The interplay between SARS-CoV-2 and the immune system has been extensively characterised in the USA and several European countries.[6–12] By contrast, there are few studies of the immune response among African people with SARS-CoV-2. The few studies that have been published show increased inflammation and neutrophil-to-lymphocyte ratios in severe COVID-19, but suggest a more tolerogenic response in mild COVID-19.[13–16]

The scarce geographical diversity in published studies prevents a complete understanding of the interplay between SARS-CoV-2 and the immune system. Therefore, the aim of this study was to comprehensively characterise the peripheral and nasal mucosal immune system in individuals with SARS-CoV-2 from cohorts in both Europe and sub-Saharan Africa in a cross-sectional manner. We hypothesised that there would be reduced COVID-19-associated pro-inflammatory responses and leukocyte perturbations in the sub-Saharan African cohort, compared with the European cohort.

Methods

Study design and participants

We did a retrospective, population-based, cross-sectional study using samples from individuals with and without SARS-CoV-2 collected between May 6, 2020, and Dec 8, 2021, in two European countries (the Netherlands and Germany) and Senegal. Additional individuals from Senegal and Gabon were included as a validation cohort for the findings of the main study.

All individuals with COVID-19 had a positive SARS-CoV-2 quantitative PCR (qPCR) test (appendix pp 3–4) and were classified as mild (symptomatic but not hospitalised) or severe (symptomatic and hospitalised with oxygen supply). Individuals without SARS-CoV-2 (controls) had no symptoms and a negative qPCR test (Senegal and Gabon) or negative serological test (the Netherlands and Germany), or were pre-pandemic samples (Senegal and the Netherlands). European and African individuals with COVID-19 were recruited through a total of seven clinical studies. Only individuals who did not report a previous SARS-CoV-2 infection were included. All infected individuals had a positive SARS-CoV-2 qPCR test, and uninfected controls had no symptoms and a negative qPCR test (Senegal) or serological test (Europe), or were pre-pandemic samples. No participants of the main study cohort received SARS-CoV-2 vaccination. The Sinopharm vaccine became available during the sampling period of the validation cohort in Senegal and four vaccinated individuals were included, as indicated in the appendix (pp 33–35).

Senegalese individuals were recruited through two studies: the SEN20/56 study and the AIDCO project (number 00000159 MSAS/CNERS/Sec). The SEN20/56 study was an observational cross-sectional study in which all patients with severe SARS-CoV-2 in Senegal were selected from one of the COVID-19 treatment centres established by the department of Health Emergency Operations Center of Ministry of

Health, between May 1, 2020 and Dec 20, 2021. Healthy controls and individuals with mild infection were recruited at their home through the AIDCO study, an observational cross-sectional study investigating the trend of the pandemic in index cases and their household contacts after systematic testing. All blood, swab, and nasal samples were taken by the same study team of the Institute of Health Research, Epidemiological Surveillance and Training, which is one the surveillance arms of the Ministry of Health, and clinical status was assessed during sample collection.

Gabonese individuals were also recruited through the AIDCO project (number 004/2020/CNER/P/SG) at the Centre de Recherches Médicales de Lambaréné, Gabon. In this observational longitudinal study, all SARS-CoV-2-positive cases in the diagnostic database at Cermel, Gabon, were contacted by the COVID-19 interventional group and people who consented to participate in the study were considered as index cases. Health status and symptoms were collected at the time of sampling. In addition, any household members who consented to participate were enrolled. All participants presented mild symptoms or were uninfected, and were collected in the period between Feb 15, 2021 and July 26, 2021.

European individuals were recruited through four clinical cohort studies: the BEAT-COVID study (NL73740.058.20), the SARS-RESPONSE study (NL74062.058.20), The Target-to-B! study (NL74974.018.20, EudraCT 2021-001102-30), and the TüCoV study (ethics numbers 247/2020BO1 and 256/2020BO2 from the University of Tübingen, Germany). The BEAT-COVID study was an observational cross-sectional study which included participants diagnosed with a positive SARS-CoV-2 PCR test, who required hospital admission (both non- intensive care unit [ICU] and ICU) at the Leiden University Medical Centre (LUMC), Netherlands, in the period between May 1, 2020 and Nov 16, 2020. Age and sex-matched healthy volunteers with a negative SARS-CoV-2 PCR test were included as healthy controls. The SARS-RESPONSE study was conducted at LUMC (Leiden, Netherlands) and was a prospective observational cohort study with the aim to monitor the cellular and serological immune response in SARS-CoV-2-infected individuals without need of hospitalisation. All participants were LUMC personnel without underlying chronic conditions, auto-immune diseases, or coagulopathy, with a BMI of less than 30, and tested positive for SARS-CoV-2 and manifested mild symptoms. Samples were collected in the period between Oct 5, 2020 and March 15, 2021. The Target-to-B! study was a prospective, observational nationwide cohort study performed at Amsterdam University Medical Centre, Netherlands. The primary objective of this study was to assess humoral and cellular immune responses after SARS-CoV-2 vaccinations in patients with immune-mediated inflammatory diseases treated with or without immunosuppressive medication. Patients with predefined types of immune-mediated inflammatory diseases and treated with predefined (combinations of) immunosuppressive medication as maintenance treatment were enrolled from outpatient clinics. Patients without immune-mediated inflammatory disorders, and patients with immune-mediated inflammatory disorders not on systemic immunosuppressants were recruited as controls. Samples that were included in this study were collected between April 16, 2021 and May 20, 2021. The TüCoV study was an observational cross-sectional study that aimed to investigate the humoral and cellular immune responses against COVID-19, specifically also to improve diagnostic testing during the pandemic. Recruitment to the study was based on self-reported SARS-CoV-2 infections including household members, as well as negative controls. Samples were collected in the period between May 1, 2020 and April 30, 2021.

For plasma and serum analysis, additional pre-pandemic samples (collected between July, 2019 and September, 2019) from the Netherlands and Senegal were collected with the GDIR study (CCMO NL66287.058.18), which was an observational cross-sectional study that aimed to compare immune cells between Europeans and Africans living in urban, semi-urban, and rural areas. Exclusion criteria included a history of diabetes, arterial hypertension, chronic kidney failure, signs or history of infection at the time of inclusion, or any history or clinical signs of inflammatory diseases.

All participants from the eight clinical studies provided written informed consent, which covered this study. Additional information on the clinical studies is available in the appendix (pp 2–3).

Procedures

At least 10 mL of peripheral blood and two nasal curettage samples were collected at the time of hospital visit at the COVID-19 treatment centre of Dakar by the Institute of Health Research team (Dakar, Senegal) and LUMC (Netherlands). Samples from control individuals and individuals with mild COVID-19 were collected at home visits or for the SARS-RESPONSE and BEAT-COVID study at the LUMC. Depending on the study, samples were either collected once or longitudinally (weekly for the AIDCO study; three-times per week for the BEAT-COVID and SARS-RESPONSE studies). For longitudinal studies, one sample per participant was selected to compare cross-sectionally, matching for days since symptom onset. Whole blood, peripheral blood mononuclear cells (PBMC), nasal cells, and serum and plasma were processed (appendix pp 4–5) and stored using standardised protocols to enable comparison of immune responses across studies. One vial of PBMC or nasal cells per donor were used for analysis.

Analysis of all samples was performed in batches to study cellular immunity using mass cytometry (cytometry by time of flight; CyTOF) and spectral flow cytometry at LUMC (Netherlands).

