Solution-Phase Crosstalk and Regulatory Interactions Between Multipotent Adult Progenitor Cells and Peripheral Blood Mononuclear Cells

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ABSTRACT

Multipotent adult progenitor cells (MAPCs) are adult adherent stromal stem cells currently being assessed in clinical trials for acute graft versus host disease with demonstrated immunomodulatory capabilities and the potential to ameliorate detrimental autoimmune and inflammation-related processes. Anti-CD3/anti-CD28 (3/28) activation of T cells within the peripheral blood mononuclear cell (PBMC) compartment was performed in the presence or absence of MAPCs. Liquid chromatography-coupled tandem mass spectrometry was used to characterize the differential secretion of proteins, and transcriptional profiling was used to monitor mRNA expression changes in both cell populations. Overall, 239 secreted and/or ectodomain-shed proteins were detected in the secretomes of PBMCs and MAPCs. In addition, 3/28 activation of PBMCs induced differential expression of 2,925 genes, and 22% of these transcripts were differentially expressed on exposure to MAPCs in Transwell. MAPCs exposed to 3/28-activated PBMCs showed differential expression of 1,247 MAPC genes. Crosstalk was demonstrated by reciprocal transcriptional regulation. Secretome proteins and transcriptional signatures were used to predict molecular activities by which MAPCs could dampen local and systemic inflammatory responses. These data support the hypothesis that MAPCs block PBMC proliferation via cell cycle arrest coupled to metabolic stress in the form of tryptophan depletion, resulting in GCN2 kinase activation, downstream signaling, and inhibition of cyclin D1 translation. These data also provide a plausible explanation for the immune privilege reported with administration of donor MAPCs. Although most components of the major histocompatibility complex class II antigen presentation pathway were markedly transcriptionally upregulated, cell surface expression of human leukocyte antigen-DR is minimal on MAPCs exposed to 3/28-activated PBMCs. Stem Cells Translational Medicine 2015;4:1436–1449

SIGNIFICANCE

This study documents experiments quantifying solution-phase crosstalk between multipotent adult progenitor cells (MAPCs) and peripheral blood mononuclear cells. The secretome and transcriptional changes quantified suggest mechanisms by which MAPCs are hypothesized to provide both local and systemic immunoregulation of inflammation. The potential impact of these studies includes development of a robust experimental framework to be used for preclinical evaluation of the specific mechanisms by which beneficial effects are obtained after treatment of patients with MAPCs.

INTRODUCTION

The bone marrow compartment contains populations of adult stromal cells that exist in a discernible hierarchy [1]. More primitive cells such as multipotent adult progenitor cells (MAPCs; MultStem; Athersys, Inc., Cleveland, OH, http://www.athersys.com) were originally described as a subpopulation of adherent stem cells that could be isolated from adult bone marrow and other adult stromal tissues [2, 3]. MAPCs, marrow-isolated adult multilineage inducible cells [4], and less primitive stromal cell populations such as mesenchymal stromal cells (MSCs) all copurify, and populations are obtained by manipulating culture conditions. MAPCs isolated from adult bone marrow meet the International Society for Cellular Therapy criteria applied to MSCs as a prototype for adherent stem and progenitor cells (i.e., expressing CD73 and CD105, negative for major histocompatibility complex [MHC] class II and CD45, and capable of differentiation to mesenchymal lineages) [5, 6]. Compared with standard MSC culture conditions, MAPCs are isolated using subconfluent culture density under hypoxic conditions, in media
supplemented with epidermal growth factor and platelet-derived growth factor [7–9]. These conditions maintain expression of telomerase, leading to increased expansion capacity prior to senescence. This finding is consistent with the reported use of forced telomerase expression in standard MSC culture [10]. We previously published a detailed comparison of MAPCs and MSCs including experiments that gauged their differential potential and transcriptome differences and the influence of cell culture conditions on cell phenotype [11]. Clinical-grade MAPCs isolated and cultured under these conditions have been used to generate a master cell bank, allowing production of uniform clinical doses without the use of multiple donors. MAPCs demonstrate a number of favorable characteristics including genetic stability, extensive expansion capacity, and low immunogenicity profiles [12]. These cells are currently being tested in phase II clinical development as an “off-the-shelf” infused cell product for ulcerative colitis, ischemic stroke, and graft versus host disease [13–16].

A single master cell bank has been sufficient to support these and previous phase I studies in acute myocardial infarction and allogeneic bone marrow transplant, in which all doses were safe and well tolerated [13, 17].

Despite ongoing evaluation in autoimmune and allogeneic solid organ transplantation settings, data supporting the immune regulatory potential of clinical-grade MAPCs are limited. Functional improvement after administration of adult-derived adherent stromal cells has been demonstrated in multiple preclinical models of injury or disease, but the mechanisms by which these outcomes are accomplished remain poorly understood [16, 18]. An understanding of the specific mechanisms by which beneficial effects are obtained after treatment with adult stromal cells will be important for refining both dosage and route of delivery of these cells in clinical development as therapeutic agents for the treatment of graft versus host disease, cardiac indications, and central nervous system injury and disease pathologies [14, 19–23].

Both MAPCs and MSCs have been shown to have strong immunomodulatory properties [9, 24–26]. Multiple soluble factors have been implicated in immunomodulation including indoleamine 2,3-dioxygenase (IDO; also known as INDO), interleukin-6, prostaglandin E2, and transforming growth factor-β1 (TGF-β1) [27–30]. Documented mechanisms include suppression of T-cell proliferation [9, 24], inhibition of monocyte-derived dendritic cell differentiation and maturation [31, 32], modulation of B-cell functions [33], modulation of natural killer cell activity [34], and promotion of the generation of regulatory T cells [35].

We previously described immune-modulatory factors secreted by MAPCs in response to interferon-γ (IFN-γ), a cytokine secreted by type 1 T-helper CD4+ cells, CD8+ natural killer cells, and monocytes/macrophages [36]. In this study, we examined transcriptional and secretoome changes induced in PBMCs and MAPCs in Transwell (Corning, Corning, NY, https://www.corning.com) following exposure to anti-CD3/anti-CD28 (3/28) that activated T cells within the PBMC milieu. Transcriptional and protein secretome crosstalk signatures for MAPCs and PBMCs were reported, and the hierarchical importance and redundancy of the various factors identified were evaluated.

