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Journal Title: PLoS ONE
Volume: Volume 11, Number 10
Publisher: Public Library of Science | 2016-10-03, Pages e0163709-e0163709
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1371/journal.pone.0163709
Permanent URL: https://pid.emory.edu/ark:/25593/rs2d6

Final published version: http://dx.doi.org/10.1371/journal.pone.0163709

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Accessed October 7, 2023 2:47 PM EDT
Respiratory Syncytial Virus-Infected Mesenchymal Stem Cells Regulate Immunity via Interferon Beta and Indoleamine-2,3-Dioxygenase

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Abstract

Respiratory syncytial virus (RSV) has been reported to infect human mesenchymal stem cells (MSCs) but the consequences are poorly understood. MSCs are present in nearly every organ including the nasal mucosa and the lung and play a role in regulating immune responses and mediating tissue repair. We sought to determine whether RSV infection of MSCs enhances their immune regulatory functions and contributes to RSV-associated lung disease. RSV was shown to replicate in human MSCs by fluorescence microscopy, plaque assay, and expression of RSV transcripts. RSV-infected MSCs showed differentially altered expression of cytokines and chemokines such as IL-1β, IL-6, IL-8 and SDF-1 compared to epithelial cells. Notably, RSV-infected MSCs exhibited significantly increased expression of IFN-β (~100-fold) and indoleamine-2,3-dioxygenase (IDO) (~70-fold) than in mock-infected MSCs. IDO was identified in cytosolic protein of infected cells by Western blots and enzymatic activity was detected by tryptophan catabolism assay. Treatment of PBMCs with culture supernatants from RSV-infected MSCs reduced their proliferation in a dose dependent manner. This effect on PBMC activation was reversed by treatment of MSCs with the IDO inhibitors 1-methyltryptophan and vitamin K3 during RSV infection, a result we confirmed by CRISPR/Cas9-mediated knockout of IDO in MSCs. Neutralizing IFN-β prevented IDO expression and activity. Treatment of MSCs with an endosomal TLR inhibitor, as well as a specific inhibitor of the TLR3/dsRNA complex, prevented IFN-β and IDO expression. Together, these results suggest that RSV infection of MSCs alters their immune regulatory function by upregulating IFN-β and IDO, affecting immune cell proliferation, which may account for the lack of protective RSV immunity and for chronicity of RSV-associated lung diseases such as asthma and COPD.
Introduction

Respiratory syncytial virus (RSV) is the most common cause of respiratory tract infection in infants and young children as well as a frequent cause of pneumonitis and death in elderly and immunocompromised adults. According to the CDC, RSV accounts for between 100,000 to 126,000 hospitalizations annually in children under one year old and each year, on average, 177,000 hospitalizations and 14,000 deaths are attributed to RSV infections in US adults over the age of 65 [1]. An enveloped single stranded RNA virus of the genus *Pneumovirus*, RSV has been associated with airway remodeling, immune system modulation and more severe airway hyper-reactivity in asthma [2–9]. Virtually all infants have been infected with RSV by the age of 2, however prior exposure does not produce protective immunity and recurrent infections even within the same RSV season are possible [10]. The molecular and immunologic basis for the lack of protective immunity after primary infections with RSV remains unclear.

The original formalin-inactivated vaccine developed in the 1960s lead to enhanced inflammatory disease and death of infants [11, 12] and despite the decades of study since there is still no FDA approved vaccine for RSV [13, 14]. A better understanding of the mechanisms of RSV immune evasion may facilitate the development of a safe and effective vaccine. Prophylaxis with Palivizumab, a monoclonal antibody against the viral fusion protein, has been used in high-risk infants since the late 1990s and is associated with reduced hospitalization due to lower respiratory tract infections, however no reduction in infant mortality or infection rate has been shown and the utility of the drug is limited due to exorbitant cost, $21.10 per mg at a recommended dose of 15 mg/kg, or approximately $1,435 per dose for a 10 lb infant excluding associated healthcare costs such as office visit fees for a pediatric pulmonary specialist [15, 16]. These injections only provide passive immunity and are required monthly throughout the RSV season, typically 5 months in most regions of the US not including Florida [8], and are often required for the first two years of life.

Until recently RSV infections were assumed to be confined to the apical airway epithelial cells, however, in vitro and animal models have shown that RSV can infect beyond the apical layer of airway epithelial cells through physical damage to the epithelium as well as epithelial cell denuding and sloughing due to the infection [17, 18]. Further, recent reports of extrapulmonary manifestations of RSV in humans have revealed that the virus is capable of infecting various immune cells of blood and bone marrow. Specifically, replicating RSV and RSV transcripts have been identified in blood neutrophils, dendritic cells, as well as human bone mesenchymal stem cells, also known as multipotent mesenchymal stromal cells (MSCs) [19–24]. Infectivity of MSCs is of particular interest since they can be found throughout the body in many tissues and are involved in immune regulation and tissue regeneration [25]. MSCs are known to mobilize to sites of injury for tissue repair [26–28] and have been identified as a major cell type responsible for regulating immune responses via a number of factors including indoleamine-2,3-dioxygenase. MSCs are found in nearly every vascularized tissue of the body including areas known to come into contact with RSV such as the lung and upper respiratory tract [29–32]. Also, the detection of RSV in marrow-derived MSCs suggests that the bone marrow may provide RSV with an immune-privileged site to evade or influence the host response and a staging area for potential subsequent RSV infections and chronic inflammatory disorders.

The increased prevalence of RSV infection in transplant patients and growing interest in utilizing MSC infusions for therapeutic purposes, including solid organ transplantation, nerve cell and tissue regeneration, as well as in control of autoimmune disorders [33–36], warrants a better understanding of the role of RSV infected MSCs in inflammation and immunity. Our initial studies revealed that RSV readily infects human MSCs; 1 MOI of virus led to nearly complete
infection (greater than 90%) of MSC cultures compared to approximately only 40% of normal human bronchial epithelial cell cultures. This led us to hypothesize that RSV infection of resident MSCs as well as those mobilized by inflammation in the lung and respiratory tract [37, 38] may play a role in increasing the spread of RSV in the lung while limiting the robustness of the innate and adaptive immune responses. To test this, we undertook a comprehensive analysis of virus replication, gene transcription and protein expression in MSCs and examined the RSV-induced expression of cytokines, viral response factors such as type I and II interferon, and immune regulatory factors, such as iNOS and IDO. We then examined whether RSV-infected MSCs affect the proliferative capability of lymphocytes. The results show that RSV-infected MSCs exhibit increased expression of immune regulatory factors and may play a role in mediating viral pathogenesis via immune tolerance.

Materials and Methods

Cell lines

Two lots of human umbilical cord blood MSCs (UCB MSC, Vitro Biopharma, Golden, CO, USA) and two separate lines of human bone marrow-derived MSCs (BM MSC, Institute for Regenerative Medicine at Scott & White Texas A&M Health Science Center College of Medicine, Temple, TX, USA) were tested. MSCs were cultured in complete MSC media: alpha-MEM with 1X Glutamax (Life Technologies, Carlsbad, CA, USA), 16.5% fetal bovine serum (FBS), 100 units/ml penicillin and 100 μg/ml streptomycin (Life Technologies). MSCs were passaged no more than 10 times before use in any experiment. For consistency, excepting Fig 1, all results shown were obtained from one of the UCB MSC lines. All four MSC lines showed similar results in triplicate experiments. Normal human bronchial epithelial (NHBE) cells (Lonza, Allendale, NJ, USA) were grown in BEGM (Lonza) and used prior to passage 5 in all experiments.

Respiratory syncytial virus strain and PFU assay

The strain of RSV used in all except the lentiviral experiments was a recombinant A2 strain expressing a red fluorescent marker, mKate2, as well as the F protein from the clinical strain Line 19 (rA2-KL19F) [39, 40]. For visualization of viral infection in lentiviral-transduced cells a recombinant green fluorescent protein-expressing RSV was used (generously provided by Dr. Mark Peeples, Nationwide Children's Hospital, Columbus, OH) [41]. Viral stocks were prepared by propagating the virus in HEp-2 cells (ATCC, Manassas, VA, USA) and purification by ultracentrifugation in a 30% glycerol, 0.1M MgSO₄ and 50mM HEPES solution for 3 hours at 24,000×g. Viral pellet was washed and resuspended in precooled buffer: 0.1M MgSO₄, 150mM NaCl and 50mM HEPES (Sigma-Aldrich, St. Louis, MO, USA). This buffer was used as mock infection media in all experiments. Viral stocks were stored in small aliquots at -80°C and never thawed more than once.

The number of infective units was determined by PFU assays performed in the HEp-2 cell line. Cells were grown to confluence in 24-well plates, washed and incubated for 90 minutes with serial dilutions of purified virus stock in Opti-MEM with 2% FBS. Cells were washed and covered in DMEM media containing 5% FBS and 0.8% methylcellulose. After 5 days cells were fixed in 4% PFA and plaques were visualized and counted with a fluorescent microscope. Results were the average of 4 wells.

RSV infection of human MSCs

To demonstrate the infection of MSCs with RSV: passage 3 cells were plated at 3 x 10⁵ cells per well in a 6 well plate with a sterilized 25mm round coverslip in the well. After 24 hours cells
were infected with $10^6$ PFU of RSV (1 MOI) in Opti-MEM with 2% FBS or mock infection media for 90 minutes at 37°C. Infection media was then aspirated and cells were washed once before incubation with complete MSC media for 48 or 72 hours. Media was aspirated, cells were washed with PBS and the coverslip was removed and fixed in cold acetone before staining.
for RSV (#5022 Mouse anti-RSV+FITC monoclonal kit; Millipore, Billerica, MA, USA). Coverslip was mounted on a slide upside down with mounting media containing 4,6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA, USA).

