Monocytes and macrophages, targets of SARS-CoV-2: the clue for Covid-19 immunoparalysis

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Conflicts of interest

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Abstract

Background: Covid-19 clinical expression is pleiomorphic, severity is related to age and comorbidities such as diabetes and hypertension, and pathophysiology involves aberrant immune activation and lymphopenia. We wondered if the myeloid compartment was affected during Covid-19 and if monocytes and macrophages could be infected by SARS-CoV-2.

Methods: Monocytes and monocyte-derived macrophages from Covid-19 patients and controls were infected with SARS-CoV-2, and extensively investigated with immunofluorescence, viral RNA extraction and quantification, total RNA extraction followed by reverse transcription and q-PCR using specific primers, supernatant cytokines (IL-10, TNF-α, IL-1β, IFN-β, TGF-β1 and IL-6), flow cytometry. The effect of M1- versus M2-type or no polarization prior to infection was assessed.

Results: SARS-CoV-2 efficiently infected monocytes and MDMs but their infection is abortive. Infection was associated with immunoregulatory cytokines secretion and the induction of a macrophagic specific transcriptional program characterized by the upregulation of M2-type molecules. *In vitro* polarization did not account for permissivity to SARS-CoV-2, since M1- and M2-type MDMs were similarly infected. In Covid-19 patients, monocytes exhibited lower counts affecting all subsets, decreased expression of HLA-DR, and increased expression of CD163, irrespective of severity.

Conclusion: SARS-CoV-2 drives monocytes and macrophages to induce host immunoparalysis for the benefit of Covid-19 progression.

Keywords: SARS-CoV-2, Covid-19, monocytes, macrophages, polarization

Background

The novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) emerged in Wuhan (China) at the end of 2019 and caused the coronavirus disease of 2019 (Covid-19) pandemic, with 85 million cases and 1,800,000 deaths to date (1). Covid-19 is characterized by a strikingly heterogeneous clinical presentation and prognosis. Most patients are pauci-symptomatic or have fever, cough and fatigue, while a minority experience progression to an acute respiratory distress syndrome or other critically severe conditions. The severity of the disease is related to underlying conditions such as hypertension, diabetes, coronary heart diseases or obesity (2). The mechanisms remain elusive, but evidence for a prominent role of the immune system is accumulating. The severity of Covid-19 pneumonia is associated with lymphopenia and a cytokine release syndrome (CRS) (3), which contributes to the massive migration of T cells into tissues, mainly the lung, and accumulation of T cells within lesions (4).

There is evidence that myeloid cells are involved in the pathophysiology of coronavirus infection, either directly, as virus target, or indirectly, as CRS effectors (5). Macrophages are susceptible to MERS-CoV and SARS-CoV-1 infection (6). Macrophage and monocyte accumulation in the alveolar lumen was demonstrated in a mouse model of SARS-CoV-2 expressing human angiotensin-converting enzyme 2 (ACE2) (7). SARS-CoV-2 nucleocapsid protein was detected in lymph nodes and spleen-associated $CD169^{+}$ macrophages from Covid-19 patients (8). Finally, single cell RNA sequencing of pulmonary tissue from Covid-19 patients revealed an expansion of interstitial macrophages and monocyte-derived macrophages (MDM) but not of alveolar macrophages (9). However, whether monocytes and/or macrophages are targets of SARS-CoV-2 and whether monocyte diversity is altered in Covid-19 patients require specific investigation since most studies are based on this hypothesis.

Monocytes are innate hematopoietic cells that maintain vascular homeostasis and ensure early responses to pathogens during acute infections. CD14 and CD16 surface antigens: delineate

three human monocyte subsets: classical $CD14^{+}CD16^{-}$, intermediate $CD14^{+}CD16^{+}$, and non-classical $CD14^{-}CD16^{+}$ (10,11). In murine models, classical monocytes are the precursors of non-classical monocytes (12). Monocyte subsets exhibit functional specialization. During bacterial infection, classical monocytes are recruited to the sites of inflammation, exert typical phagocytic functions and differentiate into inflammatory dendritic cells or macrophages. Non-classical monocytes crawl along vasculature and surveil the vascular tissue (13). Alterations of monocyte subset frequency were reported in infectious and inflammatory diseases(10). While macrophages largely arise from monocytes in acute situations such as infection, under homeostatic conditions most tissue macrophages are of embryonic origin and monocytes merely renew this population (14). Consequently, the mobilization of immune cells in Covid-19 might lead to macrophage populations of multiple origin in tissue lesions.

We show here that SARS-CoV-2 can infect human monocytes and MDMs but their infection is abortive. SARS-CoV-2 infection stimulated the production of immunoregulatory cytokines, interleukin (IL)-6 and IL-10 in both cell types and triggered in MDMs an original transcriptional program enriched with M2-type genes. MDM polarization did not account for permissivity to the virus since M1- and M2-polarized cells were similarly infected. In Covid-19 patients, the counts of classical, intermediate and non-classical monocytes were decreased, irrespective of the level of severity. CD163 expression, a molecule associated with the immunoregulatory phenotype, was significantly higher than in healthy controls, whereas that of HLA-DR was decreased. Hence, SARS-CoV-2 drives circulating monocytes and macrophages, inducing immunoparalysis of the host for the benefit of Covid-19 progression.

Methods

Patients and ethical statement

Seventy-six consecutive patients with confirmed SARS-CoV-2 infection (RT-PCR, Life Technologies) were included. Upon diagnosis, patients underwent standard of care laboratory tests. According to French law, the patients received information that excess samples and clinical data might be used

for research purposes, and retained the right to oppose (15,16). Epidemiological, demographic, clinical, laboratory and outcome data were obtained from a retrospective review of medical records (**Table 1**).

Cell isolation

Peripheral blood mononuclear cells (PBMCs) were isolated from the blood of Covid-19 patients and from buffy coats of healthy donors (Convention *N°7828*, "Etablissement Français du Sang", France) by density gradient centrifugation using Ficoll (Eurobio) (17). Monocytes were purified by CD14 selection using magnetic beads (Miltenyi-Biotec) with a purity (98%) evaluated by flow cytometry and cultured in RPMI-1640 (Life Technologies) containing 10% inactivated human AB-serum, 2mM glutamine (Sigma-Aldrich), 100U/mL penicillin and 50µg/mL streptomycin (Life Technologies). After 3 days, the medium was replaced by RPMI-1640 containing 10% fetal bovine serum (FBS, Life Technologies) and 2mM glutamine, and cells were differentiated into macrophages for 4 additional days.

THP-1 cell line were cultured in RPMI-1640 containing 10% FBS, 2mM glutamine and 100U/mL penicillin and 50µg/mL streptomycin and differentiated into macrophages after treatment with 50ng/ml phorbol-12-myristate-13-acetate (Sigma-Aldrich) for 48 hours (18,19).

Virus production and cell infection

SARS-CoV-2 strain IHU-MI3 was obtained after Vero-E6 cells (American type culture collection® CRL-1586™) infection in MEM (Life Technologies) supplemented with 4% FBS as previously described (20). In some experiment SARS-CoV-2 was heat-inactivated at 56°C during 1h (21).

