



This information is current as  
of February 24, 2022.

## Differential Efficacy of Human Mesenchymal Stem Cells Based on Source of Origin

Erin Collins, Fei Gu, Maosong Qi, Ivan Molano, Phillip Ruiz, Lingyun Sun and Gary S. Gilkeson

*J Immunol* 2014; 193:4381-4390; Prepublished online 1 October 2014;

doi: 10.4049/jimmunol.1401636

<http://www.jimmunol.org/content/193/9/4381>

**Supplementary Material** <http://www.jimmunol.org/content/suppl/2014/10/01/jimmunol.1401636.DCSupplemental>

**References** This article **cites 38 articles**, 8 of which you can access for free at:  
<http://www.jimmunol.org/content/193/9/4381.full#ref-list-1>

**Why *The JI*? Submit online.**

- **Rapid Reviews! 30 days\*** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

*\*average*

**Subscription** Information about subscribing to *The Journal of Immunology* is online at:  
<http://jimmunol.org/subscription>

**Permissions** Submit copyright permission requests at:  
<http://www.aai.org/About/Publications/JI/copyright.html>

**Email Alerts** Receive free email-alerts when new articles cite this article. Sign up at:  
<http://jimmunol.org/alerts>

# Differential Efficacy of Human Mesenchymal Stem Cells Based on Source of Origin

Erin Collins,\* Fei Gu,\*<sup>†</sup> Maosong Qi,\* Ivan Molano,\* Phillip Ruiz,<sup>‡</sup> Lingyun Sun,<sup>†</sup> and Gary S. Gilkeson\*<sup>§</sup>

Mesenchymal stem cells (MSCs) are useful in tissue repair but also possess immunomodulatory properties. Murine and uncontrolled human trials suggest efficacy of MSCs in treating lupus. Autologous cells are preferable; however, recent studies suggest that lupus-derived MSCs lack efficacy in treating disease. Thus, the optimum derivation of MSCs for use in lupus is unknown. It is also unknown which in vitro assays of MSC function predict in vivo efficacy. The objectives for this study were to provide insight into the optimum source of MSCs and to identify in vitro assays that predict in vivo efficacy. We derived MSCs from four umbilical cords, four healthy bone marrows (BMs), and four lupus BMs. In diseased MRL/lpr mice, MSCs from healthy BM and umbilical cords significantly decreased renal disease, whereas lupus BM MSCs only delayed disease. Current in vitro assays did not differentiate efficacy of the different MSCs. However, differences in MSC efficacy were observed in B cell proliferation assays. Our results suggest that autologous MSCs from lupus patients are not effective in treating disease. Furthermore, standard in vitro assays for MSC licensing are not predictive of in vivo efficacy, whereas inhibiting B cell proliferation appears to differentiate effective MSCs from ineffective MSCs. *The Journal of Immunology*, 2014, 193: 4381–4390.

Mesenchymal stem cells (MSCs) are multipotent progenitor cells readily isolated from a variety of tissues, including bone marrow (BM), umbilical cords (UCs), adipose tissue, and dental pulp. Upon stimulation, MSCs can undergo differentiation into many cell types, including, but not limited to, chondrocytes, osteoblasts, and adipocytes. Their differentiation capacity contributes to their well-studied biological niche in wound healing, because they home to sites of injury and initiate tissue repair and regeneration. Recent recognition of the immunoregulatory functions of MSCs led to exploration of their possible new therapeutic functions.

MSCs modulate the immune system by exerting regulatory effects on various immune cells, such as T and B lymphocytes, NK cells, and dendritic cells (1–4). Many factors contribute to MSCs' capacity to impact a wide array of immune cells. The abilities of MSCs to secrete anti-inflammatory cytokines, expand regulatory T cells, and downregulate costimulatory molecules on APCs are well established (4–7). The expansive immunomodulatory properties of MSCs make them an attractive candidate for the cellular

therapy of autoimmune diseases. MSC therapy has shown positive results and relative safety in uncontrolled trials of human immune diseases, including systemic lupus erythematosus (SLE) (8).

SLE is an autoimmune disease characterized by autoantibody production and subsequent immune complex formation, leading to chronic inflammation and end organ damage. Although the etiology of SLE remains unclear, numerous studies suggest that aberrant immune activation, due to ineffective immune regulation, contributes to the development of disease (9, 10). Current treatment options for SLE have broad immune effects and often interfere with critical immune functions. As such, many patients experience significant side effects, primarily infections, that may rival the manifestations of the disease itself in terms of severity (11, 12). Thus, MSC therapy may provide a novel treatment option for which benefits surpass risks.

Although results have varied depending on the source of origin, preclinical and clinical studies alike show promise in the use of MSCs for the treatment of SLE. We previously showed that allogeneic transplant of BM-derived MSCs from C57BL/6 mice and syngeneic transplant of MSCs from young lupus-prone mice improved established disease in MRL/lpr and NZB/NZW(F1) lupus-prone mice; however, no impact on disease was seen with MSC transplants derived from older, diseased lupus-prone mice (13). These results are consistent with prior work in which BM-derived MSCs from B6 mice transplanted into lupus-prone MRL/lpr mice ameliorated disease (14–16). Conversely, other groups found that syngeneic transplant of BM-derived MSCs in (NZB/NZW)F1 mice did not impact disease outcome (17, 18). Positive effects on disease also were seen when MSCs derived from healthy human BM, UC, and adipose tissue were infused into MRL/lpr mice (16, 19, 20). Moreover, uncontrolled human lupus trials suggest improvement in organ dysfunction in treatment-refractory SLE patients treated with allogeneic MSCs derived from BM or UCs. However, the more limited use of autologous BM-derived MSCs did not elicit such improvement (21–23). In addition to these limited in vivo studies, in vitro examination of lupus patient-derived MSCs suggests defective MSC differentiation and early

\*Division of Rheumatology and Immunology, Department of Medicine, Medical University of South Carolina, Charleston, SC 29425; <sup>†</sup>Department of Rheumatology and Immunology, Nanjing Drum Tower Hospital, Affiliated Hospital of Nanjing University Medical School, Nanjing, Jiangsu 210008, China; <sup>‡</sup>Department of Pathology, University of Miami School of Medicine, Miami, FL 33136; and <sup>§</sup>Medical Research Service, Ralph H. Johnson Veterans Affairs Medical Center, Charleston, SC 29403

Received for publication June 30, 2014. Accepted for publication August 26, 2014.