**Quantitative PCR assay**

To measure RSV virus replication in human MSCs cells were infected with 1 MOI RSV (or mock) as previously described. Supernatants as well as RNA were collected 72 hours post-infection. Trizol method was used to isolate RNA (Invitrogen, Carlsbad, CA, USA) that was reverse transcribed to DNA (Maxima first stand kit; Thermo Fisher Scientific, Waltham, MA, USA) for use in qPCR. RSV transcripts were measured using SYBR green (Life Technologies) and normalized to GAPDH expression (Table 1; Integrated DNA Technologies, Coralville, IA, USA).

For cytokine expression RNA from RSV (or mock)-infected MSCs was collected 72 hours post-infection as previously described. Transcripts for the cytokines IL-6, IL-1β, IL-8, and

Table 1. Real-time RT-PCR primers.

| Gene     | Ref Seq   | Primer    | Sequence (5'-3') | Amplicon | Exon |
|----------|-----------|-----------|------------------|----------|------|
| GAPDH    | NM 002046.3 | IDT 103685527 | CCACATCGCTCAGACACCAT | 87       | 2 to 3 |
| RSVN     | M74568.1 | IDT 103685155 | CACTGTGACAAATACGGACCTCA | 70       | N/A   |
| IL1B     | NM 000576 | IDT Hs.PT.51.20299051 | AGAGACACTCTCTGTATTAAG | 140      | 1 to 4 |
| IL-6     | NM 000600.3 | IDT 103685150 | GCTGGTCTAAGAGATGGACCT | 184      | 3 to 4 |
| CXCL12   | NM 000609 | IDT Hs.PT.51.2268843 | CACTGTAGCTCAGGCTAC | 89       | 1 to 2 |
| IFNA2    | NM 000605 | IDT Hs.PT.53a.24294810.g | GTGACTTTGCAGCTGAGCA | 94       | 1 to 1 |
| IFNB     | NM 002176 | IDT Hs.PT.53a.28162302.g | GCTCAAAGTCATCCTTACTTCG | 136      | 1 to 1 |
| IFNG     | NM 000619 | IDT Hs.PT.53a.2672945 | GACAGTGTCGCCGGAATCTCCTT | 113      | 3 to 4 |
| IL1RN    | NM 000577 | IDT.Hs.PT.51.4381999 | TATGACTTTGCAGCTGAGCA | 89       | 7 to 8 |
| NOS2     | NM 000625 | IDT Hs.PT.51.22593297 | GAGCTCAGAGTTCTGCTGAG | 99       | 1 to 2 |
| mGAPDH   | NM 008084 | IDT Mm.PT.39a.1 | GTGGAGCATACCTGGAACATGAG | 150      | 2 to 3 |
| mIFNB1   | NM 010510.1 | IDT Mm.PT.56a.30132453.g | GGCATCAAAGTGCTGAGCTT | 119      | 1 to 1 |
| mIDO1    | NM 008324 | IDT Mm.PT.58.42364388 | AGACACTACCATACGATG | 127      | 1 to 2 |
| mNOS2    | NM 001313922 | IDT 134627193 | CCAAGGGCTGCAGGACAG | 127      | 15 to 16 |

doi:10.1371/journal.pone.0163709.t001
stromal cell-derived factor 1 (SDF-1) as well as the viral response genes interferon (IFN)–α, IFN–β, and IFN–γ were quantified by RTqPCR and normalized to GAPDH (Table 1; Integrated DNA Technologies).

To assay expression of immune regulatory factors produced by MSCs mRNA transcript levels were assessed including indoleamine 2,3-dioxygenase (IDO), nitric oxide synthase (iNOS), and IL-1 receptor antagonist (IL-1ra) by RTqPCR at 72 hours post infection (Table 1; Integrated DNA Technologies).

PFU assay
Clariﬁed supernatants were used for PFU assays. HEp-2 cells were grown to conﬂuence before infection with serial dilutions of supernatants from infected or mock-infected MSCs in Opti-MEM with 2% FBS. After a 90-minute incubation at room temperature with rocking the supernatants were removed and cells were cultured in DMEM containing 5% FBS and 0.8% methylcellulose for ﬁve days at 37°C in 5% CO₂. Cells were ﬁxed in 4% paraformaldehyde in PBS and plaques were counted using red ﬂuorescence.

Protein isolation
Cell lysates from infected or mock infected MSCs were collected following the method of Rudolph et al [42]. Briefly, cells were washed once with cold PBS, detached by scraping, and pelleted by centrifugation before adding freeze-thaw lysis buffer containing 600 mM KCl, 20 mM Tris-Cl (pH 7.8), 20% glycerol, and 1x each of protease and phosphatase inhibitor (EMD Chemicals Inc., San Diego, CA, USA). Samples were submerged in liquid nitrogen for 5 minutes and thawed on ice for 5 minutes three times, then centrifuged at 13,200 x g for 5 minutes to pellet debris. BCA protein analysis (Pierce Biotechnology, Rockford, IL, USA) was performed on the supernatant to measure the protein concentration before use in Western blots and IDO ELISA or enzyme activity assays.

Western blotting
Cell lysates were separated using SDS-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. Membranes were blocked in Tris-buffered saline (TBS; 0.05 M Tris-HCl and 0.2 M NaCl, pH 7.4) containing 0.1% (v/v) Tween-20 (TBST) and 5% non-fat milk (w/v) for 1 hr at room temperature then probed with the appropriate primary antibody; either mouse anti-human IDO (RayBiotech, Norcross, GA, USA) or mouse anti-human beta-actin (Sigma-Aldrich, St Louis, MO, USA) diluted in TBST containing 5% non-fat milk. Probing was performed overnight at 4°C. After rinsing the membranes were incubated with HRP-conjugated anti-mouse IgG for 1 hr at room temperature, and then rinsed again. Band size was determined by comparison with a biotinylated protein ladder (Cell Signaling Technology Inc., Danvers, MA) and the density of each band was determined using ImageJ image analysis software [43] using a method detailed by Miller [44]. Results are reported as the relative abundance: a ratio of the density of the IDO band to the beta-actin band.

ELISA
Conditioned media from MSCs infected with 1 MOI of RSV as previously described was clariﬁed by centrifugation and the concentration of the immune regulator prostaglandin E₂ (PGE₂) was measured by ELISA (R&D Systems, Minneapolis, MN, USA). Cell lysates were obtained by freeze-thaw as described above for use in the ELISA kit for IDO (Cloud-Clone Corp., Houston, TX, USA).
**IDO enzymatic activity assay**

To assess the activity of IDO we performed a tryptophan catabolism assay. Briefly, freeze-thaw lysates from RSV-infected MSCs were treated with buffer containing 100mM PBS, 40mM ascorbate, 20μM methylene blue, 200μg/ml catalase, and 800μM L-tryptophan (pH 6.5). After a 30 minute incubation at 37C the reaction was stopped with 30% trichloroacetic acid and heated to 52C for 30 minutes. Precipitate was removed and kynurenine in the supernatant was detected with Ehrlich’s reagent (0.8% p-dimethylaminobenzaldehyde in acetic acid) by reading absorbance at 490nm. Results are expressed as units per μg lysate where 1 unit of IDO activity is defined as the amount of enzyme producing 1nmol per hour of kynurenine.

**Isolation of PBMCs and cell proliferation assay**

To examine the effect of RSV infection on MSC regulation of lymphocyte responses peripheral blood mononuclear cells (PBMCs) were isolated from fresh buffy coats (Florida Blood Services, St. Petersburg, FL, USA) and labeled with carboxyfluorescein succinimidyl ester (CellTrace CFSE, Life Technologies). Stained cells were then stimulated with the T cell mitogen phytohemagglutinin (PHA) in the presence of conditioned media (CM) from MSCs, infected 48hrs prior. Proliferation of lymphocytes was assessed by flow cytometry 5 days later using an LSRII flow cytometer (BD Biosciences, San Jose, CA, USA). Results were analyzed using FlowJo software (Treestar, Ashland, OR, USA).

**Inhibition of IDO in RSV-infected MSCs**

In order to reverse the effects of IDO on PBMC proliferation MSCs were infected with RSV as previously described. After removing infection media MSCs were incubated in complete MSC media containing the IDO-inhibitor 1-methyltryptophan (1-MT, Santa Cruz Biotechnology) at 1mM. After 48 hours supernatants clarified by centrifugation were used in the PBMC proliferation assay as previously described. An alternative IDO inhibitor, vitamin K₃ (menadione, Sigma-Aldrich, St Louis, MO, USA) [45] was used at 10μM to confirm our results. Additionally, to interrogate the pathways leading to IDO expression we treated RSV infected MSCs with either the endosomal pathway inhibitor chloroquine (10μg per mL, Sigma-Aldrich), the RIG-I pathway inhibitor BX795 (1μM, Invivogen, San Diego, CA, USA) or a neutralizing antibody to IFN-β (IFNb/A1, 10μg per mL, BioLegend, San Diego, CA, USA). To examine endosomal TLRs specifically we treated human MSCs with inhibitory oligodextronucleotide (ODN) 2088, an inhibitor of TLR7/8/9 [46–48] (50 μg per mL, Innaxon, Tewkesbury, UK) [49], or TLR3/dsRNA complex inhibitor (50 μM, Calbiochem, San Diego, CA, USA) [50]. With each inhibitor or vehicle, MSCs were pretreated overnight before infection with RSV. Following infection MSCs were incubated in fresh media containing the inhibitors or vehicles. After 24 hours additional inhibitor was added and at 48 hours samples were collected for RNA.