Cells were infected with 50 µl virus suspension (0.25, 0.5 or 0.1 multiplicity of infection (MOI)) for 24 or 48 hours at 37°C in the presence of 5% CO_2 and 95% air in a humidified incubator.

Immunofluorescence

After a 24 or 48-hours infection, cells were incubated in phosphate buffer saline (PBS) supplemented with 5% FBS and 0.5% Triton X-100 for 30 minutes, washed and incubated with an anti-SARS-CoV-2 spike protein (subunit 1) antibody (Life Technologies). Secondary antibody alone was used as background control. Nuclei and F-actin were stained using DAPI and Phalloidin (Life Technologies) respectively. An LSM800 Airyscan confocal microscope (Zeiss) and a 63X oil objective were used.

Viral RNA extraction and q-RTPCR

Viral RNA was extracted from infected cells using NucleoSpin® Viral RNA Isolation kit (Macherey-Nagel). Virus detection was performed using One-Step RT-PCR SuperScript™ III Platinum™ (Life Technologies). Thermal cycling was achieved at 55°C for 10 minutes for RT, pursued by 95°C for 3 minutes and then 45 cycles at 95°C for 15 seconds and 58°C for 30 seconds using a LightCycler480 system (Roche). The primers and the probes were designed against the E gene (20). Viral quantification was expressed as threshold cycle (Ct) values normalized with the actin housekeeping gene.

RNA isolation and q-RTPCR

Total RNA was extracted from cells (2.10^6 cells/well) using the RNeasy Mini Kit (Qiagen) and DNase I treatment (22) and evaluated using a spectrophotometer (Nanodrop Technologies). RT-PCR was performed using a Moloney murine leukemia virus-reverse transcriptase kit (Life Technologies) and oligo(dT) primers, Smart SYBRGreen fast Master kit (Roche Diagnostics) and a CFX Touch Detection System (Bio-Rad) using specific primers (**Table 2**). The results were normalized using the housekeeping endogenous control *ACTB* gene and expressed as $2^{-\Delta Ct}$ whith $\Delta Ct=Ct_{Target}-Ct_{Actin}$ (23).

Immunoassays

IL-10, tumor necrosis factor (TNF)-α, IL-1β, interferon (IFN)-β, transforming growth factor (TGF)-β1 (R&D Systems) and IL-6 (Clinisciences) were quantified in cell supernatants. The sensitivity was (pg/ml) 15.4 for IL-6, 3.9 for IL-10, 5.5 for TNF-α, 0.125 for IL-1β, 50 for IFN-β and 4.61 for TGF-β1.

Flow cytometry

PBMCs from healthy donors or Covid-19 patients were resuspended in PBS containing 5% FBS and 2mM EDTA (Sigma-Aldrich) for 20 minutes before staining with fluorochrome-conjugated mouse IgG1: CD3(UCHT1), CD20(B9E9), CD14(RMO52), CD16(3G8) (Beckman Coulter); HLA-DR(G46-6) and CD163(GHI/61) (BD Biosciences), and appropriate isotype controls. A minimum of 50,000 events were acquired for each sample using a BD CantoII instrument (BD Biosciences) analyzed with FlowJo software (Tree Star).

Statistical analysis

Statistical analysis was performed with GraphPad Prism, using the two-way ANOVA test for viral quantification and transcriptional analysis, nonparametric Kruskall-Wallis test for group comparison, Mann-Whitney *U* test for cytokine levels, and t-test for flow cytometry results with monocyte populations and surface marker expression. Turkey's and Sidak's tests were used for post-hoc comparisons. qRT-PCR data including principal component analysis (PCA) and hierarchical clustering of gene expression, were analyzed using the ClustVis webtool(24). Significance was set at $P<0.05$.

Results

SARS-CoV-2 infects monocytes and macrophages and stimulates cytokine release

SARS-CoV-2 strain IHU-MI3 was cultivated in Vero E6 cells that were efficiently infected (Ct=18.7) after 24 hours, but a lytic process interfered with the measurement of viral replication (**Supplementary figure 1**). Monocytes and macrophages express receptors for SARS-CoV-2 (25),

suggesting that the virus targets myeloid cells. We wondered whether SARS-CoV-2 was able to infect human monocytes and macrophages. Monocytes and MDM were incubated with SARS-CoV-2 strain IHU-MI3 for 24, 48 and 72-hours and infection level was measured by RT-PCR and immunofluorescence. Monocytes were infected after 24 hours (mean Ct/actin = 7.8); their viral load increased slightly at 48h (mean Ct/actin = 6.8) but it remained constant thereafter (mean Ct/actin = 7.9) (**Figure 1A**). Similarly, MDMs were efficiently infected with the SARS-CoV-2 strain after 24 (mean Ct/actin = 9.5) and 48 hours (mean Ct/actin = 9.8) and the viral load gradually decreased with a Ct/actin = 11.5 (mean) at 72 hours, suggesting an abortive infection. Finally, when monocytes and MDM were incubated with heat-inactivated SARS-CoV-2, they more efficiency eliminated the virus from 48 to 72 hours (**Figure 1A**). In contrast to Vero cells (**Supplementary figure 1**), monocytes and MDMs were not uniformly infected, as observed by confocal microscopy (**Figure 1A**). We next addressed the ability of SARS-CoV-2 to induce the release of soluble mediators from monocytes and MDMs. IL-6, IL-10, IL-1β, TNF-α and TGF-β1 levels were significantly increased in stimulated monocyte and MDM supernatants as compared to unstimulated conditions after 24 hours (**Figure 1B**) and were persistently increased after 48 hours (**Figure 1C**) excepted for IL-1β. The same pattern was observed in monocytes and MDMs stimulated with heat-inactivated SARS-CoV-2 for 24 hours and 48 hours. The release of IL-6 was significantly higher in MDMs stimulated with live SARS-CoV-2 than in cells stimulated with heat-inactivated virus (**Figure 1B,C**). Taken together, SARS-CoV-2 infection of monocytes and macrophages is abortive. Among pro- and anti-inflammatory cytokines, only IL-6 was preferentially induced by live SARS-CoV2

SARS-CoV-2 elicits a specific transcriptional program in macrophages

Next, the expression of genes involved in the inflammatory response (*IFNA*, *IFNB*, *IFNG*, *TNF*, *IL1B*, *IL6*, *IL8*, *CXCL10*) or immunoregulation, (*IL10*, *TGFB1*, *CD163*) was measured by qRT-PCR in monocytes and MDM incubated with the virus for 24 and 48 hours. PCA of gene expression showed that live SARS-CoV-2 stimulated a specific program in contrast to heat-inactivated SARS-CoV-2 that

stimulated transcriptional programs superimposable to that in unstimulated cells (**Figure 2A**). The hierarchical clustering revealed a clusterization dependent on infection time for monocytes and on type of agonist for macrophages; unstimulated cells were on a branch distinct from live and heat-inactivated SARS-CoV-2 stimulated cells (**Figure 2A**). Indeed, live and heat-inactivated SARS-CoV-2 stimulated the expression of similar whole gene panel in monocytes (**Figure 2B**). After 48 hours, *CXCL10* was increased in live and heat-inactivated SARS-CoV-2 stimulated monocytes compared to unstimulated cells (**Supplementary figure 2**). In contrast, *IL1B* expression was higher in live SARS-CoV-2 infected monocytes than in heat-inactivated virus-stimulated cells.

