SARS-CoV-2-induced immune activation and death of monocyte-derived human macrophages and dendritic cells

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Summary: SARS-CoV-2 exhibited ACE2-dependent abortive infection of human myeloid cells. The infection induced antiviral and pro-inflammatory cytokines and chemokines, resulting in type I IFN-mediated cell death of human macrophages.

Footnote

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Abstract

Studies of SARS-CoV-2-infected patients and experimentally infected animals indicate a critical role for augmented expression of pro-inflammatory chemokines and cytokines in severe disease. Here, we demonstrate that SARS-CoV-2 infection of human monocyte-derived macrophages (MDMs) and dendritic cells (MDDCs) was abortive, but induced the production of multiple antiviral and pro-inflammatory cytokines (IFN-α, IFN-β, TNF, IL-1β, IL-6 and IL-10) and a chemokine (CXCL10). Despite the lack of efficient replication in MDMs, SARS-CoV-2 induced profound IFN-mediated cell death of host cells. Macrophage activation and death was not enhanced by exposure to low levels of convalescent plasma, suggesting that antibody-dependent enhancement of infection does not contribute to cell death. Together, these results indicate that infection of macrophages and dendritic cells potentially plays a major role in COVID-19 pathogenesis, even in the absence of productive infection.

Keywords: COVID-19, SARS-CoV-2, macrophage, IFN, cell death
Graphical abstract

IFN-α, IFN-β, TNF, IL-1β, IL-6, IL-10

SARS-CoV-2

ACE2

Caspase-3

IFNAR

Cell death
Introduction

The global pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), COVID-19, is characterized by severe pneumonia, and multiple systemic complications (multi-organ damage including endothelialitis, thrombosis, myocardial damage, etc.) [1]. Although respiratory tract epithelial cells are the primary target cells of SARS-CoV-2 [2], other respiratory tract-resident cells, such as macrophages in alveoli and pulmonary hilum lymphoid tissue, also contain SARS-CoV-2 antigen [3]. Also, circulating monocytes migrate to multiple tissues and in the context of other infections, have been implicated in the spread of virus to distant organs and tissues [4].

In addition, myeloid cells also orchestrate host immune responses to virus infections. Alveolar macrophages sense and respond to microbial threats by producing bioactive molecules to eliminate pathogens and promote tissue repair [5], while the infiltration of circulating myeloid cells, especially pathogenic inflammatory monocyte-macrophages (IMM), may result in elevated lung cytokine/chemokine levels, vascular leakage, and even impair virus-specific T cell responses [6]. A dysregulated macrophage response may promote excessive neutrophil infiltration and tissue damage, leading to macrophage activation syndrome (MAS) and increased morbidity and mortality [7, 8]. The beneficial or deleterious role of lung-resident or infiltrating macrophages is determined largely by their secretion of cytokines and chemokines [9, 10].

Previous studies of SARS and Middle East respiratory syndrome [11] coronavirus showed that human macrophages and dendritic cells were potent sources of pro-inflammatory inflammatory mediators [4, 6, 12]. SARS-CoV abortively infects these myeloid cells [13], but whether MERS-CoV infection is abortive or productive is controversial [12, 14]. SARS-CoV-2-infected macrophages or dendritic cells could potentially produce multiple pro-
inflammatory cytokines and chemokines, such as interleukin (IL)-6, IL-8, tumor necrosis factor (TNF) and C-X-C motif chemokine ligand-10 (CXCL-10), contributing to local tissue injury and pathogenic systemic inflammatory responses (cytokine storm) [15-17]. Pro-inflammatory mediator induction (IL-6, IL-8 and type I, III interferons (IFN) among others) enhances Nuclear Factor [18]-kB-mediated activation and possibly, immune dysfunction, and results in multiple organ failure in severe COVID-19 cases [19-21].

In this study, using human circulating monocyte-derived macrophages (MDMs) and dendritic cells (MDDCs), we showed that SARS-CoV-2 abortively infected both types of cells in an angiotensin-converting enzyme (ACE) 2-dependent manner. Abortive infection induced expression of higher levels of cytokines and chemokines (IFN-α, TNF, IL-6, IL-8, IL-10 and CXCL-10), leading to type I IFN-mediated cell death. In comparison to SARS-CoV, SARS-CoV-2 induced high levels of most cytokines and chemokines and more cell death.