Immune cells were grouped according to marker expression into clusters with a similar phenotype, using hierarchical stochastic neighbour embedding. We measured inflammasome in monocytes using confocal microscopy,

	Senegal cohort (n=59)			Europe cohort (n=74)		
	Control (n=22)	Mild COVID-19 (n=25)	Severe COVID-19 (n=12)	Control (n=27)	Mild COVID-19 (n=23)	Severe COVID-19 (n=24)
Age, years*	30 (25–42)	37 (27–47)	63 (56–67)	35 (28–48)	27 (22–44)	63 (51–69)
Sex						
Female	10 (46%)	14 (56%)	3 (25%)	16 (59%)	11 (48%)	10 (42%)
Male	12 (55%)	11 (44%)	9 (75%)	11 (41%)	12 (52%)	14 (58%)
SARS-CoV-2 characteristics						
Time since symptom onset, days†	NA	9·0 (7·0–12·0)	14·5 (6·8–20·8)	NA	8·0 (6·5–10·5)	14·0 (11·0–18·0)
Viral load, Ct‡	NA	28·7 (24·0–31·1)	30·2 (27·5–33·7)	NA	26·6 (19·7–30·2)	26·7 (22·3–30·9)
Probable variant§						
Original	NA	17 (68%)	2 (17%)	NA	5 (22%)	24 (100%)
Alpha (B.1.1.7)	NA	0	0	NA	8 (35%)	0
Delta (B.1.617.2)	NA	8 (32%)	10 (83%)	NA	10 (44%)	0
Samples used from total group for analysis						
Whole blood CyTOF	17 (77%)	21 (84%)	12 (100%)	15 (56%)	23 (100%)	15 (63%)
Nasal cell CyTOF	9 (41%)	14 (56%)	7 (58%)	13 (48%)	21 (91%)	12 (50%)
Luminex plasma	14 (64%)	16 (64%)	2 (17%)	14 (52%)	11 (48%)	13 (54%)
Luminex serum	12 (55%)	7 (28%)	10 (83%)	9 (33%)	0	13 (54%)

Data are median (IQR) or n (%). Ct=Cycle threshold. CyTOF=cytometry by time of flight. NA=not applicable. *Missing data: Senegal uninfected, n=1. †Missing data: Senegal mild COVID-19, n=2. ‡Only samples with a viral load determined within 1 day from sample collection are included. Missing data: Senegal severe, n=1; Europe mild COVID-19, n=12; Europe severe COVID-19, n=2. §Based on the most dominant strain during sampling period in Senegal or the Netherlands and Germany.

Table 1: **Study cohort characteristics**

anti-SARS-CoV-2 antibodies with ELISA, and plasma and serum cytokines with Luminex, as detailed in the appendix (pp 5–10). Time of symptom onset was determined as the interval between the day of sampling and the first recorded date of SARS-CoV-2-related symptoms. The predicted variants were determined based on available reports on the strains that were circulating in the specific geographical areas during the period of sampling.

Statistical analysis

Individuals were matched to have a similar age, sex, viral cycle threshold (Ct) values, and days since symptom onset distribution when comparing between geographical regions for each of the groups (control, mild COVID-19, and severe COVID-19). Matching between severity groups was not possible as patients with severe COVID-19 were older and not enough samples were available to match for sex to the control and mild individuals.

To assess the effect of mild or severe COVID-19, two-tailed non-parametric Mann–Whitney tests comparing against controls from the same region were used without any correction for covariates, and are reported as the number of times increased or decreased. P values were multiplied by two to correct for comparing two groups to controls. Interaction terms between geographical region and disease severity were assessed with linear mixed binomial models, including individuals as random effects. CIs were extracted using the confint function. For the comparison of multiple cytokines, clusters, subsets, or lineages, Benjamini–Hochberg multiple-testing correction was used. All analyses were performed with R (version 3.6.3 and 4.0.1), using RStudio (version 1.2.5033; appendix pp 10–11).

A power calculation was not performed and sample sizes were limited by the number of individuals that could be recruited. However, previous studies comparing immune profiles using CyTOF have used equal or lower numbers, as this method has limited throughput. To overcome the limitation of a small sample size, we validated key findings in an independent cohort. No imputation was performed for missing data.

Role of the funding source

The funders of the study did not have a direct role in the study design, data collection, procedures, data analysis, data interpretation, or writing of the manuscript.

Results

Demographic parameters, such as age and sex, and infection course were matched between the 133 individuals from Senegal and western Europe (table 1; appendix pp 14–15). None of these individuals were previously vaccinated for SARS-CoV-2. All 36 patients with severe COVID-19 were on general wards, indicating a moderate or severe disease course.

From these 133 individuals, we included 103 whole blood samples and 76 nasal samples. Luminex measurements were performed on plasma (n=70) and serum (n=51) samples (table 1), which included pre-pandemic individuals from Senegal (n=5) and the Netherlands (n=12). For whole blood analysis, 13 individuals were excluded due to samples not collected (11 individuals) or being missing (two individuals). For nasal cell analysis, 40 individuals were excluded due to samples not collected (15 individuals), improper storage (five individuals), usage for a different study (six individuals),

or excluded after measurement due to insufficient cellular yield (14 individuals).

Medication differed between regions, with more dexamethasone usage in the hospitalised patients in Europe (20 of 24) and more hydroxychloroquine administered in Senegalese (11 of 12). Oxygen saturation levels (Senegal: median 95·0% [IQR 94·0–96·3] *vs* Europe: median 94·5% [93·8–95·0], p=0·36) and C-reactive protein concentrations (Senegal: median 19·0 mg/L [IQR 12·3–85·3] *vs* Europe: median 23·5 mg/L [10·3–45·5], p=1·00) were not statistically significantly different between hospitalised patients from Senegal and Europe. External oxygen flow volumes were higher in Senegal patients (median 3·0 L/min [IQR 0–15·0]) than European patients (2·0 L/min [0·3–3·6]), albeit with a marginal p value (p=0·038). Based on similarity of the above-mentioned clinical parameters, hospitalised patients from Europe and Senegal had comparable disease severity (appendix pp 14–15).

Cryopreserved whole blood was analysed using a 39-marker CyTOF panel (appendix p 16), with 17·9 million cells derived from 103 whole blood samples. A total of 130 immune cell clusters were defined, belonging to seven immune lineages: B cells, $CD4^+$ T cells, $CD8^+$ T cells, granulocytes, monocytes and dendritic cells, innate lymphoid cells, including natural killer (NK) cells, and unconventional T cells (appendix pp 37–38). Comparing control individuals from Europe and Senegal showed that 27 of the 130 immune cell clusters were significantly different between the two regions (table 2; figure 1A; appendix p 39).

We compared the global immune profile per site using t-distributed stochastic neighbour embedding and found that the three severity groups clustered separately in Europe (figure 1B). However, in Senegal, the control individuals and individuals with mild COVID-19 clustered together, and the hospitalised patients clustered separately. There were no clusters statistically significantly different between control individuals and Senegalese people with mild COVID-19 (appendix pp 20–24, 37). By contrast, in Europeans with mild COVID-19, eight immune clusters were statistically significantly different compared with control individuals, seven of which were also perturbed in hospitalised patients (figure 1C, D). When comparing hospitalised patients with control individuals from Senegal, 47 clusters differed significantly, 43 of which were decreased in hospitalised patients (appendix p 37). Hospitalised patients from Europe had 49 decreased and nine increased clusters compared with control individuals. There was a 38% (29 of 77 clusters) overlap when comparing the perturbed clusters in hospitalised patients from either Europe or Senegal (figure 1C, D). Hospitalised patients from Senegal and Europe showed many similarities, in particular decreased lymphocyte cluster frequencies (figure 1D) and an increased neutrophil-to-lymphocyte ratio (Senegal: 2·9-times, p=0·0010 *vs* Europe: 1·6-times, p=0·0046; appendix pp 40–41), as described before for severe cases in both regions.[7,14]

All the immune clusters that increased in Europeans with mild COVID-19 were reflective of an activated adaptive immune response, with increased plasmablasts (3·3-times, p=0·0096) and $CD38^+PD1^+$ T-cell subsets: $CD8^+$ EM cells (6·1-times, p<0·0001), $CD4^+$ central memory (1·9-times, p=0·0010) and EM (2·4-times, p=0·059) cells. These $CD4^+$ T-cell clusters were also statistically significantly increased in hospitalised patients (CM: 2·7-times, p=0·027, EM: 1·8-times, p=0·015) but not mild COVID-19

