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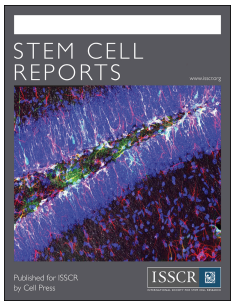

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Revision 2

Report

Human Mesenchymal Stromal Cells are resistant to SARS-CoV-2 Infection under Steady State, Inflammatory Conditions and in the Presence of SARS-CoV-2 infected Cells

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Key words: SARS-CoV-2, COVID-19, Mesenchymal Stromal Cells, Inflammation, ARDS

Summary

Previous studies reported safety and applicability of Mesenchymal Stem/Stromal Cells (MSC) to ameliorate pulmonary inflammation in ARDS. Thus, multiple clinical trials assessing the potential of MSC for COVID-19 treatment are underway. Yet, as SARS-inducing corona viruses infect stem/progenitor cells, it is unclear whether MSC could be infected by SARS-CoV-2 upon transplantation to COVID-19 patients. We found that MSC from bone marrow, amniotic fluid and adipose tissue carry angiotensin-converting enzyme 2 and transmembrane protease serine subtype 2 at low levels on the cell surface under steady state and inflammatory conditions. We did neither observe SARS-CoV-2 infection nor replication in MSC at steady state, under inflammatory conditions, or in direct contact with SARS-CoV-2 infected Caco-2 cells. Further, indoleamine 2,3-dioxygenase 1 production in MSC was not impaired in the presence of SARS-CoV-2. We show that MSC are resistant to SARS-CoV-2 infection and retain their immunomodulation potential supporting their potential applicability for COVID-19 treatment.

Introduction

The current Coronavirus disease 2019 (COVID-19) pandemic is posing substantial challenges to various medical disciplines, particularly to intensive care units where respiratory insufficient patients are being taken care of (Phua et al., 2020).

After SARS-CoV-2 transmission mainly via respiratory droplets, symptomatic patients show breathing difficulties where about 14% of the patients develop severe COVID-19, and 5% of the patients, mainly in the context of pre-existing conditions such as cardiovascular disease, diabetes, or chronic respiratory disease, are at higher risk for lethal courses of the disease featuring a case-fatality rate of 49%, when developing respiratory failure, septic shock, and/or multiple organ dysfunction (Tay et al., 2020; Wu and McGoogan, 2020). Specifically for the lung, the leading pathophysiology of COVID-19 is a severe acute respiratory distress syndrome (ARDS), and respiratory failure due to ARDS is the main cause (70%) of death (Tay et al., 2020).

Inside the airways SARS-CoV-2 targets epithelial cells and, further invasive, vascular endothelial cells and pulmonary macrophages (Tay et al., 2020). To date, two key factors for successful virus entry into the host cells have been identified, i.e. angiotensin-converting enzyme 2 (ACE2), being expressed on the surface of these cell types and acting as receptor for the S1 subunit of the viral spike protein, and the cellular serine protease TMPRSS2 for the necessary viral S protein priming (Tay et al., 2020; Hoffmann et al., 2020). The following active virus replication and its release damage the infected host cell by inducing pyroptosis which, in turn, triggers the release of pro-inflammatory cytokines and chemokines such as interleukin (IL)-6, IL-1β, interferon γ (IFNγ), IFNγ-induced protein 10 (IP-10), macrophage inflammatory protein 1α (MIP1α), MIP1β and MCP1 (Tay et al., 2020). Hereby attracted and activated monocytes, macrophages and T cells do not only maintain an inflammatory milieu but, additionally releasing IFNγ, create a pro-inflammatory feedback loop that further damages the lung tissue and induce capillary leakage (Tay et al., 2020). A sepsis-like perpetuation of the inflammatory response can create a generalized cytokine storm that eventually leads to multi-organ failure (Tay et al., 2020). The clinical relevance of the unbalanced inflammatory response is highlighted by the observation that the cytokine storm and sepsis symptoms are prominent (28%) causes for fatal COVID-19 cases (Tay et al., 2020). Thus, controlling the disruptive inflammatory responses may be considered as a substantial component for therapeutic strategies for COVID-19.

Mesenchymal Stem/Stromal Cells (MSC) are potent immune regulators secreting immunomodulatory factors and interacting with a variety of immune cell types such as T cells, B cells, dendritic cells and macrophages (Fontaine et al., 2016). To date, the major sources for manufacture of MSC therapeutics are bone marrow (BM) and adipose tissue (Schäfer et al., 2016). Clinical applications of MSC proved efficacy for immunopathologies such as Graft-versus-Host Disease (GvHD), organ graft rejection, as well as for autoimmune diseases (Schäfer, 2019). Previous preclinical and clinical studies showed the safety and applicability of MSC therapies to ameliorate the pulmonary inflammation in the context of ARDS. In addition to their immunomodulation potential, currently discussed mechanisms how MSC exert their beneficial effects in ARDS pathology include preservation of the epithelial and endothelial barrier, reduced impairment of alveolar fluid clearance, as well as possible antimicrobial activity (Walter et al., 2014; Chan et al., 2016).