We next investigated the transcriptional program induced by SARS-CoV-2 in macrophages. Pro- (*TNF*, *CXCL10*, *IFNA*) and anti-inflammatory genes (*CD163*, *TGFB1*) were increased in live SARS-CoV-2 infected MDMs compared to unstimulated cells (**Figure 2B**). The increase in *TGFB* and *CD163* gene expression was also observed after 48 hours (**Supplementary figure 2**). Live and heat-inactivated SARS-CoV-2 stimulated similar transcriptional program in MDMs at 24 and 48 hours. Infection of MDMs lead to expression of genes associated with M1/M2 profile, suggesting that SARS-CoV-2 does not induce clear polarization at the onset of the infection but rather a delayed shift toward a M2-type.

Macrophage polarization and SARS-CoV-2 infection

As SARS-CoV-2 induced an early M1/M2 followed by a late M2 program in macrophages, we investigated the effect of macrophage polarization status on infection. MDM polarization was induced by IFN-γ and lipopolysaccharide (M1), IL-4 (M2) or was kept at a resting state without polarization (M0). The polarization status was confirmed by measuring the expression of M1 and M2 genes. PCA and hierarchical clustering confirmed the induction of three distinct activation statuses (**Supplementary figure 3**). The expression of polarization-related genes was investigated after 24 and 48 hours of SARS-CoV-2 stimulation. Unstimulated and SARS-CoV-2-stimulated-MDM (M0, M1, M2) were present on two distinct branches but the discrimination of responses as a function of

polarization was not possible (**Supplementary figure 4**). Regarding pro- and anti-inflammatory cytokines, SARS-CoV-2 stimulation significantly increased the release of both cytokine groups in M1- and M2-polarized MDMs after 24 and 48 hours (**Figure 3A,B**). In addition, no differences were observed in the viral load of M0, M1 or M2 macrophages (**Figure 3C**). We next performed the same experiment using M0, M1 or M2 THP-1-macrophages to minimize variations. The viral load of M1 polarized THP-1- macrophages was similar to that of non-polarized (M0) counterparts. In contrast, SARS-CoV-2 load was significantly decreased in M2-polarized macrophages as compared with M0 macrophages (**Figure 3C**). Although a type 2 immune response was associated with lesser infection of macrophages, their polarization did not appear critical for SARS-CoV-2 infection.

Monocyte subsets are altered in SARS-CoV-2-infected patients

We next wondered if the frequency of monocyte subsets was affected in Covid-19 patients. Monocyte subsets were analyzed for CD14 and CD16 expression by flow cytometry in 76 Covid-19 patients and compared to those of 41 healthy blood donors. Covid-19 patients were classified in mild (n=41,5%), moderate (n=21,3%), and severe (n=14,2%, including 8 Covid-19-related deaths). Median age was 58 (range 18–95) in Covid-19 patients and 40 (range 18–68) in healthy donors. Complete demographic data are available in **table 1**. In healthy donors, classical monocytes were the best represented monocyte subset (9.17% of total PBMCs), while intermediate and non-classical monocytes accounted for 0.42% and 0.60%, respectively. In Covid-19 patients, classical (2.03%), intermediate (0.23%) and non-classical monocytes (0.22%) were significantly lower than in healthy controls (**Figure 4A**). Hence, the relative monocytopenia previously reported in Covid-19 patients (26) affected all three monocyte subsets. We wondered if circulating monocytes displayed changes in the expression level of activation-associated membrane markers: HLA-DR, a canonical marker of monocyte activation, and CD163, an immunoregulatory marker. All three monocyte subsets expressed HLA-DR and CD163 (**Figure 4B**). In Covid-19 patients, intermediate and non-classical monocytes, but not classical monocytes, expressed decreased level of HLA-DR. In contrast, the

CD163 expression was significantly increased in classical and non-classical monocytes (**Figure 4B**). This opposite expression suggests that their activation status was shifted to an immunoregulatory program. This phenotypic profile of Covid-19 monocytes was partly recapitulated by incubating control monocytes with SARS-CoV-2 (data not shown). Finally, no significant differences in monocyte HLA-DR and CD163 expression were observed among mild, moderate, and severe patients (**Figure 5**). Hence variation of monocyte HLA-DR and CD163 expression in Covid-19 patients was induced by SARS-CoV-2 infection but was not related to subsequent disease severity.

Discussion

We showed that SARS-CoV-2 efficiently infects human monocytes and macrophages. This is reminiscent of previous reports about SARS-CoV-1 that infects human macrophages but does not replicate within (27). Macrophages infected by SARS-CoV-1 were detected in lungs of SARS patients (5). *Post-mortem* examination of lymph nodes and spleen revealed the presence of SARS-CoV-2 in macrophages $CD169^{+}$, a maker of macrophages from the splenic marginal zone (8). Using an unsupervised computational pipeline that can detect viral RNA in any scRNA-seq data set, an enrichment of SARS-CoV-2 reads in macrophages expressing secreted phosphoprotein-1 was observed (28). Although monocytes and macrophages express the molecular machinery to recognize and internalize SARS-CoV-2 such as ACE and, TMPRSS2 (25), the ability of the virus to replicate within these cells is not fully understood. Our results SARS-CoV-2 virus replication favor the hypothesis of an abortive infection similar to SARS-CoV-1 (29) but clearly distinct from MERS-CoV replication in macrophages(30).

The infection of monocytes and macrophages exhibit a common secretory profile of IL-6, IL-10, TNF, IL-1β, TGF-β1 and the absence of IFN-β for both live and heat-inactivated SARS-CoV-2 stimulation. Impaired IFN production is consistent with the reported inhibition of type I IFNs by SARS-CoV-1 and the lack of IFN regulatory factor-3 activation in macrophages and dendritic cells (5). In addition to preventing IFN-α/β responses, SARS-CoV downregulated IFN-related genes in THP-1 cell line (31). At least three SARS-CoV proteins (N, OrfB3, Orf6) are known to antagonize the IFN-β

response (32). In our hands, both monocytes and macrophages released IL-10 and TGF-β1, suggesting that anti-inflammatory cytokines are also involved in cell responses to infection. TGF-β release may be associated with tissue repair and fibrosis complicating Covid-19 (33). Our results suggest that the early response of infected cells is inflammatory whereas the delayed response promotes tissue repair. This model is in line with the immune response unfolding in Covid-19 patients, in whom myeloid cells interact with innate and adaptive immune partners able to redirect immune responses towards an inflammatory status.