Methods

Human subjects approval.

Written informed consent was obtained from subjects to obtain plasma for this study. The study was approved by the Institutional Review Board of the University of Iowa (IRB #202003554 and #201402735).

Cells and Virus

Human peripheral blood samples were obtained from anonymous donors at the DeGowin Blood Center at the University of Iowa. Consent forms were approved by the University of Iowa’s Institutional Review Board. To obtain monocytes, PBMCs were isolated by density gradient centrifugation through Ficoll-Paque PLUS density gradient media.
(Cytiva) and cultured at a seeding density of $1 \times 10^6$ cells/ml in RP-10 media (RPMI-1640 medium (Invitrogen) supplemented with 10% FBS (Atlanta Biologicals), 2mM l-glutamine) and 5ng/ml M-CSF (eBioscience) (macrophages) or 100ng/ml GM-CSF (PeproTech)+50ng/ml IL-4 (PeproTech) (MDDCs) at 37°C with 5% CO2. After 96 hours, the plates were washed with Hanks’s balanced salt solution devoid of divalent cations (Invitrogen) to remove nonadherent cells. Adherent cells were then trypsinized, pelleted and cultured for 10 days.

The 2019n-CoV/USA-WA1/2019 strain of SARS-CoV-2 (Accession number: MT123290) used in these studies was passaged on Calu-3 2B4 cells. SARS-CoV were obtained from Dr. Kanta Subbarao (National Institutes of Health) and passaged on Vero E6 cells. Calu-3 2B4 cells were grown in MEM (GIBCO, Grand Island, NY) supplemented with 20% fetal bovine serum (FBS). Vero E6 cells were grown in DMEM (GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS).

**Virus infection and titration**

Human PBMC, MDMs or MDDCs were infected with SARS-CoV-2 or SARS-CoV at MOI=2 in Dulbecco's modified Eagle's medium (DMEM, GIBCO, Grand Island, NY), 37°C. 1 hour later, the culture medium was replaced with 10% FBS-DMEM with or without washing with PBS. In some experiments, virus was inactivated by UV. Inactivation was confirmed by plaque assay as described below.

Virus titers in culture medium were determined using Vero E6 cells (grown in 10% FBS-DMEM). For plaque assays, cells were fixed with 10% formaldehyde 3 days after infection and stained with crystal violet.
Confocal microscopy

Cultured MDMs were plated in Chamber Slide (Thermo Fisher) before infection. At the indicated time points post infection, supernatants were removed, and cells were fixed with 4% PFA. For staining, sections were blocked with goat serum for 2 hours in humidity chambers at room temperature (RT). Sections were then treated with primary antibodies (rabbit anti-SARS-CoV2 N protein (Sino Biological) and mouse anti-human caspase-3 (Abcam)) at 4°C overnight. After washing with PBS, samples were treated with secondary antibodies (Alexa 488-goat anti-rabbit IgG, Alexa 562-goat anti-mouse IgG, Abcam) for 30 min at RT. Finally, slides were overlaid with DAPI (Vector) and examined using a confocal microscope (Zeiss 710).

Transmission Electron Microscopy

After fixation with 2.5% glutaraldehyde in 0.1M Na cacodylate, the cells monolayers were treated with 1% OsO4 containing 1.5% potassium ferrocyanide followed by 2.5% uranyl acetate. Samples were dehydrated using increasing concentrations of ethanol, and embedded in Eponate 12 resin (Ted Pella, Redding, CA). Resin was polymerized in a 65°C oven overnight. Ultrathin sections (70–90 nm) were cut with a Leica UC6 ultramicrotome (Leica Microsystems). Sections were collected on copper grids and sequentially stained with 5% uranyl acetate and Reynold’s lead citrate. The sections were viewed using a Jeol 1230 transmission electron microscope (Jeol USA, Peabody, MA) equipped with a Gatan Ultrascan 2k × 2k CCD camera.
Flow cytometry assays

The following monoclonal antibodies were used: APC-eFluor780-conjugated mouse anti–human CD8 (SK1, eBioscience), FITC-conjugated mouse anti–human CD3 (HIT3a), APC-conjugated mouse anti–human CD19 (4G7), PE-conjugated mouse anti–human CD14 (63D3), Pacific Blue-conjugated mouse anti–human CD56 (5.1H11), PerCP Cy5.5-conjugated mouse anti–human CD4 (RPA-T4, Biolegend), rabbit anti-SARS-CoV2 N protein (Sino Biological), Alexa 488-goat anti-rabbit IgG (Abcam). For surface staining, 1×10^6 cells were stained with the indicated antibodies at 4°C. For SARS-CoV-2 N protein staining, cells were fixed and permeabilized using Cytofix/Cytoperm Solution (BD Biosciences) after washing and stained with the indicated antibodies. A LIVE/DEAD cell viability assay kit (Invitrogen) was used for gating live cells. Flow cytometric data were acquired using a FACSVerse and were analyzed using FlowJo software (Tree Star).