Specifically, intravenously applied BM-MSC reduced static lung elastance, interstitial edema, and collagen fiber content in a murine ARDS model (Silva et al., 2019), and human umbilical cord-derived MSC reduced the mortality in a rat ARDS model (Lee et al., 2017). Moreover, intravenous single-dose infusions of allogeneic, BM-derived human MSC were well tolerated in patients with moderate to severe ARDS, as recently assessed in a multi-center study (Wilson et al., 2015; Matthay et al., 2019). Thus, multiple clinical trials evaluating the potential of MSC for COVID-19 treatment are currently underway.

Yet, SARS-CoV were shown to infect and replicate in ACE2 expressing pulmonary progenitor cells, eventually killing them (Ling et al., 2006), and it is unclear if MSC could be infected by SARS-CoV-2 upon transplantation to COVID-19 patients. Recently, we established an *in vitro* model for infection with SARS-CoV-2 from clinical isolates (Bojkova et al., 2020). In this model we now tested the potential of SARS-CoV-2 to infect human MSC from different sources and to replicate within these cells.

Results

First, we confirmed the typical surface characteristics of the MSC from the three sources BM, AF and adipose tissue. BM-MSC, AF-MSC and ASC showed positive marker expressions such as CD73, CD90, and CD105, and lacked CD45 on the cell surface (**Figure 1A**). As expected, under steady state the HLA-DR expression on BM-MSC and AF-MSC was very low, but was strongly increased upon exposure to inflammatory condition such validating the *in vitro* inflammation model for the MSC (**Figure 1B**).

Next, we investigated the presence of both to date identified key entry factors for SARS-CoV-2, i.e. angiotensin-converting enzyme 2 (ACE2) and TMPRSS2, on the MSC surface. As *ACE2* is an interferon-stimulated gene (Ziegler et al., 2020), and to model the inflammatory environment in COVID-19 we tested the ACE2 and TMPRSS2 expressions also under inflammatory conditions in our MSC-PBMNC co-culture system. In contrast to KG1a control cells both ACE2 and TMPRSS2 proteins could be detected at very low levels on BM-MSC, AF-MSC and ASC with similar percentages and mean fluorescence intensities (**Figure 2**). Exposure to inflammatory condition did not increase the low expression of ACE2 or TMPRSS2 on the surface of BM-MSC and AF-MSC (**Figure 2**).

Next, we tested the potential of SARS-CoV-2 to infect MSC under different conditions. Control Caco-2 cells showed high SARS-CoV-2 infection rates with massive intracellular S-Protein accumulation indicating virus entry, and analysis by qPCR confirmed SARS-CoV-2 replication in Caco-2 cells (**Figure 3 A-C**). In contrast, neither BM-MSC nor ASC could be infected with SARS-CoV-2 (**Figure 3 C, D**). Moreover, BM-MSC remained resistant to SARS-CoV-2 infection under inflammatory condition and even at close contact to highly infected Caco-2 cells (**Figure 3 E, F**).

We further investigated if, even when not being infected, the presence of SARS-CoV-2 would affect the IDO-1 production in MSC which is the main surrogate factor for MSC´s immunomodulation capacities. The production of IDO-1 is triggered by pro-inflammatory cytokines such as TNF-α, IL-1β and IFN-γ1b. Indeed, we did not detect IDO-1 protein in BM-MSC lysates under steady state or in the presence of SARS-CoV-2 without cytokines. In contrast and as expected, the BM-MSC produced IDO-1 after cytokine exposure. Notably, we did not observe an impairment of MSC´s IDO-1 production after cytokine exposure in the presence of SARS-CoV-2 (**Figure 4**).

Discussion

Severe courses of COVID-19 are characterized by ARDS together with cytokine storm-mediated multi-organ failure, and to date vaccinations are still under development. Consequently, therapeutic approaches include the evaluation of immunosuppressive drugs. Recently released intermediate data from the RECOVERY trial (NCT04381936) showed a reduced mortality of about 20% in patients on ventilators who received dexamethasone (https://clinicaltrials.gov/ct2/show/NCT04381936 2020).