**Materials and Methods**

**MAPC Culture and Characterization**

Clinical-grade MAPCs, generated by Atherys, were isolated from donor bone marrow aspirate from a black women aged 24 years and cultured in fibronectin-coated tissue culture plastic flasks, as described previously [37–39]. Human MAPCs were isolated from a single bone marrow aspirate obtained with consent from a healthy donor and seeded onto fibronectin precoated vessels at 2,000 cells/cm². Cell cultures were maintained under low oxygen tension in a humidified atmosphere of 5% CO₂. Cells were cultured subconfluent in MultiStem culture medium comprising low-glucose Dulbecco’s modified Eagle’s medium (Invitrogen, Life Technologies; Thermo Fisher Scientific, Waltham, MA, https://www.thermofisher.com) supplemented with fetal bovine serum (FBS; Atlas Biologicals, Fort Collins, CO, http://atlasbio.com), insulin-transferrin-selenium liquid media supplement (Sigma-Aldrich, St. Louis, MO, https://www.sigmaaldrich.com), epidermal growth factor (R&D Systems, Minneapolis, MN, https://www.rndsystems.com), hepatic growth factor (R&D Systems), dexamethasone (Sigma-Aldrich), penicillin/streptomycin (Invitrogen, Life Technologies; Thermo Fisher Scientific), 2-phospho-L-ascorbic acid (Sigma-Aldrich), and linoleic acid-albumin (Sigma-Aldrich). Cells were passaged every 3–4 days, when cultures reached 50%–60% confluence, and harvested using trypsin/EDTA (Invitrogen, Life Technologies; Thermo Fisher Scientific). Subconfluent cultures were maintained for up to 40–70 population doublings (PDs). The PD was calculated according to the number of cells initially seeded (C₀) and the number of cells harvested (Cₙ) using the following equation: PDₙ = PD₀ + log(C₀ / Cₙ) / log 2. Flow cytometric analysis of surface-expressed antigens confirmed that MAPCs used in this study were a homogenous population. The cells were positive (>80%) for CD90, CD123, and CD105 and negative (<5%) for MHC class II and CD45 (antibodies were from BD Biosciences, San Jose, CA, http://www.bdbiosciences.com). Cells were cryopreserved in Plasma-Lyte A (Baxter Healthcare, Washington, DC, http://www.baxter.com) with dimethyl sulfoxide and human serum albumin. Immediately prior to their use for in vitro assays, MAPCs were thawed and washed twice in complete culture medium.

G0 banding was performed after 3 hours of colcemid treatment of the cells, which were then harvested according to a standard cytogenetic protocol. The resulting metaphase cells were evaluated by G banding, and 20 metaphases were completely analyzed at a 400- to 475-band-level resolution. Endothelial differentiation was performed by plating 20,000 cells/cm² in fibronectin-coated wells in MultiStem culture medium. The next day, medium was changed to basal medium (MultiStem culture medium without serum and growth factors) containing 10 ng/ml vascular endothelial growth factor (VEGF; R&D Systems) for 7 days. Half of the medium was replaced every 3–4 days. Hepatic differentiation was performed by plating 30,000 cells/cm² in 2% Matrigel-coated wells (Corning) in MultiStem culture medium. The next day, medium was changed to basal medium containing 20 ng/ml hepatocyte growth factor (HGF) and 10 ng/ml FGF4 (both from R&D Systems) for 14 days. Half of the medium was replaced every 3–4 days. Differentiation toward neurons was initiated by plating 7,000 cells/cm² on fibronectin-coated wells in MultiStem culture medium. The next day, medium was changed to basal medium containing 20 ng/ml hepatocyte growth factor (HGF) and 10 ng/ml FGF4 (both from R&D Systems) for 14 days. Half of the medium was replaced every 3–4 days. Differentiation toward neurons was initiated by plating 7,000 cells/cm² on fibronectin-coated wells in MultiStem culture medium. The next day, medium was changed to neurobasal A medium containing SHH (100 ng/ml) and FGF8 (10 ng/ml) (both from R&D Systems). At the end of each differentiation, total RNA was extracted from the cells, and cDNA was generated by reverse transcription.
and PBMCs were harvested 18 hours later for quantification of the bottom wells, and 1.5 ml of human PBMCs at 4 milliliter (6 \* 10^6 cells) per well was labeled with a total of 20 \* 3/28 stimulation of PBMCs was started immediately. After 3 days, MAPC-CM samples were immunodepleted using a MARS-14 column (4.6 x 50 mm) designed to deplete 14 abundant proteins (albumin, IgG, antitrypsin, IgA, transferrin, haptoglobin, fibrinogen, \( \alpha_2 \)-macroglobulin, \( \alpha_1 \)-acid glycoprotein, IgM, apolipoprotein A1, apolipoprotein AII, complement C3, and transthyretin; Agilent Technologies) that comprise 94% of the total protein in serum prior to characterization by liquid chromatography-coupled tandem mass spectrometry (LC-MS/MS). The immunoaffinity-column antibodies and buffer components are designed to interact with the major serum components in a denaturing but nonreducing buffer A, resulting in the removal of the major serum components without removing secretome components that potentially bind these major serum proteins.

T-Cell Activation and Transwell Assays

The immunosuppressive properties of MAPCs were evaluated in T-cell activation assays using whole-PBMC cultures obtained from healthy consenting donors. T cells were panactivated in vitro by incubation for 1–5 days with 10 ng/ml anti-CD3ε (clone HIT3a; murine IgG2a, \( \kappa \)) and 5 \( \mu \)g/ml anti-CD28 (clone CD28.2, IgG1, \( \kappa \)) monoclonal antibodies (Becton Dickinson/PharMingen, East Rutherford, NJ, http://www.bd.com). T-cell activation assays were performed in semipermeable Transwells using human MAPC growth medium containing 18%-FBS. In addition, 2.5 ml of human MAPCs at 4 \* 10^6 cells per milliliter (10 \* 10^6 cells) were seeded in the bottom wells, and 1.5 ml of human PBMCs at 4 \* 10^6 cells per milliliter (6 \* 10^6 cells) were incubated on the upper side of each Transwell insert of 6-well 0.4- \( \mu \)m Transwell dishes. On seeding, 3/28 stimulation of PBMCs was started immediately. After 3 days, each well was labeled with a total of 20 \( \mu \)Ci \(^{3}H\)-thymidine per well, and PBMCs were harvested 18 hours later for quantification of \(^{3}H\)-thymidine incorporation. Duplicate plates were used from which cells were harvested for mRNA analysis, and cell-conditioned medium was harvested for secretome analysis. All reported results are from triplicate measurements. Supernatants from the Transwell assays were spun down at 400g for 5 minutes at 4°C to separate cells and debris, and the supernatants were transferred to new 50-ml conical tubes. Conditioned medium samples were concentrated 50-fold with an Amicon Ultra-15 centrifugal filter with a 3,000-dalton molecular-weight cutoff (Millipore, Billerica, MA, http://www.emdmillipore.com), snap frozen on dry ice and stored at \(-80^\circ\)C until analysis. For the determination of the percentage of cells positive for human leukocyte antigen (HLA), MAPCs were cultured in Transwell with 3/28-activated PBMCs in RPMI containing 5% inactivated human serum, 2 mM ultraglutamine, and 100 U penicillin/streptomycin. After 3 days, MAPCs were harvested, and flow cytometry was performed.