This work was supported by grants from the Lupus Foundation; the National Institutes of Health (UL1 RR029882 [National Center for Research Resources] and UL1 TR000062 [National Center for Advancing Translational Sciences]); and the Medical Research Service, Ralph H. Johnson Veterans Affairs Medical Center.

Address correspondence and reprint requests to Dr. Erin L. Collins, Department of Medicine, Medical University of South Carolina, 96 Jonathan Lucas Street, Suite 912, Charleston, SC 29425. E-mail address: collinel@musc.edu

The online version of this article contains supplemental material.

Abbreviations used in this article: BM, bone marrow; HBM, healthy BM; hMSC, human MSC; LBM, lupus BM; MSC, mesenchymal stem cell; SLE, systemic lupus erythematosus; UC, umbilical cord.

Copyright © 2014 by The American Association of Immunologists, Inc. 0022-1767/14/\$16.00

signs of cellular senescence (14, 22). Together, these results show variance in the disease-modulating capacity of MSCs based on their origin, with questions remaining regarding the most beneficial source to be used for treatment of SLE.

In any cellular therapy in humans, use of autologous cells is preferred to allogeneic cells, matched or unmatched for immune markers, to prevent allogeneic responses. The aim of this study was to compare the *in vivo* and *in vitro* immunoregulatory capacity of lupus patient-derived MSCs with MSCs from healthy control BM or UCs to determine the differential efficacy of human MSCs (hMSCs) based on their source of origin. We also determined whether current *in vitro* assays used for “licensing” MSC lots for human use are predictive of efficacy *in vivo*. Finally, we used *in vitro* assays, which were more lupus specific, to predict *in vivo* disease-modulating capacity.

## Materials and Methods

### Mice

MRL/*lpr* mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were maintained in the specific pathogen-free animal facility of the Ralph H. Johnson Veterans Affairs Medical Center, and the Institutional Animal Care and Use Committee approved all animal procedures. When the mice developed 30 mg/dl proteinuria (Chemstrip 7), the 15–17-wk-old MRL/*lpr* mice were randomized into four groups: UC MSC transplantation group ( $n = 8$ ), healthy BM (HBM) MSC transplantation group ( $n = 8$ ), lupus BM (LBM) MSC transplantation group ( $n = 16$ ), and PBS injection group ( $n = 8$ ). Three UC MSC lines were injected into two or three mice, three HBM MSC lines were injected into two or three mice, and three LBM MSC lines were injected into five or six mice. A total of  $1 \times 10^6$  cells/mouse was administered *i.v.* in a single dose, and all mice were sacrificed at 8 wk after treatment. Mice were euthanized and organs were collected for processing if they demonstrated signs of distress, such as lack of grooming, eating, or drinking; weight loss  $> 15\%$ ; or proteinuria  $> 500$  mg/dl.

### Isolation and culture of hMSCs

The use of human UC MSCs and BM MSCs was approved by the Institutional Review Board of the Medical University of South Carolina, and all donors provided informed consent. The MSCs were isolated and cultured as previously described, with slight modifications (3, 24). Briefly, human UC MSCs harvested from the Wharton's Jelly in the UCs of healthy babies born by cesarean section at the Medical University of South Carolina hospital were expanded in  $\alpha$ -MEM (Life Technologies) supplemented with 10% human platelet lysate (Emory University), 2 mM L-glutamine (Lonza BioWhittaker), and antibiotics (100 U/ml penicillin and 100  $\mu$ g/ml streptomycin; Lonza BioWhittaker) and placed in a humidified cell culture incubator containing 5% CO<sub>2</sub> at 37°. Nonadherent cells were removed by changing medium every 3 d after the initial plating. After 7 d of incubation, when UC MSC colonies took up  $\geq 60$ –80% of the total surface area, the cells were subcultured.

Both HBM and LBM mononuclear cells were collected from BM aspirates, separated by gradient centrifugation, and seeded at a density of  $1 \times 10^6$  cells/cm<sup>2</sup> in the same medium used for UC MSCs. After 3 d of culture, nonadherent cells were removed, and the medium was changed twice weekly thereafter. Once 60–80% confluence was reached, adherent cells were replated at a density of  $10^4$  cells/cm<sup>2</sup> in medium for expansion. Human BM MSCs were derived from fresh whole BM of healthy donors (purchased from ALLCELLS) and four lupus patients. Lupus patients consisted of four African Americans (three females and one male) between the ages of 28 and 41 y. Two patients had active disease, with SLE Disease Activity Index scores  $> 6$ , and two patients had inactive disease, with an SLE Disease Activity Index score  $< 2$ . All patients were on prednisone (dose  $\leq 5$  mg), as well as either azathioprine or mycophenolate mofetil.

After three passages, the cells were harvested. MSCs were identified according to the criteria of the International Society for Cellular Therapy (25). Briefly, flow cytometric analysis confirmed that the cells expressed CD44, CD90, and CD105 but not CD31, CD45, or HLA-DR. The capacity of MSCs to differentiate along adipogenic and osteogenic lineages was evaluated, as described previously (26). Cells of passages 3–6 were used in the experiments. Initial MSC characterization confirmed the purity of each cell line. However, MSC purity in cultures postcryopreservation and prior to use was determined by an additional flow confirmation of MSC-specific markers and absence of non-MSC markers. In all experiments, analyses indicated  $> 95\%$  purity.

### Urine albumin excretion

All mice were placed in metabolic cages for 24-h urine collection before treatment and every 2 wk after treatment. Urinary albumin excretion was determined by ELISA using a standard curve of known concentrations of mouse albumin (Bethyl Laboratories), as previously described (26). Results are expressed as milligrams of albumin/mouse/d.

### Measurement of serum anti-dsDNA Ab levels

Serum was collected from each mouse before treatment and every 2 wk after treatment. Anti-dsDNA Ab levels were measured by ELISA, as previously described (27, 28). Briefly, 96-well ELISA plates were coated with 5  $\mu$ g/ml double-stranded calf thymus DNA (Sigma-Aldrich) in sodium salt citrate buffer at 37°C overnight. After washing, sera were added in serial dilutions starting at 1/100. HRP-conjugated goat anti-mouse IgG ( $\gamma$ -chain specific) was added, followed by 3,3', 5,5'-tetramethylbenzidine (both from Sigma-Aldrich) for color development. OD at 450 nm was measured by a microtiter plate reader (Multiskan Ascent; Thermo Electron).