In line with this, MSC, being proven as clinically potent immunomodulating cell therapeutics as shown for GvHD (Voermans and Hazenberg, 2020), are currently evaluated for their applicability to treat COVID-19 with to date more than 40 registered clinical MSC trials at ClinicalTrials.gov. Moreover, first clinical data suggested that MSC applications to patients suffering from non-COVID ARDS were safe (Matthay et al., 2019). Yet, SARS-inducing corona viruses were shown to infect progenitor cells (Ling et al., 2006) and thus, it is reasonable to investigate the possibility of MSC SARS-CoV-2 infection and its potential implications. On the one hand, SARS-CoV-2 infection of MSC could lead to rapid clearance of the transplanted MSC thus hampering their therapeutic efficacy. On the other hand, SARS-CoV-2 could induce apoptosis of MSC that could further exert desired immunomodulation effects like induction of IDO-1 production as shown previously for GvHD (Galleu et al., 2017). In addition, SARS-CoV-2 infected MSC might provoke unknown, and thus uncontrollable, side effects such as aggravation of COVID-19 symptoms, complications, or transformation (Cason et al., 2017; Oberstein and Shenk, 2017).

To date the only available data addressing the question whether or not human MSC would carry ACE2 and/or TMPRSS2 was reported in a recent study on 7 patients who received allogeneic MSC of undisclosed source for the treatment of COVID-19. Here, RNAseq analysis showed low expression of both *ACE2* and *TMPRSS2* gene transcripts (Leng et al., 2020). In a hallmark paper Ziegler *et al.* (Ziegler et al., 2020) screened multiple human and non-human tissues with single-cell RNAseq and found *ACE2* and *TMPRSS2* genes co-expressing type II pneumocytes, ileal absorptive enterocytes, and nasal goblet secretory cells, but this dataset did not include human BM, AF, adipose tissue, or *ex vivo* cultured MSC, a prerequisite for their clinical use. Moreover, substantial discrepancies of up to 40% between transcript and protein are common due to post-transcriptional regulation or protein degradation (Schwanhausser et al., 2011; Kuci et al., 2019).

Therefore, we analyzed the expression of both key entry factors on the surface of human MSC from sources that have been mainly used for clinical MSC therapeutic manufacture, *i.e.* BM-MSC and ASC (Schäfer et al., 2016), as well as MSC isolated from AF, another promising source (Moraghebi et al., 2017). Confirming the above mentioned transcript data on protein level we did not detect noteworthy numbers of ACE2+ or TMPRSS2+ cells within the BM-MSC and AF-MSC preparations, and only very few (<10%) ACE2+ or TMPRSS2+ cells amongst the ASC. Under inflammatory conditions we observed the expected up-regulation of HLA-DR on the MSC surface, but we did not detect changes of ACE2 or TMPRSS2 expressions. This is of relevance, as *ACE2* gene expression is up-regulated by interferon (IFN)-α2 and IFN-γ, yet, as it appears to date, in a cell-type-specific manner, *i.e.* mainly affecting epithelial cells (Ziegler et al., 2020). However, pathways independent of IFNs that regulate *ACE2* or *TMPRSS2* expression may exist, and it is not clear if the cell populations with IFN-dependent *ACE2* regulation are actually sensitive for SARS-CoV-2 infection (Su and Jiang, 2020), or if ACE2 and TMPRSS2 are the exclusive ports of entry for non-epithelial cells. The roles of cytokines and IFNs in COVID-19 pathogenesis and for the development of therapeutic options are still under debate (Chu et al., 2020; Su and Jiang, 2020), whereas it is clear that the exposure to (pulmonary) inflammatory conditions impacts substantially the gene expression of MSC besides the above mentioned change of HLA-DR expression (Andrzejewska et al., 2019; Abreu et al., 2019). Therefore, we used our allogeneic co-culture system to model the inflammatory environment for the MSC, *i.e.* the MSC share the media with pooled allogeneic PBMNC that are additionally stimulated with PHA, thus exposing the MSC to the complete repertoire of the cytokines and IFNs being secreted by the PBMNC.

We used our *in vitro* SARS-CoV-2 infection model (Bojkova et al., 2020) to evaluate the potential of SARS-CoV-2 to infect human BM-MSC and ASC. Specifically, we tested three conditions: a. steady state, *i.e.* without additional stimuli, b. inflammatory condition as described above, and c. co-culture with infected Caco-2 cells. The BM-MSC and ASC could not be infected with SARS-CoV-2 in steady state, and the further tested BM-MSC remained resistant under inflammatory conditions as well as in co-culture with SARS-CoV-2 infected Caco-2 cells. We aimed to model the local inflammatory environment and the generalized cytokine storm in COVID-19. Moreover, we analyzed the IDO-1 production of MSC which is an accepted surrogate for their immunomodulation potential (Galleu et al., 2017). Thus, the observed resistance of MSC to SARS-CoV-2 infection in condition b. as well as their unimpaired IDO-1 production in the presence of SARS-CoV-2 and cytokines suggest that

clinically applied MSC would deliver their expected immunomodulation function in COVID-19 as they did in various preclinical ARDS models (Johnson et al., 2017).