Flow Cytometric Analysis

Fluorescence-activated cell sorting (FACS) was performed with antibodies purchased from Becton Dickinson, including anti-HLA-DR monoclonal antibody (clone G46-6; catalog no. 555812) and mouse IgG2a isotype control monochlonal antibodies. Analysis was performed on a MACSQuant flow cytometer (Miltenyi Biotec, Bergisch Gladbach, Germany, http://www.miltenyibiotec.com). For some analyses, MAPCs were cultured in the presence of 25 ng/ml IFN-\( \gamma \) (catalog no. 285IF100; R&D Systems) for 3 days prior to flow cytometry.

Processing of Medium and Conditioned Medium Samples, Immunodepletion of Major Serum Components

Samples were thawed and assayed for protein content using a bicinchoninic acid assay (BCA) and bovine serum albumin standard (Thermo Fisher Scientific) [40]. MAPC-conditioned media (MAPC-CM) samples were buffer exchanged into Agilent buffer A (proprietary media formulation; Agilent Technologies, Santa Clara, CA, http://www.agilent.com) concentrated, and the total amount of protein present in the samples was determined.

Recognizing that the presence of even 1% serum limits the depth of coverage and identification of secreted cell products, MAPC-CM samples were immunodepleted using a MARS-14...
respectively. Cysteine had a static modification mass of +57 Da, and trypsin specificity was used. An in-house suite of programs [42] was used to identify peptides using sequence-reversed matches to estimate the false discovery rate (FDR). Of the 798,803 acquired MS2 scans from the 24 samples, there were 132,333 that passed score thresholds with 6,649 matches to reversed sequences, giving an estimated overall peptide FDR of 5.3%. Protein identification lists were prepared using standard parsimony principles. Proteins were required to have two or more fully tryptic peptides with distinct sequences. The number of identified proteins per sample ranged from 59 to 287 (nonredundant proteins, excluding contaminants), and the protein FDR per sample ranged from 1.2% to 6.4%. An in-house algorithm was used to compare identified peptide sets between proteins having peptides in common and to group together highly homologous identifications into families. Shared and unique peptide status was recomputed across all proteins after family grouping. Shared peptide counts were fractionally split based on relative total unique counts of the proteins that had those peptides in common. These corrected total spectral counts per protein were used in the quantitative comparisons described below. At equimolar concentrations, a large protein will generate more peptides and have higher spectral counts than a small one of similar composition and structure. To correct for this effect, we divided the spectral count for a given protein by the molecular mass of that protein so the abundance more closely resembled molar concentrations rather than weight concentrations. We recognize that the relationships may not be strictly linear and accordingly use the term “apparent abundance” when comparing the signals from two proteins. Proteins identified by LC-MS/MS analysis were considered to be PBMC or MAPC secretome candidates if their normalized, corrected, molecular-weight-scaled spectral counts were greater than 2.5. Spectral counts observed for each protein are indicative of the relative abundance of each protein in the sample.

Bioinformatics

Swiss protein identifiers (more human-informative but less stable identifiers) were mapped to their Swiss protein accessions for subsequent analysis when needed. The potential for MAPCs to manufacture the secretome components identified by LC-MS/MS was confirmed by microarray analysis, described below. Conversion of Illumina identifiers (Illumina_ID) to Uniprot IDs and removal of redundancies was performed using DAVID Bioinformatics resource 6.7 software (http://david.abcc.ncifcrf.gov/) [Da-Wei Huang, personal communication]. An exploratory data analysis by means of principal components analysis and hierarchical clustering of the secretome candidate spectral count changes was done to find potential outliers and patterns in the data.

Global mRNA Analysis

The mRNA was isolated from MAPCs or PBMCs using the RNeasy Mini Prep (Qiagen, Valencia, CA, https://www.qiagen.com) and Ambion DNASe (Invitrogen, Life Technologies: Thermo Fisher Scientific) treatment. After confirmation of mRNA quality, samples were run in duplicate on Human WG-6 V2 gene chips using the Illumina platform (Illumina Inc., San Diego, CA, https://www.illumina.com). Data analysis was performed using Illumina Bead Studio software, Microsoft Excel (Microsoft, Redmond, WA, http://www.microsoft.com), and Invitrogen Linnea Pathway software (Thermo Fisher Scientific). The differential expression analysis parameters used were an Illumina custom error model, rank invariant normalization, false-discovery computation, and a twofold differential expression cutoff. A 95% confidence interval was used as the threshold for statistical significance. The resulting lists of differentially expressed genes were used as input for data analysis. Following microarray analysis, several targets were selected for quantitative PCR analysis. Primers were designed to the target mRNA sequences using ABI Prism Primer Design software (Applied Biosystems, Inc., Carlsbad, CA). These primers were then validated against Human Reference RNA (Stratagene, Santa Clara, CA, http://www.stratagene.com), Human Brain RNA (Stratagene), Human Liver RNA (Stratagene), Human Umbilical Vein RNA (Stratagene), 100 ng genomic DNA (Roche, Basel, Switzerland, http://www.roche.com), and water.

RESULTS

MAPC Characterization

Figure 1A shows the visual morphology of MAPC between 30 and 35 PDs taken by phase-contrast microscopy (>40). MAPCs showed normal karyotype 46, XX (G-banding) (Fig. 1B). FACS analysis was used to monitor cell surface markers. MAPCs were positive (>80%) for CD90, CD130, and CD105 and negative (<5%) for MHC class II and CD45 (Fig. 1C). The differentiation potential of MAPCs was monitored using different stimulation protocols (described in the Methods section). MAPCs showed a significant increase in endothelial (vWF, PECAM), hepatic (albumin, CYP2B6), and neuronal (MBP, Tau) transcripts at day 7 or 14 of differentiation (Fig. 1D). Anti-CD3/anti-CD28 treatment-induced T-cell proliferation was inhibited using different PBMC:MAPC effector ratios (Fig. 1E).