### Evaluation of IgG and C3 deposition in glomeruli

At the time of sacrifice, one kidney was snap-frozen in liquid nitrogen and placed in OCT medium (Tissue-Tek), and 4-mm-thick frozen sections were stained with fluorescein-conjugated anti-mouse IgG or anti-mouse C3 (both from MP Biomedical). The average intensities of deposits in five independent fields of one kidney section/animal were quantitated by NIS-Elements BR 3.0.

### Histology and pathology assessment of kidney

Kidneys were removed when the mice were sacrificed at 8 wk after treatment. One kidney was fixed with 10% buffered formalin, embedded in paraffin, and then sectioned. The sections were stained with H&E. Photos were taken at 40 $\times$  using a Nikon Eclipse 80i microscope equipped with a Nikon digital sight color camera and NIS-Elements BR 3.0. For pathology assessment, kidney slides were examined in a blinded fashion and graded for hypercellularity, mesangial expansion, necrosis, crescents, and membrane thickening. Scores from 0 to 3+ (0, none; 1+, mild; 2+, moderate; and 3+, severe) were assigned for each of these features and then added together to yield a final renal pathology score. The scores for crescent formation and necrosis were doubled to reflect the severity of those lesions.

### Flow cytometry

BM cells were incubated with unlabeled mouse anti-CD16/CD32 for blocking FcRs and stained with mouse PE-labeled anti-CD138, PerCP-labeled anti-B220, allophycocyanin-labeled anti-TCR- $\beta$ , and unlabeled anti-mouse Ig $\kappa$ . Spleen cells were incubated with unlabeled mouse anti-CD16/CD32 for blocking FcRs. The following anti-mouse Abs were incubated with the spleen cells to characterize spleen cell subsets: allophycocyanin-labeled anti-CD3, PerCP-labeled anti-CD4, FITC-labeled anti-CD8, and PE-labeled anti-Foxp3. Flow samples were analyzed on a flow cytometer (FACSCalibur; Becton Dickinson) and analyzed using BD CellQuest Pro 5.2.1. All Abs were purchased from BD Biosciences or eBioscience.

### Urine cytokine analysis

IL-17A, CSF1, IFN- $\gamma$ , and MCP1 cytokine ELISAs were performed on urine from MSC-injected mice. ELISA kits were obtained from eBioscience (CSF1) or BioLegend (IL-17A, IFN- $\gamma$ , and MCP1), and the procedures were performed following the manufacturer's specifications. Plates were read using a Multiskan Ascent at 450 nm.

### PBMC-proliferation assay

The proliferation assay was performed in 24-well plates (Costar) in a total volume of 0.5 ml  $\alpha$ -MEM (Cellgro) supplemented with 5% human platelet lysate, 2 mM L-glutamine, 55  $\mu$ M 2-ME (Life Technologies), 100 U/ml penicillin, and 100  $\mu$ g/ml streptomycin. PBMCs were isolated from healthy human blood using lymphocyte separation medium (Cellgro) and were labeled with 2  $\mu$ M CFSE (Invitrogen). A total of  $5 \times 10^5$  PBMCs was stimulated with 1  $\mu$ g/ml anti-human CD3/CD28 Abs in the presence or absence of varying concentrations of MSCs. Three UC MSC lines, two HBM MSC lines, and three LBM MSC lines were tested one or two times. Plates were incubated for 72 h at 37°C in a humidified atmosphere with 5% CO<sub>2</sub>. PBMCs were collected and stained with anti-human allophycocyanin-labeled CD3, PerCP-labeled CD4, or PE-labeled CD8, and the proliferation status was analyzed by flow cytometry. All Abs were purchased from BioLegend.

### Cytokine-secretion analysis

Human IFN- $\gamma$  and IL-17A ELISAs were performed on supernatants from PBMC-proliferation assays. ELISA kits were obtained from BioLegend, and the procedure was performed following the manufacturer's specifications. Plates were read using a Multiskan Ascent at 450 nm.

### B cell-proliferation assay

PBMCs from the blood of healthy donors ( $n = 3$ ) were isolated using lymphocyte separation medium (Cellgro). CD19<sup>+</sup> B cells were isolated by CD19 MicroBeads (Miltenyi Biotec), according to the manufacturer's instructions. B cells were labeled with 2  $\mu$ M CFSE (Invitrogen). A total of  $5 \times 10^4$  CD19<sup>+</sup> cells was cocultured with  $5 \times 10^4$  MSCs from various sources in a 96-well flat-bottom plate (Costar) with a total volume of 200  $\mu$ l RPMI 1640 (Cellgro) supplemented with 10% FBS, 2 mM L-glutamine, 55  $\mu$ M 2-ME (Life Technologies), 100 U/ml penicillin, and 100  $\mu$ g/ml streptomycin. B cells received the following stimuli: 2.5  $\mu$ g/ml CpG oligonucleotide 2006 (Miltenyi Biotec), 1  $\mu$ g/ml soluble CD154 (CD40L; BioLegend), 2.5  $\mu$ g/ml F(ab')<sub>2</sub> anti-human IgM/IgA/IgG (Jackson ImmunoResearch), and 1000 U/ml IL-2 (BioLegend). Three UC MSC lines, two HBM MSC lines, and three LBM MSC lines were tested one or two times. Plates were incubated for 4 d at 37°C in a humidified atmosphere with 5% CO<sub>2</sub>. CD19<sup>+</sup> cells were collected and stained with anti-human PE-labeled CD19 (Miltenyi Biotec), allophycocyanin-labeled IgG (BioLegend), or allophycocyanin-labeled IgA (Miltenyi). Cell-proliferation status was analyzed by flow cytometry.