We observed in steady state as well as under inflammatory condition very low expression of the current known entry molecules ACE2 and TMPRSS2 on the MSC surface, as well as resistance of MSC to SARS-CoV-2 infection. Both observations do likely confirm ACE2 and TMPRSS2 as relevant factors for SARS-CoV-2 infection not only for epithelial cells, but also for mesenchymal cells. However, we cannot exclude alternative routes of SARS-CoV-2 entry particularly for non-epithelial cells.

It is reasonable to hypothesize that the transplanted MSC may interact with at least some of the various cell types in the lung tissue from the endothelial side via the stroma to the epithelial side. The results obtained from the MSC-Caco-2 co-culture system in the presence of SARS-CoV-2 (condition c.) showed that even the direct contact to infected Caco-2 cells with massive virus replication did not lead to MSC infection with SARS-CoV-2. This sheds an interesting light on possible interactions of infected cells with MSC, yet more detailed investigations on possible interactions of MSC with pulmonary cells are warranted.

After systemic administration MSC were shown to accumulate in the lung and such could be augmented by pulmonary inflammation with local deposition of factors acting as chemo-attractants for MSC (Krueger et al., 2018). This might lead to side effects such as pulmonary artery thrombosis, but reports of hemodynamically relevant impact of MSC entrapment in the lung are inconsistent (Nystedt et al., 2013). Moreover, MSC were reported to express tissue factor and such they were reported to have pro-coagulant activity (Christy et al., 2017), an issue that needs further investigations particularly in the context of the relevant involvement of the coagulation in COVID-19 pathogenesis (Jose and Manuel, 2020).

Altogether, we show that clinically relevant MSC entities are resistant to SARS-CoV-2 infection under conditions being pathophysiological relevant for COVID-19. Moreover, our data suggest that MSC keep their immunomodulation potential in the presence of SARS-CoV-2, thus supporting their ongoing clinical evaluation for the treatment of COVID-19. However, further investigations are warranted to better understand the possible impact of MSC interactions with different cell types and their systemic effects including possible pro-coagulant activity in presence of SARS-CoV-2 or in COVID-19 respectively.

Experimental Procedures

MSC isolation and cultivation

Human BM samples were obtained from three healthy donors undergoing bone marrow donation after informed consent and ethical committee approval. Trapped cells were removed from the three-way filter-system used to process the BM aspirate by retrograde flushing with PBS (Thermo Fisher Scientific, Waltham, MA, USA), and BM mononuclear cells (MNC) were isolated by density gradient centrifugation on lymphocyte separation medium (Lonza, Basel, Switzerland). The BM-MSC isolation was performed as described previously (Siegel et al., 2013). Briefly, the MNC were re-suspended in standard cell culture medium, composed of Alpha MEM (Lonza), 10% human platelet lysate (hPL; manufactured in-house), 2 IU/mL Heparin (Ratiopharm, Ulm, Germany), and 1% Penicillin-Streptomycin (Thermo Fisher Scientific) and seeded into cell culture flasks at a density of $1.0x10^5$ cells/cm^2. Non-adherent cells were washed away after 24 hours and the persistent cells were maintained in cell culture medium at 37°C in humidified atmosphere with 5% CO_2. When MSC reached subconfluency, they were detached using 0.05% Trypsin-EDTA (Thermo Fisher Scientific) and either cryopreserved or seeded at a density of 1,000 cells/cm^2.

Human adipose-derived adult Mesenchymal Stromal Cells (ASC) were isolated from lipoaspirates from three donors undergoing cosmetic liposuction in accordance to the local ethical committee and isolated as described previously (Baer et al., 2013). Briefly, Dulbecco's modified Eagle's medium (DMEM; Sigma, Taufkirchen, Germany) was used with a physiologic glucose concentration (100 mg/dL) supplemented with 10% fetal bovine serum (FBS; PAA, Cölbe, Germany) as the culture medium. The medium was replaced every three days. Subconfluent cells (85-90% confluency) were passaged by trypsinization.

For the isolation of AF-MSC amniotic fluid was collected by amniocentesis from three polyhydramnios after informed consent and ethical committee approval. The AF-MSC isolation was performed with modifications as described previously (De et al., 2007). Briefly, after centrifugation of the amniotic fluid the cells were re-suspended in standard cell culture medium, composed of Alpha MEM (Lonza), 15% ES-FBS (Thermo Fisher Scientific), 18% Chang B medium (Irvine Scientific, Santa Ana, CA, USA), 2% Chang C medium supplement (Irvine Scientific), 1% Glutamine (Thermo Fisher Scientific), and 1% Penicillin-Streptomycin (Thermo Fisher Scientific) and seeded into cell culture flasks at a density of $1.6\text{-}3.6x10^5$

cells/cm^2. Non-adherent cells were washed away after 24 hours and the persistent cells were maintained in cell culture medium at 37°C in humidified atmosphere with 5% CO_2. When AF-MSC reached subconfluency, they were detached using 0.05% Trypsin-EDTA (Thermo Fisher Scientific) and either cryopreserved or seeded at a density of 1,000 cells/cm^2.