	Senegal	Europe	P values
B cells			
Plasmablasts	0·072% (0·042–0·116)	0·035% (0·021–0·053)	p=0·048
Memory B cells			
Cluster 2	1·036% (0·747–1·287)	0·526% (0·353–0·594)	p=0·0012
Cluster 3	0·373% (0·325–0·482)	0·139% (0·096–0·167)	p=0·0012
Naive B cells			
Cluster 1	2·872% (2·307–4·661)	1·726% (1·030–2·084)	p=0·0045
NK cells			
$CD56^{dim}$ NK cells			
Cluster 4	0·312% (0·151–0·628)	0·094% (0·033–0·128)	p=0·0097
Cluster 6	0·212% (0·073–0·358)	0·032% (0·023–0·079)	p=0·0045
$CD16^+$ NK cells			
Cluster 5	1·026% (0·487–1·402)	2·395% (1·614–3·028)	p=0·017
Cluster 6	0·194% (0·112–0·411)	0·749% (0·455–0·947)	p=0·0058
Cluster 9	0·396% (0·238–0·554)	1·700% (1·120–1·966)	p=0·0002
$CD4^+$ T cells			
$CD4^+$ T EM cells			
Cluster 10	0·784% (0·563–0·979)	0·404% (0·337–0·638)	p=0·048
Cluster 17	1·604% (1·013–2·474)	0·858% (0·730–1·424)	p=0·048
$CD161^+$ $CD4^+$ T EM cells			
Cluster 9	0·969% (0·829–1·455)	0·488% (0·313–0·691)	p=0·045
Cluster 18	0·814% (0·487–1·013)	0·288% (0·236–0·374)	p=0·0002
$CD27^+$ $CD4^+$ T EM cells			
Cluster 6	0·299% (0·143–0·349)	0·713% (0·445–0·988)	p=0·0063
Cluster 8	0·295% (0·219–0·557)	0·807% (0·507–1·317)	p=0·012
$CD8^+$ T cells			
$CD161^+$ $CD8^+$ T cells	0·040% (0·028–0·094)	0·149% (0·059–0·252)	p=0·024
$CD8^+$ T EM cells			
Cluster 3	0·244% (0·159–0·797)	0·058% (0·042–0·266)	p=0·025
Cluster 4	0·104% (0·048–0·144)	0·037% (0·021–0·055)	p=0·0081
EMRA cells	0·100% (0·053–0·226)	0·010% (0·002–0·020)	p=0·0012
Unconventional T cells			
$CD56^+$ T cells			
Cluster 4	0·226% (0·101–0·313)	0·029% (0·010–0·080)	p=0·0088
Cluster 5	0·044% (0·008–0·119)	0·004% (<0·001–0·025)	p=0·035
γδ T cells			
Cluster 10	0·010% (0·004–0·016)	0·001% (<0·001–0·004)	p=0·017
Cluster 14	0·181% (0·074–0·390)	0·008% (0·004–0·104)	p=0·024
Monocytes			
Classical monocytes			
Cluster 3	0·263% (0·128–0·401)	1·251% (0·901–1·816)	p=0·0045
Cluster 5	1·149% (0·629–1·444)	1·836% (1·626–2·353)	p=0·030
Cluster 6	0·014% (0·011–0·065)	0·427% (0·247–0·522)	p=0·0005
Cluster 7	0·010% (0·007–0·013)	0·054% (0·018–0·066)	p=0·028

Data are median (IQR). Only populations with statistically significant differences are shown (all clusters shown in appendix pp 20–24). EM=effector memory. EMRA=EM re-expressing CD45RA. NK=natural killer.

Table 2: **Immune clusters among control individuals in Senegal and Europe**

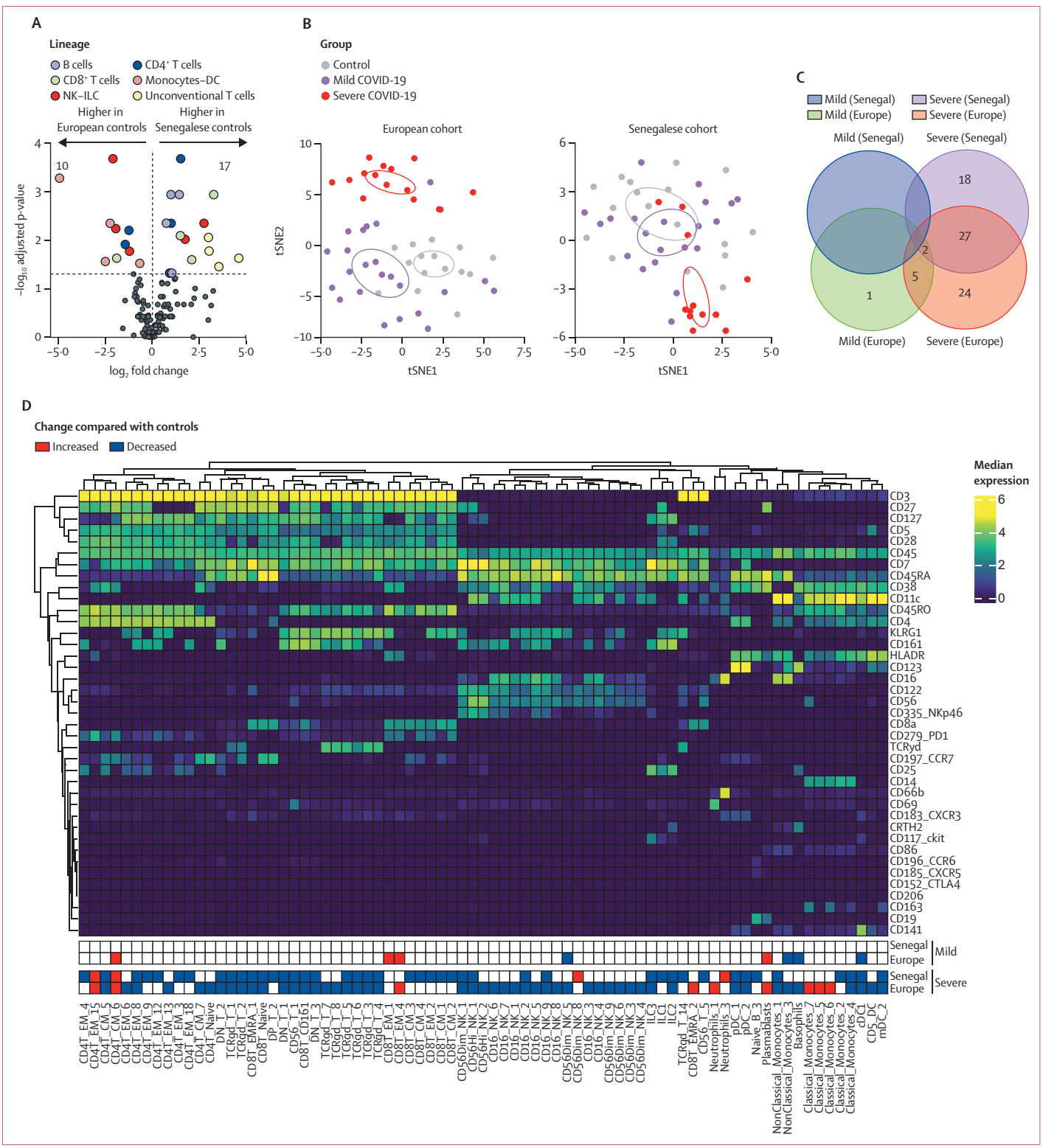


Figure 1: **CyTOF on whole blood from patients with COVID-19 in Senegal and Europe**

(A) Volcano plot comparing clusters between individuals without SARS-CoV-2 (controls) from Senegal and Europe. Statistical test Mann–Whitney, with Benjamini–Hochberg correction for multiple cluster testing. (B) tSNE plots of immune profiles per country, including all z-scaled immune cluster percentages. Individuals are depicted as symbols coloured per group, with 25% CIs shown. (C) Venn diagram showing the overlap in significant immune clusters. (D) Heatmaps depicting all statistically significantly different clusters in any patient group compared with controls from the same region. The top heatmap

(CM: 1·5-times, p=1·00, EM: 1·3-times, p=1·000) from Senegal (figure 2A, appendix pp 20–24). Individuals from Europe showed reduced frequencies of conventional dendritic cell 1 (3·4-times, p=0·034 [individuals with mild COVID-19] and 2·8-times, p=0·0032 [hospitalised patients]), $CD56^{dim}$ NK cluster 5 (2·9-times, p=0·038 and 12·1-times, p=0·0001), non-classical monocytes (3·1-times, p=0·022 and 4·6-times, p=0·0007), and basophils (2·4-times, p=0·0013 and 5·4-times, p=0·0004), similar to previous findings;[17] however, in Senegalese individuals with mild COVID-19, none of these clusters and subsets were affected.

