**ARTICLE**

# Highly restricted SARS-CoV-2 receptor expression and resistance to infection by primary human monocytes and monocyte-derived macrophages

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**Abstract**

Severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV2), which causes the disease COVID-19, has caused an unprecedented global pandemic. Angiotensin-converting enzyme 2 (ACE2) is the major cellular receptor for SARS-CoV2 entry, which is facilitated by viral Spike priming by cellular TMPRSS2. Macrophages play an important role in innate viral defense and are also involved in aberrant immune activation that occurs in COVID-19, and thus direct macrophage infection might contribute to severity of SARS-CoV2 infection. Here, we demonstrate that monocytes and monocyte-derived macrophages (MDM) under in vitro conditions express low-to-undetectable levels of ACE2 and TMPRSS2 and minimal coexpression. Expression of these receptors remained low in MDM induced to different subtypes such as unpolarized, M1 and M2 polarized. Untreated, unpolarized, M1 polarized, and M2 polarized MDM were all resistant to infection with SARS-CoV2 pseudotyped virions. These findings suggest that direct infection of myeloid cells is unlikely to be a major mechanism of SARS-CoV2 pathogenesis.

Summary sentence: Monocytes and macrophages express minimal ACE2 and TMPRSS2 and resist SARS-CoV-2 Spike-mediated infection, suggesting direct myeloid cell infection is unlikely a major contributor to pathogenesis.

**KEYWORDS**
ACE2, COVID-19, TMPRSS2

## 1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19) is caused by the newly discovered severe acute respiratory syndrome coronavirus 2 (SARS-CoV2). The virus was first reported in Wuhan, China and since then has spread globally causing millions of deaths worldwide and unprecedented social and economic disruption.[1] Cellular entry of SARS-CoV2 depends on the binding of the viral Spike (S) protein to angiotensin-converting enzyme 2 (ACE2) receptor and priming by TMPRSS2 protease activity in target cells.[2] Coexpression of ACE2 and TMPRSS2 genes has been detected by single-cell RNA-sequencing analyses on multiple cell types relevant to infection and pathogenesis, including goblet secretory cells (nasal mucosa), type-2 pneumocytes (lungs), oral epithelial cells,[3,4] and intestinal absorptive enterocytes,[5] characterizing potential target sites for SARS-CoV-2 replication in humans.[6] Macrophages are a key component of innate immune activity, and lungs alveolar macrophages are a first line of defense to

**Abbreviations:** SARS-CoV2:, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 2019; MDM, monocyte-derived macrophages; ACE2, Angiotensin-Converting; Enzyme 2 TMPRSS2, transmembrane serine protease 2; GUSB, glucuronidase Beta; TMPRSS4, transmembrane serine protease 4; TMPRSS11, transmembrane serine protease 11; VSVg, vesicular stomatitis virus glycoprotein; HSV-1, herpes simplex virus type 1; FMO, fluorescence-minus-one; LPS, lipopolysaccharide; IFN, interferon; IL, interleukin; M-CSF, macrophage colony stimulating factor; IMDM, Iscove's modified Dulbecco's medium; DMEM, Dulbecco's modified eagle medium; FBS, fetal bovine serum.

initially contact and recognize pathogens. It has been hypothesized that by virtue of polarization toward either an M1 or M2 phenotype, lung macrophages contribute to differential consequences following SARS-CoV-2 infection.[7] Autopsies of patients who died of COVID-19 reveal extensive cellular infiltration in lungs with a predominance of macrophages. The severity of the respiratory syndrome positively correlates with macrophage numbers.[8] However, little is known about ACE2 and TMPRSS2 expression on macrophages, whether macrophages may be direct targets for SARS-COV2 infection, or whether polarization to M1 and M2 subtypes affects ACE2 and TMPRSS2 expression or susceptibility to infection.

In the present study, we investigate the expression of ACE2 and TMPRSS2 in monocytes and MDM during differentiation in in vitro culture and in macrophage subject to different polarization conditions, and susceptibility of differently polarized macrophages to SARS-CoV2 Spike pseudotype virion infection.