Western Blot assays

Cell lysates were collected from cells lysed in Nonidet P-40 cell lysis buffer [22] supplemented with a protease inhibitors cocktail [22] and a phosphatase inhibitor cocktail [22]. Protein samples were run on 4%-20% SDS/PAGE gels and transferred to activated PVDF membranes. Membranes were incubated overnight with isotype control IgG antibody or SARS-CoV-2 (2019-nCoV) nucleocapsid antibody (Sino Biological; 1:1000 in Intercept Blocking Buffer (IBB, Li-Cor)). Membranes were then incubated with IRDye® 680RD Goat anti-Rabbit IgG Secondary Antibody (Li-Cor) at 1:15,000 for 1 h in IBB. Membrane was imaged using a LI-COR odyssey Imaging System.
Human plasma treatment

High titer COVID-19 convalescent plasma (IC$_{50}$=1:1480, determined by a neutralizing titer assay using a luciferase-expressing SARS-CoV-2 S protein pseudovirus system as previously described[23]) and control plasma were collected (IRB approved protocols #202003554 and #201402735). The plasma was diluted at 1:5 (final concentration: 10%) or 1:50 (final concentration: 1%) in DMEM and mixed 1:1 with SARS-CoV-2 for 1 hour at 37°C. The virus-antibody mixtures were then adsorbed to MDMs for 1 hour at 37°C.

Blocking assay

To block viral entry, MDMs were incubated with α-ACE2 monoclonal antibody (AF933, R&D) or isotype control IgG at a final concentration of 10µg/ml for 1 hour prior to SARS-CoV-2 infection.

To block type I IFN signaling, α-IFNAR monoclonal antibody (clone TAB-722, Creative Biolabs) or isotype control IgG was added to cells at a final concentration of 10µg/ml at 12 hours after SARS-CoV-2 infection.

RT-PCR assays

Total RNA was extracted from MDMs using Trizol (Invitrogen) according to the manufacturer’s protocol. Following DNase treatment, 200 ng of total RNA was used as a template for first strand cDNA. The resulting cDNA was subjected to amplification of selected genes by real-time qPCR using Power SYBR Green PCR Master Mix (Applied Biosystems). Average values from duplicate samples were used to calculate the relative abundance of transcripts normalized to HPRT and presented as $2^{-\Delta CT}$. The following primers used for cytokine and chemokines detection: IFN-α: 5’-AGA AGG CTC CAG CCA TCT G TG T-3’, 5’-TGC TGG TAG AGT TCG GTG CAG A-3’; IFN-β: 5’-CTT GGA TTC CTA

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CAA AGA AGC AGC-3’, 5’-TCC TTC TTC TGG AAC TGC TGC A-3’; TNF: 5’- CTCTC TTC TGC CTG CTG CAG TTC TGG AAC TGC TGC A-3’, 5’- ATG GGC TAC AGG CTT GTC ACT C-3’; IL-1β: 5’- CCA CAG ACC TTC CAG GAG AAT G-3’, 5’- GTG CAG TTC AGT GAT CGT ACA GG -3’; IL-6: 5’- AGA CAG CCA CTC ACC TCT TCA G-3’, 5’- TTC TGC CAG TGC CTC TTT GCT G-3’; IL-8: 5’- GAG AGT GAT GAT TGA GAG TGG ACC AC-3’, 5’- CAC AAC CCT CTG CAC CCA GTT T-3’; IL-10: 5’- TCT CCG AGA TGC CTT CAG CAG A-3’, 5’- TCA GAC AAG GCT TGG CCC A-3’; CCL2: 5’- CAG GTG ACA GAG ACT CTT GGG A-3’, 5’- GCC AAT CCT ACA GCC AAG AGC T-3’; CXCL10: 5’- GGT GAG AAG AGA TGT CTG AAT CC-3’, 5’- GTC CAT CCT TGG AAG CAC TGC A-3’. For detection of viral genomes, the following primers were used (ORF1ab): 5’- CCC TGT GGG TTT TAC ACT TAA-3’, 5’- ACG ATT GTG CAT CAG CTG A-3’, 5’- CCG TCT GCG GTA TGT GGA AAG GTT ATG G-3’ and sub-genomic RNA (N protein RNA): SARS-CoV-2: 5’- GAC CCC AAA ATC AGC GAA AT-3’, 5’- TCT GGT TAC TGC CAG TGC ATG AAT CTG-3’; SARS-CoV: 5’- CAG AAC GCT GTA GCT TCA AAA ATC T-3’, 5’- CAG AAC CCT GTG ATG AAT CAA CAG-3’.