For preparation of infection experiments, cryopreserved MSC passage (P) 0 were thawed and seeded at a density of 2,000 cells/cm^2 in modified cell culture medium without Heparin and replacement of hPL by FBS (Sigma-Aldrich, St. Louis, MO, USA).

SARS-CoV-2 infection and immunostaining

The Caco-2 cell line was obtained from DSMZ (Braunschweig, Germany). The cells were grown at 37°C in minimal essential medium (MEM) supplemented with 10% FBS, 100 IU/mL penicillin, and 100 µg/mL of streptomycin. All culture reagents were purchased from Sigma (Munich, Germany). Cells were authenticated by short tandem repeat (STR) analysis and tested for mycoplasma contamination. The isolate SARS-CoV-2/1/Human/2020/Frankfurt (Hoehl et al., 2020) was cultivated in Caco-2 cells as previously described for SARS-CoV strain FFM-1 523 (Cinatl, Jr. et al., 2004). Virus titers were determined as TCID50/mL in confluent cells in 96-well microtiter plates (Cinatl et al., 2003; Cinatl, Jr. et al., 2004). MSC were incubated with virus at MOI 1 for 2h. After this period, cells were washed, supplemented with fresh medium and cultured for five days. Immunostaining of SARS-CoV-2 was performed as previously described (Bojkova et al., 2020). Cells were fixed with acetone/methanol (40:60) solution and incubated with primary antibody anti-spike (1:1500, Sino Biological, catalogue number 40150-R007, Singapore) which was detected with a peroxidase conjugated anti-rabbit secondary antibody (1:1000, Dianova, catalogue number SKU:111-035-045), followed by addition of AEC substrate. All work with infectious viruses was performed in a biosafety level 3 facility.

Quantification of SARS-CoV-2 RNA

RNA from cell culture supernatant was isolated with the QIAamp Viral RNA Kit (Qiagen) according to the manufacturer's instructions. SARS-CoV-2 RNA was detected by OneStep qRT-PCR analysis using the Luna Universal One-Step RT-qPCR Kit (New England Biolabs) and primers directed for RNA-dependent RNA polymerase (RdRp): SARSr-F2 (GTG ARA

TGG TCA TGT GTG GCG G) and RdRP_SARSr-R1 (CAR ATG TTA AAS ACA CTA TTA GCA TA). Standard curves were created using plasmid DNA (pEX-A128-RdRP) harboring the corresponding amplicon regions for RdRP target sequence according to GenBank Accession number NC_045512. The amount of RNA was calculated as RNA copies/mL.

Flow cytometry

MSC were analyzed at P1 by flow cytometry (LSRFortessa™; Becton-Dickinson, Heidelberg, Germany) with regard to the ISCT MSC criteria. Cells were stained with 7-AAD viability dye (BioLegend, San Diego, CA, USA) and antibodies against CD45 (catalogue number 560777), CD73 (catalogue number 563199), CD90 (catalogue number 561558), CD105 (catalogue number 562380), HLA-ABC (catalogue number 555555), or HLA-DR (catalogue number 562804) (all from BD Biosciences unless otherwise noted).

ASC (P1) were analyzed by flow cytometry under steady state, BM-MSC (P2) and AF-MSC (P4) under steady state and inflammatory condition for expression of ACE2 and TMPRSS2, respectively. Cells were stained with 7-AAD viability dye and antibodies against ACE2 (catalogue number sc-390851) and TMPRSS2 (catalogue number sc-515727) (Santa Cruz Biotechnology, Dallas, TX, USA), IgG1 kappa light chain (catalogue number 555749, BD Biosciences) served as isotype control, and the cell line KG1a as positive control.

Flow cytometry data were analyzed using FCS Express 6 Flow Software (De Novo Software, Pasadena, CA, USA).

Inflammatory condition

BM-MSC were seeded at a density of 96,000 cells per well into 6-well plates and allowed to adhere overnight. For indirect co-culturing permeable cell culture inserts with 0.4µm pores (Greiner Bio-One, Frickenhausen, Germany) were placed into the 6-well plates. Peripheral blood mononuclear cells (PBMNC; derived from buffy coats from 5 healthy donors, pooled and cryopreserved), were seeded at a density of $1x10^6$ cells per insert, stimulated with 10 µg/mL phytohemagglutinin (PHA; Sigma-Aldrich) and co-cultured for 72 h. Effective inflammation was monitored flow cytometric by upregulation of HLA-DR.

Quantification of indoleamine 2,3-dioxygenase 1 production

Production of IDO-1 was stimulated in BM-MSC with or without exposure to SARS-CoV-2 by TNF-α, IFN-γ1b (Miltenyi Biotec, Bergisch Gladbach, Germany) and IL-1β (PeproTech, Hamburg, Germany) each 20 ng/mL for 48h. BM-MSC exposed to SARS-CoV-2 without cytokine stimulation were analyzed as well. MSC grown in media containing 10% FBS were stimulated as well and served as positive controls.