## 2 | MATERIALS AND METHODS

### 2.1 | Cells

Monocytes were obtained from healthy blood donors by leukapheresis followed by negative selection (Rosette-Sep; Stemcell Technologies). Monocytes were plated at $2 \times 10^6$ cells per well in 6-well plates using IMDM containing 10% human serum (Valley Biomedical), 100 U/ml penicillin, and 100 $\mu$g/ml streptomycin. Cells cultured in IMDM will be referred to as untreated monocyte-derived macrophages (MDMs) hereafter. For macrophage polarization, monocytes were cultured for 6 days in RPMI 1640 medium (Gibco) supplemented with 10% FBS (GeminiBio), 5% human serum, 100 U/ml penicillin, 100 $\mu$g/ml streptomycin, 2 mM L-glutamine and 25 ng/ml M-CSF (Stemcell Technologies) to differentiate them into nonpolarized macrophages. M1 polarization was achieved by supplementation with IFN-$\gamma$ (Stemcell Technologies) at 10 ng/ml and LPS from *Escherichia coli* (LPS; Sigma) at 100 ng/ml for 48 h. M2 polarization was achieved by supplementation with 20 ng/ml IL-4 (Stemcell Technologies) for 48 h.[9] The 293T-ACE2cl.22 cell line engineered to express human ACE2 was a gift from Dr. P. Bieniasz.[10] 293T-ACE2 cells were cultured in DMEM supplemented with 10% FBS.

### 2.2 | Flow cytometry

Freshly isolated monocytes (day 0) and MDM following in vitro culture were stained with an antibody cocktail containing CD14-Pacific Blue, CD16-APC-Cy7 and CD163-BV711 (BD Biosciences), CD3-BV570 and CD4-Pe-Cy5 (BioLegend), CD8-PeTxRd (Invitrogen), and ACE2-AF488 (R&D Systems; clone # 535919) at room temperature for 20 min in the dark. In additional experiments, cells were incubated first with AquaBlue (ThermoFisher) to stain for cell viability. Cells were then washed, permeabilized using the BD Cytofix/Cytoperm Fixation/Permeabilization Kit (cat# 554714), and incubated with polyclonal anti-TMPRSS2 primary antibody (rabbit anti-hu TMPRSS2;

cat# PA5-14264; Invitrogen) diluted 1:25 for 30 min in the dark at 4°C. Cells were washed and APC-conjugated secondary antibody (goat anti-rabbit IgG (H+L)-APC; cat# A-10931; Invitrogen) diluted 1:400 was added and incubated for 30 min. Because the TMPRSS2 is a polyclonal antibody, negative controls included primary-only, secondary-only, and in additional experiments also included an irrelevant rabbit polyclonal antibody (anti-HSV-1; Abcam; Cat #ab9533) for gating. ACE2 was gated using fluorescence-minus-one (FMO). Gating strategy is shown in Figures S1 and S2. Cells were washed and suspended in FACS buffer for acquisition. In some stains, 5% human serum was added to FACS buffer (beyond the human serum in which cells were cultured) to confirm Fc receptor blocking. A similar protocol was followed for ACE2-293T cells, which served as a positive control (Figure S3). FACS data were acquired on a modified LSRII (BD Biosciences) using bead compensation tubes and analyzed using FlowJo (TreeStar) software. For TMPRSS2, negative staining controls included a primary antibody only and secondary antibody only, and for ACE2 receptor an FMO tube was included as a control to establish gating. A minimum of 300,000 events were collected for each analysis, and cells from 4 to 9 donors were analyzed for each time/condition.

### 2.3 | ACE2 and TMPRSS2 mRNA expression by RT-PCR

Total RNA was extracted from cells using RNeasy Plus Kit (Qiagen) and 100 ng total RNA was reverse transcribed to cDNA using random hexamers and high-capacity reverse transcription kit (Thermo Fisher). ACE2 was amplified using primer-probe set Hs01085333_m1 and TMPRSS2 was amplified using primer-probe set Hs00237175_m1 as described[11–13] (both from Applied Biosystems) using Taqman universal mastermix (Thermo Fisher). qPCR for housekeeping GUSB gene was performed using Powerup SYBR green mastermix (Thermo Fisher) using primers GUSB_F: CGCCCTGCCTATCTGTATTC and GUSB_R: TCCCCACAGGGAGTGTGTAG.[14] qPCR reactions were run at 50°C for 2 min, 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min on an ABI PRISM 7500 FAST instrument.