Overview of Secretome Analysis and Transcriptional Changes

Protein secretomes and transcriptional changes and were monitored in PBMCs, 3/28-activated PBMCs, and 3/28-activated PBMCs exposed to MAPCs for 72 hours in a Transwell system that prevented direct cell-cell contact. An overview of the experimental design is presented in Figure 1F (left). Ficoll-purified PBMCs used in our studies consisted primarily of lymphocytes (CD4+ and CD8+ T cells, CD56+ natural killer cells, CD19+ B cells) and monocytes (CD11b+). The number of secretome proteins identified and the overlap between treatment groups is presented in Figure 1F (center). The number of genes showing differential transcriptional activity induced by 3/28 treatment and following solution-phase interaction between 3/28-activated PBMCs and MAPCs is presented in Figure 1F (right). The 3/28 activation of T cells within the PBMC milieu induced differential expression of 2,925 genes. Overall, 650 (22%) of these transcripts were differentially expressed when 3/28-activated PBMCs were exposed to MAPCs in Transwell, and 622 (96%) were cross-correlated (i.e., mRNAs upregulated in 3/28-activated PBMCs were downregulated following exposure to MAPCs, and mRNAs downregulated in 3/28-activated PBMCs were upregulated following exposure to MAPCs). MAPCs exposed to 3/28-activated PBMCs showed differential expression of 1,247 MAPC genes. Crosstalk was demonstrated by this reciprocal transcriptional regulation.

Secretome Overview

A wide range of functionally distinct classes of molecules were identified by LC-MS/MS. Diverse families including matrix
metalloproteases such as the zymogen-form of MMP2 (supplemental online Fig. 1), protease inhibitors, proteins that regulate extracellular divalent cation concentrations (S100A8, S100A9), and CXCR2 ligands CXCL1 and CXCL7. Our data set included classically secreted proteins, glycosylphosphatidylinositol-linked proteins, single-pass type 1 membrane proteins, and proteins classically identified as endoplasmic reticulum or cytoplasmic residents but that have been identified from previous studies as being secreted via various nonclassical routes, including components of extracellular microvesicles such as exosomes, or by ectodomain-shedding mechanisms [36].

A total of 26 proteins were identified above detection thresholds in the 3/28-activated PBMC-MAPC interactome (Fig. 2), and the complete data set of 239 proteins identified is presented in supplemental online Table 1. Decorin (encoded by the PGS2 gene) showed the greatest increase in spectral counts in the 3/28 PBMC-MAPC interactome, followed by QSOX1, MMP2, and PCOC1 (Fig. 2). Biglycan (encoded by the PGS1 gene) was also detected. All of these molecules are involved in extracellular matrix (ECM) remodeling. Decorin and biglycan function as secreted “ligand traps” that sequester TGF-β1, preventing TGF-β1 binding to TGF-β receptors until released from decorin. Decorin (and biglycan) effectively interfere with downstream signal transduction activity through canonical TGF-β1 receptors, redirecting TGF-β1 toward cells and ECM components bearing decorin- and biglycan-binding domains.

Figure 1. Characterization of human MAPCs. (A): Morphology of the cells between population doublings 30–35 taken by phase-contrast microscopy (×40). (B): Normal karyotype 46,XX (G-banding). (C): Cell-surface marker staining of MAPCs show that MAPCs are positive (>80%) for CD90, CD130, and CD105 and negative (<5%) for major histocompatibility complex class II and CD45. (D): MAPCs show significant transcriptional increase in endothelial (vWF, PECAM), hepatic (albumin, CYP2B6), and neuronal (MBP, Tau) markers at day 7 or 14 of differentiation. Data are shown as relative expression levels ± SEM compared with undifferentiated cells. (E): Anti-CD3/CD28-induced T-cell proliferation inhibition using different PBMC:MAPC effector ratios, as indicated. (F): Design of the Transwell system (left). Ficoll-purified PBMCs and MAPCs were exposed to each other for 72 hours in the presence or absence of anti-CD3 and anti-CD28. The total number of secreted proteins (minimum of two peptides/protein) detected and the number of genes with differential mRNA expression between samples (up- or downregulated twofold or more) are indicated (right). Abbreviations: 3/28, anti-CD3/anti-CD28-activated; HLA, human leukocyte antigen; MAPCs, multipotent adult progenitor cells; PBMCs, peripheral blood mononuclear cells.
MAPCs Induce Cell Cycle Arrest in 3/28 PBMCs

We previously documented that addition of human MAPCs to 3/28-activated PBMCs resulted in a dose-dependent inhibition of T-cell proliferation occurring at a T cell:MAPC ratio of 8:1 [43]. In an effort to further understand the mechanisms by which this block of proliferation occurred, we used a Transwell interaction assay and documented that a substantial contribution to the block of PBMC proliferation was the result of MAPC-secreted soluble factors [43]. In the present studies, we documented that MAPCs in Transwell altered transcription of a number of genes involved in the cell cycle (Fig. 4; Table 1). The 3/28-activated PBMCs showed a 14.7-fold increase in transcription of cyclin D (CCND2; from 183 to 2,765) and a 14.8-fold increase in cyclin D’s cyclin dependent kinase (cdk) partner cdk6 (from 93 to 1,415) (Fig. 4). Solution-phase interaction between 3/28-activated PBMCs and MAPCs in Transwell maintained cyclin D mRNA transcription 66% lower and prevented more than 50% of its cdk6 partner’s mRNA transcription (Fig. 4). In addition, 3/28 activation of PBMCs induced a 37.8-fold increase (from 41 to 1,600) in transcription of cyclin A (CCNA2) and a 2.14-fold increase (from 180 to 395) in transcription of cyclin C (CCNC). Exposure to MAPC in Transwell resulted in 50% less cyclin A transcription and completely prevented the increase in transcription of cyclin C induced by 3/28 activation (Fig. 4). The 3/28 activation of PBMCs induced a 94.8-fold increase (from 50 to 4,917) in transcription of CDC20, a key component of the anaphase-promoting complex. The E3 ubiquitin ligase activity of this complex polyubiquitinates targeted cell cycle proteins, marking them for degradation by the 26S proteasome [44]. Exposure to MAPCs in Transwell blocked more than 50% of CDC20 transcription (Fig. 4).