**Knockdown of IDO in RSV-infected MSCs**

Human MSCs were transduced with lentiviral particles containing the pCRISPR-LvSG03 expression plasmid for mCherry, CRISPR associated protein 9 (Cas9), and one of three human IDO-specific single guide (sg)RNAs or a scrambled control (LPPHCPL-2554751L03-3-a-100 and LPPCCPCCTR01L03-100, GeneCopia Inc., Rockville, MD, USA). MSCs were seeded into 6 well plates at a density of 10,000 cells per cm². Cells were allowed to attach overnight and were transduced with one of 4 lentiviral constructs the following day in the presence of 10 μg per mL polybrene for 12 hours, per manufacturer’s instructions. The cells were incubated for 48 hours
prior to infection with rgRSV. RSV infection and lentiviral transduction were visualized by fluorescent microscopy for GFP (green) and mCherry (red) respectively.

RSV infection of IDO-deficient mice
Wild type C57Bl/6 mice (WT) as well as mice deficient in IDO (IDO-KO) were purchased from Jackson Laboratories (B6.129-Ido1<sup>tm1Alm</sup>/J, # 005867) and infected with RSV when they were 6–8 weeks old by intranasal administration of 1 million PFU. Five days post infection the mice were euthanized and lungs were collected and processed for histology, plaque forming unit assay, and RNA quantification as previously described [51]. The right bronchus was clamped shut while the left lung was perfused with 4% paraformaldehyde (PFA) in PBS via the trachea, then removed to a vial containing 4% PFA for histology. The right lobes were removed, minced, portioned into two 1.5 mL tubes, and snap-frozen on dry ice for RNA and plaque forming unit assays. All animal procedures were reviewed and approved by the University of South Florida's Institutional Animal Care and Use Committee.

RSV infection of mouse MSCs
MSCs were isolated from the bones of wild type C57Bl/6 mice using the method of Nardi, et al. [52, 53]. Hind leg bones were excised, cleaned, and flushed with PBS containing 1% FBS. Contaminating red blood cells were lysed and adherent cells were grown for 3 passages. Cells were characterized by staining for the MSC markers CD105, CD29, and Sca-1 as well as the CD45 lineage surface markers (Mouse Mesenchymal Stem Cell Multi-Color Flow Kit, R&D Systems) as well as their capacity to differentiate, as described previously [54]. Mouse MSCs were infected as previously described, with 1 MOI RSV. RNA was collected 72 hours post-infection and the transcripts for mouse IFN-β, IDO, and iNOS were measured (Table 1, Integrated DNA Technologies).

Statistical analysis
All experiments were repeated at least twice depending upon the type of experiments. Statistical significance for each experiment was determined by Student's t test or one-way analysis of variance (ANOVA) as appropriate with an alpha of 5%. Calculations were performed and graphs produced using Prism 6.0 software for Mac OS (Graphpad Software, San Diego, CA, USA). Graphs of results show the mean and error bars depict the standard deviation.

Results
RSV infects and replicates in human MSCs
Live cell imaging of the infected cells for m-Kate2 expression at 24, 48 and 72 hours post-infection demonstrated RSV was capable of infecting four different MSC lots including two UCB MSC lines and two BM MSC lines (Fig 1). FITC-labeled anti-RSV antibody detected the viral products in MSCs at 48 and 72 hours post-infection (Fig 2A). The antibody used was a mixture of 4 monoclonal antibodies derived from mice against the nucleocapsid protein (N), glycoprotein (G), and fusion protein (F, 2 antibodies), all tagged with FITC. RTqPCR detected RSV specific transcripts for the nucleocapsid gene at 5.8 million-fold in the RSV infected MSCs after 72 hours compared with uninfected A549 control (P = 0.0498 by one sample t test) while levels in mock infected MSCs were not significantly different (Fig 2B). Culture supernatants isolated from RSV infected MSCs 72 hours after infection and used in PFU assays with HEP-2 target cells contained a mean of 3.8 X 10<sup>5</sup> PFU/ml in the infected cultures compared to mock, where no plaques were detectable (p < 0.0001 by Wilcoxon signed-rank test; Fig 2C).
RSV infects and replicates in human MSCs. Human MSCs grown on coverslips, infected with 1 MOI of RSV, and immunostained at 48 or 72 hours post-infection with a monoclonal antibody to RSV tagged with FITC (green), Evan's blue dye (red) and DAPI (blue). Total magnification of images is 200X (inset 1000X) and scale bar is 50 μm (A). RSVN transcripts detected in MSCs at 6, 12, 24 and 72 hours post infection normalized to mock (B). RSV titers (PFU/ml) isolated from the culture medium of infected and mock-infected MSCs is shown (C). Results representative of at least duplicate experiments. * p < 0.05, ** p < 0.01, *** p < 0.001.

doi:10.1371/journal.pone.0163709.g002
RSV infection induces differential cytokine expression in MSCs and epithelial cells

RNA from RSV infected NHBE cells and MSCs was examined for the expression of pro-inflammatory cytokines, IL-1β, IL-6, IL-8, SDF-1, and type I and type II interferons. At 72 hours post-infection RSV-infected NHBE cells had increased expression of IL-6 (4.6-fold, p = 0.0220) and SDF-1 (2-fold, p = 0.0457) transcripts compared to mock infected cells (Fig 3A). Transcripts for these cytokines were unchanged in RSV infected MSCs, however a 2.85-fold increase in IL-8 mRNA (p = 0.0291) was seen. An increase in IFN-β transcripts was observed for both RSV infected NHBE cells (2.409-fold, p = 0.0334) and MSCs (106.9-fold, p = 0.0036) compared to mock (Fig 3B). However, no changes in either IFN-α or IFN-γ expression were seen for either cell type.

RSV infection increases expression of indoleamine-2,3-dioxygenase by MSCs and epithelial cells

RNA from RSV infected NHBE cells and MSCs was examined for the expression of the immune regulatory factors IL-1 receptor antagonist (IL-1ra), inducible nitric oxide synthase (iNOS) and IDO (Fig 4A). RSV infection increased IDO transcripts 68.9-fold compared to mock infected (p = 0.0023) in MSCs. A significant but less dramatic 5.9-fold increase was also observed for in RSV infected NHBE cells (p = 0.0120). No changes in IL-1ra or iNOS were seen. As another potential immune regulatory pathway, prostaglandin E₂ (PGE₂) level was measured by ELISA (Fig 4B). RSV-infection was not associated with any change in PGE₂ levels in MSC culture supernatant.

IDO activity was detected in RSV-infected MSCs as 50.35 and 52.31 units per μg protein at 24 and 48 hours respectively, while it was undetectable at 24 hours and just 2.03 units per μg protein at 48 hours in mock-infected MSCs (Fig 4C). IDO expression in RSV-infected MSCs was confirmed by Western blot (Fig 4D). Analysis showed that IDO protein was detected 6-fold and 2-fold higher in RSV-infected MSCs compared with mock at 24 and 48 hours post infection, respectively. Cell lysates were also examined for IDO enzymatic activity by tryptophan catabolism assay.

![Fig 3. RSV infection increases IFN-β transcription in human MSCs.](http://example.com/fig3.png)

Cytokine transcription in RSV-infected NHBE cells and MSCs (n = 6) after 72 hours post infection by RTqPCR reported as fold expression compared to mock-infected NHBE and MSCs. (A) Interferon transcript expression in RSV-infected NHBE and MSCs compared to mock infected cells (n = 6) (B). Results of a representative experiment. * p < 0.05, ** p < 0.01.

doi:10.1371/journal.pone.0163709.g003
RSV induces IDO-mediated inhibition of PBMC expansion by MSCs

To examine the immunological consequences of RSV-infected MSCs we isolated fresh human PBMCs and treated them with varying amounts of CM from MSCs. We observed a dose-dependent decrease in mitogen-stimulated proliferation of PBMCs treated with the CM from either RSV- or mock-infected MSCs (Fig 5). Additionally, the cells treated with CM from MSCs infected with RSV had significantly lower proliferation than those treated with mock at all concentrations tested (Fig 5B). PBMCs cultured with 25% CM from RSV-MSCs showed 40% less proliferation than those cultured with CM from mock-MSCs (46% vs. 86%, p = 0.0001).

**Fig 4. RSV infection of MSCs modulates expression of the immune regulatory factor IDO.** Expression of IL-1Ra, IDO and iNOS were examined in RSV- or mock-infected NHBE cells and MSCs after 72 hours post infection by RTqPCR, reported as fold expression compared to mock (A). PGE2 expression was assessed by ELISA (B). (C-D) Tryptophan catabolism was measured in MSC lysates at 0, 24 and 48hrs after infection with RSV or mock. IDO activity (C) and expression by Western blot (D) is shown. * p < 0.05, ** p < 0.01.

doi:10.1371/journal.pone.0163709.g004
Similarly, PBMCs cultured with 50% or 75% CM showed a reduction in PHA-stimulated proliferation, 37% less (37% vs. 74%, \( P < 0.0001 \)) and 15% less (18% vs. 33%, \( P = 0.0234 \)), respectively. These results showed that RSV infection increased the regulatory effect of MSCs via factors in the CM. Since the lowest p-value was seen between the groups treated with 50% MSC CM we chose to use this concentration for future experiments.