### 2.4 | Pseudotyped viruses and infections

Pseudotype virion particles comprised of an HIV-1 nanoluciferase reporter backbone carrying SARS-CoV-2 Spike (G614 variant with a 19 amino acid cytoplasmic tail deletion, kindly provided by Dr. P. Bieniasz[10]) were generated by cotransfection in HEK293T cells as previously described.[15] Particles pseudotyped with VSVg and particles generated in the absence of envelope glycoproteins (bald virus) served as positive and negative controls, respectively.

For virus infection, monocytes were plated at $7.5 \times 10^4$ cells/well in 96-well plates and allowed to differentiate to macrophages for 8 days. One day prior to infection, 293T-ACE2 cells were plated at $7.5 \times 10^4$ cells/well in 96-well plates. Cells were infected by spinoculation (1200×*g* for 2 h at 25°C) using 100 $\mu$l of 1:2 and 1:10 diluted

**TABLE 1** Expression of ACE2 and TMPRSS2 by monocytes and MDM

**A. Differentiation time in culture**

| Marker | D.0 mono untreated (%) | D.3 mono/MDM untreated (%) | D.8 MDM untreated (%) | 293T-ACE2 cells (%) |
|---|---|---|---|---|
| ACE2+ | 0.51 ± 0.27 | 0.59 ± 0.35 | 2.65 ± 0.87 | 25.37 ± 14.60 |
| TMPRSS2+ | 0.10 ± 0.04 | 0.46 ± 0.30 | 2.11 ± 1.02 | 21.12 ± 14.11 |
| TMPRSS2+ACE2+ | 0.01 ± 0.01 | 0.02 ± 0.01 | 0.36 ± 0.28 | 3.02 ± 1.70 |

**B. Differentiation conditions**

| Marker | Untreated D.8 MDM (%) | Unpolarized D.8 MDM (%) | M1 polarized D.8 MDM (%) | M2 polarized D.8 MDM (%) |
|---|---|---|---|---|
| ACE2+ | 2.65 ± 0.87 | 0.40 ± 0.30 | 0.49 ± 0.33 | 0.32 ± 0.26* |
| TMPRSS2+ | 2.11 ± 1.02 | 0.29 ± 0.18 | 0.11 ± 0.01 | 0.11 ± 0.03 |
| TMPRSS2+ACE2+ | 0.36 ± 0.28 | 0.00 ± 0.00 | 0.01 ± 0.01 | 0.01 ± 0.01 |

Data are expressed as mean ± SEM from 5 to 9 independent monocyte/macrophage donor cells for each condition. Marker expression in monocytes and MDM are on gated CD14+ cells. ACE2-overexpressing 293T cells serve as a positive control. *$p = 0.044$ versus D.8 untreated MDM (Mann–Whitney 2-tailed $U$ text); no other marker was significantly different in D.3 or D.8 MDM compared with D.0 monocytes (Table 1(A)) or in D.8 unpolarized, M1 or M2 MDM compared with D.8 untreated MDM (Table 1(B)) (details in Table S1).

SARS-CoV2 Spike pseudotype particles, 1:10 and 1:200 diluted VSVg pseudotypes, and 1:10 diluted particles lacking viral envelop glycoprotein (background control). Uninfected cells served as negative control. After infection, cells were cultured for 72 h and luciferase expression measured by NanoGlo luciferase assay (Promega).

## 2.5 | Statistical analysis

Experiments were carried out on monocyte/macrophage cells isolated from independent donors. Statistical analysis comparing expression under different conditions employed nonparametric Mann–Whitney two-tailed $U$ test and Prism software (GraphPad).