When comparing the reduced lymphocyte subsets observed in hospitalised patients in Senegal and Europe, the T-cell lineages ($CD4^+$, $CD8^+$, and unconventional) were similarly decreased in the two cohorts, although Senegalese patients showed a significant decrease of $CD8^+$ T central memory (3·5-times, p=0·0034) and EMRA cells (1·5-times, p=0·038), which were more preserved in European patients (figure 2B). Plasmacytoid dendritic cells (Senegal: 5·8-times, p=0·0069; Europe: 10·9-times, p<0·0001), innate lymphoid cell 2 (Senegal: 6·3-times, p=0·0013; Europe: 4·8-times, p=0·0071), and innate lymphoid cell 3 (Senegal: 6·7-times, p=0·0013; Europe: 11·2-times, p<0·0001) subsets were decreased in both regions. NK cell subsets were statistically significantly decreased in Europe ($CD16^+$: 5·3-times, p<0·0001; $CD56^{dim}$: 3·3-times, p<0·0001; $CD56^{high}$: 4·1-times, p<0·0001), but not in Senegal. One $CD56^{dim}CD38^+CD11c^+$ NK cell cluster was increased in hospitalised patients from Senegal (5·4-times, p=0·014; appendix pp 40–41). A statistical model testing the interaction between infection severity and geographical location confirmed the differential effect of COVID-19 on NK cells in hospitalised European and Senegalese patients ($estimate_{interaction}$ 1·17 log_{10} [95% CI 0·55 to 1·80], p=0·0007; appendix p 25). This also revealed that $CD8^+$ T cells were more strongly decreased in hospitalised Senegalese than European patients ($estimate_{interaction}$ –0·80 log_{10}, [95% CI –1·35 to –0·26], p=0·0085).

Although neutrophil frequencies were increased in hospitalised patients from both Senegal (2·0-times, p=0·0044) and Europe (1·3-times, p=0·026), compared with healthy controls, this increase was due to distinct phenotypes (figure 2C; appendix pp 40–41). Neutrophil cluster 1 ($CD16^{low}CD69^{high}$) was significantly increased in European patients (6·5-times, p=0·0002), and cluster 3 ($CD16^+CD69^+$) was increased in Senegalese patients (2·5-times, p=0·0079). The expansion of immature $CD16^{low}$ neutrophils has been associated with severe COVID-19 previously.[9,10] Severe COVID-19 had opposite effects on classical monocytes: these increased by 1·5-times in European patients (p=0·017), but displayed a 2·2-times decrease in Senegalese patients (p=0·0382; figure 2D; appendix pp 40–41). The monocyte expansion in Europe was due to three clusters with low HLA-DR expression. These reflect a state of activated immature monocytes that others have described and functionally characterised as monocytic myeloid-derived suppressor cells (M-MDSC), able to suppress T-cell proliferation and IFN-γ production.[18] We confirmed this by manually gating that the $HLA\text{-}DR^-CD14^+$ population increased in hospitalised European patients (median 2% of CD45 [IQR 1·4–5·4], p<0·0001), but not in other groups (appendix pp 40–41). Two of these expanded M-MDSC clusters expressed CD163, CD56, or both. While CD56 is a classical NK cell marker, $HLA\text{-}DR^{low}CD56^+$ monocytes have been reported in severe COVID-19 previously, and might be hyperinflammatory.[19] In hospitalised Senegalese patients, the decrease in total classical monocytes was mostly due to monocyte cluster 2 ($HLA\text{-}DR^+CD45RO^+$), which decreased only in this patient group (2·2-times, p=0·025).

Although the respiratory tract is the main site of SARS-CoV-2 infection and replication, it remains understudied compared to blood. Here we used curettage to collect nasal cells in a minimally invasive manner and performed CyTOF analysis to extensively phenotype the local immune cells. We analysed 1·2 million nasal immune cells, deriving 32 cell clusters (figure 3A). To correct for the variable yield of nasal curettage, while overcoming the compositional nature of the immune cells, we normalised immune cluster counts to epithelial counts per sample. Comparing control individuals from both regions, one cluster of $CD66b^+CD16^+CD11c^+$ neutrophils was increased in European individuals (43·4-times, p=0·042), and a cluster of $Lineage^-CD16^+CD25^+CCR6^+CD45^+CD9^+$ cells was increased in Senegalese individuals (1038-times, p=0·010, appendix p 42).

Considering all nasal clusters, the control cohort, individuals with mild COVID-19, and hospitalised patients from Europe clustered separately from another, but in Senegal only the hospitalised patients clustered separately from the control individuals and individuals with mild COVID-19 (figure 3B). 17 immune cell clusters were increased in the nasal mucosa of Europeans with mild COVID-19, only four of which were also found in hospitalised patients (figure 3A; appendix pp 26–27, 42). Europeans with mild, but not severe COVID-19, showed higher plasmacytoid dendritic cell numbers at the nasal mucosa compared to control individuals (26·6-times, p=0·0004; figure 3C). Such a plasmacytoid dendritic cell recruitment could be responsible for the previously described interferon responses in individuals with mild COVID-19.[6,8] This increased plasmacytoid dendritic cell number was accompanied by increased lymphocyte populations with potential antiviral roles, such as $Tbet^+CD4^+$ T EM cells (14·5-times, p<0·0001), $CD8^+$ T effector (9·1-times, p=0·034) and tissue-resident memory cells (TRM, 6·4-times, p=0·0001), and $CD69^+$ NK cells (20·6-times, p=0·0034; figure 3C; appendix pp 42–43).

shows median marker expression per cluster. The bottom heatmap indicates statistically significant changes per cluster per group. There were 17 controls, 21 with mild COVID-19, and 12 with severe COVID-19 from Senegal and 15 controls, 23 with mild COVID-19, and 15 with severe COVID-19 from Europe. cDC=conventional dendritic cell. CM=central memory. DN=double negative ($CD4^-CD8^-$). DP=double positive. ($CD4^+CD8^+$). EM=effector memory. EMRA=effector memory re-expressing CD45RA. CyTOF=cytometry by time of flight. ILC=innate lymphoid cell. NK=natural killer. pDC=plasmacytoid dendritic cell. tSNE= t-distributed stochastic neighbour embedding.