Cell cycle arrest is coupled to metabolic stress such as tryptophan depletion. In 3/28-activated PBMCs, IDO was transcriptionally upregulated 5.6-fold (from 36.3 to 207.7) (Fig. 3C). On exposure of 3/28-activated PBMCs to MAPCs, IDO transcription was further increased 3.3-fold (from 207.7 to 704.8) (Fig. 3C). IDO was also transcriptionally upregulated 11.6-fold (from 33.3 to 384.9) in MAPCs exposed to 3/28-activated PBMCs (Fig. 3C). Depletion of tryptophan can be compensated for in part by increased tryptophan uptake. In mammalian cells, transporter-mediated tryptophan uptake occurs mainly via the ubiquitously expressed neutral amino acid transporter system L, a heterodimer composed of a heavy glycoprotein chain (CD98, encoded by SLC3A2) and one of two catalytic light chains LAT1 or LAT2, encoded by SLC7A5 and SLC7A8 genes [45–48]. SLC7A5 was transcriptionally upregulated 14-fold (from 279 to 3,924) in 3/28-activated PBMCs.

The cytoplasmic general control nonderepressible GCN2 kinase (also termed eukaryotic translation initiation factor 2-α kinase 4 [EIF2AK4]) serves as a metabolic monitor for uncharged transfer RNAs (tRNAs). Depletion of the essential amino acid tryptophan results in an increase of uncharged tryptophan tRNA (tRNA(try); activating GCN2, which phosphorylates eukaryotic translation initiation factor 2α (eIF2α) and blocks protein synthesis, arresting cell growth. This pathway is critical for T-cell suppression by IDO because the genetic deletion of EIF2AK4 (Gcn2) abolishes this response [49, 50]. In 3/28-activated PBMCs exposed to MAPCs, GCN2 reporter genes DDIT3, GADD45α, and IFNGR2 [50] were transcriptionally upregulated 8.1-fold (from 69.25 to 563.7), 2.1-fold (from 145.3 to 307.7), and 3.8-fold (from 123.8 to 478.6), respectively. Increased GCN2 kinase activity could lead to phosphorylation of eIF2α, inhibition of cyclin D1 translation, and subsequent cell cycle arrest. These events are reflected in the transcriptional signatures presented in our study. Of note, MAPCs do not show transcriptional changes in any of the GCN2 kinase reporter genes, suggesting that GCN2 kinase is not activated within MAPCs exposed to 3/28-activated PBMCs.

Figure 2. The 3/28 PBMC-MAPC interactome. Twenty-six proteins were identified with spectral counts above detection thresholds (minimum of two peptides/protein) in the 3/28 PBMC-MAPC interactome. Abbreviations: 3/28, anti-CD3/anti-CD28-activated; MAPCs, multipotent adult progenitor cells; PBMCs, peripheral blood mononuclear cells.

Genes of Interest Up- and Downregulated in 3/28-Activated PBMCs Exposed to MAPCs

Overall, 2,925 genes were differentially expressed following 3/28-activation of PBMCs. MAPCs alter a diverse array of genes involved in broad aspects of PBMC metabolic activity and clusters of genes regulating innate and adaptive immune responses. A selection of the 365 genes with transcriptional upregulation in 3/28-activated PBMCs that was blocked by MAPCs in Transwell is presented in Figure 3A, and a selection of the 257 genes with transcriptional downregulation in 3/28-activated PBMCs that was blocked by MAPCs in Transwell is presented in Figure 3B. There is also a small set of genes with transcriptional upregulation in 3/28-activated PBMCs that was further increased by exposure to MAPCs in Transwell (Fig. 3C). Granzyme B (GZMB) is a proapoptotic serine protease that proteolytically processes decorin, disrupting decorin sequestration of TGF-β1 [40]. GZMB is upregulated 43-fold (from 65.2 to 2,902) in 3/28-activated PBMCs, and MAPCs in Transwell substantially prevented this upregulation (from 65.2 to 913.6), a 3-fold inhibition of transcription (Fig. 3C).

T-cell proliferation with a consistent half-maximal block of
A number of eukaryotic translation initiation factors were transcriptionally upregulated in 3/28-activated PBMCs. Exposure to MAPCs in Transwell blocked transcriptional upregulation of key regulatory components including EIF1AX, EIF2B3, EIF3B, EIF4A3, EIF4G1, and EIF5B. In addition, 3/28-activated PBMCs downregulated EIF4EBP3 3-fold (from 191.3 to 62), and exposure to MAPCs in Transwell prevented 70% of this transcriptional inhibition. EIF4EBP3 binds EIF4E and regulates its activity by preventing EIF4E assembly into the EIF4F complex.

Figure 3. Genes of interest up- and downregulated in 3/28-activated PBMCs exposed to MAPCs. (A): MAPCs in Transwell blocked transcriptional upregulation of 365 genes in 3/28-activated PBMCs. (B): MAPCs in Transwell prevented transcriptional downregulation of 257 genes in 3/28-activated PBMCs. (C): MAPCs promote further increase in a small set of genes transcriptionally upregulated in 3/28-activated PBMCs. PBMC transcriptional signatures are organized and categorized to demonstrate the range of impact of the MAPC secretome. Abbreviations: 3/28, anti-CD3/anti-CD28-activated; co-stim TCR, T cell receptor costimulation-related molecules; MAPCs, multipotent adult progenitor cells; PBMCs, peripheral blood mononuclear cells.
Genes of Interest Up- and Downregulated in MAPCs Exposed to 3/28-Activated PBMCs

MAPCs exposed to 3/28-activated PBMCs showed differential expression of 1,247 MAPC genes, with crosstalk demonstrated by this reciprocal transcriptional regulation (Fig. 1F, right). A selection of the 829 genes with transcription that was upregulated in MAPCs exposed to 3/28-activated PBMCs in Transwell is presented in Figure 5A, and a selection of the 418 genes with transcription that was downregulated is presented in Figure 5B. In MAPCs exposed to 3/28-activated PBMCs, IDO was transcriptionally upregulated 11.6-fold (from 33.3 to 384.9) and SLC7A5 was transcriptionally upregulated 14-fold (from 279 to 3,924) (Fig. 5A).