### IFN- $\gamma$ -licensing assay

The IFN- $\gamma$ -licensing assay was performed in 24-well plates (Costar) in a total volume of 0.5 ml  $\alpha$ -MEM (Cellgro) supplemented with 5% human platelet lysate, 2 mM L-glutamine, 55  $\mu$ M 2-ME (Life Technologies), 100 U/ml penicillin, and 100  $\mu$ g/ml streptomycin. A total of  $5 \times 10^4$  MSCs was stimulated with 0, 5, 10, or 50 ng/ml of recombinant human IFN- $\gamma$ . Plates were incubated for 24 h at 37°C in a humidified atmosphere with 5% CO<sub>2</sub>. MSCs were collected, stained with anti-human allophycocyanin-labeled CD274 (B7-H1) and PerCP-labeled HLA-ABC, and analyzed by flow cytometry. All Abs were purchased from BioLegend.

### Quantitative RT-PCR

One microgram of total RNA was isolated from cultured MSCs and mouse kidney tissue. RNA was used to generate cDNA (SA Biosciences) for real-time RT-PCR. iQ SYBR Green Supermix (Bio-Rad, Hercules, CA) and gene-specific primers (SABiosciences) at 200 nM were used to amplify relative amounts of cDNA on a CFX Connect Real-Time System (Bio-Rad). The amplification was performed by one 5-min cycle at 95°C, which was required for enzyme activation, followed by 39 cycles of denaturation (95°C, 15 s), annealing (55°C, 30 s), and extension (72°C, 30 s). Melting curve analysis was performed to confirm amplicon specificity. The relative expression was calculated using the double  $\Delta C_T$  method (i.e., using the Equation  $2^{-\Delta\Delta C_T}$ ) with Bio-Rad software.

### Statistical analysis

All data were analyzed using Prism version 5.0 software (GraphPad, San Diego, CA). Survival significance was determined via log-rank (Mantel-Cox) analysis of the survival curve. Two-way ANOVA was used for proteinuria curve and in vitro assay analyses. One-way ANOVA (Tukey posttest) and the Kruskal-Wallis test (Dunn posttest) were used to assess significance between groups in single-group comparisons. A 95% confidence limit, defined by  $p$  values  $\leq 0.05$ , was considered statistically significant.

## Results

### Improved survival of lupus-prone mice receiving hMSCs

hMSC transplantation, regardless of source, significantly enhanced the survival of MRL/lpr mice when given at disease onset compared with the PBS control group. By 60 wk of age, 62% mortality was observed in the PBS group compared with 0–15% in the hMSC-treated groups (Fig. 1A). Furthermore, there was a significant increase in the body weight of hMSC mouse groups in comparison with the PBS control mice (Fig. 1B). Trends toward lower spleen weights in hMSC-treated mice corresponded with increased body weight. However, this correlation did not reach significance (Fig. 1C).

Next, we sought to determine whether improved survival of hMSC treated mice was due to effects on renal disease. Urine was collected before and every 2 wk post-MSC transplantation to assess 24-h urine albumin excretion. As expected, the PBS mouse group experienced increasing proteinuria over time. In contrast, mice transplanted with healthy donor MSCs from UC or BM had significantly less proteinuria. However, mice treated with lupus patient MSCs (LBM MSC) had delayed onset of proteinuria (Fig. 1D). Mice that died prior to sacrifice had elevated proteinuria, implicating renal disease as causative in their mortality. Because of the role of anti-dsDNA Abs in lupus nephritis, we then assessed serum anti-dsDNA Ab levels before and every 2 wk after MSC transplantation. Anti-dsDNA Ab levels were not impacted by hMSC treatment (Fig. 1E), suggesting that the effect of MSCs on lupus nephritis is post autoantibody production/immune complex deposition.

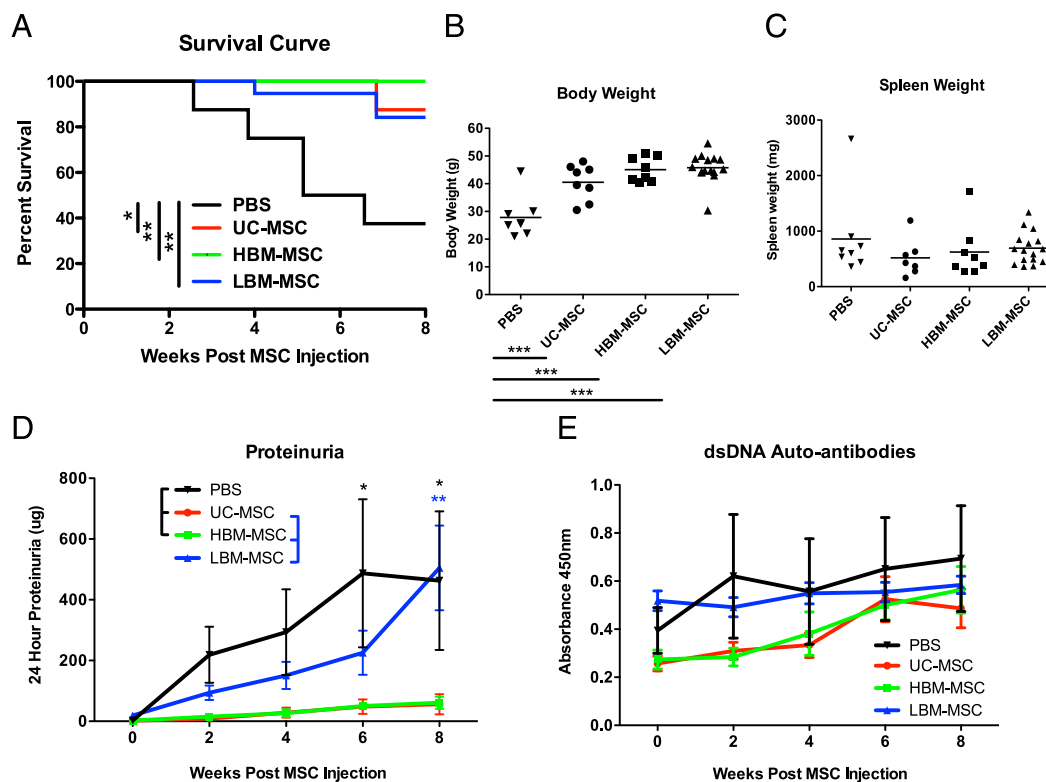
### Effect of hMSCs on kidney pathology varies based on source of origin