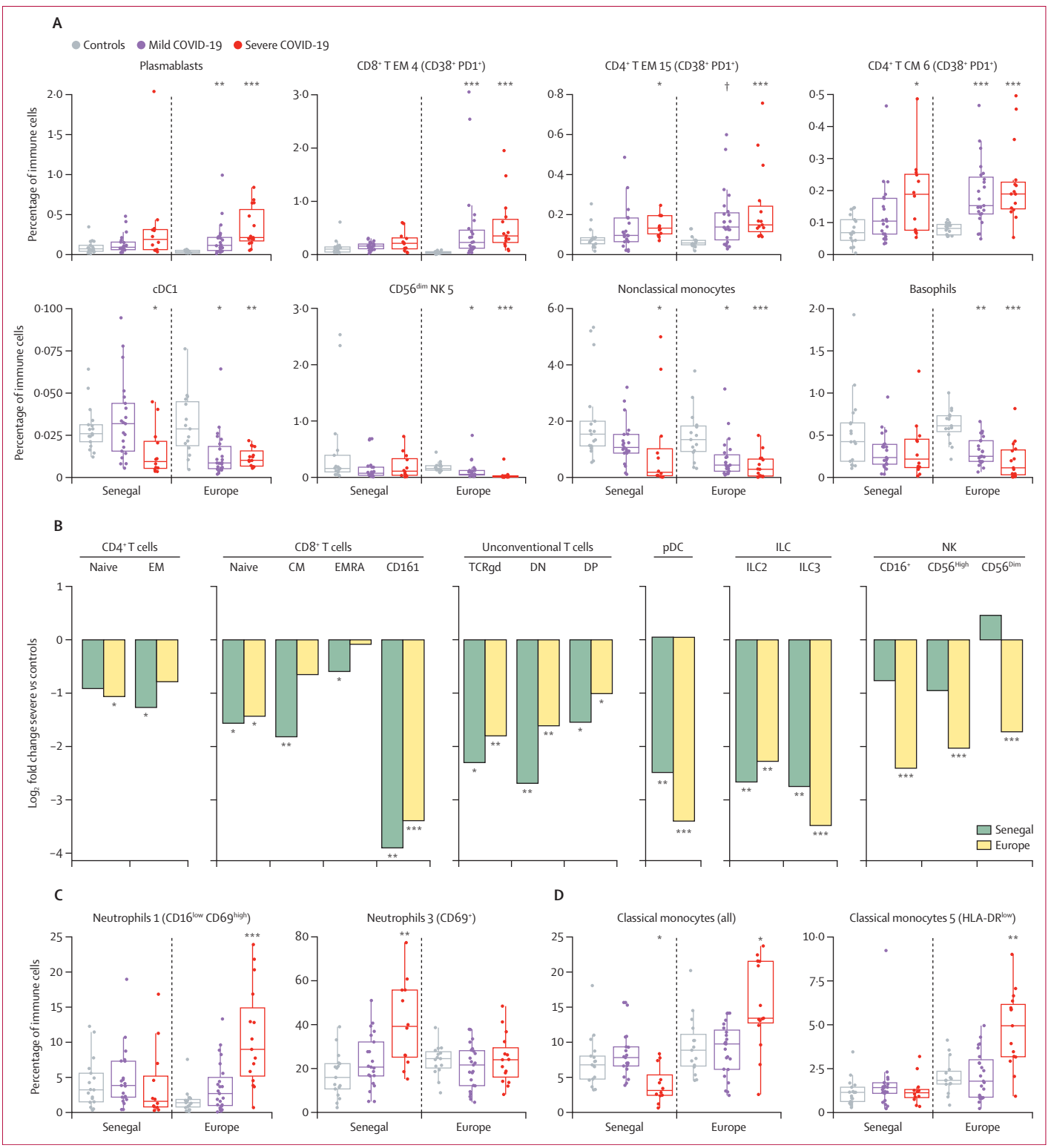


Figure 2: **Differential effects of COVID-19 on the peripheral immune system**

(A) Clusters and subsets perturbed during mild COVID-19 in Europe but not Senegal. The patient groups are indicated by colour and countries are separated by the dashed line. (B) Bar plots showing $\log_2$ median fold-change between patients with severe COVID-19 and individuals without SARS-CoV-2 (controls) in Senegal (green) and Europe (yellow). All clustered lymphocyte subsets with statistically significant changes in at least one location are shown, except for ILC1 for which the median was 0% in both Senegal and Europe. Frequency of neutrophil (C) and classical monocyte (D) subsets and clusters

The recruitment of neutrophil populations was generally absent in Europeans with mild COVID-19. Only one cluster of $CD16^{low}$ neutrophils was increased (7·4-times, p=0·012), and to a lesser degree compared with hospitalised patients (392-times, p<0·0001; figure 3D). $CD163^{+}$ monocytes were increased at the nasal mucosa in both individuals with mild (10·1-times, p=0·0001) and severe (17·9-times, p<0·0001) COVID-19 from Europe, and these cells expressed reduced HLA-DR levels in hospitalised patients (p<0·0001) but not in individuals with mild COVID-19 (figure 3D; appendix p 42). Analysing a subset of mild and hospitalised European patients matched for infection duration showed that the differences between the groups were not related to differences in the time since infection onset (appendix p 44). Hospitalised patients from Senegal also showed increased $CD66b^{+}CD16^{low}$ neutrophils at the nasal mucosa compared with healthy Senegalese controls (9·9-times, p=0·045). Monocytes or $CD206^{+}CD163^{+}$ macrophages at the nasal mucosa were not increased in hospitalised Senegalese patients compared with healthy Senegalese controls.

To understand how the limited cellular perturbations in blood and nasal mucosa of Senegalese individuals with mild COVID-19 were linked to peripheral blood inflammatory responses, we quantified 19 soluble immune proteins in serum and plasma. In the plasma of control European individuals compared with Senegalese controls, seven proteins were lower (S100A8: 1593-times, p=0·0012; C5a: 1·9-times, p=0·0068; HGF: 1·9-times, p=0·0068; CCL20: 2·5-times, p=0·0085; IL-6: 2·8-times, p=0·015; CCL8: 1·6-times, p=0·033; IL-18: 1·5-times, p=0·038) and one was higher (CCL2: 3·8-times, p=0·0068; appendix p 45). Mild COVID-19 in European individuals led to a strong induction of cytokines, such as CCL2 (1·8-times, p=0·0008), CXCL10 (4·1-times, p=0·0024), IL-18 (1·9-times, p=0·0017), IL-6 (2·8-times, p=0·021), and IL-10 (26-times, p=0·0024; figure 4A; appendix pp 28–29, 45). By contrast, Senegalese individuals with mild COVID-19 had no statistically significantly increased pro-inflammatory or anti-inflammatory cytokines compared with control individuals in plasma or serum. In the blood of hospitalised patients from Senegal, concentrations of 12 cytokines were significantly increased, seven of which overlapped with European patients, including CCL2, IL-6, CXCL10 and IL-10 (figure 4B; appendix pp 28–29, 46).

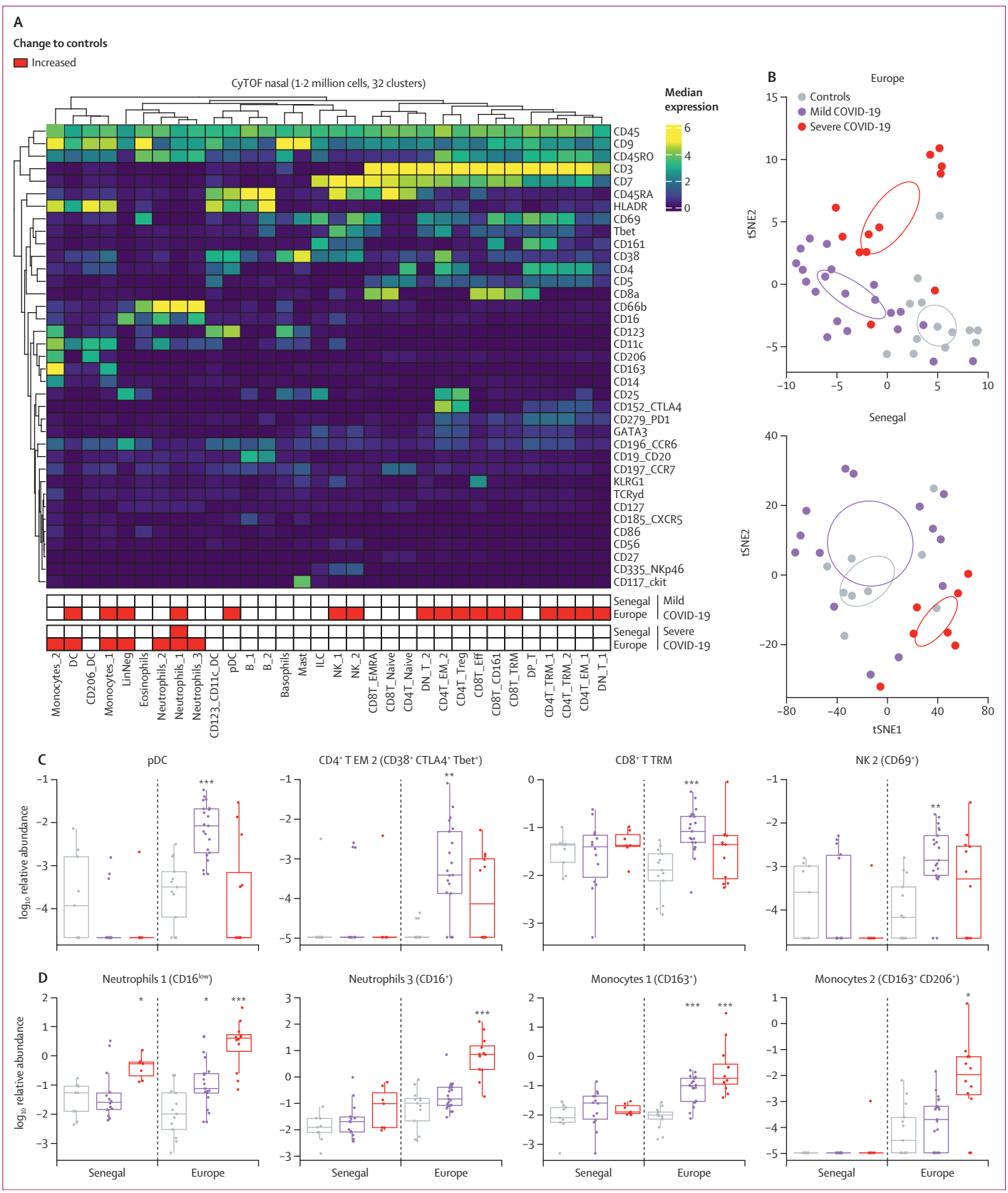
Monocytes and macrophages are important drivers of inflammation in SARS-CoV-2 infection through pyroptosis and inflammasome activation, leading to the processing and subsequent extracellular release of the inflammatory cytokines IL-1β and IL-18.[20] The analysis of circulating cytokines revealed a statistically significant increase of inflammasome-induced cytokine IL-18 in Europeans with mild (median 321·5 pg/mL [IQR 295·3–371·0], p=0·0017) or severe (median 351·1 pg/mL [214·1–439·3], p=0·010) COVID-19, compared with control individuals (median 166·1 pg/mL [117·9–232·3]; figure 4A; appendix pp 45–46). However, IL-18 levels between Senegalese control individuals and individuals with mild or severe COVID-19 were not statistically significantly different. To confirm this with a different technology, we assessed inflammasome activation in PBMC using confocal fluorescent microscopy, confirming the lack of inflammasome-activated monocytes in hospitalised Senegalese patients (figure 4C–D; appendix pp 30, 47).