Clinical reports have highlighted the prognostic value of IL-6 for the occurrence of Covid-19 associated complications (34). The investigation of *post-mortem* samples revealed that SARS-CoV-2 induces IL-6 production more efficiently than other cytokines (8). We reported that IL-6 release was significantly increased in macrophages stimulated by live SARS-CoV-2 compared to heat-inactivated virus. IL-6 release is consistent with previous reports on the ability of SARS-CoV-1 to stimulate IL-6 secretion in MDM (27). We also reported an M2-like polarization of stimulated-macrophage. This finding was in accordance with previous study reporting that M2-like infiltrating macrophages were found the major source of IL-6 in a fibrosis mice model (35).

We found that in monocytes, SARS-CoV-2 elicited a transient program, while macrophages exhibited a more diversified transcriptional program associating inflammatory and anti-inflammatory genes, which shifted to an anti-inflammatory program of M2 type at 48 hours. Hence, SARS-CoV-2 affected macrophage polarization according to the kinetics of infection. Previous reports on SARS-CoV-1 infection directly affected macrophage activation. SARS-CoV-1 polarized pulmonary monkey macrophages in a M1 profile associated with decreased viral load but persistence of inflammation (36). SARS-CoV-1 infection, alveolar murine macrophages were repolarized to limit T cell activation (37). Moreover, SARS-CoV-1 induced non-protective M2 polarization in lung macrophages from infected mice (38). Whether macrophage polarization affected their capacity to control SARS-CoV-2 replication was not addressed. Using polarized macrophage, we found that non-polarized and M1 macrophages were permissive to SARS-CoV-2. This may explain why obesity and

diabetes, conditions associated with M1 macrophage polarization, are critical comorbidities in Covid-19 (39). In our hands, M2 type macrophages tended to be less permissive to SARS-CoV-2. As estrogens favor M2 polarization (40), this may explain why women are less affected than men by Covid-19. In addition, patients with allergic asthma seem to be less susceptible to the virus (41). Our results suggest that, instead of inducing a clear polarization, SARS-CoV-2 exacerbates macrophage responses whatever the type of polarization.

We show that Covid-19 monocytopenia affects all monocyte subsets. Consensus about the variations of monocyte counts in Covid-19 is lacking, probably because of the diversity of measurement tools and cohort outcome. A single-cell RNA sequencing study reported depletion of $CD16^+$ monocytes, including intermediate and non-classical monocytes (42). Expansion of IL-6 producing $CD14^+CD16^+$ monocytes was reported in Covid-19 patients hospitalized in intensive care units (ICU) as compared with patients not requiring ICU care (33).

Monocytes of Covid-19 patients exhibited HLA-DR down-modulation and CD163 upregulation. HLA-DR down-modulation is in agreement with previous studies (39, 41), including a disease-severity-associated signature in MDM with down-modulated MHC II and type I IFN genes (27, 42). Previously unreported CD163 upregulation in Covid-19 patients suggests monocyte polarization toward M2-type. Immunohistochemical staining of SARS pneumonia demonstrated $CD163^+$ M2 macrophages *in situ* (45). M2 polarization is the consequence of the release of immunoregulatory cytokines, but also of the interaction with the virus. IL-6 antagonizes HLA-DR expression and the addition of the specific inhibitor of IL-6 pathway, tocilizumab, partly restores it in Covid-19 patients (44). SARS-CoV-2 and IL-6 likely synergize to down-modulate the expression of HLA-DR and to disarm microbicidal competence of monocytes and macrophages.

Monocyte and macrophage response to SARS-CoV-2 is more complex than expected from the observation of CRS, to which they poorly contribute. The viability of SARS-CoV-2 has a limited impact on the response of monocytes, but not for macrophages. It would be interesting to investigate other variants with different levels of virulence and the use of alveolar macrophages,

more natural target cells of SARS-CoV-2, to assess immune response and polarization status of myeloid cells. The investigation of monocytes suggested that massive migration to tissues had occurred and remaining blood monocytes exhibit a repairing profile. This observation may help understand the risk of post-Covid-19 complication including fibrosis. Indeed, a subset of macrophages with a pro-fibrotic program has been described in patients with Covid-19 (33). The lack of correlation between monocyte count and monocyte functional polarization with severity stages suggest that monocytes are markers of SARS-CoV-2 infection. One limitation is that a majority patient exhibited moderate clinical expression of the disease, which interferes with our evaluation of monocyte and macrophage activation in the progression of the disease. Taken together, our study showed that monocytes and macrophages are targets of SARS-CoV-2, and their manipulation may open the way for therapeutic perspectives.

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Tables

Table 1. Clinical and demographic data of the study population. Seventy-six consecutive Covid-19 patients and 41 healthy controls were analyzed. Demographic data were available for 40 healthy controls. Nonparametric Kruskall-Wallis test was used for group comparison. HC, healthy control; F, female, M, male.

Clinical status	**Covid-19 patients**				**Healthy controls**	***P value***
	Severe	**Moderate**	**Mild**	**All**		
Sample size (%)	14 (18)	21 (28)	41 (54)	76 (100)	40	
Median age (range)	73 (45-95)	56 (29-82)	53 (18-85)	58 (18-95)	40 (18-68)	<0.0001 (with HC) 0.006 (COVID groups only)
Gender (M/F)	12/2	8/13	19/22	39/37	22/18	0.03 (with HC) 0.02 (COVID groups only)
Deceased	8	0	0	0	0	<0.0001 (COVID groups only)

Table 2. List of primers used for q-RTPCR.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
ACTB	GGAAATCGTGCGTGACATTA	AGGAGGAAGGCTGGAAGAG
TNF	AGGAGAAGAGGCTGAGGAACAAG	GAGGGAGAGAAGCAACTACAGACC
CXCL10	GGAAATCGTGCGTGACATTA	AGGAAGGAAGGCTGGAAGAG
IL1B	CAGCACCTCTCAAGCAGAAAAC	GTTGGGCATTGGTGTAGACAAC
IL6	CCAGGAGAAGATTCCAAAGATG	GGAAGGTTCAGGTTGTTTTCTG
IL10	GGGGGTTGAGGTATCAGAGGTAA	GCTCCAAGAGAAAGGCATCTACA
TGFB	GACATCAAAAGATAACCACTC	TCTATGACAAGTTCAAGCAGA
IFNA	ACAACCTCCCAGGCACAAGGGCTGTATTT	TGATGGCAACCAGTTCCAGAAGGCTCAAG
IFNB	GTTCCTTAGGATTTCCACTCTGACTATGGTCC	GAACTTTGACATCCCTGAGGAGATTAAGCAGC
IFNG	GTTTTGGGTTCTCTTGGCTGTTA	ACACTCTTTTGGATGCTCTGGTC
IL8	CTGGCCGTGGCTCTCTTG	TTCCACGTCAAAACGGTTCC
CD163	CGGTCTCTGTGATTTGTAACCAG	TACTATGCTTTCCCCATCCATC

Figure legends

Figure 1. SARS-CoV-2 infects monocytes and macrophages and stimulates cytokine release. (A) MDM macrophages were incubated with live- or heat-inactivated-SARS-CoV-2 IHU-MI3 strain (0.1 MOI) for 24, 48 or 72 hours (n = 11). (**B**) SARS-CoV-2 was quantified by RT-PCR, expressed as Ct values normalized with the actin housekeeping gene and observed by immunofluorescence: virus in red,nucleus in blue and F-actin in green (n = 11). Images were acquired using a confocal microscope (63x). ****$P < 0.0001$ using two-way ANOVA and Turkey's test for post-hoc comparisons. (**B, C**) Pro-(IFN-β, IL-6, TNF-α, IL-1β) and anti-inflammatory (TGF-β, IL-10) cytokines release was evaluated in supernatants from live- or heat-inactivated-SARS-CoV-2-stimulated monocytes and macrophages at (**B**) 24 and (**C**) 48 hours (n = 11). Results are expressed as mean ± standard error of the mean. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$ using Mann-Whitney *U* test.