We then assessed whether the different sources of hMSC had a differential effect on histopathological changes in the kidneys of MRL/lpr mice. We found that IgG, but not C3, deposition in the glomeruli was decreased in UC MSC- and BM MSC-treated mice compared with PBS controls (Fig. 2A, 2B). In contrast, LBM MSC treatment significantly increased C3 and IgG deposition in the glomeruli compared with all other treatment groups, including PBS controls (Fig. 2A, 2B). H&E-stained kidney slides were scored in a blinded fashion. The following renal pathology measures make up the overall renal pathology score: hypercellularity, mesangial expansion, necrosis, crescents, and membrane thickening (Fig. 2C). We observed lower necrosis and crescents in UC MSC-treated and HBM MSC-treated mice compared with LBM MSC-treated mice, which were not different from PBS-treated mice (Fig. 2C). Significantly lower total pathology scores were seen in HBM MSC-treated mice—with UC MSC-treated mice approaching significance ( $p = 0.0706$ )—but not in LBM MSC-treated mice compared with PBS controls (Fig. 2D). Together, these results suggest that UC MSCs and HBM MSCs, but not LBM MSCs, are effective in reducing kidney pathology in MRL/lpr mice. In fact, these data suggest potential exacerbation of kidney disease by LBM MSCs.

### LBM MSCs are not effective in reducing inflammatory markers

Upon finding differences in the ability of hMSCs to modulate kidney pathology, we examined inflammatory cytokines in the kidney and urine of treated mice. CSF1, MCP1, IL-17, and IFN- $\gamma$  are all cytokines indicative of immune activation in active nephritis (27–30). As shown in Fig. 3A, UC MSC-treated mice experienced reduced MCP1 expression compared with LBM MSC-treated mice. Mice treated with UC MSCs ( $p = 0.4351$ ) or HBM MSCs ( $p = 0.6216$ ) appeared to have lower IFN- $\gamma$  expression in the kidney compared with PBS controls, although the difference was not significant (Fig. 3A). CSF1 and IL17A gene expression was undetectable in the kidneys, regardless of treatment group (data not shown). We then looked at the presence of these cytokines in the urine of mice. Over the course of treatment, there was less urinary MCP1 in UC MSC- and HBM MSC-treated mice compared with LBM MSC- and PBS-treated mice, although this finding did not reach statistical significance (Fig. 3B). However, at 6 wk post-MSC transplantation, we observed significantly higher levels of urinary IFN- $\gamma$  in LBM MSC-treated mice compared with all other treatment groups, including controls, consistent with the kidney pathology results (Figs. 2, 3C). Urinary IL-17A was not detected, and no differences in urinary CSF1 were found at any time point post-MSC transplant





**FIGURE 1.** Survival curve, body and spleen weight, urinary albumin excretion, and serum dsDNA autoantibody levels of MRL/*lpr* mice. **(A)** Kaplan-Meier survival curve of MRL/*lpr* mice receiving hMSCs. Mortality was recorded until the time of sacrifice (8 wk posttransplant). **(B)** Body weight of MRL/*lpr* mice at the time of sacrifice. **(C)** Spleen weights (mean  $\pm$  SEM) of MRL/*lpr* mice at the time of sacrifice. **(D)** Urinary albumin excretion over time post-MSC transplant. Data are mean  $\pm$  SEM. **(E)** Serum dsDNA autoantibodies over time post-MSC transplant. Data are mean  $\pm$  SEM. PBS ( $n = 8$ ), UC MSCs ( $n = 8$ ), HBM MSCs ( $n = 8$ ), LBM MSCs ( $n = 16$ ). Statistical comparisons were performed using the log-rank test, one-way ANOVA with the Tukey posttest, or two-way ANOVA with the Bonferroni posttest. \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ .

(data not shown, Supplemental Fig. 1). The inability of LBM MSCs to inhibit inflammatory cytokines in the kidney and urine of MRL/*lpr* mice further support that lupus patient MSCs are not as effective as healthy donor MSCs at modulating disease.

#### Reduced human gene expression in the kidney of LBM MSC-treated mice

Because of the inability of LBM MSCs to inhibit kidney pathology and inflammatory cytokine production, we hypothesized that they were not homing to the site of inflammation. To assess the presence of hMSCs in the kidneys of MRL/*lpr* mice, we looked for the expression of human IDO1 and CFH, which are expressed by hMSCs. MSC presence in the kidney of hMSC-treated mice is associated with higher gene expression than that of PBS control mice. Our results show positive gene expression for human IDO1 and CFH only in the UC MSC- and HBM MSC-treated groups (Fig. 3D). Together, these results suggest that LBM MSCs have lower expression of IDO1/CFH or that there are fewer hMSCs in the kidney of mice treated with LBM MSCs, either due to decreased migration capacity or decreased survival.