MAPCs Maintain Low Levels of MHC Class II Cell Surface Expression: Potential Transcriptional Mechanisms Include Upregulation of CD74, Ubiquitin, and Ubiquitin E3 Ligase MARCH3

In MAPCs exposed to 3/28-activated PBMCs, some of the highest transcriptional upregulation was observed in genes classically thought of as part of the MHC class II antigen-processing system. Transcriptional regulation of the MHC class II system is controlled predominantly by IFN-γ, and 3/28 activation of PBMCs transcriptionally upregulated IFN-γ 46.7-fold (from 37.2 to 1,788.9). Although exposure of 3/28-activated PBMCs to MAPCs blocked transcriptional upregulation of IFN-γ substantially (−6.8-fold), it did not eliminate it (Fig. 3A). The MHC class II allele HLA-DRA was transcriptionally upregulated 130-fold (from 31 to 4,046.2), HLA-DPA1 was upregulated 36-fold (from 64.9 to 2,316.6), and HLA-DQA1 was upregulated 83-fold (from 37.1 to 3,066.7). CD74, also known as the MHC class II invariant chain, provides a scaffold on which the heterodimeric MHC class II molecules fold during protein synthesis in the endoplasmic reticulum. CD74 also chaperones MHC class II through the complex trafficking machinery required for MHC class II loading of antigenic peptides for presentation of antigenic peptides at the plasma membrane for antigen-specific programming of CD4+ T cells that regulate adaptive immunity. CD74 was upregulated 62-fold (from 42.5 to 2,650.7) in MAPCs exposed to 3/28-activated PBMCs—the sixth highest upregulated gene. Genes encoding many of the molecules regulating antigen processing were upregulated in a similar fashion (Fig. 6A).

Cell surface expression of HLA-DR on MAPCs was monitored by flow cytometry. After exposure of MAPCs to 3/28-activated PBMCs in Transwell, less than 5% of the cells showed plasma membrane surface expression of HLA-DR (Fig. 6B). After 3-day exposure to 25 ng/ml IFN-γ in culture, only a modest ∼6% of MAPCs showed cell surface expression of HLA-DR (Fig. 6C). These data suggested the existence of an active mechanism that kept cell surface expression of MHC class II low. Transcriptional upregulation of the MHC class II system on MAPCs was accompanied by a 70.1-fold increase (from 30.8 to 2,157.4) in ubiquitin and a 2.5-fold increase (from 54.4 to 135.4) in the E3 ligase ubiquitin ligase MARCH3 (Fig. 6A). MARCH3 is a homolog of MARCH1 that polyubiquitinates Lys-225 on the cytoplasmic carboxy-terminal tail of the β chain of the MHC class II heterodimer [51]. Lys-225 ubiquitination tags MHC class II molecules for trafficking off the plasma membrane and into endosomal multivesicular bodies [52]. A concomitant decrease in transcription of positive costimulatory inputs and increased transcription of negative costimulatory molecules was also observed. A distinct absence of transcriptional upregulation of positive costimulatory molecules such as CD80 (B7.1) and CD86 (B7.2) that provide costimulatory input to CD4+ T cells via CD28 was maintained in MAPCs, whereas negative costimulatory molecules CTLA-4, were transcriptionally upregulated. Negative costimulatory molecule CTLA-4 was transcriptionally upregulated 6.9-fold (from 37.4 to 256.1) in MAPCs exposed to 3/28-activated PBMCs (Fig. 6A). Moreover, negative costimulatory molecule TNFRSF14 was transcriptionally upregulated 5-fold (from 634.2 to 3,272.8) in MAPCs exposed to 3/28-activated PBMCs (Fig. 6A). In addition, ICAM-1 was transcriptionally upregulated 11.9-fold (from 41.3 to 493) in MAPCs.
| Cyclin-CDK complex | Cyclin, cdk | 3/28 PBMCs | 3/28 PBMC-MAPC | Function |
|-------------------|------------|-----------|---------------|----------|
| S-Cdk             | Cyclin A (CCNA2) | 37.8     | (-2.3) | Cyclin A/Cdk2 promotes DNA replication |
|                   | Cdk2       | 5.1       | NC            |          |
| M-Cdk             | Cyclin B   | NC        | NC            | Destruction of cyclin B1 inactivates Cdk1, allowing M-phase exit and cell division |
|                   | Cdk1 (CDC2) | 19.84     | NC            |          |
| G1-Cdk            | Cyclin C (CCNC) | 2.14     | (-2.15) | Cyclin C/cdk3 promotes G0 exit |
|                   | Cdk3 (CDKN3) | 8.79      | NC            |          |
| G2-Cdk            | Cyclin D (CCND2) | 14.7     | (-3.0) | Cyclin D/Cdk6 accumulation promotes G2 exit to S phase |
|                   | Cdk6       | 14.8      | (-2.2) |          |
| G1/S-Cdk          | Cyclin E   | NC        | NC            | Cyclin E/cdk2 promotes transition from the G1 to the S phase |
|                   | Cdk2       | 5.1       | NC            |          |

**Cell cycle regulators**

| Cyclin-CDK complex | Cyclin, cdk | 3/28 PBMCs | 3/28 PBMC-MAPC | Function |
|-------------------|------------|-----------|---------------|----------|
| AK1               | (-2.44)   | 3.20     |               | Adenylate cyclase, involved in maintenance of energy homeostasis |
| BCCIP             | 2.67       | (-2.42) |               | Cdk-interacting protein |
| BOP1              | 4.85       | (-3.95) |               | Cell cycle regulation |
| C10orf119         | 2.68       | -2.37    |               | MCMBP, component of the MCM complex that acts as a regulator of DNA replication |
| CCG1              | (-4.18)   | 4.67     |               | Acts as an assembly platform for p protein signaling complexes involved in cell cycle regulation |
| CDC123            | 3.01       | (-2.30) |               | Required for S phase entry |
| CDC20             | 94.75      | (-2.0)  |               | Activates anaphase promoting complex |
| CDC25A            | 8.54       | (-2.57) |               | DNA damage monitor |
| CDC2L6 (Cdk19)    | (-2.44)   | 2.02     |               | Cdk8 paralogue |
| Cdk5R1            | (-2.99)   | 2.12     |               | Cdk5 regulatory protein |
| CDKN2D (p19)      | NC         | 2.16     |               | Cyclin-dependent kinase 4 (CDK4) inhibitor |
| Cks2              | 11.20      | (-2.31) |               | Modulates p27 degradation |
| DUSP1             | (-5.58)   | 3.63     |               | Negative regulation of cell cycle |
| GSPT1             | 2.61       | (-2.22) |               | G1/S transition of cell cycle |
| MCM4              | 15.12      | (-2.06) |               | Replicase helicase |
| MCM10             | 12.26      | (-2.30) |               | Replication fork assembly |
| PBK               | 17.43      | (-2.6)  |               | G2/M checkpoint |
| POLD4             | (-2.69)   | 2.98     |               | Required for optimal DNA polymerase (delta) activity involved in cell cycle regulation |
| PPAN              | 3.60       | (-3.25) |               | Regulates cell growth |
| PSMD1             | 2.35       | (-3.31) |               | Regulation of ubiquitin-protein ligase activity involved in cell cycle |
| PWP1              | 2.77       | (-2.13) |               | Unknown function |
| PWP2              | 2.61       | (-2.16) |               | Unknown function |
| RAN               | 2.85       | (-2.05) |               | The trimeric complex (Ran-GTP-RANBP1) regulates the transport of protein and nucleic acids across the nuclear membrane, controlling progression through the cell cycle |
| RANBP1            | 7.08       | (-2.43) |               | Forms a trimeric complex (Ran-GTP-RANBP1) regulating the transport of protein and nucleic acids across the nuclear membrane, controlling progression through the cell cycle |
| RFC3              | 6.75       | (-2.05) |               | Cell cycle regulation |
| RUVBL1            | 5.21       | (-2.79) |               | Cell cycle regulation |
| RUVBL2            | 4.36       | (-2.25) |               | Cell cycle regulation |
| Skp2              | 3.4        | (-2.45) |               | Substrate recognition component of the multimeric Skp, Cullin, F-box containing complex (SCF), an E3 ubiquitin ligase that marks targeted cell cycle proteins for proteasomal degradation |
exposed to 3/28-activated PBMCs (Fig. 6A). We previously described ectodomain shedding of ICAM-1 detectable in the secretome of MAPCs exposed to IFN-γ and its potential role in dampening inflammation [36].