As cellular perturbations and inflammation were controlled during SARS-CoV-2 infection in Senegalese individuals with COVID-19, we examined the induction of adaptive immunity by measuring anti-spike IgG in peripheral blood. Among individuals with mild COVID-19, seven (35%) of 20 individuals were seropositive in Senegal and five (28%) of 18 in Europe (figure 5A). This limited seroconversion reflects the relatively short time since infection for the individuals with mild COVID-19 in Senegal (median 9·0 days since symptom onset [IQR 7·0–12·0]) and Europe (median 8·0 days since symptom onset [IQR 6·5–10·5]). Antibodies were induced in hospitalised patients, with ten (83%) of 12 patients being seropositive in Senegal and 14 (93%) of 15 in Europe.

To assess cellular adaptive immunity, we isolated PBMC from hospitalised individuals (appendix pp 31–32) and performed antigen-specific tetramer staining and peptide pool stimulations to detect SARS-CoV-2-specific B and T cells, respectively (appendix pp 48–49). Similar to antibody titres, spike-specific B cells were comparable in frequency among Senegalese and European patients (median 0·092% of B cells [IQR 0·07–0·12] *vs* 0·073% [0·04–0·09], respectively, p=0.2636; figure 5B). Based on 25 phenotypic markers, we divided the 3624 spike-specific B cells into seven clusters (appendix p 48). Cluster distribution was comparable between Senegalese and European patients, albeit with inter-individual heterogeneity (figure 5C).

With respect to T-cell immunity, frequencies of spike-specific $CD4^{+}$ T cells (Senegal: median 0·27% [IQR 0·20–0·43] *vs* Europe: 0·49% [0·14–0·87], p=0·5364) and membrane or nucleocapsid-specific $CD4^{+}$ T cells (Senegal: 0·24% [0·19–0·41] *vs* Europe: 0·34% [0·11–0·54], p=0·803) were similar between hospitalised patients from Senegal and Europe (figure 5D). $CD8^{+}$ T cells were not increased compared with controls (appendix p 50). Comparing functional markers, SARS-CoV-2-specific $CD4^{+}$ and $CD8^{+}$ T cells from European patients showed a trend towards increased activation (CD38 and HLA-DR), and higher IFN-γ production compared with Senegalese patients (appendix p 50). Thus, SARS-CoV-2-specific adaptive immunity developed in both Senegalese and Europeans, despite the restrained

per group. N=17 controls, 21 with mild COVID-19, and 12 with severe COVID-19 from Senegal and n=15 controls, 23 with mild COVID-19, and 15 with severe COVID-19 from Europe. The statistical test was based on Mann–Whitney test per group compared with controls of the same country, with correction for multiple patient groups testing. EM=effector memory. EMRA=effector memory re-expressing CD45RA. DN=double negative ($CD4^{-}CD8^{-}$). DP=double positive ($CD4^{+}CD8^{+}$). ILC=innate lymphoid cell. NK=natural killer. *p<0·05, **p<0·01, ***p<0·001 with correction for testing multiple clusters using Benjamini–Hochberg, †p<0·01 without correction for testing multiple clusters.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Change to controls
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CyTOF nasal (1·2 million cells, 32 clusters)
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CD161
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CD4
CD5
CD8a
CD66b
CD16
CD123
CD11c
CD206
CD163
CD14
CD25
CD152_CTLA4
CD279_PD1
GATA3
CD196_CCR6
CD19_CD20
CD197_CCR7
KLRG1
TCRγd
CD127
CD185_CXCR5
CD86
CD56
CD27
CD335_NKp46
CD117_ckit
Senegal
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Mild COVID-19
Senegal
Europe
Severe COVID-19
Monocytes_2
DC
CD206_DC
Monocytes_1
LinNeg
Eosinophils
Neutrophils_2
Neutrophils_1
Neutrophils_3
CD123_CD11c_DC
pDC
B_1
B_2
Basophils
Mast
ILC
NK_1
NK_2
CD8T_EMRA
CD8T_Naive
CD4T_Naive
DN_T_2
CD4T_EM_2
CD4T_Treg
CD8T_Eff
CD8T_CD161
CD8T_TRM
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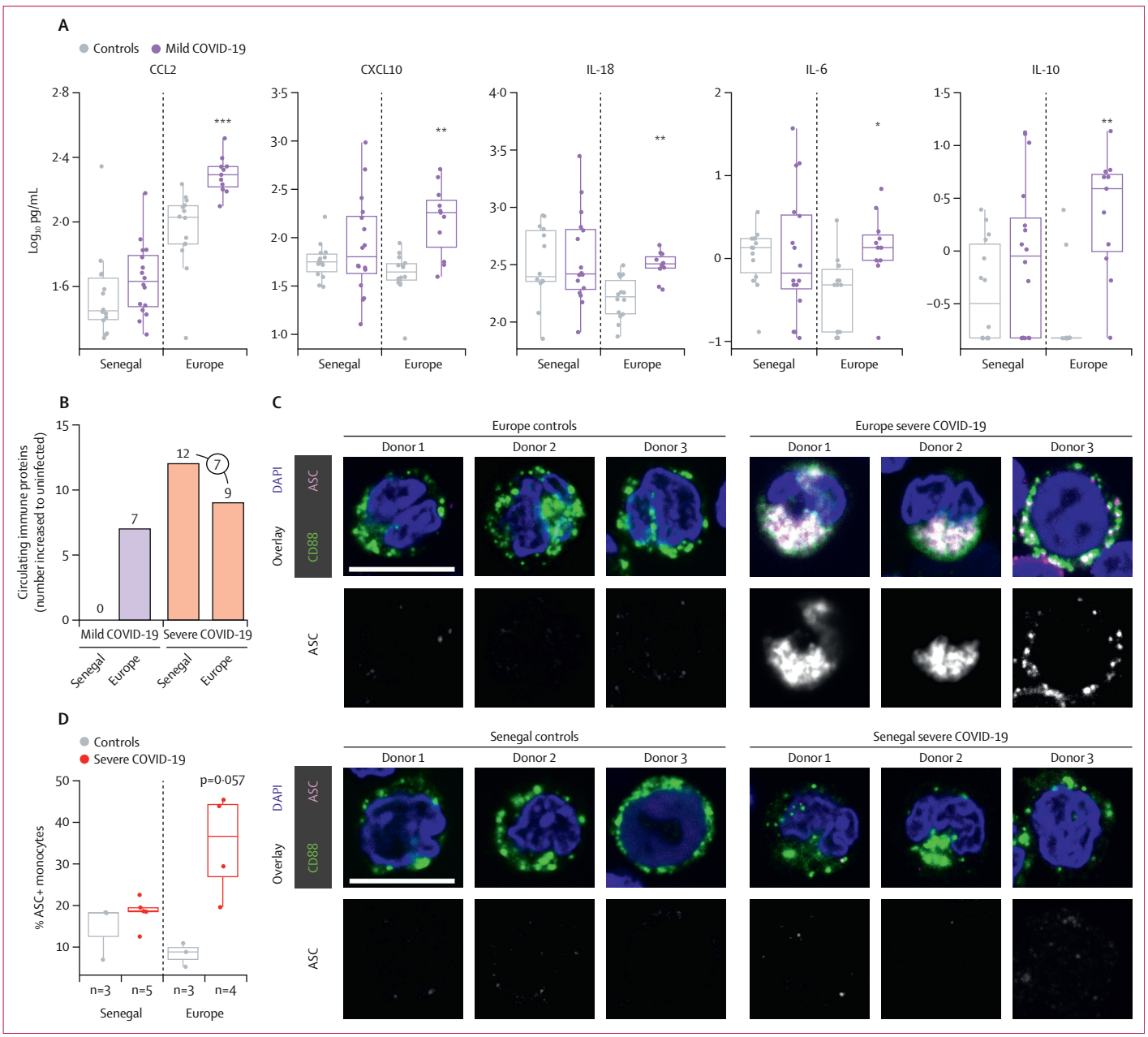