Figure 2. SARS-CoV-2 elicits a specific transcriptional program in macrophages. Monocytes and macrophages were stimulated with live- or heat-inactivated-SARS-CoV-2 IHU-MI3 strain (0.1 MOI) for 24 or 48 hours (n = 11). The expression of genes involved in the inflammatory response (*IFNA*, *IFNB*, *IFNG*, *TNF*, *IL1B*, *IL6*, *IL8*, *CXCL10*) or immunoregulation (*IL10*, *TGFB1*, *CD163*) was investigated by qRT-PCR after normalization with housekeeping actin gene as endogenous control. (**A**) Data are illustrated as principal component analysis and hierarchical clustering obtained using ClustVis webtool. (**B**) Relative quantity of investigated genes at 24 hours of stimulation was evaluated for monocytes (left panel) and macrophages (right panel). Values represent mean ± standard error of the mean. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ using two-way ANOVA and Sidak's test for post-hoc comparisons.

Figure 3. Investigation of polarized macrophages in the SARS-CoV-2 response. Macrophages and PMA-differentiated THP-1 cells were polarized by treatment with IFN-γ (20 ng/ml) and lipopolysaccharide (100 ng/ml) (M1), IL-4 (20 ng/ml) (M2) or without agonist (M0).

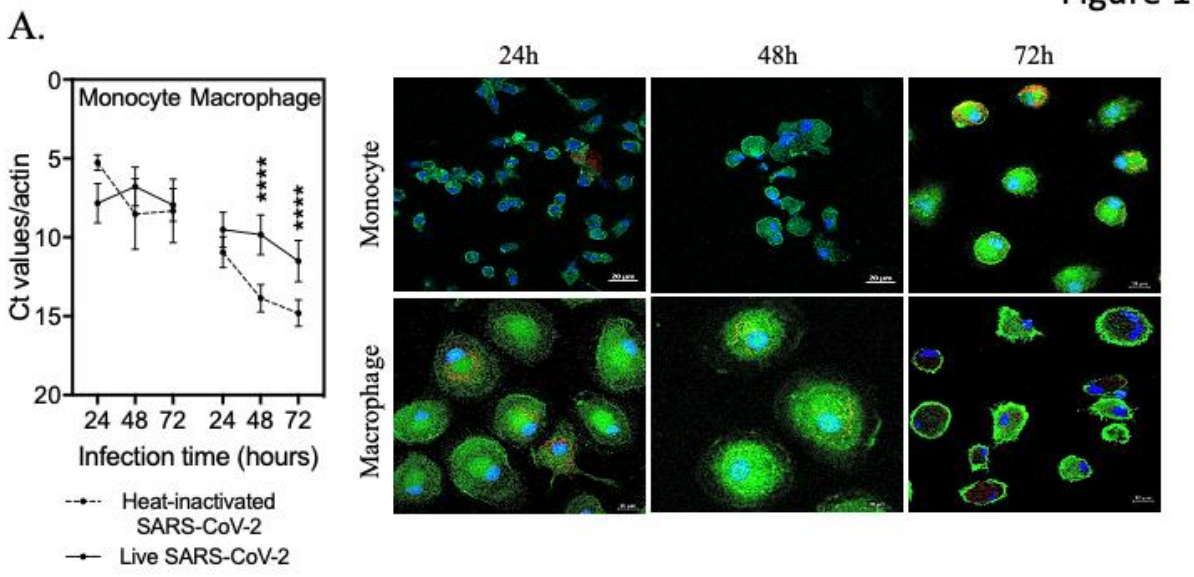
Polarized macrophages were stimulated for (**A**) 24 or (**B**) 48 hours with IHU-MI3 SARS-CoV-2 strain and IL-6, IL-10, TNF-α and TGF-β release were evaluated in the culture supernatants by ELISA (n = 3). **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 using Mann-Whitney *U* test. (**C**) Virus quantification was assessed by the evaluation of the Ct values for polarized SARS-CoV-2-infected macrophages (n = 6) and PMA-differentiated THP-1 cells (n = 6) at 24 hours post-infection. Values represent mean ± standard error of the mean. **P* < 0.05 using two-way ANOVA and Turkey's test for post-hoc comparisons.

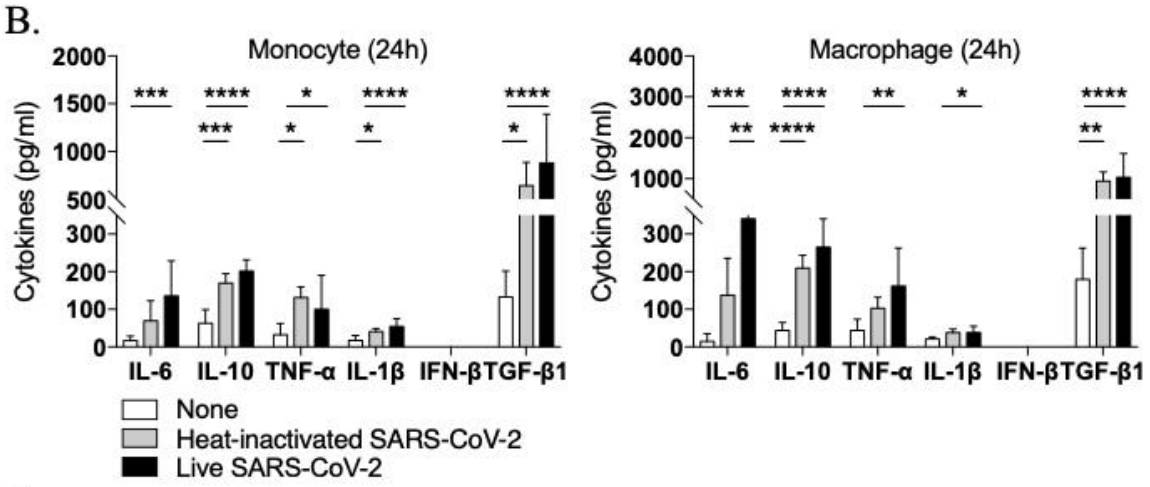
Figure 4. Monocyte subsets are altered in SARS-CoV-2-infected patients. PBMCs from healthy donors and Covid-19 patients were isolated and monocyte sub-populations were investigated by flow cytometry (**A**) Representative flow cytometry plot showing the gating strategy to investigate non-classical, classical and intermediate HLA-DR$^+$ monocytes from Covid-19 patients and healthy donors as control. (**B**) Mean fluorescence intensity (MFI) of HLA-DR and CD163 expression was investigated for CD14$^+$, CD14$^+$/CD16$^+$ and CD16$^+$ monocyte populations from healthy and Covid-19 patients in PBMC population.