Statistics

A Student’s t test was used to analyze differences in mean values between groups. All results are expressed as mean±SEM. P-values of <0.05 were considered statistically significant. * P < 0.05; ** P < 0.01, *** P < 0.001.
Results

Abortive infection of human MDMs and MDDCs by SARS-CoV-2

To determine SARS-CoV-2 infectivity of human immune cells, peripheral blood mononuclear cells (PBMCs), and in vitro-differentiated myeloid cells (MDMs or MDDCs) from healthy donors were infected with SARS-CoV-2 at MOI=2. 24 hours later, the expression of SARS-CoV-2 nucleoprotein (N protein) in immune cell subsets was determined by flow cytometry. As shown in Figure 1A and B, SARS-CoV-2 N protein could be detected in CD14+ monocytes, MDMs and MDDCs, but not CD3+ T cells, CD19+ B cells or CD56+ NK cells (gating strategy shown in Supplementary Figure 1). MDM and MDDC infection required entry through ACE2, the SARS-CoV-2 receptor, since ACE2 blockade prevented virus entry (Figure 1C-E).

To further examine replication in human MDMs and MDDCs, RNA was harvested from infected cells at the indicated time points post infection (p.i.) and evaluated for viral RNA by qPCR. Viral N protein RNA could be detected in both MDMs and MDDCs at 12 hours p.i. (hpi) and decreased 10 (MDMs) and 100 (MDDCs) fold over the following 96 hours (Figure 1D and E). Notably, viral genomic RNA (ORF1ab, gRNA) levels were much lower in MDMs and MDDCs compared to both Vero E6 and Calu-3 (See Supplementary Figure 2A and B). Expression levels of viral N protein were lower in MDMs compared to Vero E6 cell as determined by western blot assay (See Supplementary Figure 2C). In addition, while virus could be detected in culture medium of MDMs within 24 hpi (Figure 1F), it represented residual virus inoculum because washing cells with PBS after virus adsorption reduced levels in the culture medium to nil, but did not affect N protein RNA expression in infected cells (Figure 1G). Taken together, these data showed that human MDMs and MDDCs could be infected by SARS-CoV-2 but failed to support the production of infectious virus. Similarly
and consistent with previous studies [13, 24], SARS-CoV also resulted in abortive infection of MDMs and MDDCs.

**Convalescent plasma blocked SARS-CoV-2 infection of MDMs**

To further distinguish ACE2-mediated entry from non-specific virus uptake by MDMs, virus was incubated with media containing 10% or 1% human COVID-19 convalescent plasma (CP, original titer: 1:1480) or control plasma from a healthy donor before infection. CP treatment decreased the percentage of SARS-CoV-2 N protein+ MDMs (Figure 2A and B), and viral RNA expression (Figure 2C). In addition, we found no evidence of enhanced macrophage infection when cells were treated with a low dose of CP (final concentration: 1%, neutralizing titer 1:14.8) (Figure 2C).