Figure 4: **Inflammation during SARS-CoV-2 infection in Senegal and Europe**

(A) Multiplex protein measurement in plasma from individuals without SARS-CoV-2 (controls; n=14) and individuals with mild COVID-19 (n=16) from Senegal and controls (n=14) and individuals with mild COVID-19 (n=11) from Europe. Log_{10}-transformed concentrations of selected proteins that increased in individuals with mild COVID-19 from Europe but not Senegal are depicted. *p<0·05, **p<0·01, ***p<0·001 with Mann–Whitney test per group compared to controls of the same site, with correction for comparing multiple groups and multiple proteins using Benjamini–Hochberg. (B) Number of immune proteins statistically significantly induced in individuals with mild (purple, plasma) or severe (red, serum) COVID-19 from Senegal and Europe, respectively. Comparison against controls was done, matching per sample type, with statistical testing as described above. The number of overlapping immune proteins between severe patients from Senegal and Europe is depicted. (C) Representative confocal images from three individual donor samples per group, fixed and immunostained against monocyte marker CD88 (green) and inflammasome marker ASC (magenta). Colour overlays with DAPI (blue) are shown, along with the corresponding single channel signals for ASC (white). Scale bar=10 μm. (D) Quantification of monocytes with ASC specks, indicative of inflammasome activation. Boxplots and individuals are shown. Number of analysed individuals per group and p value from Mann–Whitney test is depicted.

Figure 3: **CyTOF on nasal cells from patients with COVID-19 in Senegal and Europe**

(A) Heatmaps showing all defined immune clusters from the nasal CyTOF. The top heatmap shows median marker expression per cluster. The bottom heatmap indicates significant changes per cluster per group. (B) tSNE plots of nasal immune profiles per country, including all z-scaled clusters. Individuals are depicted as symbols coloured per group, with 25% CIs shown. Relative abundances of selected clusters increased in patients in Europe with mild COVID-19 (C) or severe COVID-19 (D). Samples from 76 individuals were included in the analysis: n=9 controls, 14 with mild COVID-19, and seven with severe COVID-19 from Senegal and n=13 controls, 21 with mild COVID-19, and 12 with severe COVID-19 from Europe. Statistical testing using Mann–Whitney test comparing either controls from Senegal or Europe, or comparing per location each patient group to controls, with correction for multiple patient groups testing. Benjamini–Hochberg correction was then applied to address testing multiple clusters. An adjusted p value <0·05 was considered significant. CyTOF=cytometry by time of flight. LinNeg=lineage-negative cell. pDC=plasmacytoid dendritic cell. NK=natural killer. TRM=tissue-resident memory. *p<0·05, **p<0·01, ***p<0·001 after correcting for multiple testing.