Fig 5. Conditioned media from RSV-infected MSCs reduces PBMC proliferation. Freshly isolated PBMCs stained with CFSE, treated with either RSV or mock conditioned media, and grown in triplicates with (line) or without PHA (shaded) for 5 days. CFSE fluorescence was measured by flow cytometry as an indication of proliferation (A). Dose-dependent reduction in proliferation of PBMCs treated with conditioned media from RSV-infected MSC (B). Result of a representative experiment is shown. * \( p < 0.05 \), ** ** \( p < 0.001 \), **** \( p < 0.0001 \).

\[ \text{doi:10.1371/journal.pone.0163709.g005} \]
In order to investigate whether increased IDO production by RSV-infected MSCs was responsible for modulating PBMC expansion we used the IDO inhibitor 1-MT. MSCs were infected as previously, however in the treatment group infection media was replaced with MSC media containing 1mM 1-MT. The rest of the experiment was performed as described previously with PBMCs being treated with 50% MSC CM or MSC media as a control. PBMCs treated with CM from RSV-infected MSCs showed a significant reduction in mitogen-stimulated proliferation (38.77%) compared with mock (72.64%; P < 0.0001) while treatment of MSCs with 1-MT during RSV infection restored proliferative capacity of PBMCs (66.27%; versus mock CM P = 0.1657, versus RSV CM P < 0.0001) (groups compared by one-way ANOVA with Fisher’s LSD test, Fig 6A). Similar results were found when MSCs were treated with the
alternative IDO inhibitor vitamin K₃ (71.40%; versus mock CM P = 0.7771, versus RSV CM P < 0.0001). Flow cytometry, plaque assay and RTqPCR were used to show that the reversal was not due to inhibition of viral replication by the IDO inhibitor (Fig 6B–6D). These results demonstrate the potential immune regulatory role of IDO production by MSCs in RSV infection, an effect that was reversed in the presence of either of the IDO inhibitors.

CRISPR-mediated knockout of IDO prevents anti-proliferative effects of RSV-infected MSCs

As an alternative method to the use of IDO inhibitors, which may have off target effects, we leveraged the CRISPR/Cas9 system to knockout the IDO gene from the human MSCs prior to infection with RSV. Three separate plasmids expressing different IDO-specific guide RNAs as well as a control plasmid expressing a non-targeted scrambled guide RNA were transfected individually into human MSCs. Expression of the plasmid was evident by fluorescent microscopy for mCherry (red) expression in cells (Fig 7A). Similarly, infection with rgRSV was

![Fig 7. Knockout of IDO eliminates the immune regulatory effect of RSV-infected MSCs. Transfected MSCs were observed by fluorescent microscopy for lentiviral delivery of the plasmid (mCherry, red) as well as infection with rgRSV (GFP, green) (A). Proliferation of PBMCs treated with conditioned media from MSCs was detected by CFSE. Lv Scrambled represents media from MSCs transfected with Lv Scrambled construct; LvA, LvB, and LvC represent media from MSCs transfected with each of the three IDO-knockout constructs (B). N = 3, **** p < 0.0001 vs. PHA control, ‡ p < 0.0001 vs. Lv Scrambled.](image-url)
observed by GFP (green) fluorescence (Fig 7A). Conditioned media from IDO-knockout and control MSCs was used to treat PBMCs during a CFSE proliferation assay as previously described. PBMCs treated with conditioned media from infected MSCs that received the scrambled guide RNA (Lv Scrambled) showed a similar pattern of decreased proliferation due to RSV infection of the MSCs (Fig 7B). However, conditioned media from MSCs which lacked IDO expression (LvA, LvB, and LvC) showed no effect on PBMC proliferation regardless of RSV infection (Fig 7B). These results further demonstrate the role of IDO in immune regulation by RSV-infected MSCs.

IFN-β neutralizing antibody reduces IDO expression

To examine the role autocrine IFN-β signaling may play in IDO expression RSV-infected MSCs were treated with a neutralizing antibody against IFN-β (anti-IFN-β). Treated MSCs had reduced IDO expression compared with untreated cells, which was not due to a reduction in IFN-β expression (Fig 8A). Further, treating the cells with either chloroquine or anti-IFN-β reduced IDO protein detected by ELISA as well as enzymatic activity in lysates from infected cells (Fig 8B). The decreased IDO was not due to an effect of the inhibitors on RSV infectivity or replication, measured by PFU production by infected cells (Fig 8C).

Endosomal TLR but not RIG-I pathway inhibitors reduce IFN-β and IDO expression

To examine the role of pathogen associated molecular pattern (PAMP) receptor signaling in IDO expression during RSV infection we treated infected MSCs with either the RIG-I signaling inhibitor BX795 or the endosomal TLR inhibitor chloroquine. Chloroquine treatment but not BX795 reduced IFN-β expression in RSV-infected human MSCs (Fig 9A). This reduction in IFN expression was associated with a reduction in IDO expression in RSV infected MSCs treated with chloroquine and was not seen in MSCs treated with BX975 (Fig 9B). Neither inhibitor was shown to effect the replication of the virus in MSCs (Fig 9C).
Inhibition of TLR3 reduces IFN-β and IDO expression

RSV stimulation of MSCs, either with live virus or UV-inactivated virus, induced endosomal TLR expression (Fig 10A); specifically TLR3, TLR7, and TLR9 were upregulated versus mock. However, only TLR3 was increased with live virus when compared to UV-inactivated control, suggesting its role in productive RSV infection. To examine the endosomal pathway or pathways responsible for the IFN-β response to RSV in MSCs we used two specific inhibitors; ODN 2088, an inhibitor of TLR7/8/9 activity [46–48], and TLR3/dsRNA Complex Inhibitor [50]. Treatment with ODN 2088 showed no effect on either IFN-β expression or IDO expression compared with vehicle treated cells (Fig 10B), suggesting that the TLR7, 8, and 9 pathways were not involved. However, when MSCs were treated with the TLR3/dsRNA complex inhibitor during RSV-infection they had reduced IFN-β and IDO (Fig 10C), suggesting a role for TLR3 in the observed response to RSV in MSCs. The effect of the inhibitor did not appear to be due to a change in viral replication, measured by RSV gene expression.

RSV infection of IDO-deficient mice

After ensuring mouse MSCs could be infected in vitro with human RSV (Fig 11A) we infected WT and IDO-KO mice with 3 x 10^6 PFU RSV intranasally. After 5 days infection we did not see any difference in viral load between WT and IDO-KO mice, as measured by PFU assay and RSVN mRNA in the lungs of infected mice (Fig 11B and 11C). There was also no difference in IFN-β expression (Fig 11D).

RSV infection of mouse MSCs induces iNOS and not IDO

After the previous result we wanted to determine whether the mouse presented an appropriate model for examination of IDO expression by MSCs in response to RSV infection. Similar to human MSCs, wild type mouse bone marrow-derived MSCs infected with RSV exhibited a 128.2-fold increase of IFN-β transcripts (p = 0.0029), however IDO expression was unchanged (p = 0.23, Fig 11D). Interestingly, iNOS was highly transcribed following RSV infection at 594.1-fold the mock level (p = 0.0015). These results stand in contrast to what has been

Fig 9. Endosomal and not RIG-I pathway inhibitors block IFN-β induction and IDO expression in RSV-infected MSCs. IFN-β levels were examined in infected MSCs treated with the endosomal pathway inhibitor chloroquine, vehicle control (No Tx), or with RIG-I pathway inhibitor BX795 (A). IDO protein expression was measured in cells treated with chloroquine versus BX795 (B), and cells were examined for RSV PFUs (C). Significance between groups determined by one-way ANOVA with Fisher’s LSD test; n = 3. ** p < 0.01, *** p < 0.001, **** p < 0.0001.

doi:10.1371/journal.pone.0163709.g009
reported above in human MSCs (Fig 4) and suggest that RSV infection in mice, both in vitro and in vivo, may be an unsuitable model for examining the role of MSCs in RSV infections, at least as it pertains to IDO activity.

**Discussion**

To date most RSV research has focused on the viral modulation of innate immune signaling within immortalized epithelial cell lines such as HEp-2 and A549 although recent reports of RSV infecting and replicating in cells of the immune system including blood and lung neutrophils [22, 55–57], macrophages [24, 58, 59], and dendritic cells [20, 21, 60] suggest that RSV is capable of infecting cells of non-epithelial origin. Though extrapulmonary RSV infection of MSCs in humans was reported [23, 61], little is known about its role in virus-mediated immune regulation and the lack of protective immunity during secondary RSV infection. Our study addresses the role of MSCs as a target cell for RSV and the consequences of this extra-epithelial infection and is the first to report an increase in the expression of the immune regulatory factors IFN-β and IDO.

Our findings demonstrate that RSV infects MSCs in vitro and infective virus progeny are produced by the MSCs. This observation is similar to the other reports [23, 61]. Infection of MSCs would require the virus to infect beyond the apical epithelial layer of the airway, a phenomenon that has been demonstrated in ovine and baboon animal models [17, 18]. In vitro air-liquid culturing of NHBE cells has allowed researchers to examine this further, demonstrating that RSV can gain access to and infect the basal progenitor cells beneath by epithelial denuding or physical damage to the apical cells [62, 63] as well as via transport by infected alveolar macrophages [64]. Similar mechanisms may permit RSV to gain access to airway stromal cells such as MSCs.