**SARS-CoV-2 induces cytokine and chemokine production by MDMs and MDDCs**

Since MDMs-derived cytokines and chemokines play critical roles in limiting viral infection and in pathogenic processes, we evaluated cytokine and chemokine mRNA production by virus-infected MDMs (Figure 3A) and MDDCs (Figure 3B) at several time points after infection. To provide a framework for interpreting the results, we infected parallel cultures with SARS-CoV and UV-inactivated SARS-CoV-2. Infectious SARS-CoV-2, but not UV-inactivated SARS-CoV-2, induced higher expression of IFN-α, TNF, IL-6, IL-8, IL-10, and CXCL-10 and similar levels of IL-1β compared to SARS-CoV. SARS-CoV preferentially induced IFN-β while SARS-CoV-2 induced higher and prolonged expression of IFN-α. In addition, cytokine and chemokine production was dynamic after infection with expression of some molecules peaking at 24 hours post SARS-CoV-2 infection (e.g.,
CXCL10, IL-10) or earlier (TNF) while maximal levels of IL-6 were reached at 48 hpi. As expected, treatment with low amounts of CP (final concentration: 1%) decreased the expression of these cytokines and chemokines, again inconsistent with antibody disease enhancement. Compared to those of MDMs, the expression levels of cytokines and chemokines were lower in SARS-CoV-2-treated MDDCs, implying distinct roles of these two cell types in infection.

**SARS-CoV-2 infection results in type I IFN-mediated cell death of MDMs**

To determine whether abortive infection in combination with elevated expression of pro-inflammatory cytokines and chemokines resulted in additional phenotype changes such as cell death, MDMs were infected and examined for ultrastructural changes by transmission electron microscopy [18]. The nucleus and cytoplasm of infected macrophages showed progressive electron-lucency with dilation of the cyto-cavitary network. At 48 hpi, cells exhibited early signs of death, which progressed to frank cell death by 96 hpi. Consistent with these changes, mitochondria, which were normal in appearance prior to infection (left panel, inset), were dysmorphic by 48 hpi (center panel, inset). By 96 hpi, mitochondria exhibited extensively blebbing (right panel, inset), indicative of cell death. Of note, we found no virus particles or virus replication structures such as DMVs (double membrane vesicles) in these infected cells. To gain further insight into the mechanism of cell death, we analyzed MDMs for the co-expression of SARS-CoV-2 N protein and caspase-3 (a marker of cell apoptosis) by confocal microscopy at 96 hpi (Figure 4B). Nearly all of the cells expressed caspase-3, consistent with extensive apoptosis. To determine whether IFN-α/β contributed to the development of cell death, we treated infected cells with IFN-α/β receptor (IFNAR) blocking antibody. As shown in Figs. 4B, 4C and S3A, blocking IFNAR at 12 hpi decreased the percentage of dead MDMs, determined using a Live/Dead cell viability assay kit, when cells
were examined at 96 hpi. Treatment with IFNAR blocking antibody did not change the percentage of infected cells or levels of virus RNA (Figure 4D, Supplementary Figure 3B), suggesting that type I IFN contributed to SARS-CoV-2-induced cell death. Moreover, treatment with low concentrations (1%) of CP did not increase cell death (Figure 4C). In contrast to results obtained with MDMs, a smaller fraction of MDDCs underwent death after infection with SARS-CoV-2 (See Supplementary Figure 3C).