## 3 | RESULTS

We first assessed ACE2 and TMPRSS2 staining in fresh monocytes (Table 1 and Figure 1(A)), using the gating strategy as shown in Figure S1 and S2, with 293T-ACE cells as a positive control (Figure S3). Monocytes at day 0 expressed minimal detectable ACE2 (0.51 ± 0.27%) and TMPRSS2 (0.10 ± 0.04%), and virtually no cells were dual-positive (0.01 ± 0.01%). In contrast, 293T-ACE2 cells expressed robust levels of each molecule and had a clear population of dual positive cells.

We then performed a time-course study to determine whether expression of ACE2 and TMPRSS2 changed as cells differentiated into MDM in standard culture conditions ("untreated") over 8 days in vitro (Table 1(A)) and Figures 1(B) and 1(C)). ACE2 expression was unchanged at day 3 (0.59 ± 0.35%) and increased slightly at day 8 (2.65 ± 0.87%), and TMPRSS2 increased slightly at day 3 (0.58 ± 0.46%) and day 8 (2.11 ± 1.02%), though these changes were not statistically significant (Table S1). There remained minimal detectable coexpression of the receptors (0.02 ± 0.01 and 0.36 ± 0.28 at days 3 and 8, respectively).

Given that myeloid cells are exposed to polarizing stimuli in vivo, we asked whether SARS-CoV2 receptors were up-regulated on MDM subject to distinct in vitro differentiation conditions, including unpolarized (M-CSF), M1 polarized (IFN-γ/LPS), and M2 polarized (IL4). As shown in Table 1(B) and Figure 2, day 8 MDM in all 3 culture conditions expressed levels of both ACE2 and TMPRSS2 that were even lower than standard conditions; the decrease in ACE2 on M2 polarized MDM was statistically significant (Tables 1 and S1; $p = 0.044$; Mann–Whitney). Finally, while only 0.36 ± 0.28% of untreated day 8 MDM showed receptor coexpressions, there was essentially no detectable coexpression of ACE2 and TMPRSS2 in any of the other culture conditions (Table 1(B)).

We then investigated ACE2 and TMPRSS2 gene expression by RT-PCR in day 8 macrophages maintained in standard, unpolarized, and M1 and M2 differentiation culture conditions (Figure 3). 293T-ACE2 cells expressed robust levels of both ACE2 and TMPRSS2 RNA. In contrast, ACE2 transcripts were nearly undetectable (Ct ≥ 38), whereas TMPRSS2 levels were exceedingly low (5–10 Ct higher than 293T cells). Importantly, there were no major differences in expression under different differentiation conditions.