**DISCUSSION**

Our previous studies documented the effects of individual compounds such as IFN-γ, lipopolysaccharide (LPS), or the CD74 ligand RTP1000 on proteins secreted by MAPCs [36]. In this study, we mapped transcriptional and proteomic signatures for MAPCs exposed to a more complex solution-phase signal: that of anti-CD3/anti-CD8 panactivated T cells within the PBMC milieu (3/28-exposed to 3/28-activated PBMCs). The signatures observed were not random; rather, they suggest a coordinated series of molecular events.

The secreted molecules showing the greatest increase in spectral counts in the 3/28 PBMC-MAPC interactome were decorin (encoded by the PGDS2 gene) followed by QSOX1, MMP2, and PCOC1 (Fig. 2; supplemental online Table 1). All of these molecules are involved in extracellular matrix remodeling. Decorin binds tropoelastin, collagen VI, and TGF-β. Decorin serves as a ligand trap for TGF-β, sequestering and protecting an extracellular pool of active TGF-β. The physiological relevance of ligand-trapped TGF-β now becomes dependent on decorin. Bound in the form of a decorin:TGF-β in a 1:1 stoichiometric complex, TGF-β activity can be targeted to bind cells expressing receptor tyrosine kinases (RTK) such as hepatocyte growth factor receptor (HGF/ MET) or epidermal growth factor receptor [53], and release of TGF-β from decorin would be regulated by the extracellular proteolytic activity of GZMB and MMP2 that cleave decorin with concomitant release of TGF-β [42, 54]. GZMB is transcriptionally upregulated 43-fold (from 65.2 to 2,902) in 3/28-activated PBMCs, and MAPCs in Transwell induced a 3-fold increase in spectral counts of all secretome proteins identified in the 3/28 PBMC-MAPC interactome (Fig. 2). A combination of increased decorin secretion coupled with decreased proteolytic processing of decorin by MMP2 and GZMB would be expected to dramatically attenuate TGF-β activity. Decorin also induces rapid secretion of thrombospondin-1 (TSP1) via inhibition of the RTK-coupled Ras kinase [53]. Increased spectral counts for thrombospondin were observed in MAPCs exposed to 3/28-activated PBMCs (supplemental online Table 1). Thrombospondins are potent endogenous inhibitors of angiogenesis through direct effects on endothelial cell migration, proliferation, survival, and apoptosis and by antagonizing the activity of VEGF [58].

Thezymogen form of MMP2 (pro-MMP2) forms a stable complex with the KISS1 protein, preventing its proteolytic processing by furin to release the bioactive decapetide metastin while active MMP2 digests metastin [59]. Metastin binds KISS1R (GPR54), with signaling via this G-protein-coupled receptor inducing focal adhesion formation and actin stress fiber rearrangement in cells expressing the metastin receptor (KISS1R) [60]. MAPCs exposed to 3/28-activated PBMCs transcriptionally upregulated KISS1R 11.2-fold (from 34.5 to 387.4), in support of the hypothesis that MAPCs are poised to respond to metastin present in the media. Increased pro-MMP2 in the secretome would inhibit release of bioactive metastin and dampen activities downstream from metastin signaling.

QSOX1 is a sulfhydryl oxidase required for incorporation of laminin into the ECM, and ECM produced without QSOX1 is defective in supporting cell-matrix adhesion [61]. QSOX1 crosslinks the collagen fibrils of the ECM with laminin, mediating the attachment, migration, and organization of cells into tissues via binding with high affinity to cell surface receptors including epidermal growth factor receptor, α6β4 integrin, and syndecan 1 [62]. The PCOC1 gene encodes the procollagen C-endopeptidase enhancer protein 1, which binds to the C-terminal propeptide of type I procollagen and enhances BMP1 procollagen C-proteinase activity ∼20-fold [63, 64]. The C-terminal processed portion of PCOC1 inhibits MMP2 activity with a half-maximal inhibitory concentration of ∼560 nM [65].

Our data support a molecular mechanism for the ability of MAPCs to block T-cell proliferation via tryptophan depletion and metabolic reprogramming tied to cell cycle arrest. MAPCs in Transwell blocked the upregulation and reversed the downregulation of cell cycle genes transcriptionally regulated in 3/28-activated PBMCs (Fig. 4; Table 1). The signature data are consistent with MAPCs promoting cell cycle arrest in G0 and transcriptional blockade of 3/28-induced proliferation of PBMCs. Arrest is hypothesized to be due in part to metabolic depletion of tryptophan via expression and secretion of IDO. IDO is transcriptionally upregulated in 3/28-activated PBMCs, further upregulated in 3/28-activated PBMCs following exposure to MAPCs, and in MAPCs themselves (Fig. 3C). Increased autophagy, reduces cell migration by regulating the stress response, dual regulator of transcription and autophagy, reduces cell migration by regulating the expression of SPARC (SPRC), consistent with SPRC being detected in 3/28 PBMC and MAPC interactome.