Figure 5.

Peripheral blood mononuclear cells from Covid-19 patients were isolated and monocyte sub-populations were investigated by flow cytometry. (**A**) Non-classical, classical and intermediate HLA-DR$^+$ monocytes were evaluated from moderate, mild and severe Covid-19 clinical population. (**B**) Mean fluorescence intensity (MFI) of HLA-DR and CD163 expression was investigated for CD14$^+$, CD14$^+$/CD16$^+$ and CD16$^+$ monocyte populations from moderate, mild and severe Covid-19 patients using t-test.

Figure 1


A.
24h
48h
72h
Monocyte
Macrophage
Monocyte Macrophage
Ct values/actin
0
5
10
15
20
****
****
24 48 72 24 48 72
Infection time (hours)
Heat-inactivated SARS-CoV-2
Live SARS-CoV-2


B.
Monocyte (24h)
Macrophage (24h)
Cytokines (pg/ml)
IL-6 IL-10 TNF-α IL-1β IFN-β TGF-β1
None
Heat-inactivated SARS-CoV-2
Live SARS-CoV-2

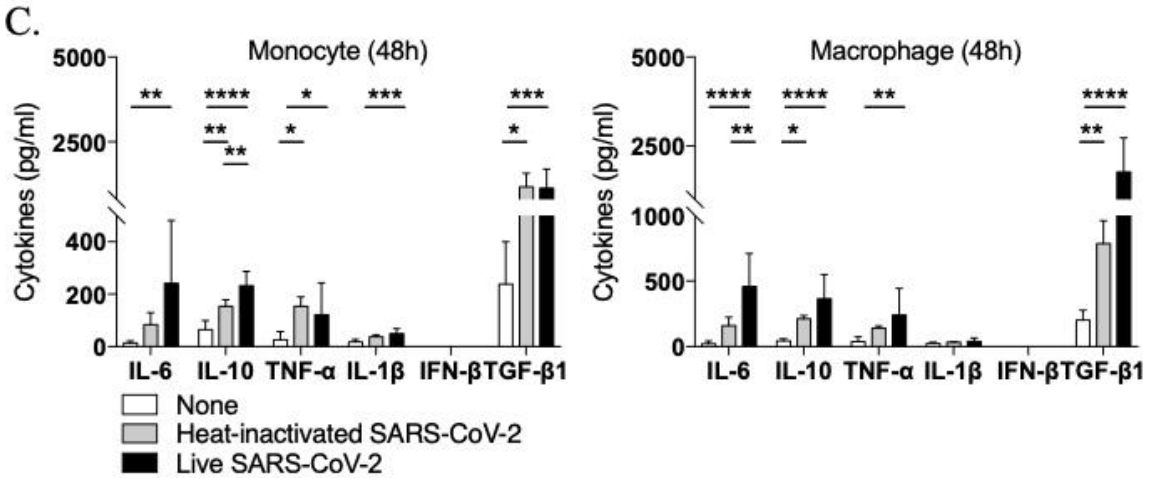

C.
Monocyte (48h)
Macrophage (48h)
Cytokines (pg/ml)
IL-6 IL-10 TNF-α IL-1β IFN-β TGF-β1
None
Heat-inactivated SARS-CoV-2
Live SARS-CoV-2

Figure 2

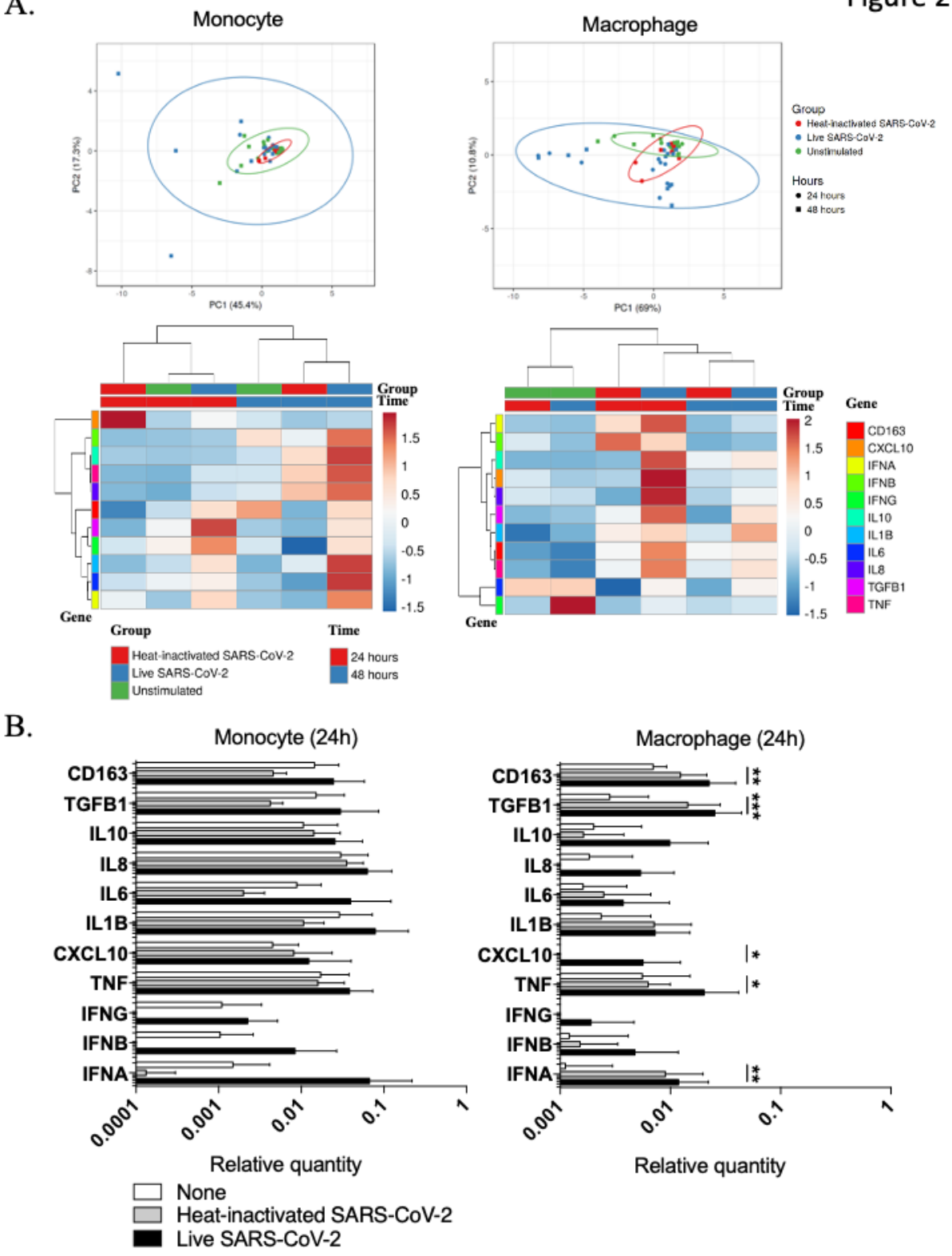
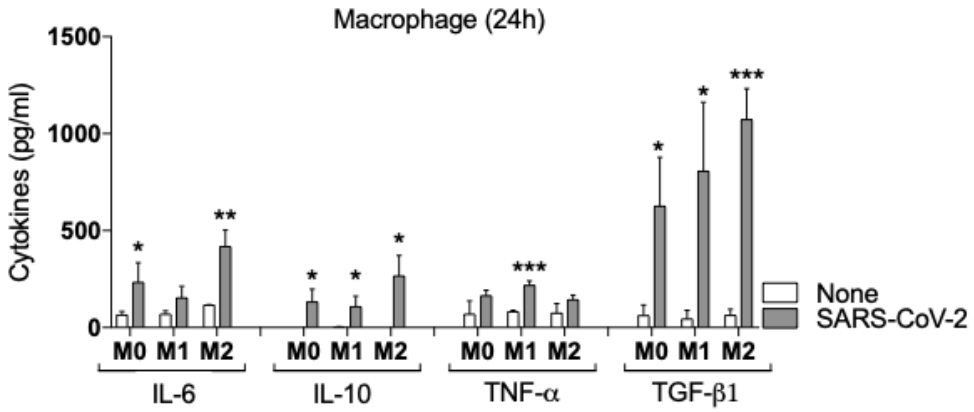
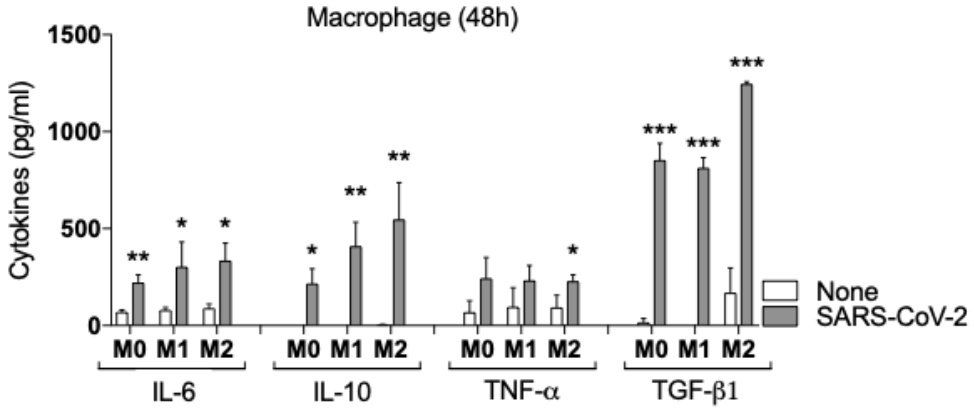