Finally, we investigated if macrophages were susceptible to infection by SARS-CoV2, using Spike protein pseudotyped virus particles carrying a luciferase reporter gene (Figure 4). As a positive control, we used virions carrying the VSVg protein, and virions lacking any glycoprotein ("bald") served as a negative control. Both SARS-CoV2 and VSVg pseudotypes infected 293T-ACE2 cells, yielding a robust luminescence signal. MDM cultured under standard conditions were readily infected by VSVg pseudotypes but were resistant to Spike pseudotypes, with luciferase levels indistinguishable from virions lacking envelope glycoproteins. Similarly, neither unpolarized, M1 nor M2 MDM were susceptible to Spike-mediated infection. M1 and especially M2 polarized cells had reduced infection by VSVg pseudotypes compared with standard conditions, so it is possible that postentry blocks could be operative in the pseudotype assay in these cells.
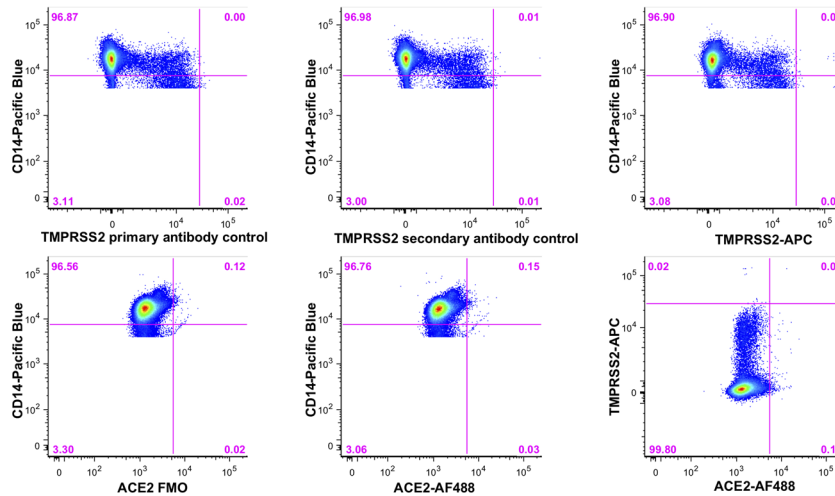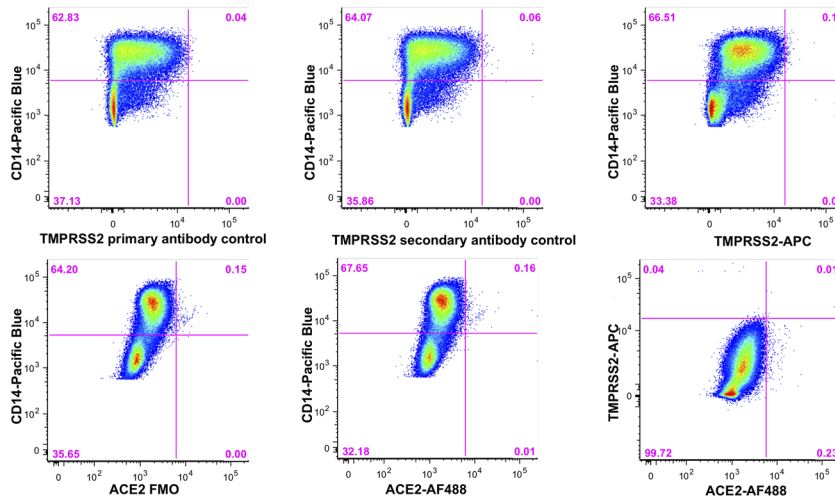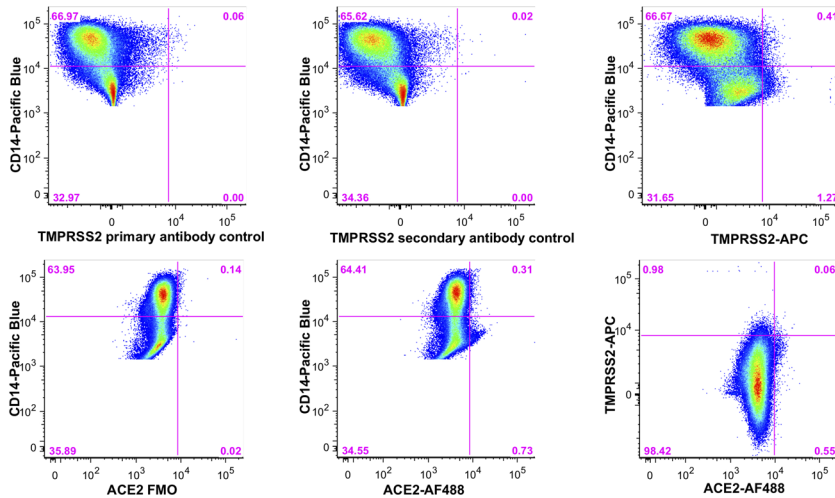
## A. D0 Untreated monocyte