We sought to identify factors that could be important in mediating the effects of RSV in MSCs by examining differential expression of chemokines and cytokines involved in the inflammatory response. Unlike with RSV-infected NHBE cells, RSV-infected MSCs showed no change in the mRNA of the proinflammatory cytokines IL-6 or SDF-1 (CXCL12). However, there was a significant increase in the expression of the chemokine IL-8 (CXCL8), which is known for neutrophil recruitment and may be involved in mediating the inflammation and neutrophilia associated with severe RSV infections. Also, since neutrophils are susceptible to RSV [22] and are known to return to the bone marrow upon senescence [65, 66] they may contribute to the extrapulmonary RSV infection of bone MSCs observed by others [23, 61].

A major finding from our studies relates to modulation of interferon expression and signaling by RSV-infected MSCs. Our results showed that when infected with RSV both NHBE and MSCs displayed significant upregulation of IFN-β, but not in gamma or alpha interferon. Of note, MSCs showed a more than 100-fold increase in IFN-β transcript compared to mock as determined by RTqPCR. The functional relevance of this finding is unclear as RSV continues to replicate in MSCs and blocking IFN-β signaling with a neutralizing antibody did not increase virus replication. It is possible that the IFN-β mRNAs are sequestered in exosomes, or
microvesicles, that are exported out of MSCs [44, 67, 68]. Alternatively, IFN-β-signaling simply may not be protective in the case of RSV infection of MSCs.
Another major finding from our studies is that although MSCs produce a number of immune regulatory factors including iNOS, IL-1Ra, and PGE₂ [27, 28, 33, 69], none of these were altered in their expression during RSV infection. However, RSV-infected MSCs did show a significant increase in IDO transcripts and protein, as well as activity of the enzyme. Further, RSV-infected MSCs exerted a greater inhibition of mitogen-stimulated proliferation of human PBMCs through IDO activity. Antagonizing IDO activity with a competitive inhibitor such as 1-MT or vitamin K₃ [45] reduced kynurenine levels in conditioned media of RSV infected MSCs and reversed the suppressive effect of MSC CM on lymphocyte proliferation, although these inhibitors did not affect RSV infective rate or proliferation in MSCs. These results suggest that the use of an IDO inhibitor during respiratory viral infection could result in a boosted immune response. This idea is consistent with a report which demonstrated that pretreatment of influenza-infected mice with 1-MT, increased inflammatory markers, such as IL-6, TNF-α and IFN-β [70], and Th1 cell numbers and boosted the secondary immune response [71].

Of special importance is our finding that there exists an autocrine signaling pathway in the regulation of IDO by IFN-β, both of which are significantly produced in RSV-infected MSCs and are also present in the proinflammatory environment of the lung during a respiratory viral infection. This notion is supported by our results that blocking IFN-β signaling with a neutralizing antibody resulted in reduced IDO expression and enzymatic activity in infected MSCs and agrees with reports in other immune cells showing an IFN-γ-independent pathway in the production of IDO involving IFN-β [72, 73]. It is possible this IFN-β-dependent pathway is also active in other cell types during RSV infection, such as dendritic cells and apical cells of the airway epithelium, which also play a role in regulation of the immune system and could be a target for blocking immune regulation by IDO during viral infection.

Regulation of immunity by MSCs has been linked to active TLR signaling [74–79] and products of RSV replication are known TLR ligands [80–82]. The results of our study showed chloroquine treatment of infected MSCs was sufficient to block IFN-β expression, which suggests that endosomal TLR recognition of viral products plays a key role in the MSC response to RSV infection. These results are similar to those reported for adenovirus and baculovirus, which induce an increased expression and TLR signaling in MSCs as well as increased IFN-β production [83]. Since RSV also stimulates immunity via the RNA helicase RIG-I through activation of the mitochondrial antiviral signaling complex (MAVS), TBK-1/IKKe, and IRF3 [9, 84], the role of this pathway in RSV infected MSCs was examined. Our results that the TBK1/IKKe inhibitor BX795 did not affect RSV-stimulated IFN-β expression suggest that the RIG-I pathway is not involved in this aspect of the MSC response to the virus. Further examination of the endosomal TLR pathway using ODN 2088, an antagonist of the endosomal TLR7/8/9 pathways, implied that while TLR7 and TLR9 were each upregulated versus mock infection with either UV-inactivated or live RSV neither played a role in the IFN-β or IDO expression we observed. However, MSCs treated with the TLR3/dsRNA complex inhibitor did show a reduction in IFN-β and IDO following RSV infection and TLR3 was the only TLR we observed to have significantly increased expression when comparing live infection to UV-inactivated virus. These results together suggest a role for endosomal TLR3 recognition of the virus in the expression of IDO, via IFN-β.

Our findings with MSCs differ from a recent report on IDO expression in recombinant green RSV-infected monocyte-derived dendritic cells (DCs) [42], which showed that the induction of IDO and IFN-γ were due to RIG-I pathway activity and not endosomal TLRs. Interestingly, in contrast to DCs RSV-infected MSCs showed an increase in IFN-β, not in IFN-γ. This result is similar to those previously reported in epithelial cells where RSV has been found to antagonize IFN-γ expression by inhibition of RIG-I pathway molecules via RSV nonstructural proteins [63, 85]. These discrepancies suggest that the cell types may differ in their activation
of molecular pattern recognition receptors and subsequent signaling, perhaps due to their differential susceptibility to RSV. The convergence of both pathways in the induction of IDO is an interesting finding, regardless. Future work could focus on the different viral response pathways triggered by classical antigen presenting cells such as monocytes and DCs compared to non-classical immune regulators like epithelial and stromal cells.

While our results suggest novel considerations in the development of prophylactics and therapeutics for RSV, perhaps involving IDO antagonism, how these findings will translate to human infection is unclear, in part due to the results gathered from IDO-knockout mouse experiments. This work found no difference in the number of RSV PFU in the lungs of IDO-knockout versus wild type C57Bl/6 mice. One possible reason could be the reported disparity between mice and men in the immune-regulatory role of IDO. For example, in humans IDO has been shown to be induced in listeriosis and tuberculosis, however murine models of each of these diseases suggest that iNOS and not IDO is induced in mice [86]. In line with this, our results here as well as other reports [87, 88] suggest that activated murine MSCs utilize iNOS in their immune regulatory function, while human MSCs exert their effects via IDO under the same stimuli. This difference in IDO and iNOS expression by stromal cells may play a role in the dissimilarities seen in RSV replication and response between mice and humans and also may account for the inability of the murine model of RSV infection to fully recapitulate the human disease [89–91]. Recently a group reported the use of a “humanized” mouse MSC model in which MSCs produced IDO under the control of the iNOS promoter [92]. Given these results showing the importance of MSCs in RSV pathogenesis and anti-RSV immunity a similar strategy may be useful to further elucidate the importance of IDO produced by RSV-infected MSCs.

Overall, these results provide the first report that RSV infection of bronchial epithelial cells and MSCs results in expression of the immune regulatory molecule IDO, which may be involved in disrupting the development of protective immunity against subsequent infections with the virus. Inhibition of virus-induced IDO production or activity may be a viable target for developing more effective therapies to respiratory viral pathogens. These results have implications beyond RSV-induced lung diseases, since the use of MSCs is being investigated in several areas of medicine including reducing rejection rates in solid organ transplantation, as cell-based therapy for autoimmune disease, as well as use in regenerative medicine [28, 33, 37, 69, 93]. A database search at ClinicalTrials.gov for the terms “Mesenchymal Stem Cells” returned 304 open studies (searched 10/14/15) demonstrating the high level of interest in the use of these cells in a clinical setting. This underscores the importance of understanding the consequences of RSV infection in this cell population in addition to understanding the mechanisms behind viral enhancement of their immune regulatory function, which may lead to procedures that optimize this feature for regulatory or regenerative applications.

**Acknowledgments**

We would like to thank Dr. Gary Hellerman, Dr. Sandhya Boyapalle, and Dr. Terianne Wong for their input and advice during the process of conceiving and carrying out the experiments described in this manuscript.

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### References

1. Respiratory Syncytial Virus Activity—United States, July 2011–January 2013. Centers for Disease Control and Prevention, Office of Surveillance E, and Laboratory Services; 2013. Report No.: Contract No.: 8.

2. Fields BN, Griffin DE, Howley PM, Knipe DM. Fields’ virology / editors-in-chief, Knipe David M, Peter M. Howley; associate editors, Griffin Diane E. . . . [et al.]. 4th ed. ed. Philadelphia: Lippincott Williams & Wilkins; 2001.

3. Piedimonte M. The association between respiratory syncytial virus infection and reactive airway disease. Respir Med. 2002; 96 Suppl B:S25–9. Epub 2002/05/09. doi: 10.1053/rmed.2002.1297 PMID: 11996401.

4. Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE. Respiratory syncytial virus infection in elderly and high-risk adults. The New England journal of medicine. 2005; 352(17):1749–59. Epub 2005/04/29. doi: 10.1056/NEJMoa043951 PMID: 15858184.

5. You D, Becler D, Wang K, Ripple M, Daly M, Cormier SA. Exposure of neonates to respiratory syncytial virus is critical in determining subsequent airway response in adults. Respir Res. 2006; 7:107. Epub 2006/08/09. doi: 10.1186/1465-9921-7-107 PMID: 16893457; PubMed Central PMCID: PMC1563465.