A.
Monocyte
Macrophage
PC1 (45.4%)
PC2 (17.9%)
PC1 (69%)
PC2 (10.8%)
Group
Heat-inactivated SARS-CoV-2
Live SARS-CoV-2
Unstimulated
Hours
24 hours
48 hours
Group
Time
Gene
CD163
CXCL10
IFNA
IFNB
IFNG
IL10
IL1B
IL6
IL8
TGFB1
TNF
Group
Heat-inactivated SARS-CoV-2
Live SARS-CoV-2
Unstimulated
Time
24 hours
48 hours
B.
Monocyte (24h)
Macrophage (24h)
CD163
TGFB1
IL10
IL8
IL6
IL1B
CXCL10
TNF
IFNG
IFNB
IFNA
0.0001
0.001
0.01
0.1
1
Relative quantity
None
Heat-inactivated SARS-CoV-2
Live SARS-CoV-2

Figure 3

A.


Macrophage (24h)
Cytokines (pg/ml)
1500
1000
500
0
M0 M1 M2
IL-6
IL-10
TNF-α
TGF-β1
None
SARS-CoV-2

B.


Macrophage (48h)
Cytokines (pg/ml)
1500
1000
500
0
M0 M1 M2
IL-6
IL-10
TNF-α
TGF-β1
None
SARS-CoV-2

C.