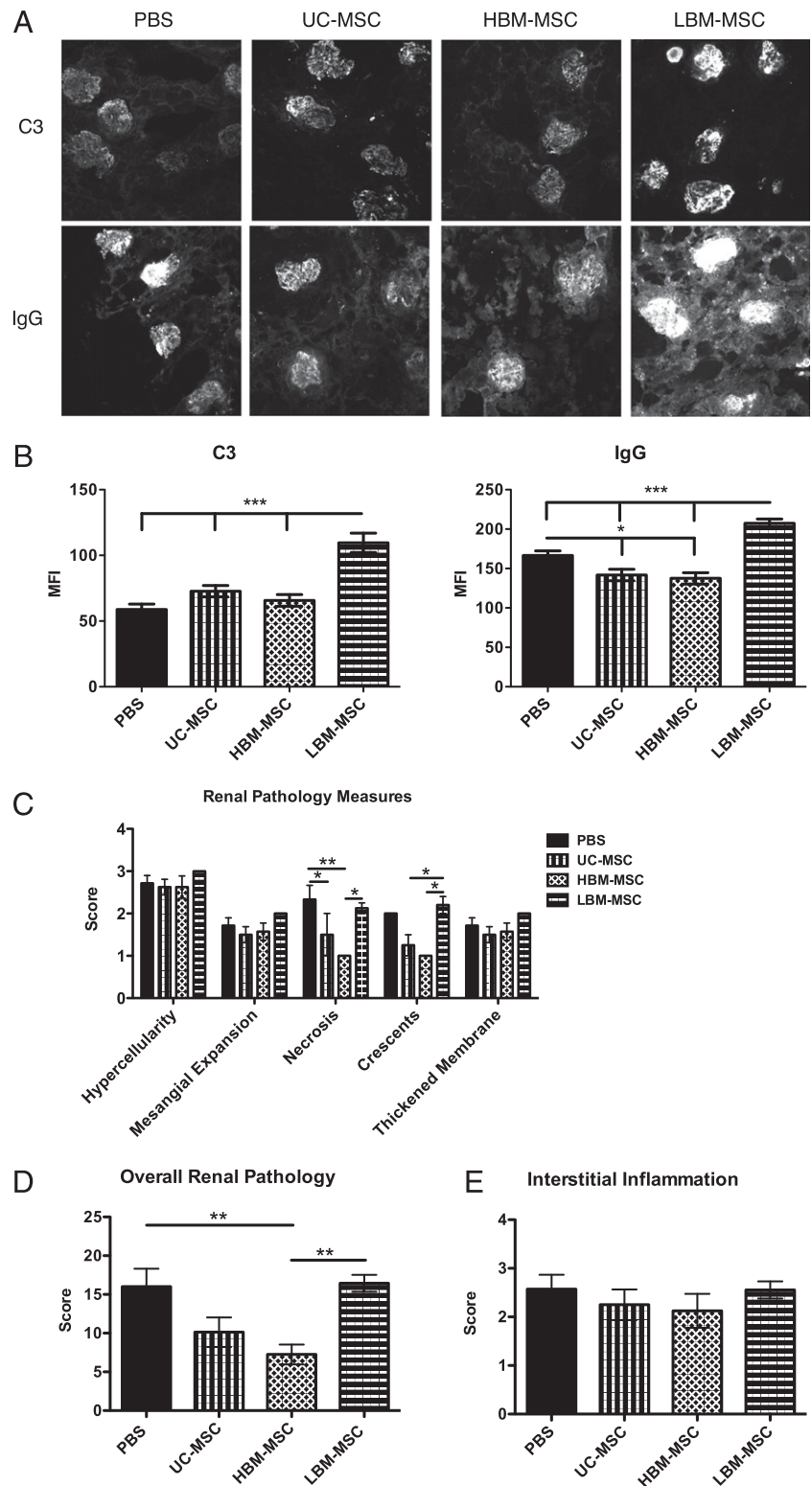
#### Increased T and B cells in the BM of mice treated with lupus patient MSCs

Our results support an exacerbation of kidney disease in LBM MSC-treated mice, although these mice still experienced improved survival, compared with PBS controls. To assess potential mechanisms by which MSCs from the various sources impact lupus disease activity, we characterized various immune cell populations in the spleen and BM of mice from each group via flow cytometry. When examining the spleen, no differences were

seen in the percentages of CD8<sup>+</sup> T cells, CD4<sup>+</sup> T cells, activated CD4<sup>+</sup>CD25<sup>+</sup> T cells, or Foxp3<sup>+</sup> T cells between mouse groups (Fig. 4A, data not shown). In the BM, no significant changes in TCRβ<sup>+</sup> cells, B220<sup>+</sup> cells, or plasma (CD138<sup>+</sup>) cells were observed in the mice treated with UC MSCs ( $p = 0.2286$ ,  $p = 0.0571$ ,  $p = 0.0571$ ) or HBM MSCs ( $p = 0.0714$ ,  $p = 1$ ,  $p = 0.5714$ ), although the percentages trended lower in the hMSC treatment groups compared with PBS controls. However, a significant increase in the percentage of all three cell subsets was seen in LBM MSC-treated mice compared with healthy hMSC-treated mice (Fig. 4B).

#### In vitro differences in MSCs are dependent on tissue origin rather than donor

Inhibition of T cell and B cell proliferation in vitro is a known effect of MSCs and is proposed as a measure of in vivo effectiveness. Because of our results showing the inability of LBM MSCs to inhibit progression of disease in MRL/*lpr* mice, we next tested the in vitro efficacy assays. We were only able to detect suppression of mouse CD3<sup>+</sup> T cells when cocultured at a 1:20 dilution with UC MSCs, but not HBM MSCs or LBM MSCs, although this effect was not enhanced at higher concentrations (Supplemental Fig. 2). Because of our inability to potently suppress mouse splenocytes, we examined the effects of hMSCs on healthy human PBMCs. As shown in Fig. 5A and 5B, human CD3<sup>+</sup> T cell proliferation was reduced in a dose-dependent fashion by all MSC groups. However, when examining IFN-γ cytokine production, we observed that MSCs derived from the BM were not able to inhibit IFN-γ production to the same extent as were UC MSCs (Fig. 5C). Moreover, we examined IL-17A production because it was previously shown that coculture with MSCs can increase IL-17 production by