6. Hall CB, Weinberg GA, Iwane MK, Blumkin AK, Edwards KM, Staat MA, et al. The Burden of Respiratory Syncytial Virus Infection in Young Children. New England Journal of Medicine. 2009; 360(6):588–98. doi: 10.1056/NEJMoa0804877 PMID: 19196675.

7. Sigurs N, Aljassim F, Kjellman B, Robinson PD, Sigurbergsson F, Bjarmason R, et al. Asthma and allergy patterns over 18 years after severe RSV bronchiolitis in the first year of life. Thorax. 2010; 65 (12):1045–52. Epub 2010/06/29. [pii] doi: 10.1136/thx.2009.121582 PMID: 20581410.

8. Respiratory syncytial virus—United States, July 2007–June 2011. Centers for Disease Control and Prevention, 2011 Sep 9. Report No.: Contract No.: 35.

9. van Drunen Littel-van den Hurk S, Watkiss ER. Pathogenesis of respiratory syncytial virus. Current Opinion in Virology. 2012; 2(3):300–5. doi: 10.1016/j.coviro.2012.01.008 PMID: 22709517.

10. Collins PL, Graham BS. Viral and Host Factors in Human Respiratory Syncytial Virus Pathogenesis. Journal of Virology. 2008; 82(5):2040–55. doi: 10.1128/JVI.01625-07 PMID: PMC2258918.

11. Kapikian AZ, Mitchell RH, Chanock RM, Shvedoff RA, Stewart CE. An epidemiologic study of altered clinical reactivity to respiratory syncytial (RS) virus infection in children previously vaccinated with an inactivated RS virus vaccine. American journal of epidemiology. 1969; 89(4):405–21. Epub 1969/04/01. PMID: 4935197.

12. Opitz CA, Litzenburger UM, Opitz U, Sahm F, Ochs K, Lutz C, et al. The Indoleamine-2,3-Dioxygenase (IDO) Inhibitor 1-Methyl-D-tryptophan Uregulates IDO1 in Human Cancer Cells. PLoS ONE. 2011; 6 (5):e18982. doi: 10.1371/journal.pone.00189823 PMID: PMC3998827.

13. Jorquera PA, Oakley KE, Tripp RA. Advances in and the potential of vaccines for respiratory syncytial virus. Expert review of respiratory medicine. 2013; 7(4):411–27. Epub 2013/08/24. doi: 10.1586/17476348.2013.814409 PMID: 23964629.
14. Kim E, Okada K, Beeler JA, Crim RL, Piedra PA, Gilbert BE, et al. Development of an Adenovirus-Based Respiratory Syncytial Virus Vaccine: Preclinical Evaluation of Efficacy, Immunogenicity, and Enhanced Disease in a Cotton Rat Model. Journal of Virology. 2014; 88(9):5100–8. doi: 10.1128/JVI.03194-13 PMID: PMC3993798.

15. Diseases Col, Committee BG. Updated Guidance for Palivizumab Prophylaxis Among Infants and Young Children at Increased Risk of Hospitalization for Respiratory Syncytial Virus Infection. Pediatrics. 2014; 134(2):415–20. doi: 10.1542/peds.2014-1665 PMID: 25070315

16. Borse RH, Singleton RJ, Bruden DT, Fry AM, Hennessy TW, Meltzer MI. The Economics of Strategies to Reduce Respiratory Syncytial Virus Hospitalizations in Alaska. Journal of the Pediatric Infectious Diseases Society. 2014; 3(3):201–12. doi: 10.1093/jpids/pit072 PMID: 26625383

17. Meyerholz DK, Grubor B, Fach SJ, Sacco RE, Lehmkuhl HD, Gallup JM, et al. Reduced clearance of respiratory syncytial virus infection in a preterm lamb model. Microbes and Infection. 2004; 6(14):1312–9. http://dx.doi.org/10.1016/j.micinf.2004.08.006. doi: 10.1016/j.micinf.2004.08.006 PMID: 15355538

18. Papin JF, Wolf RF, Kosanke SD, Jenkins JD, Moore SN, Anderson MP, et al. Infant baboons infected with respiratory syncytial virus develop clinical and pathologic changes that parallel those of human infants 2013 2013-04-15 00:00:00. L530-L9 p.

19. Eisenhut M. Extrapulmonary manifestations of severe respiratory syncytial virus infection—a systematic review. Crit Care. 2006; 10(4):R107. Epub 2006/07/25. [pii] doi: 10.1186/cc4984 PMID: 16859512; PubMed Central PMCID: PMC1751022.

20. Hobson L, Everard ML. Persistent of respiratory syncytial virus in human dendritic cells and influence of nitric oxide. Clinical and Experimental Immunology. 2008; 151(2):359–66. Epub 2007/12/08. doi: 10.1111/j.1365-2249.2007.03560.x PMID: 18062796; PubMed Central PMCID: PMC2276949.

21. Ajamian F, Wu Y, Davoine F, Moqbel R, Adamko DJ. Respiratory syncytial virus replication induces Indoleamine 2,3-dioxygenase (IDO) activation in human dendritic cells. Allergy, Asthma & Clinical Immunology. 2010; 6:1–2. doi: 10.1186/1710-1492-6-s3-p8

22. Halfhide CP, Flanagan BF, Brearey SP, Hunt JA, Fonceca AM, McNamara PS, et al. Respiratory Syncytial Virus Binds and Undergoes Transcription in Neutrophils From the Blood and Airways of Infants With Severe Bronchiolitis. Journal of Infectious Diseases. 2011; 204(3):451–8. doi: 10.1093/infdis/jir280 PMID: 21742845

23. Rezaee F, Gibson LF, Piktel D, Othumpangat S, Piedimonte G. Respiratory Syncytial Virus Infection in Human Bone Marrow Stromal Cells. Am J Respir Cell Mol Biol. 2011; 45(2):277–86. Epub 2010/05/11. doi: 10.1165/rcmb.2010-0121OC PMID: 20971893

24. Arrevilla C, Caspe A, Rodriguez V, Garcia A, Moya P. The role of soluble factors associated with immune regulatory properties of mesenchymal stem cells. Stem Cell Rev. 2012; 8(2):375–92. doi: 10.1007/s12015-011-0312-0 PMID: WOS:000304614900008.

25. Basri EJ, de Almeida DC, Moraes-Vieira PM, Camara NO. Exploring the role of soluble factors associated with immune regulatory properties of mesenchymal stem cells. Stem Cell Rev. 2012; 8(2):329–42. Epub 2011/09/02. doi: 10.1007/s12015-011-0311-1 PMID: 21881832

26. Jakob M, Hemeda H, Janeschik S, Bootz F, Rotter N, Lang S, et al. Human nasal mucosa contains tissue-resident immunologically responsive mesenchymal stromal cells. Stem Cells and Development. 2010; 19(5):635–44. Epub 2009/09/19. doi: 10.1089/scd.2009.0245 PMID: 19761404.

27. Cho KS, Park HY, Roh HJ, Bravo DT, Hwang PH, Nayak JV. Human ethmoid sinus mucosa: a promising novel tissue source of mesenchymal progenitor cells. Stem cell research & therapy. 2014; 5(1):15. Epub 2014/01/28. doi: 10.1186/s13286-014-0082-9; PubMed Central PMCID: PMC4055077.

28. Delorme B, Nivet E, Gaillard J, Haupl T, Ringe J, Devezee A, et al. The human nose harbors a niche of olfactory ectomesenchymal stem cells displaying neurogenic and osteogenic properties. Stem Cells and Development. 2010; 19(6):853–66. Epub 2009/11/13. doi: 10.1089/scd.2009.0267 PMID: 19905894.
32. Girard SD, Deveze A, Nivet E, Gepner B, Roman FS, Feron F. Isolating nasal olfactory stem cells from rodents or humans. Journal of visualized experiments: JoVE. 2011;(54):. Epub 2011/08/31. doi: 10.3791/2762 PMID: 21876529; PubMed Central PMCID: PMC3217619.

33. Haddad NE. Mesenchymal Stem Cells: Immunology and Therapeutic Benefits. 2011 2011-08-23.

34. Larsen S, Lewis ID. Potential therapeutic applications of mesenchymal stromal cells. Pathology. 2011; 43(6):592–604. doi: 10.1097/PAT.0b013e32834ab72d PMID: WOS:000295538700007.

35. Tasso R, Ilengo C, Quarto R, Cancedda R, Caspi RR, Pennesi G. Mesenchymal Stem Cells induce functionally active T regulatory lymphocytes in a paracrine fashion and ameliorate Experimental Autoimmune Uveitis. Investigative Ophthalmology & Visual Science. 2012. doi: 10.1167/iovs.11-8211 PMID: 22234235

36. Mesentier-Louro LA, Zaverucha-do-Valle C, da Silva-Junior AJ, Nascimento-dos-Santos G, Gubert F, Haddad NE. Mesenchymal Stem Cells: Immunology and Therapeutic Benefits. 2011 2011-08-23.

37. Ringe J, Strassburg S, Neumann K, Endres M, Notter M, Burmester GR, et al. Towards in situ tissue repair: human mesenchymal stem cells express chemokine receptors CXCR1, CXCR2 and CCR2, and migrate upon stimulation with CXCL8 but not CCL2. J Cell Biochem. 2007; 101(1):135–46. Epub 2007/02/14. doi: 10.1002/jcb.21172 PMID: 17295203.