### Table 1. (Cont’d)

| Cyclin-CDK complex | Cyclin, cdk | 3/28 PBMCs | 3/28 PBMC-MAPC | Function |
|--------------------|------------|------------|---------------|----------|
| TIPIN              | 6.77       | (−2.25)    |               | Required for normal progression of S phase |
| TPS3INP1           | (−6.21)    | 3.82       |               | Antiproliferative and proapoptotic protein involved in cell stress response, dual regulator of transcription and autophagy, reduces cell migration by regulating the expression of SPARC (SPRC), consistent with SPRC being detected in 3/28 PBMC and MAPC interactome. |
| TBRG4              | 2.35       | (−2.09)    |               | TGF-β regulator-4; cell cycle progression restoration protein 4 |

Abbreviations: 3/28, anti-CD3/anti-CD28-activated; MAPCs, multipotent adult progenitor cells; NC, no change; PBMCs, peripheral blood mononuclear cells; TGF-β, transforming growth factor β.
transcription, translation, and secretion of IDO would deplete the essential amino acid tryptophan, resulting in an increase of uncharged tRNA(trp) due to decreased supply of tryptophan. In human 3/28-activated PBMCs exposed to MAPCs, we noted a number of transcriptional signature features consistent with tryptophan depletion, including transcriptional upregulation of GCN2 reporter genes DDIT3 (CHOP), GADD45a, and IFNGR2 [50] and decreased transcription of molecular constituents of the eIF2 complex and increased transcription of eIF4EBP3 binding protein that prevents eIF4E assembly into the eIF4F complex. In addition, transcriptional upregulation of the IFN-γ-inducible tryptophanyl-tRNA synthetase (WARS) by 3/28-activated PBMCs (Fig. 3C) reflects a commitment to increased cytokine production in effector T cells and increased metabolic load. Further transcriptional upregulation of WARS in 3/28-activated PBMCs exposed to MAPCs (Fig. 3C) is postulated to reflect an attempt by 3/28-activated PBMCs exposed to MAPCs to capture remaining tryptophan for protein production. In the absence of tryptophan, uncharged tRNA(trp) accumulates and activates GCN2 kinase, triggering downstream signaling and cell cycle arrest. Because MAPCs do not show transcriptional changes in any of the GCN2 kinase reporter genes, we hypothesize that MAPCs tolerate the medium conditions in our study without activating GCN2. Increased tryptophan uptake could account for the ability of MAPCs to overcome the effects of tryptophan shortage, and a component of the transporter-mediated tryptophan uptake system SLC7A5 was transcriptionally upregulated 9.2-fold (from 134.1 to 1,229.7) in MAPCs exposed to 3/28-activated PBMCs. Our results suggest that increased IDO expression is correlated with

Figure 5. Genes of interest up- and downregulated in MAPCs exposed to 3/28-activated PBMCs. (A): The 3/28-activated PBMCs in Transwell upregulated transcription of mRNA for 829 genes in MAPCs. A subset of genes of interest that showed upregulation ≥10-fold is shown. (B): The 3/28-activated PBMCs in Transwell downregulated transcription of mRNA for 418 genes in MAPCs. A subset of genes of interest downregulated more than fourfold is shown. MAPC transcriptional signatures are organized and categorized to demonstrate the range of impact of the 3/28-activated PBMC secretome on the MAPC transcriptional signature. Abbreviations: 3/28, anti-CD3/anti-CD28-activated; ECM, extracellular matrix; MAPCs, multipotent adult progenitor cells; MHC, major histocompatibility complex; PBMCs, peripheral blood mononuclear cells.
increased expression of transporter system components in MAPCs and highlight a potential mechanism by which IDO-secreting MAPCs can both survive under conditions of low tryptophan concentration and further contribute to depletion of tryptophan from the extracellular milieu.

Donor MAPCs are considered to have immune privilege [66]. Our studies document that MAPCs exposed to 3/28-activated PBMCs transcriptionally upregulate the MHC class II system without increased cell surface expression of MHC class II (Fig. 6). In addition, MAPCs transcriptionally upregulate negative costimulatory molecules CTLA-4 and TNFRSF14. CTLA-4 is an inhibitory receptor acting as a major negative regulator of T-cell responses [67, 68]. TNFRSF14, also known as herpes virus entry mediator (HVEM), is a bidirectional switch regulating T-cell activation in a costimulatory or coinhibitory fashion with an outcome that depends on the ligand engaged [69, 70]. CTLA-4 is transcriptionally upregulated 6.8-fold (from 37.4 to 256.1), and TNFRSF14 was transcriptionally upregulated 5-fold (from 634.2 to 3,272.8) in MAPCs exposed to 3/28-activated PBMCs (Fig. 6A). ICAM-1 was transcriptionally upregulated 11.9-fold (from 41.3 to 493) in MAPCs exposed to 3/28-activated PBMCs (Fig. 6A). We previously described ectodomain shedding of ICAM-1 detectable in the secretome of MAPCs exposed to IFN-γ and its potential role in dampening inflammation [36]. Transcriptionally upregulated ubiquitin and the E3 ubiquitin ligase MARCH3 in combination with upregulation of negative costimulatory molecules CTLA-4 and TNFRSF14, we postulate that the net balance of antigen-specific and antigen-independent costimulatory inputs drives tolerance, providing a mechanistic explanation for the “immune-privileged” status of MAPCs.

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CONCLUSION

We quantified transcriptional and secretome changes induced in PBMCs and MAPCs communicating in Transwell before and after exposure to anti-CD3 and anti-CD28 mononclonal antibodies, a panactivation of all CD3+ T cells within the PBMC milieu. This information was used to construct crosstalk signatures for MAPCs and PBMCs. The hierarchical and redundant manner by which the various factors identified are hypothesized to provide both local and systemic immune regulation of inflammation provides a framework for logically evaluating and refining our understanding of the specific mechanisms by which beneficial effects are obtained after treatment with MAPCs.

AUTHOR CONTRIBUTIONS

G.G.B.: conception and design, collection and assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; W.V.H.: conception and design, data interpretation, manuscript writing; A.P.R. and L.L.D.: collection of data, data analysis and interpretation, manuscript writing; P.A.W. and L.T.: assembly of data, data analysis, manuscript writing; A.R.: collection of data, manuscript writing; A.B., L.P., V.D.R., and R.J.D.: data interpretation, manuscript writing; R.T.M.: conception and design, financial support, data analysis and interpretation, manuscript writing, final approval of manuscript.

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