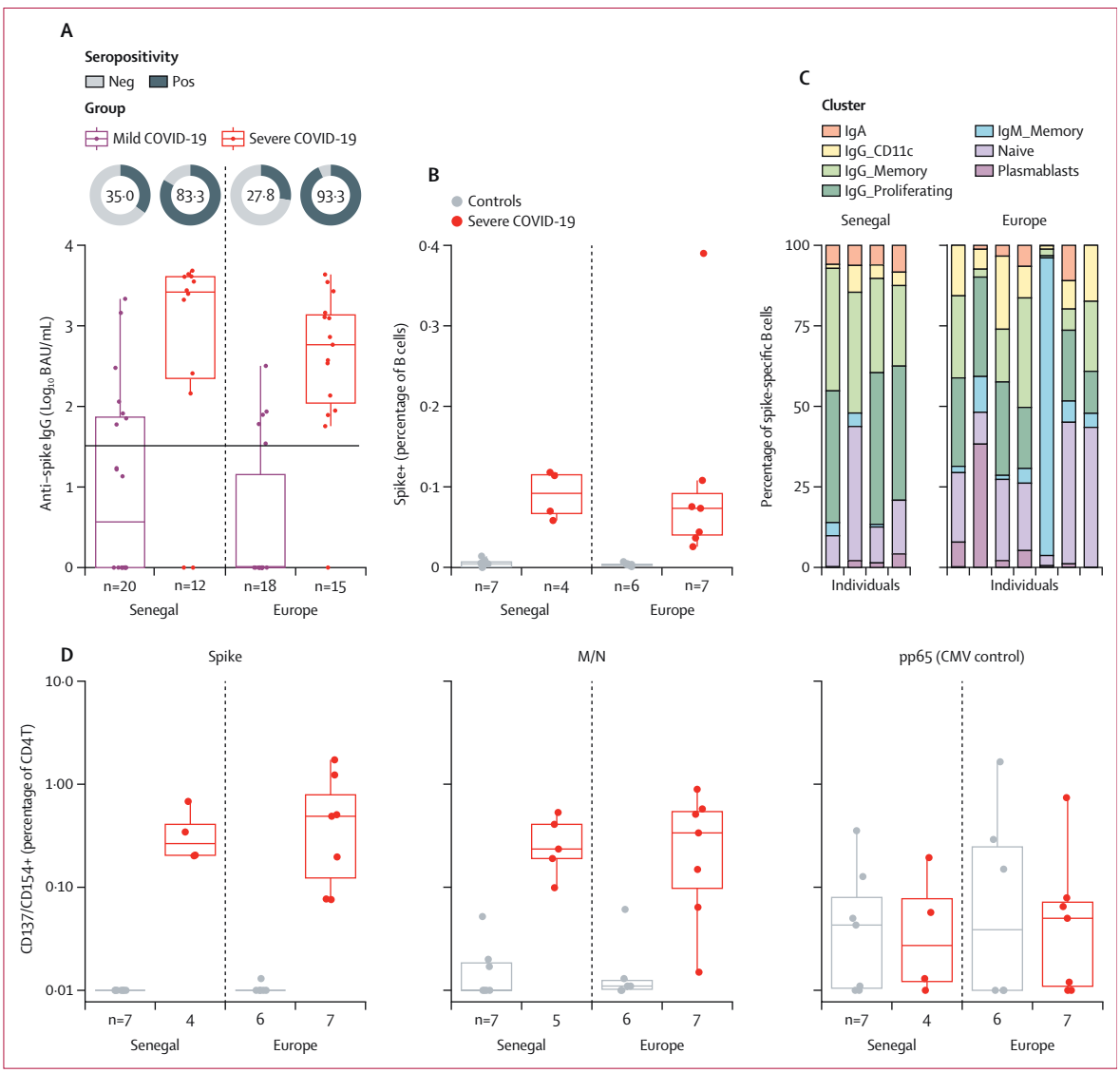

Figure 5: **Adaptive immunity against SARS-CoV-2**

(A) Spike-specific IgG seropositivity in individuals with mild (purple) and severe (red) COVID-19 from Senegal and Europe. Sample sizes are depicted. Boxplots and symbols indicate IgG titres and donut plots the frequency of individuals above the validated seropositivity threshold of the ELISA assay (indicated by a bold line). (B) Frequency of spike-specific B cells. Individuals and boxplots are shown. (C) Stacked bar plots showing the phenotype of spike-specific cells per individual, group per site. (D) $CD4^+$ T-cell frequencies after peptide pool stimulation with SARS-CoV-2 spike, M/N, or control peptide pool (cytomegalovirus pp65). Specific cells were defined as $CD137^+/CD154^+$ T cells. Boxplots and individual samples shown for controls (grey) and hospitalised patients (red). Donor numbers indicated below the plot. One Senegalese patient had only enough cells for the M/N pool and not for spike-pool or pp65-pool, nor for B cell measurements. M/N=membrane or nucleocapsid.

inflammatory responses observed in Senegalese individuals.

To assess the reproducibility of our findings, we analysed a validation cohort, including controls and individuals with mild and severe COVID-19 from Senegal and Gabon, which confirmed our key findings (appendix pp 11–12).

Discussion

Striking differences were observed when comparing the immune responses in both the nasal and systemic compartments of individuals from sub-Saharan Africa and western Europe, with either mild or severe COVID-19. In Senegalese individuals, mild COVID-19 did not trigger strong inflammatory responses, nor nasal infiltration of immune cells with an antiviral nature, including plasmacytoid dendritic cells, NK cells, and $CD8^+$ T cells. This was despite a similar infection pattern and equivalent SARS-CoV-2-specific antibodies and cellular immunity. The lack of inflammation and nasal recruitment was, therefore, likely to be linked to a restrained immune system rather than direct viral control. Although the underlying cause of this reduced response is likely to be multifactorial,

the function and phenotype of the immune system are strongly influenced by environmental exposures.[21,22] An epidemiological study from Ethiopia showed that helminth infection was negatively associated with COVID-19 severity.[23] This supports the notion that increased exposure to microorganisms and parasites might influence the response to SARS-CoV-2 infection. Mechanistically, epigenetics might confer such tolerance as suggested by a study of individuals from India and Europe receiving BCG vaccination followed by in vitro stimulation.[24]

Inflammatory cytokines and chemokines, including IL-6, CCL2, and CXCL10, were increased in hospitalised patients with COVID-19 from both Senegal and Europe, as was the anti-inflammatory protein IL-10. IL-10 was previously shown to accompany the hyperinflammatory response during COVID-19, likely as a compensatory mechanism.[25] However, hospitalised Senegalese patients differed from European patients as they controlled inflammasome activation. Another major difference was that hospitalised Senegalese patients, in contrast to European patients, lacked the expansion of M-MDSC and CD16^{low} neutrophils. The lack of inflammasome activation and the lack of expansion of M-MDSC and immature neutrophils in Senegalese patients indicates that additional pathways likely affect the severity of COVID-19. Previously, nasal neutrophil levels were found to be associated with oxygen saturation during severe COVID-19.[12] As we observed a nasal neutrophil influx in hospitalised patients from both Europe and Senegal, the ability to control neutrophil recruitment to the mucosa might be key in preventing severe disease.

There are limitations to this study. The treatment of hospitalised COVID-19 patients was different between sites, with most the European patients receiving dexamethasone. Dexamethasone is more efficient than hydroxychloroquine in reducing pro-inflammatory cytokine release, but does not have a substantial effect on IL-10 or immune cell populations.[26] Despite this treatment, the immune perturbations in hospitalised patients from the Netherlands strongly resembled those observed earlier in a cohort without dexamethasone treatment,[12] suggesting that dexamethasone treatment does not underlie the differences we observed. Hospitalised patients in Senegal received almost all hydroxychloroquine or chloroquine, an immunosuppressant that might induce M-MDSC apoptosis.[27,28] However, in an earlier European cohort in which 70% of patients received hydroxychloroquine, M-MDSC expansion was still observed.[29] Of the Senegalese patients with mild COVID-19, only a minority (20%) received this treatment, indicating that other factors were underlying the observed control of inflammation in this group. Regardless, an effect of the differential treatment on altered immune responses cannot be excluded. Another limitation is the absence of information on vaccine history, as heterologous T-cell immunity or trained immunity might influence SARS-CoV-2 immune responses. We have also not confirmed the variants responsible for the infection within our cohorts.

The observed restrained inflammatory responses during SARS-CoV-2 infection could help explain the lower mortality rates of COVID-19 in sub-Saharan Africa. Moreover, the inclusion of a diverse population allowed us to generate a better understanding of the immune populations and pathways involved during SARS-CoV-2 infection. Finally, the comparison of immune responses to a newly emerged infection highlighted clearly how much the response to an infection differs between geographical regions.

Contributors

Conceptualisation and study design: MM, AHER, TWK, AAA, LJW, FE, PGK, TPV, SPJ, and MY. Sample collection and processing: MM, YJH, MC, ID, YAD, MZ, SA, ACdK, TT, YCMK, FK, JLHZ, and CP. Measurements: DH, MHK, WH, CRP, ACdK, LTKL, IB, and MLMJ. Data analysis: DH, WH, CP, IB, MLMJ, and SPJ. Supervision of work: MM, AD, SM, LW, JJCdV, HHS, MHMH, TPV, MY, and SPJ. Logistical support and administrative coordination: MM, SM, AD, MY, and SPJ. SPJ wrote the first draft of the manuscript, MM, MY, and DH contributed to subsequent versions. SPJ, DH, and MM verified all the data reported in this study. All authors read and approved the manuscript. All authors had access to the data used in the study and accept responsibility for the decision to submit the manuscript for publication.

Declaration of interests

FE reports research grants from ZonMw (a Dutch governmental agency) for vaccination-induced immunity against SARS-CoV-2 in patients with immune-mediated diseases. SPJ reports grants from ZonMw, BMGF, the EU, and the Dutch Research Council; and travel fees covered by the Dutch Lung Foundation for attending advisory board meetings. TPV is a member of PAN-ASEAN Coalition for Epidemic and Outbreak Preparedness (PACE-UP; German Academic Exchange Service [DAAD] Project ID: 57592343). All other authors declare no competing interests.

Data sharing

De-identified data of all cohorts are provided in the figures and supplementary files. Any other additional data not included in the paper may be available upon request to the corresponding author and ethical review. If granted, the information will be made available through a data transfer agreement, as even pseudonymised data can be related back to individuals in some cases.

Acknowledgments

The work performed in this study was funded by the European and Developing Countries Clinical Trials Partnership 2 (EDCTP2) programme, which supports AIDCO and provides funding to AAA, MM, and MY; the LUMC Gisela Thier fellowship (received by SPJ); the NWO Spinoza Award and the European Research Council Advanced Grant (received by MY); and Wake Up To Corona crowdfunding by Leids Universitair Fonds (received by AHER). We thank Rolf Fendel from the University of Tübingen, Germany, who led the TüCoV study and kindly allowed for the inclusion of samples used in this study. We thank all patients and healthy volunteers for taking part in this study. The authors acknowledge the Flow Cytometry Core Facility at LUMC (Leiden, Netherlands; https://www.lumc.nl/research/facilities/fcf) for technical support in the mass cytometry and flow cytometry studies as well as clinical staff of COVID-19 treatment centres in Senegal and Gabon. We would like to thank Nathalia M de Vasconcelos for advising on the inflammasome and microscopy experiments.

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