38. Bentley JK, Popova AP, Bozyk PD, Linn MJ, Baek AE, Lei J, et al. Ovalbumin sensitization and challenge increases the number of lung cells possessing a mesenchymal stromal cell phenotype. Respir Res. 2010; 11:127. Epub 2010/09/23. doi: 10.1186/1465-9921-11-127 PMID: 20858250; PubMed Central PMCID: PMC2949728.

39. Moore ML, Chi MH, Luongo C, Lukacs NW, Polosukhin VV, Huckabee MM, et al. A chimeric A2 strain of respiratory syncytial virus (RSV) with the fusion protein of RSV strain line 19 exhibits enhanced viral load, mucus, and airway dysfunction. J Virol. 2009; 83(9):4185–94. Epub 2009/02/13. [pii] doi: 10.1128/JVI.01853-08 PMID: 19211758; PubMed Central PMCID: PMC2668460.

40. Hotard AL, Shaikh FY, Lee S, Yan D, Teng MN, Plemper RK, et al. A stabilized respiratory syncytial virus reverse genetics system amenable to recombination-mediated mutagenesis. Virology. 2012; 434 (1):129–36. Epub 2012/10/16. doi: 10.1016/j.virol.2012.09.022 PMID: 23062737; PubMed Central PMCID: PMC3492879.

41. Hallak LK, Spillmann D, Collins PL, Peeples ME. Glycosaminoglycan Sulfation Requirements for Repair: human mesenchymal stem cells express chemokine receptors CXCR1, CXCR2 and CCR2, and migrate upon stimulation with CXCL8 but not CCL2. J Cell Biochem. 2007; 101(1):135–46. Epub 2007/02/14. doi: 10.1002/jcb.21172 PMID: 17295203.

42. Ajamian F, Wu Y, Ebeling C, Ilarraza R, Odemuyiwa SO, Moqbel R, et al. Respiratory syncytial virus induces indoleamine 2,3-dioxygenase activity: a potential novel role in the development of allergic disease. Clin Exp Allergy. 2015; 45(3):644–59. Epub 2015/01/30. doi: 10.1111/cea.12498 PMID: 25627660.

43. Rasband WS. ImageJ. U S National Institutes of Health, Bethesda, Maryland, USA. 1997–2014.

44. Miller L. Analyzing gels and western blots with ImageJ. Available: http://lukemiller.org/index.php/2010/11/analyzing-gels-and-western-blots-with-image-j/

45. Kumar S, Malachowski WP, DuHadaway JB, LaLonde JM, Carroll PJ, Jaller D, et al. Indoleamine 2,3-Dioxygenase Is the Anticancer Target for a Novel Series of Potent Naphthoquinone-Based Inhibitors. Journal of medicinal chemistry. 2008; 51(6):1706–18. doi: 10.1021/jm7014155 PMID: PMC4384695.

46. Hackstein H, Knoche A, Nockher A, Poeling J, Jurk M, et al. The TLR7/8 ligand resiquimod targets monocyte-derived dendritic cell differentiation via TLR8 and augments functional dendritic cell generation. Cellular Immunol. 2011; 271(2):401–12. Epub 2011/09/06. doi: 10.1016/j.cellimm.2011.08.006 PMID: 21889130.

47. Lenert PS. Classification, mechanisms of action, and therapeutic applications of inhibitory oligonucleotides for Toll-like receptors (TLR) 7 and 9. Mediators of inflammation. 2010; 2010:986596. Epub 2010/05/22. doi: 10.1155/2010/986596 PMID: 20490286; PubMed Central PMCID: PMCPMC2873634.

48. Stunz LL, Lenert P, Peckham D, Yi A-K, Haxhinasto S, Chang M, et al. Inhibitory oligonucleotides specifically block effects of stimulatory CpG oligonucleotides in B cells. European journal of immunology. 2002; 32(5):1212–22. doi: 10.1002/eji.200131411 PMID: 11981808.

49. Qi J, Buzas K, Fan H, Cohen JL, Wang K, Mont E, et al. Painful Pathways Induced by TLR Stimulation of Dorsal Root Ganglion Neurons. The Journal of Immunology. 2011; 186(1):647–26. doi: 10.4049/jimmunol.1001241 PMID: 21515789.

50. Cheng K, Wang X, Yin H. Small-Molecule Inhibitors of the TLR3/dsRNA Complex. Journal of the American Chemical Society. 2011; 133(11):3764–7. doi: 10.1021/ja111312h PMID: 21355588.
51. Wong TM, Boyapalle S, Sampayo V, Nguyen HD, Bedi R, Kamath SG, et al. Respiratory Syncytial Virus (RSV) Infection in Elderly Mice Results in Altered Antiviral Gene Expression and Enhanced Pathology. PLoS ONE. 2014; 9(2):e88764. doi: 10.1371/journal.pone.0088764 PMID: 24558422

52. da Silva Meirelles L, Nardi NB. Murine marrow-derived mesenchymal stem cell: isolation, in vitro expansion, and characterization. British journal of haematology. 2003; 123(4):702–11. Epub 2003/11/18. doi: 10.1046/j.1365-2141.2003.04669.x PMID: 14616976.

53. Beyer Nardi N, Silva Meirelles L, Wobus A, Boheler K. Mesenchymal Stem Cells: Isolation, In Vitro Expansion and Characterization. Stem Cells. Handbook of Experimental Pharmacology. 174: Springer Berlin Heidelberg; 2006. p. 249–82. PMID: 16370331

54. Mallela J, Ravi S, Jean Louis F, Mulaney B, Cheung M, Sree Garapati U, et al. Natriuretic peptide receptor A signaling regulates stem cell recruitment and angiogenesis: a model to study linkage between inflammation and tumorigenesis. Stem cells (Dayton, Ohio). 2013; 31(7):1321–9. Epub 2013/03/28. doi: 10.1002/stem.1376 PMID: 23533187.

55. Yui I, Hoshi A, Shigeta Y, Takami T, Nakayama T. Detection of human respiratory syncytial virus sequences in peripheral blood mononuclear cells. J Med Virol. 2003; 70(3):481–9. doi: 10.1002/jmv.10421 PMID: WOS:000183211000022.

56. O'Donnell DR, McGarvey MJ, Tully JM, Balfour-Lynn IM, Openshaw PJM. Respiratory syncytial virus RNA in cells from the peripheral blood during acute infection. J Pediatr. 1998; 133(2):272–4. doi: 10.1016/s0022-3476(98)70234-3 PMID: WOS:000075300500024.

57. Lindemans CA, Coffer PJ, Schellens IM, de Graaff PM, Kimpen JL, Koenderman L. Respiratory syncytial virus inhibits granulocyte apoptosis through a phosphatidylinositol 3-kinase and NF-kappaB-dependent mechanism. J Immunol. 2006; 176(9):5529–37. Epub 2006/04/20. 176/9/5529 [pii]. doi: 10.4049/jimmunol.176.9.5529 PMID: 16622022.

58. Guerrero-Plata A, Ortega E, Gomez B. Persistence of respiratory syncytial virus in macrophages alters phagocytosis and pro-inflammatory cytokine production. Viral immunology. 2001; 14(1):19–30. Epub 2001/03/29. doi: 10.1089/08828240151061347 PMID: 11270594.

59. Guerrero-Plata A, Ortega E, Ortiz-Navarrete V, Gomez B. Antigen presentation by a macrophage-like cell line persistently infected with respiratory syncytial virus. Virus research. 2004; 99(1):95–100. Epub 2003/12/23. doi: 10.1016/j.virusres.2003.10.005 PMID: 14687952.

60. de Graaff PM, de Jong EC, van Capel TM, van Dijk ME, Roholl PJ, Boes J, et al. Respiratory syncytial virus infection of monocyte-derived dendritic cells decreases their capacity to activate CD4 T cells. J Immunol. 2005; 175(9):5904–11. Epub 2005/10/21. 175/9/5904 [pii]. doi: 10.4049/jimmunol.175.9.5904 PMID: 16237083.

61. Rezaee F, Gibson L, Piktel D, Othumpangat S, Samsell L, Fortney J, et al. Respiratory Syncytial Virus Alters Human Bone Marrow Stromal Cell Function. Am J Respir Crit Care Med. 2010; 181(1_MeetingAbstracts):A2502–.

62. Lin H, Li H, Cho HJ, Bian S, Roh HJ, Lee MK, et al. Air-liquid interface (ALI) culture of human bronchial epithelial cell monolayers as an in vitro model for airway drug transport studies. Journal of pharmaceutical sciences. 2007; 96(2):341–50. Epub 2006/11/03. doi: 10.1002/jps.20803 PMID: 17080426.

63. Ling Z, Tran KC, Teng MN. Human respiratory syncytial virus nonstructural protein NS2 antagonizes the activation of beta interferon transcription by interacting with RIG-I. J Virol. 2009; 83(8):3734–42. Epub 2009/02/06. doi: 10.1128/jvi.02434-08 PMID: 19193793; PubMed Central PMCID: PMCPMC2663251.