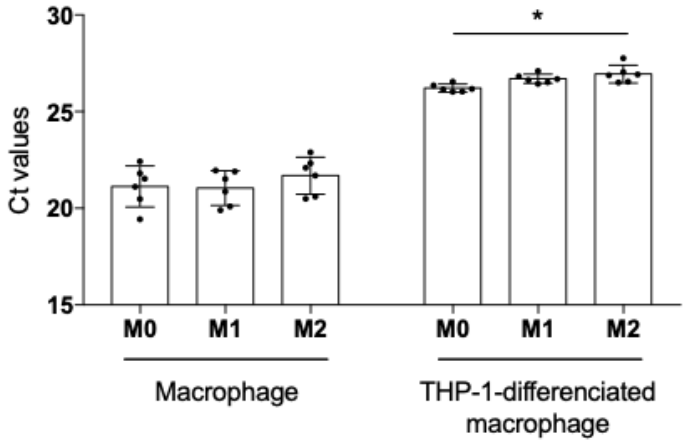

Ct values
30
25
20
15
M0
M1
M2
Macrophage
THP-1-differenciated macrophage

Figure 4

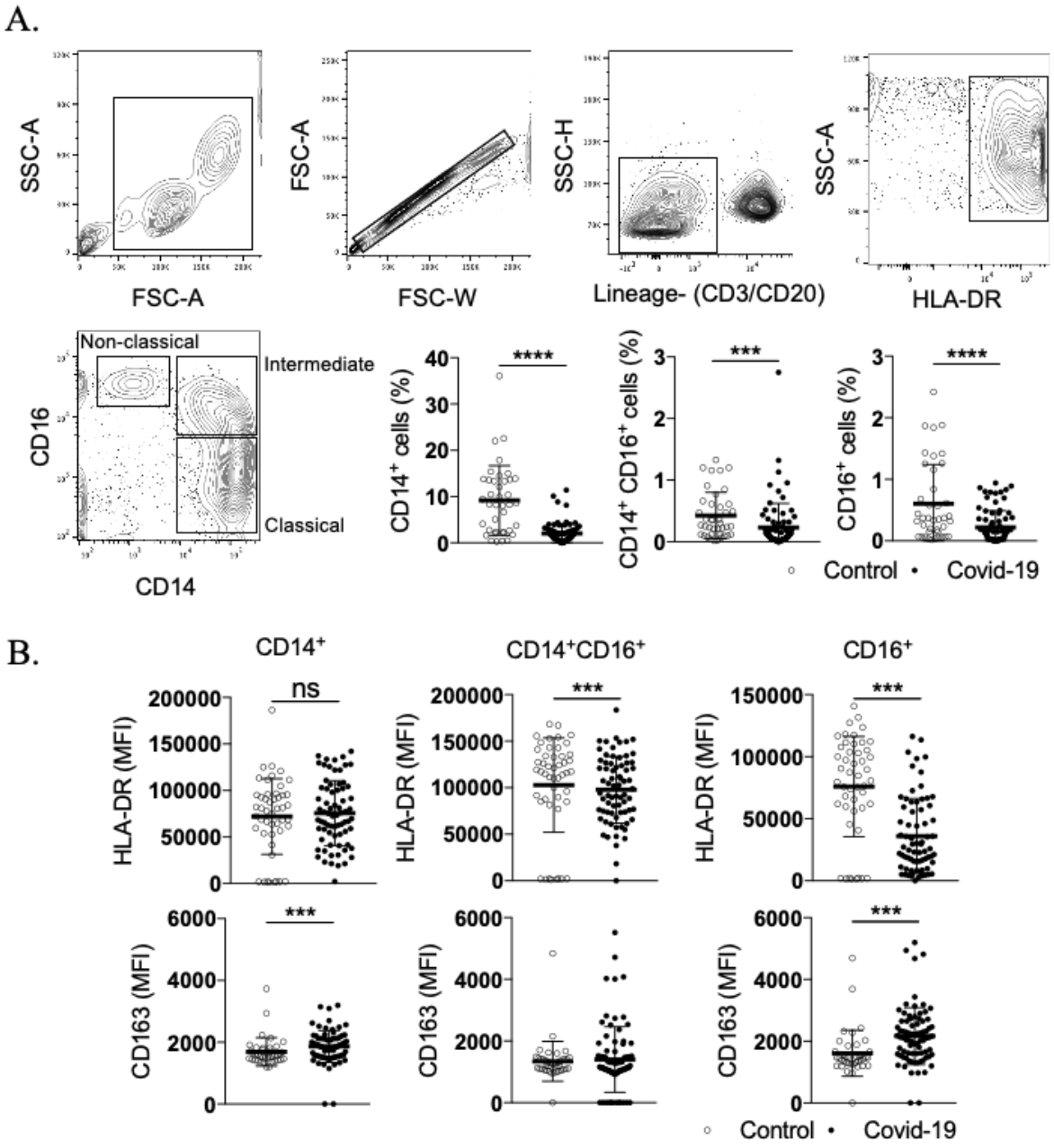
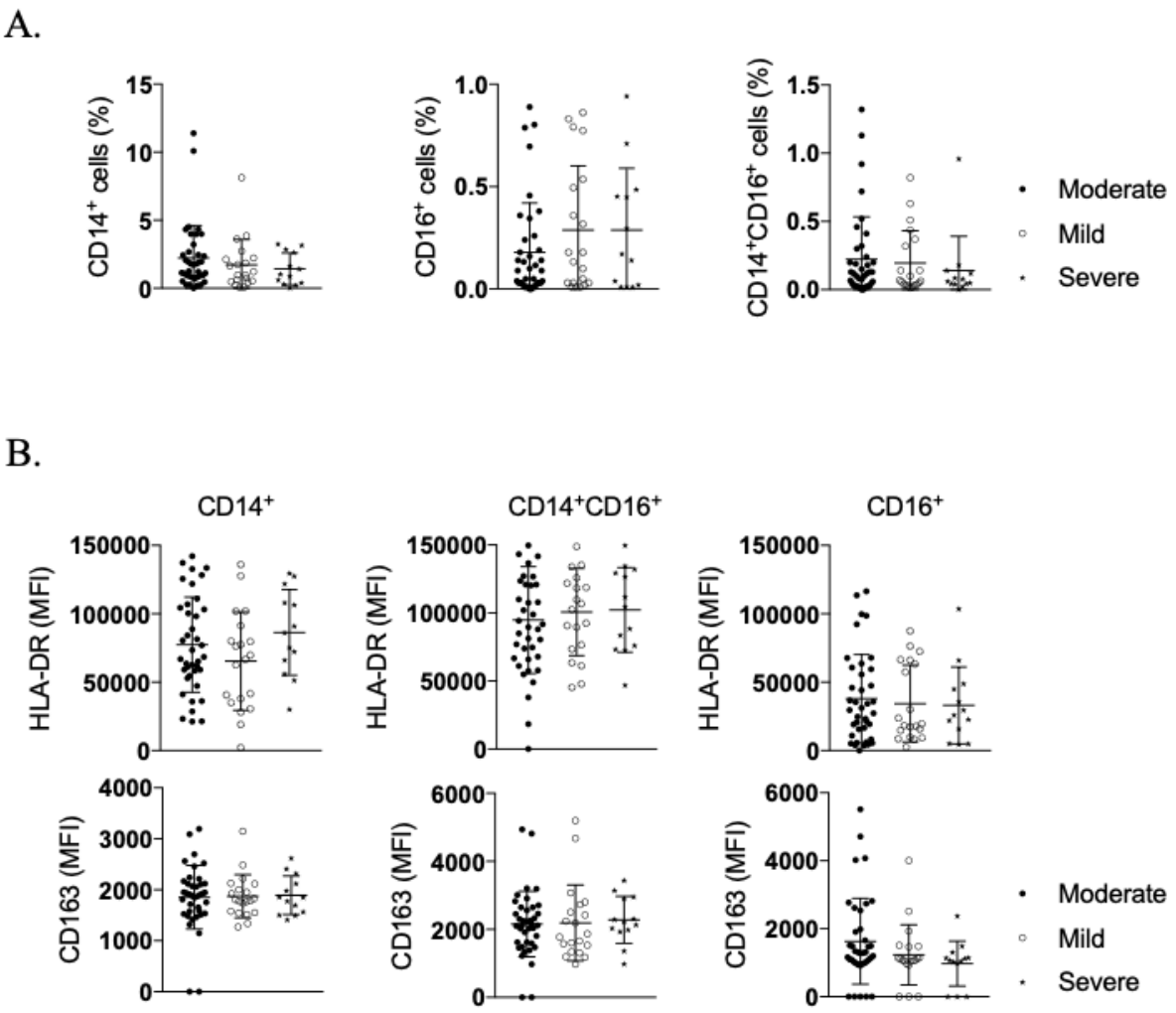

A.
SSC-A
FSC-A
FSC-A
FSC-W
SSC-H
Lineage- (CD3/CD20)
SSC-A
HLA-DR
Non-classical
Intermediate
Classical
CD16
CD14
CD14+ cells (%)
CD14+ CD16+ cells (%)
CD16+ cells (%)
****
***
****
Control • Covid-19
B.
CD14+
CD14+CD16+
CD16+
ns
***
***
***
HLA-DR (MFI)
CD163 (MFI)
Control • Covid-19

Figure 5


A.
CD14+ cells (%)
CD16+ cells (%)
CD14+CD16+ cells (%)
Moderate
Mild
Severe
B.
CD14+
CD14+CD16+
CD16+
HLA-DR (MFI)
CD163 (MFI)
Moderate
Mild
Severe