## B. D3 Untreated mono/MDM



## C. D8 Untreated MDM



**FIGURE 1** Expression of ACE2 and TMPRSS2 by monocytes and macrophages. Representative FACS stain of ACE2 and TMPRSS2 on (A) freshly isolated monocytes (day 0), and after 3 (B) and 8 days (C) in culture under standard conditions. Top panels show TMPRSS2 negative controls with specific staining. Bottom panels show ACE2 FMO negative control, ACE2 staining, and dual TMPRSS2/ACE2 staining. Data are representative of 5–9 replicate experiments using cells from different donors
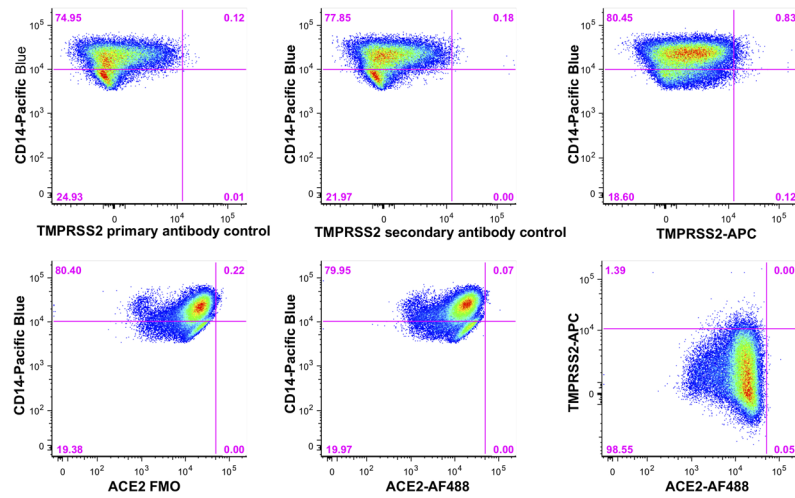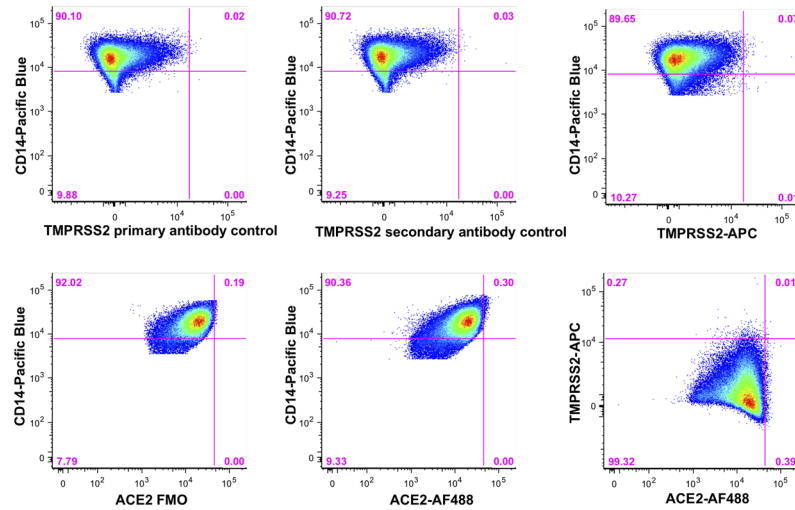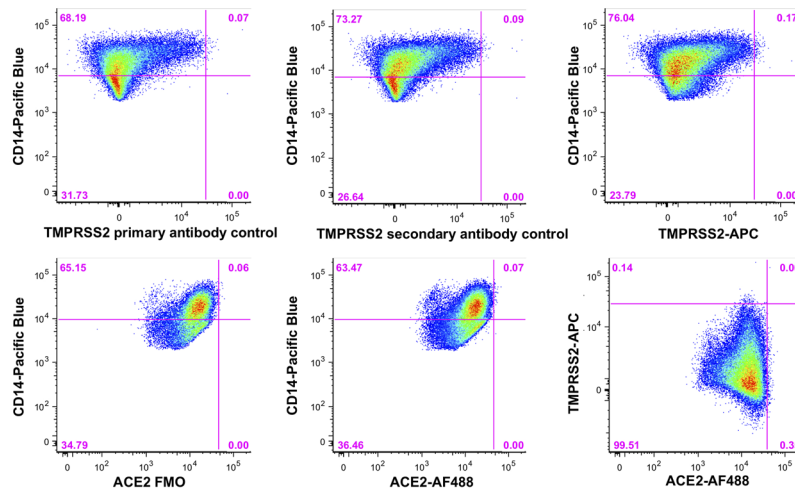