Subsequently, the MSC were harvested, lysed and analyzed in triplicates with an ELISA specific for IDO-1 (Cloud-Clone Corp., Katy, TX, USA).

Statistical analyses

Quantitative data are presented as means ± standard error of the means (SEM). For comparison of selected data sets the two-tailed t-test was employed (Figure 2 and 4) using GraphPad Prism (La Jolla, CA, USA). Exact *P* values are reported in the Figures, and *P* values of <0.05 were considered as statistically significant.

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Contributors

R.S. and J.C. designed the research, analyzed the data and wrote the manuscript. G.S., M.B., and D.B. performed the experiments, analyzed the data and reviewed the manuscript. P.C.B. and S.K. provided human ASC, anti-ACE2 and anti-TMPRSS2 antibodies and reviewed the manuscript. E.S. and S.C. discussed the results and reviewed the manuscript.

Declaration of interests

The authors declare no competing interests.

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Figure Titles and Legends

Figure 1 Human MSC characterization.

Human MSC characterization by flow cytometry under steady state (**A**) and inflammatory condition (**B**). Histograms show fluorescence of specific antibodies (red lines) compared to unstained cells (black lines).

Figure 2 ACE2 and TMPRSS2 expression on human MSC.

ACE2 (**A, B**) and TMPRSS2 (**C, D**) expression assessed by flow cytometry. On the controls (KG1a cells) ACE2 and TMPRSS2 was highly expressed, whereas very low ACE2 and TMPRSS2 expressions were detected on the surface of MSC; Two-tailed paired t test; data presented as means of 3 donors (BM-MSC, AF-MSC), or 1 donor/cell line (ASC, KG1a) respectively; error bars: SEM; dot plots show viable cells with gate set according to isotype control for the respective condition.

Figure 3 Evaluation of SARS-CoV-2 infection of MSC.

Evaluation of SARS-CoV-2 infection of MSC under steady state and inflammatory condition and in the presence of SARS-CoV-2 infected Caco-2 cells. SARS-CoV-2 infection is identified by SARS-CoV-2 S-Protein staining (red).

All MSC and Caco-2 cells experiments were repeated in three independent settings from three BM-MSC donors and three ASC donors, and were performed in three biological replicates each. One representative picture is shown for each condition. A: Caco-2 cells without SARS-CoV-2; B: Caco-2 cells with SARS-CoV-2 MOI1; C: SARS-CoV-2 replication quantified by qPCR detecting high copy numbers in Caco-2 cells infected by SARS-CoV-2; D: BM-MSC steady state with SARS-CoV-2 MOI1; E: ASC steady state with SARS-CoV-2 MOI1; F: BM-MSC inflammatory condition with SARS-CoV-2 MOI1; G: Co-culture BM-MSC:Caco-2 cells (10:1) with SARS-CoV-2 MOI1; BM-MSC (black star) + Caco-2 cells (white star); Scale bars: 100 µm except for the magnified section in G (20 µm).

Figure 4 Evaluation of IDO-1 production by MSC.

BM-MSC lysates analyzed by IDO-1 ELISA. Two-tailed paired t test; N = 3 biological replicates (3 individual BM-MSC donors); data are presented as means of 3 technical replicates per donor; error bars: SEM; n.d. = not detectable.