64. Ugonna K, Bingle CD, Plant K, Wilson K, Everard ML. Macrophages Are Required for Dendritic Cell Uptake of Respiratory Syncytial Virus from an Infected Epithelium. PLoS ONE. 2014; 9(3):e91855. doi: 10.1371/journal.pone.0091855 PMID: 24651119

65. Furze RC, Rankin SM. The role of the bone marrow in neutrophil clearance under homeostatic conditions in the mouse. The FASEB Journal. 2008; 22(9):3111–9. doi: 10.1096/fj.08-109876 PMID: 18509199

66. Rankin SM. The bone marrow: a site of neutrophil clearance. Journal of Leukocyte Biology. 2010; 88(2):241–51. doi: 10.1189/jlb.0210112 PMID: 20483920

67. Baglio SR, Rooijers K, Koppers-Lauck D, Verweij FJ, Pérez Lanzón M, Zini N, et al. Human bone marrow- and adipose-mesenchymal stem cells secrete exosomes enriched in distinctive miRNA and tRNA species. Stem cell research & therapy. 2015; 6(1):127. doi: 10.1186/s13287-015-0116-z PMID: PMC4529699.

68. Chen J, Li C, Chen L. The Role of Microvesicles Derived from Mesenchymal Stem Cells in Lung Diseases. BioMed Research International. 2015; 2015:985814. doi: 10.1155/2015/985814 PMID: PMC4443645.
69. Bassi EJ, Aita CA, Camara NO. Immune regulatory properties of multipotent mesenchymal stromal cells: Where do we stand? World journal of stem cells. 2011; 3(1):1–8. Epub 2011/05/25. doi: 10.4252/wjsc.v3.i1 PMID: 21607131; PubMed Central PMCID: PMC3097934.

70. Fox JM, Sage LK, Poore S, Johnson S, Tompkins SM, Tripp RA. Drug analog inhibition of indoleamine 2,3-dioxygenase (IDO) activity modifies pattern recognition receptor expression and proinflammatory cytokine responses early during influenza virus infection. Journal of Leukocyte Biology. 2014. doi: 10.1189/jlb.3A B0114-046RR PMID: 24799604

71. Sage LK, Fox JM, Mellor AL, Tompkins SM, Tripp RA. Indoleamine 2,3-dioxygenase (IDO) activity during the primary immune response to influenza infection modifies the memory T cell response to influenza challenge. Viral immunology. 2014; 27(3):112–23. Epub 2014/04/08. doi: 10.1089/vim.2013.0105 PMID: 24702323; PubMed Central PMCID: PMC3995207.

72. Pallotta MT, Orabona C, Volpi C, Vacca C, Belladonna ML, Bianchi R, et al. Indoleamine 2,3-dioxygenase is a signaling protein in long-term tolerance by dendritic cells. Nat Immunol. 2011; 12(9):870–8. http://www.nature.com/ni/journal/v12/n9/abs/ni.2077.html - supplementary-information. doi: 10.1038/ni.2077 PMID: 21804957.

73. Chen W. IDO: more than an enzyme. Nat Immunol. 2011; 12(9):809–11. doi: 10.1038/ni.2088 PMID: 21852775.

74. Cassatella MA, Mosna F, Micheletti A, Lisi V, Tamassia N, Cont C, et al. Toll-like receptor-3 activated human mesenchymal stromal cells significantly prolong the survival and function of neutrophils. Stem cells (Dayton, Ohio). 2011; 29(6):1001–11. Epub 2011/05/13. doi: 10.1002/stem.651 PMID: 21632379.

75. Delarosa O, Dalemans W, Lombardo E. Toll-like receptors as modulators of mesenchymal stem cells. Front Immunol. 2012; 3:182. Epub 2012/07/12. doi: 10.3389/fimmu.2012.00182 PMID: 22783256; PubMed Central PMCID: PMC3387651.

76. Giuliani M, Bennacere-Griscelli A, Nanbaksh A, Oudhiri N, Chouaib S, Azzarone B, et al. TLR ligands stimulation protects MSC from NK killing. Stem cells (Dayton, Ohio). 2014; 32(1):290–300. Epub 2013/10/15. doi: 10.1002/stem.1563 PMID: 24123639.

77. Le Blanc K, Mougiakakos D. Multipotent mesenchymal stromal cells and the innate immune system. Nature reviews Immunology. 2012; 12(5):383–96. Epub 2012/04/26. doi: 10.1038/nri2909 PMID: 22531326.

78. Optit CA, Litzenburger UM, Lutz C, Lanz TV, Tritschler I, Köppel A, et al. Toll-Like Receptor Engagement Enhances the Immunosuppressive Properties of Human Bone Marrow-Derived Mesenchymal Stem Cells by Inducing Indoleamine-2,3-dioxygenase-1 via Interferon-β and Protein Kinase R. Stem cells (Dayton, Ohio). 2009; 27(4):909–19. doi: 10.1002/stem.7 PMID: 19353159.

79. Raicevic G, Rouas R, Najjar M, Stordeur P, Boufker HI, Bron D, et al. Inflammation modifies the pattern and the function of Toll-like receptors expressed by human mesenchymal stromal cells. Human immunology. 2010; 71(3):235–44. Epub 2009/12/26. doi: 10.1016/j.humimm.2009.12.005 PMID: 20034529.

80. Haynes LM, Moore DD, Kurt-Jones EA, Finberg RW, Anderson LJ, Tripp RA. Involvement of Toll-Like Receptor 4 in Innate Immunity to Respiratory Syncytial Virus. Journal of Virology. 2001; 75(22):10730–7. doi: 10.1128/jvi.75.22.10730-10737.2001 PMID: 11602714.

81. Kurt-Jones EA, Popova L, Kwinin L, Haynes LM, Jones LP, Tripp RA, et al. Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. Nat Immunol. 2000; 1(5):398–401. http://www.nature.com/ni/journal/v1/n5/abs/ni1100_398_S1.html. doi: 10.1038/80833 PMID: 11062499.

82. Murawski MR, Bowen GN, Cerny AM, Anderson LJ, Haynes LM, Tripp RA, et al. Respiratory Syncytial Virus Activates Innate Immunity through Toll-Like Receptor 2. Journal of Virology. 2009; 83(3):1492–500. doi: 10.1128/jvi.00671-08 PMID: 19019963.

83. Chen G-Y, Shiah H-C, Su H-J, Chen C-Y, Chuang Y-J, Lo W-H, et al. Baculovirus Transduction of Mesenchymal Stem Cells Triggers the Toll-Like Receptor 3 Pathway. Journal of Virology. 2009; 83(20):10548–56. doi: 10.1128/jvi.01250-09 PMID: 19656899.

84. Takeuchi O, Akira S. Pattern Recognition Receptors and Inflammation. Cell. 2010; 140(6):805–20. doi: http://dx.doi.org/10.1016/j.cell.2010.01.022 PMID: 20303872.

85. Boyapalle S, Wong T, Garay J, Teng M, San Juan-Vergara H, Mohapatra S, et al. Respiratory syncytial virus NS1 protein colocalizes with mitochondrial antiviral signaling protein MAVS following infection. PLoS One. 2012; 7(2):e29386. Epub 2012/03/03. doi: 10.1371/journal.pone.0029386 PMID: 22383950; PubMed Central PMCID: PMC3288005.

86. Schmidt SV, Schultz J. New insights into IDO biology in bacterial and viral infections. Frontiers in Immunology. 2014; 5. doi: 10.3389/fimmu.2014.00384 PMID: 25157255.

87. Meisel R, Brockers S, Heseler K, Degistirici O, Bulle H, Woite C, et al. Human but not murine multipotent mesenchymal stromal cells exhibit broad-spectrum antimicrobial effector function mediated by...
indoleamine 2,3-dioxygenase. Leukemia: official journal of the Leukemia Society of America, Leuke-
mia Research Fund, UK. 2011; 25(4):648–54. Epub 2011/01/19. doi: 10.1038/leu.2010.310 PMID:
21242993.

88. Ren G, Su J, Zhang L, Zhao X, Ling W, L’Huillie A, et al. Species variation in the mechanisms of mes-
enchymal stem cell-mediated immunosuppression. Stem cells (Dayton, Ohio). 2009; 27(8):1954–62.
Epub 2009/06/23. doi: 10.1002/stem.118 PMID: 19544427.

89. Bern RA, Domachowske JB, Rosenberg HF. Animal models of human respiratory syncytial virus dis-
ease. American journal of physiology Lung cellular and molecular physiology. 2011; 301(2):L148–56.
Epub 2011/05/17. doi: 10.1152/ajplung.00065.2011 PMID: 21571908; PubMed Central PMCID:
PMC3154630.

90. Domachowske JB, Bonville CA, Rosenberg HF. Animal models for studying respiratory syncytial virus
infection and its long term effects on lung function. The Pediatric infectious disease journal. 2004; 23
(11 Suppl):S228–34. Epub 2004/12/04. doi: 10.1097/01.inf.0000144672.81955.a4 PMID: 15577578.

91. Kong X, Hellermann GR, Patton G, Kumar M, Behera A, Randall TS, et al. An immunocompromised
BALB/c mouse model for respiratory syncytial virus infection. Virology journal. 2005; 2:3. Epub 2005/
02/11. doi: 10.1186/1743-422x-2-3 PMID: 15701174; PubMed Central PMCID: PMC549044.

92. Ling W, Zhang J, Yuan Z, Ren G, Zhang L, Chen X, et al. Mesenchymal stem cells use IDO to regulate
immunity in tumor microenvironment. Cancer research. 2014; 74(5):1576–87. Epub 2014/01/24. doi:
10.1158/0008-5472.can-13-1656 PMID: 24452999; PubMed Central PMCID: PMCPmc3959857.

93. Lee RH, Oh JY, Choi H, Bazhanov N. Therapeutic Factors Secreted by Mesenchymal Stromal Cells
and Tissue Repair. J Cell Biochem. 2011; 112(11):3073–8. doi: 10.1002/jcb.23250 PMID:
WOS:000296423000001.