**FIGURE 2** Expression of ACE2 and TMPRSS2 by day 8 monocyte-derived macrophages (MDM) under unpolarized (A), M1 polarized (B), and M2 polarized (C) culture conditions. Panels are as described in Figure 1. Data are representative of 5 replicate experiments using cells from different donors
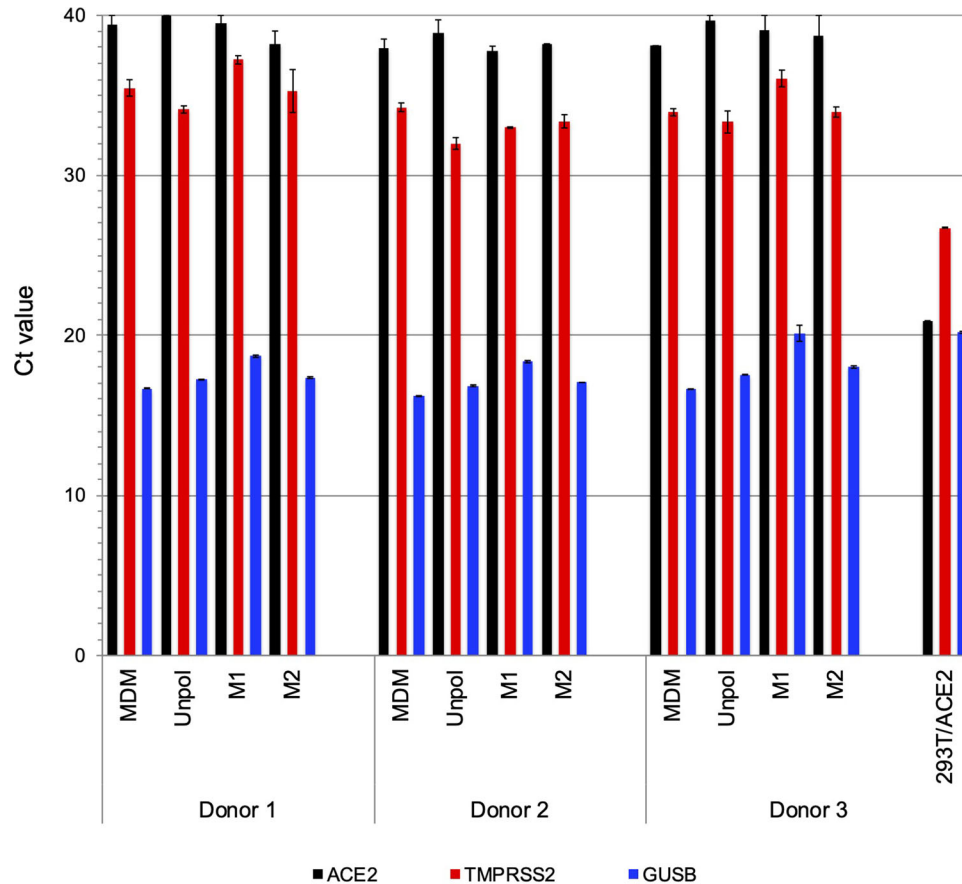
**FIGURE 3** RT-PCR detection of ACE2 and TMPRSS2 gene expression. Day 8 MDM were maintained under different culture conditions and subject to RT-qPCR for ACE2 and TMPRSS2, with GUSB serving as a housekeeping gene. 293T-ACE2 cells served as a positive cell control. No-RT controls were carried out in parallel for each sample and were all undetectable (>40 Ct). Data are means ± SEM of triplicate qPCR reactions, and each of 3 independent macrophage donors are shown
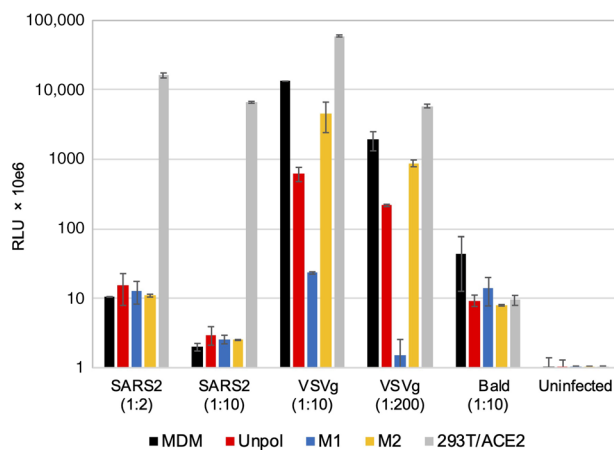