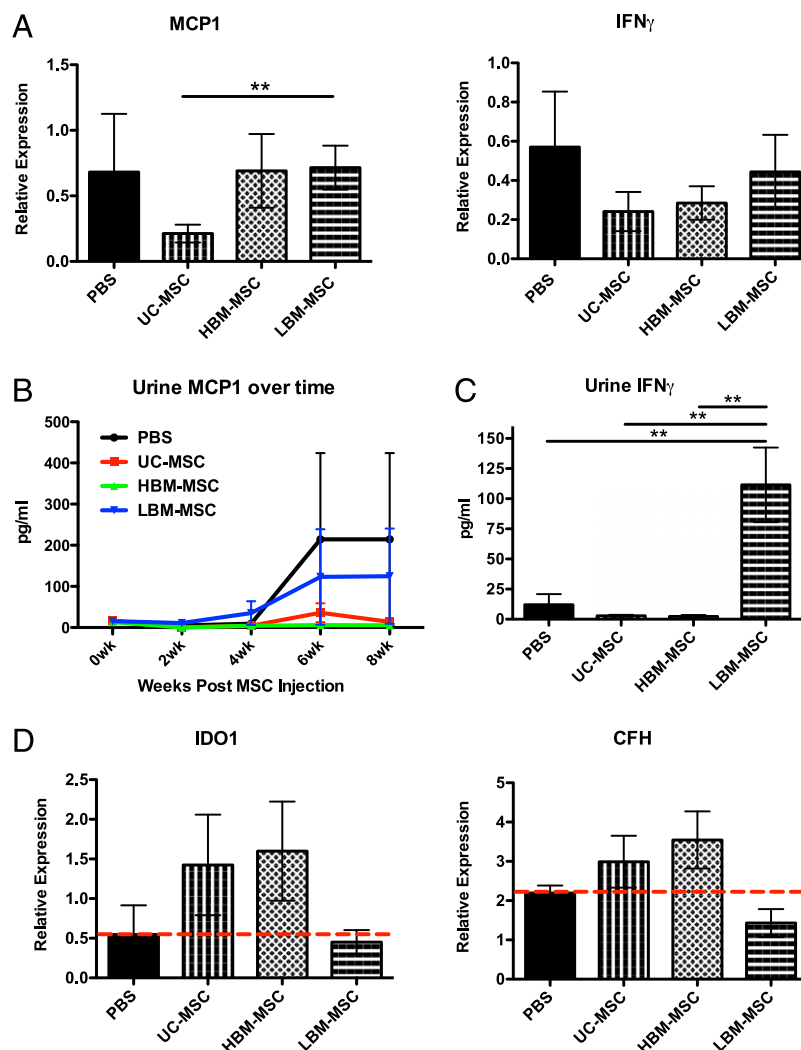


**FIGURE 2.** Glomerular C3 intensity, IgG intensity, and overall glomerular pathology score in MRL/lpr mice. At the time of sacrifice, kidneys were embedded in OCT or paraffin for sectioning. OCT-embedded kidney was labeled with fluorescein-conjugated anti-mouse C3 or IgG. **(A)** Representative images of C3 and IgG deposits in glomeruli (original magnification  $\times 20$ ). **(B)** Mean fluorescence intensity of C3 and IgG deposition. **(C)** H&E-stained paraffin-embedded kidney were examined and graded for hypercellularity, mesangial expansion, necrosis, crescents, and membrane thickening. **(D)** Scores were added together to yield the overall renal pathology score. **(E)** Interstitial inflammation scores. Data are mean  $\pm$  SEM. PBS ( $n = 8$ ), UC MSC ( $n = 8$ ), HBM MSC ( $n = 8$ ), and LBM MSC ( $n = 9$ ). \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ , one-way ANOVA with Tukey posttest.

PBMCs (31). Our findings did not show increased IL-17 production; rather, the presence of MSCs slightly reduced IL-17 production, although only UC MSCs at the highest concentration provided a significant decrease (Fig. 5C). There are varied results in the literature regarding B cell suppression by MSCs. One study examining the effects of BM MSCs on CD19<sup>+</sup> B cells found suppression of B cell proliferation, whereas another reported expansion (2, 32). To explain these results and determine whether variances in suppression could be detected in our various MSC

lines, we conducted similar coculture assays using the same cell stimulants and cell ratios. Our study showed expansion of CD19<sup>+</sup> B cells; however, we found that UC MSCs and HBM MSCs, but not LBM MSCs ( $p = 0.1119$ ), were able to significantly reduce B cell proliferation (Fig. 5D, 5E).

We next turned to the examination of MSC surface markers and gene expression to explain the inconsistency in suppressive trends in vitro. IFN- $\gamma$  licensing was shown to enhance and be indicative of suppressive function and is an inflammatory cytokine associ-



**FIGURE 3.** Inflammatory markers and human gene expression in MRL/lpr mice. At the time of sacrifice, kidneys were isolated for quantitative PCR analysis. **(A)** Expression of mouse MCP1 and IFN- $\gamma$  in the kidney of MRL/lpr mice relative to GAPDH. **(B)** Urinary MCP1 measured over time post-MSC transplant by ELISA. **(C)** Urinary IFN- $\gamma$  at 6 wk post-MSC transplant measured by ELISA. **(D)** Human IDO1 and CFH expression relative to GAPDH from the kidney of MRL/lpr mice at the time of sacrifice (8 wk post-transplant). Data are mean  $\pm$  SEM. PBS ( $n = 4$ ), UC MSC ( $n = 8$ ), HBM MSC ( $n = 8$ ), and LBM MSC ( $n = 12$ ). Statistical comparisons were performed using one-way ANOVA with the Tukey posttest and two-way ANOVA with the Bonferroni posttest.  $**p \leq 0.01$ .