Figure 1

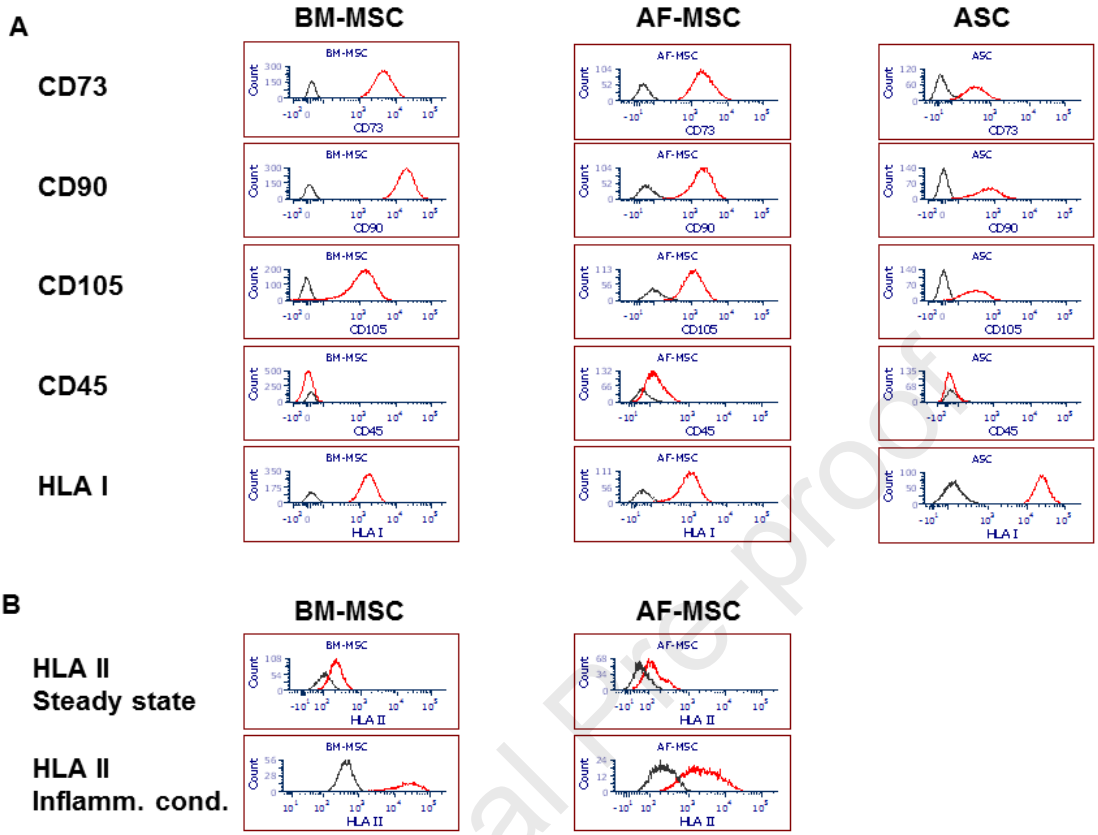


Figure 2

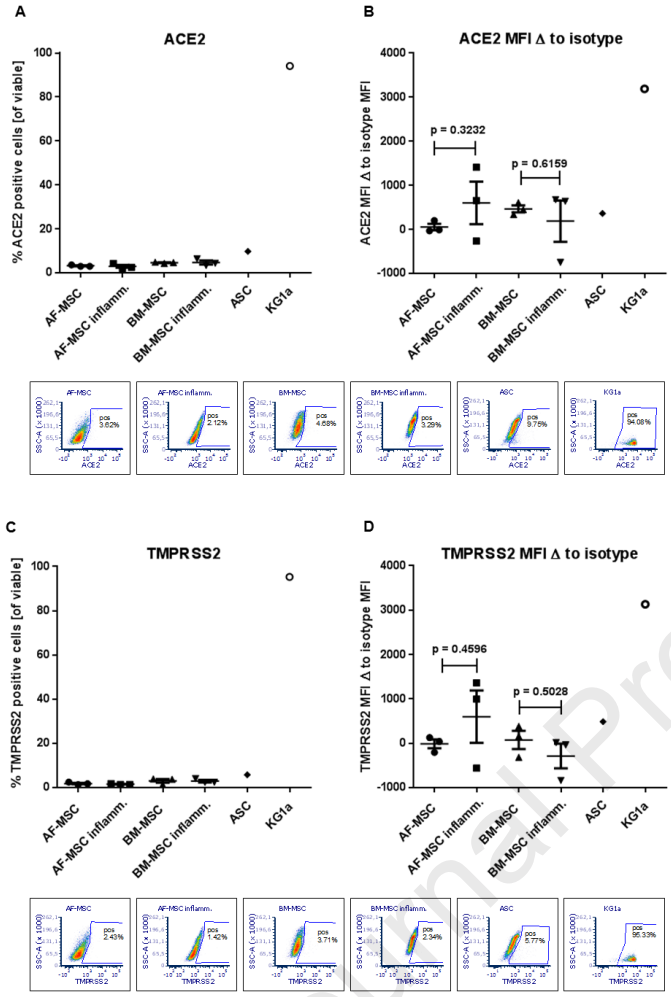


Figure 3

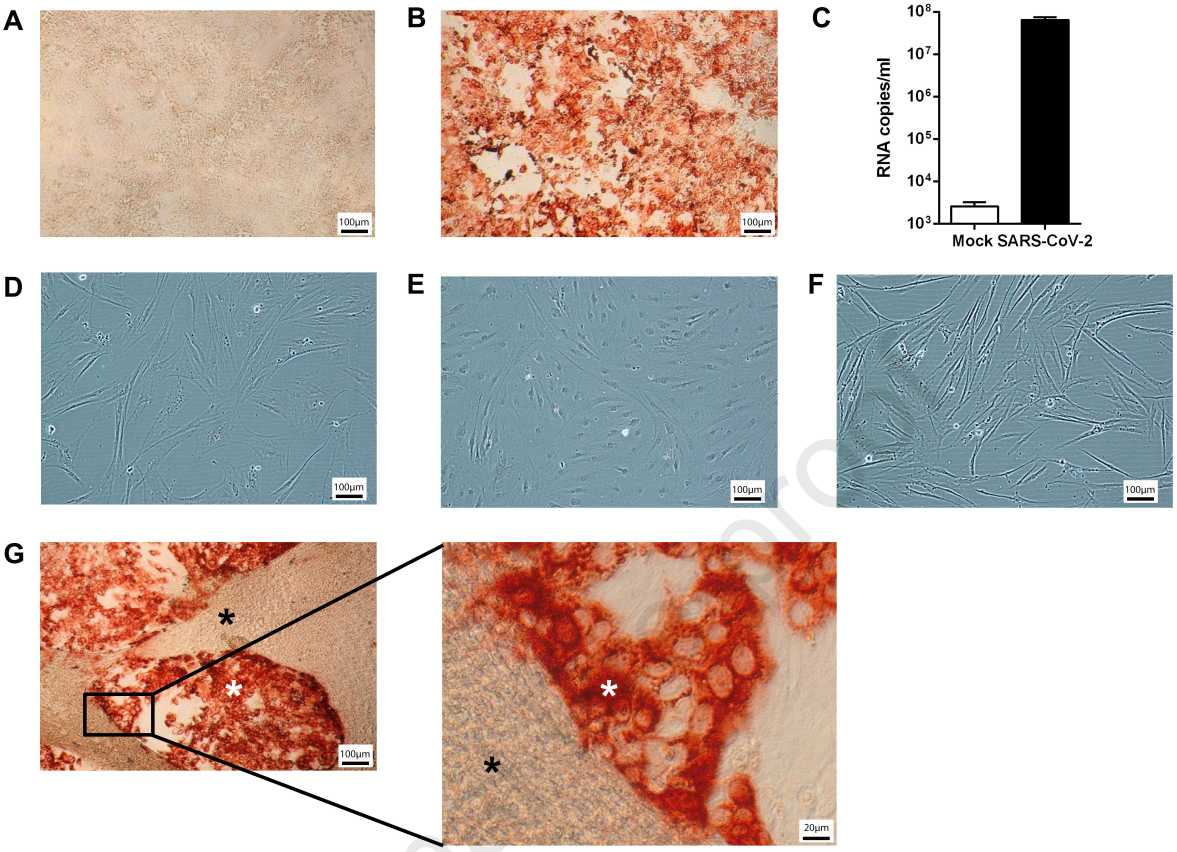
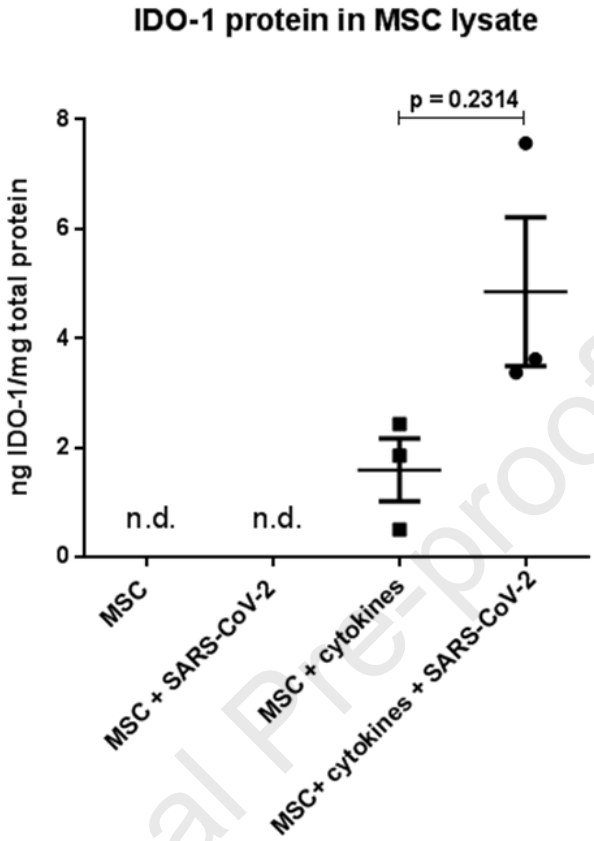


Figure 4



Highlights

- MSC carry ACE2 and TMPRSS2 only at very low levels on the cell surface
- Inflammatory conditions do not change ACE2 and TMPRSS2 expression on MSC
- MSC are resistant to SARS-CoV-2 infection
- MSC retain their immunomodulation potential in the presence of SARS-CoV-2

eTOC Blurb

Schäfer and colleagues found that human MSC carry ACE 2 and TMPRSS2 at very low levels on the cell surface under steady state and inflammatory conditions. Using their in vitro model for infection with SARS-CoV-2 from clinical isolates they show that MSC are resistant to SARS-CoV-2 infection and retain their immunomodulation potential supporting their potential applicability for COVID-19 treatment.