**FIGURE 4** Infection of day 8 MDM cultured under various conditions using pseudotype viruses expressing the SARS-CoV2 Spike protein, with VSVg as a positive control and no-glycoprotein (bald) virions as a negative control. Dilution of virus stock used is indicated. Uninfected cells were all ≤$1 \times 10^6$ but were set at $1 \times 10^6$ for clarity of visualization. Data are means ± SEM of triplicate infections using cells from different donors

Nevertheless, combined with the absence of ACE2 and TMPRSS2 coexpression, these findings indicate that macrophages are resistant to infection with SARS-CoV2 under all conditions tested.

## 4 | DISCUSSION

Abundant evidence implicates macrophage activation and dysregulation in the hyperinflammatory immune response seen in COVID-19,[7,16,17] which is believed to be a major contributor to pathogenesis of severe disease. An important question therefore is whether this reflects direct viral infection or indirect bystander effects. Our findings show that the major cellular components ACE2 and TMPRSS2 that enable SARS-CoV2 infection in human cells are sparsely expressed in primary human monocytes and MDMs in vitro and under several polarization conditions, and coexpression is essentially undetectable. Coexpression of these receptors is required for susceptibility of target cells to infection by SARS-CoV2, and consistent with this observation, macrophages were resistant to SARS-CoV-2 Spike-mediated pseudotype infection under multiple culture conditions.

In contrast to our findings in vitro, several studies have reported SARS-CoV-2 antigens or nucleic acids in myeloid cells from pathology or autopsy tissue of infected patients. Immunostaining of postmortem tissue from patients who died from COVID-19 revealed lymph node subcapsular and splenic marginal zone macrophages that expressed ACE2 and contained SARS-CoV2 nucleoprotein.[18] SARS-CoV-2 has been described in alveolar macrophages obtained from infected individuals by bronchoscopy.[19] In some cases, signals in vivo may result from macrophage phagocytosis of virions or infected cells actively replicating virus. Alternatively, it is possible that culture conditions in vitro do not fully mirror all in vivo conditions and body compartments. Levels of both ACE2 and TMPRSS2 expression in different tissues and organs of the body are regulated in various proinflammatory conditions such as obesity, diabetes, local, and autoimmune diseases.[20] While some reports found ACE2 expression to be absent from lung macrophages,[21] others identified ACE2 by single-cell sequencing in macrophages from heart and lung.[22] Thus, it is possible that macrophages from different organs may express different molecules under specific circumstances.

While we found little evidence for ACE2 and TMPRSS2 in MDMs, other entry mechanisms have been proposed. CD147 has been implicated as a pathway for SARS-CoV-2 entry,[23] but this is controversial.[24] Antibody-dependent enhancement can lead to macrophage infection by some viruses in the absence of receptor expression, although evidence for this in SARS-CoV-2 is currently lacking.[25] TMPRSS2 is the principal protease that cleaves the S2' Spike site required for fusion, but other proteases such as TMPRSS4 and TMPRSS11 may also function in that role.[26,27] Nevertheless, the lack of infection in our model argues against alternative entry pathways, and is consistent with findings reported by others using different macrophage cell models.[21,25] Finally, it has been reported that SARS-CoV-2 can cause abortive infection of macrophages.[28,29] However, our findings that cellular receptor expression is lacking and SARS-CoV-2 Spike fails to mediate pseudovirus entry would argue against entry and abortive infection in these cells.

In summary, we find no evidence of SARS-CoV-2 receptor expression or infection susceptibility of human MDMs. Better understanding of the mechanisms by which monocyte/macrophages contribute to disease pathogenesis will be important for the development of future therapeutic interventions.

## AUTHORSHIP
U. Z., A. Y., and Y. Y. performed experiments. U. Z. and R. G. C. conceptualized experiments and wrote the manuscript. B. H. H. provided reagents and guided experiments. All authors reviewed and approved the manuscript. U. Z. and A. Y. are co-first author and have contributed equally to this article.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.