**Discussion**

Here, we demonstrate that human MDMs are abortively infected by SARS-CoV-2, in agreement with a previous report [24]. We additionally show that MDDCs do not support productive SARS-CoV-2 infection. Levels of intracellular viral RNA and protein were very low in both cell types and we detected no virus or viral replicative structures by TEM. We also show that entry is ACE2 and S protein-dependent, since anti-ACE2 antibody (Figure 1C-E) and CP (Figure 2) from a COVID-19 patient blocked the entry of SARS-CoV-2 into MDMs and MDDCs. Further, a lower dose of CP (final concentration: 1%) did not enhance viral entry, cytokine expression or cell death (Figs. 2C, 3A, 4C), suggesting that antibody-dependent enhancement of macrophage infection did not occur. Although we observed no antibody-dependent enhancement, it will still be important to carefully evaluate patients for disease enhancement in clinical trials of vaccination and antibody treatment and in patients who show evidence of re-infection [25]. Of note, in disagreement with a recent report [26], we found no evidence that T cells, B cells or NK cells (Figure 1A) were infected by the virus.
Remarkably, given the low expression level of viral RNA and protein, SARS-CoV-2 infection invoked potent cytokine and chemokine production by human MDMs and less by MDDCs (Figure 3). Chemokine and cytokine expression were not induced solely by virus binding to the ACE2 receptor because they were not induced by UV-inactivated virus (Figure 3A). Whether viral RNA or protein expressed during abortive replication signal through toll-like receptors (TLRs) or retinoic acid-inducible gene (RIG)-I-like receptors to activate IFN- and NF-kB-dependent pathways will require further investigation. Several SARS-CoV-2 proteins have been reported to activate or counter the innate immune [18, 27] but at this point, it is not known whether these proteins, double stranded viral RNA or the intrinsic process of virus replication (e.g., membrane distortion to form sites of RNA replication[28]) are most important in this process. Further, we assessed cytokine/chemokine RNA expression longitudinally, and found that infection of MDMs induced higher expression of IFN-α, TNF, IL-6, IL-8, IL-10, CXCL-10 and similar expression of IFN-β and IL-1β when compared to SARS-CoV (Figure 3A). Of note, neither virus was as potent inducer of proinflammatory molecule expression as H5N1, H1N1pdm, or MERS-CoV [24]. Since mortality in COVID-19 patients is associated in some cases with dysregulated and elevated pro-inflammatory molecule expression [1, 3], this observation suggests that other cells, such as lung epithelial cells, or uninfected bystander myeloid cells are major contributors to expression of these molecules. Nonetheless, since macrophage-derived cytokines and chemokines contribute to both host defense and tissue damage, these results suggest that even abortive infection of myeloid cells could contribute to a hyperinflammatory milieu. For example, CXCL10 plasma levels are highly associated with COVID-19 severity and may predict disease progression [29, 30]. TNF levels were found to correlate with the abundance of pro-inflammatory CXCL10⁺CCL2⁺ macrophages in severe COVID-19 [31], which might exacerbate inflammation in a positive feedback loop.
IL-6 and IL-1β expressions were also shown to correlate with disease severity [16, 17, 30]. Although blocking IL-6 therapy was postulated to improve clinical outcome [32-34], recent controlled trials did not support its efficacy in treatment of COVID-19 patients [35]. IL-1β signaling blockade using Anakinra, was also found to provide benefit in some COVID-19 cases [36-39]. However, confirmation of IL-1β blockade efficacy will require larger controlled trials.

We also demonstrate that human MDMs-derived IFN-α/β participated in cell death in an autocrine or paracrine manner after SARS-CoV-2 infection (Figure 4C). Similarly, IFN-α, β, γ treatment of human alveolospheres [2] cells resulted in cell death [40]. Induction of caspase 3 suggests that MDMs underwent apoptosis. However, this conclusion needs further validation because caspase-3 is also associated with necrotic/pyroptotic cell death in a few settings [41]. These results are in contrast to studies of apoptosis in MDMs infected with a common cold human coronavirus (HCoV-229E), which is dependent on viral replication, and not TNF-related apoptosis-inducing ligand (TRAIL), FasL, TNF or caspase activity. HCoV-229E infection of PBMC-derived myeloid cells shows preferential killing of dendritic cells but not macrophages [42]. The lower expression of pro-inflammatory cytokines in SARS-CoV-2-infected MDDCs, compared to that of MDMs, might contribute to their lower rates of death (Figure S4C), suggesting that the role of IFN signaling in different myeloid cell types is variable. In addition to effects on expression of pro-inflammatory mediators, enhanced myeloid cell death may contribute to adaptive immune responses. Despite the lack of viral particles and DMV structures in infected cells (Figure 4A), dying macrophages might contain sufficient viral antigen so that they can be phagocytosed by antigen-presenting cells (APCs) to induce T cell- or B cell-mediated immune responses.
In summary, these results illustrate some of the complexities of the pathogenesis of infections caused by SARS-CoV-2 and other human respiratory CoV. Even when the infection is abortive, the consequence may be expression of pro-inflammatory mediators that contribute to pathogenic outcomes. These results reinforce the need for anti-viral agents since infectious but not UV-treated virus causes macrophage activation and cell death and for agents that dampen the host immune response, diminishing the exuberant inflammatory response, to which dying macrophages contribute.
Competing interests:

The authors have no competing interests, financial or non-financial.

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Figure Legend

Figure 1. SARS-CoV-2 abortive infection of human MDMs and MDDCs. A and B.
Human PBMCs, MDMs, MDDCs were infected with SARS-CoV-2 at MOI=2. Infected immune cell subsets were identified by flow cytometry using an anti-SARS-CoV-2 N protein antibody. C. SARS-CoV-2 N protein expression in SARS-CoV-2-infected MDMs treated with an anti-hACE2 antibody or its isotype control. D-G. MDMs (D, F and G) or MDDCs (E) were infected with SARS-CoV-2 or SARS-CoV at MOI=2, followed by washing with PBS (G) or not (D-F), before addition of 10% FBS-DMEM. At indicated time points, culture media were collected, and cells were treated with TRIZOL for RNA extraction. SARS-CoV-2 N RNA expression were determined by Q-PCR (D, E and G). Infectious viral particles in culture medium were detected by plaque assays (F and G). (B, D-G) Data are shown as mean±SEM and are representative of 3 independent experiments, n=4-8, * p<0.05, ** p<0.01.

See also Supplementary Figure 1-2.

Figure 2. COVID-19 CP blocks the entry of SARS-CoV-2 in human MDMs. SARS-CoV-2 was incubated with CP or control plasma as described in Methods before infecting human MDMs. A and B. N protein expression in SARS-CoV-2-infected MDMs treated with CP or control plasma was determined by flow cytometry at indicated time points. C. SARS-CoV-2 N RNA expression in MDMs were detected by Q-PCR. (B and C) Data are shown as mean±SEM and are representative of 3 independent experiments, n=4-6, * p<0.05, ** p<0.01, *** p<0.001.
Figure 3. SARS-CoV-2-infected human MDMs and MDDCs produce multiple cytokines/chemokines. MDMs (A) or MDDCs (B) were exposed to SARS-CoV or SARS-CoV-2 (with or without CP treatment), or UV-inactivated SARS-CoV-2. RNA was extracted at the indicated time points post infection. Cytokines/chemokines levels were determined by Q-PCR as described in Methods. Data are shown as mean±SEM and are representative of 3 independent experiments, n=4-6, * (p<0.05) stands for difference between SARS-CoV-2 and SARS-CoV groups.

Figure 4. SARS-CoV-2 infection of human MDMs results in IFN-dependent cell death.

A. Representative transmission electron micrographs of SARS-CoV-2-infected human macrophages from mock and SARS-CoV-2 groups. N: nucleus, C: cytosol, bar = 1µm. Images shown are representative of 3 independent donors. Insets show progressive loss of mitochondrial integrity as infection proceeds. B. SARS-CoV-2-infected MDMs were fixed at 96 hpi and prepared for confocal microscopy as described in Methods. Some wells were treated with α-IFNAR antibody at 12 hpi. The expression and co-localization of SARS-CoV-2 N protein and caspase-3 are shown. C, D. Virus was incubated with CP (final concentration: 1%) or cultures were treated with α-IFNAR antibody at 12 hpi. Isotype matched antibodies were included as controls. Dead MDMs were identified at 96 hpi using a Live/Dead cell viability assay kit with flow cytometry as shown in Figure S3A. (C). SARS-CoV-2 N protein RNA expression in MDMs was detected by Q-PCR at the indicated time points post infection (D). (C, D) Data are shown as mean±SEM and are representative of 3 independent experiments, n=5, *** p<0.001.

See also Supplementary Figure 3.
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Figure 1

A

CD3  CD19  CD56  CD14  MDMs  MDDCs

SARS-CoV-2

0.01  0.00  0.00  0.00  0.01  0.00

APC-SARS-CoV-2-N protein

B

% of N protein+ cells

CD3  CD19  CD56  CD14  MDMs  MDDCs

SARS-CoV-2

SARS-CoV-2+ ctrl IgG

SARS-CoV-2+ anti-ACE2

C

N RNA in MDMs

Expression relative to HPRT

hours post infection

** p<0.01

N RNA in MDDCs

Expression relative to HPRT

hours post infection

** p<0.01

D

Infection with virus

Addition of FBS-DMEM

1h culture

SARS-CoV-2

SARS-CoV

Infection with virus

Wash with PBS

Addition of FBS-DMEM

N RNA

pU/ml

hours post infection

LOD
Figure 3

A MDMs

- **IFN-α**
  - SARS-CoV-2
  - SARS-CoV
  - SARS-CoV-2+CP
  - UV-activated SARS-CoV-2

- **IFN-β**
- **TNF**

B MDDCs

- **IFN-α**
- **IFN-β**
- **TNF**

- **IL-6**
- **IL-8**
- **IL-10**

- **IL-1β**
- **CCL2**
- **CXCL10**

Expression relative to HPRT

hours post infection