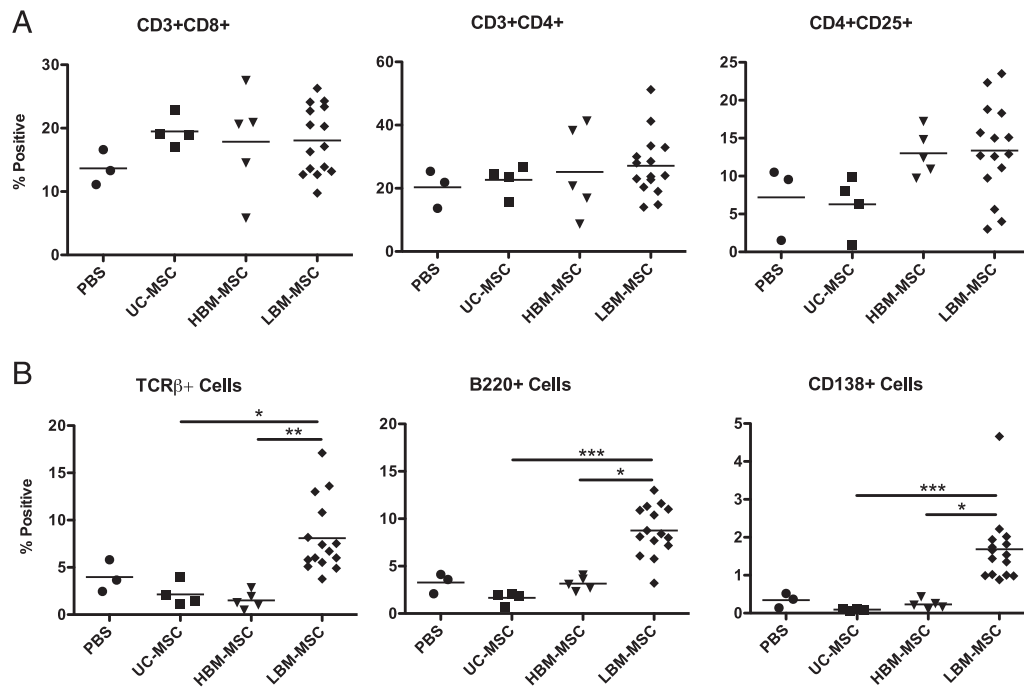
ated with lupus. Thus, we examined the effects of IFN- $\gamma$  stimulation on MSCs from all three sources (33–35). Although no difference was observed in surface expression of HLA-DR (data not shown), we saw a dose-dependent increase in HLA-ABC to IFN- $\gamma$ , with significantly higher surface expression on MSCs derived from BM (Fig. 6B). Heightened basal expression of HLA-ABC on BM-derived MSCs could suggest higher immunogenicity of these cells compared with MSCs derived from UC. Significantly higher surface expression of B7-H1 was seen in BM-derived MSCs over UC MSCs at the highest dose of IFN- $\gamma$  (Fig. 6B). B7-H1, IDO1, and CFH are highly expressed in early-passage MSCs with suppressive function, and this expression increases with IFN- $\gamma$  licensing (36–38). Our findings show that IFN- $\gamma$  licensing significantly increased the expression of B7-H1 in HBM MSCs compared with UC MSCs. Furthermore, IDO1 and CCL9 expression was increased in HBM MSCs and LBM MSCs compared with UC MSCs. However, only LBM MSCs experienced a significant increase in CFH expression compared with both UC MSCs and HBM MSCs (Fig. 6C). Our findings show heightened IFN- $\gamma$  responsiveness in MSCs from BM, regardless of the disease status of the donor, compared with UC MSCs. Together, our in vitro results suggest that IFN- $\gamma$  licensing is not a sufficient measure for predicting suppressive capacity in vivo.

## Discussion

Although many uncontrolled studies investigating the use of MSC therapy in various diseases were effective and well-tolerated,

studies in SLE patients are missing. Our present study directly compares the immunoregulatory capacity, both in vivo and in vitro, of MSCs from lupus patients and healthy controls. Additionally, we investigated the capacity of in vitro MSC assays to predict in vivo efficacy. To examine whether lupus patient-derived BM MSCs are effective as disease modulators, we compared them directly with healthy donor BM MSCs and UC MSCs. We observed improved survival and increased body weight in the groups of mice that received hMSCs, regardless of their source of origin or whether the cells were derived from a lupus patient or healthy control. Supporting previous studies examining healthy human or mouse MSCs in murine lupus, HBM MSCs and UC MSCs were significantly more effective than were LBM MSCs in improving lupus disease manifestations, including proteinuria, renal pathology, and urinary inflammatory markers (13–16, 19, 20). Although our study strongly supports the efficacy of healthy hMSCs to modulate disease in murine lupus, we uncovered a negative impact on renal disease manifestations in mice treated with lupus patient-derived MSCs.

Consistent with our previous study on murine MSCs, lupus patient MSCs were unable to prevent renal disease progression, including their inability to reduce proteinuria and inhibit renal pathology, both of which were impacted positively by UC MSCs and HBM MSCs (13). LBM MSC-treated mice experienced increased proteinuria compared with HBM MSC- or UC MSC-treated mice at 8 wk posttreatment. Although no differences were detected in serum anti-dsDNA Ab levels in the various mouse



**FIGURE 4.** Percentages of inflammatory cells in the spleen and BM of MRL/*lpr* mice. Flow cytometry analysis was performed at the time of sacrifice on spleen and BM isolated from mice injected with MSCs. (A) Percentages of CD3<sup>+</sup>CD8<sup>+</sup>, CD3<sup>+</sup>CD4<sup>+</sup>, and CD3<sup>+</sup>CD4<sup>+</sup>CD25<sup>+</sup> T cells in the spleen. (B) Percentages of TCRβ<sup>+</sup>, B220<sup>+</sup>, and CD138<sup>+</sup> cells in the BM. Data are mean ± SEM. PBS (*n* = 3), UC MSC (*n* = 4), HBM MSC (*n* = 5), and LBM MSC (*n* = 15). \**p* ≤ 0.05, \*\**p* ≤ 0.01, \*\*\**p* ≤ 0.001, Kruskal–Wallis test with Dunn multiple comparison posttest.

groups, we observed significantly higher C3 and IgG glomerular deposition in LBM MSC-treated mice compared with all other treatment groups, including the control. Moreover, overall renal pathology was not impacted in LBM MSC-treated mice. The discrepancy in autoantibody levels in the serum compared with IgG deposition in the kidney may be explained by the active renal disease activity in the LBM MSC-treated mice at the time of sacrifice. Anti-dsDNA Abs may decrease in the serum of patients with renal flares as a result of their deposition into the tissue (39). Thus, serum levels may remain constant or decrease as Abs are deposited in the kidney.

Despite the active kidney disease at the time of sacrifice, we observed improved survival of mice in the LBM MSC treatment groups compared with PBS-treated controls. As in all similar murine studies, we anticipate that the mice that died had severe renal disease, which falsely lowered the renal disease scores of the control mice. Improved survival of LBM MSC-treated mice, despite active renal disease, reflects a time delay in the onset of disease but no effect on the overall severity of disease. Between 4 and 6 wk post-MSC transplant, PBS control mice developed increased proteinuria, followed shortly thereafter by death. Although UC MSC- and HBM MSC-treated mice did not have an increase in proteinuria, LBM MSC-treated mice developed increased proteinuria at 8 wk, 2–4 wk after the PBS-treated group. If the experiment was allowed to proceed for an additional 2–4 wk, we likely would have observed increased mortality in the LBM MSC-treated mice. Moreover, LBM MSC-treated mice sacrificed at 8 wk were at peak disease activity, explaining the heightened kidney pathology and increased inflammatory cell populations in the BM. It is notable that not all aspects of lupus nephritis pathology were impacted by the MSC treatments; mesangial proliferation is one example. The significant impact of MSC treatment was in preventing the more severe manifestations of renal disease: crescents and necrosis. This is in contrast to the effect of using control murine MSCs to treat disease, in which all

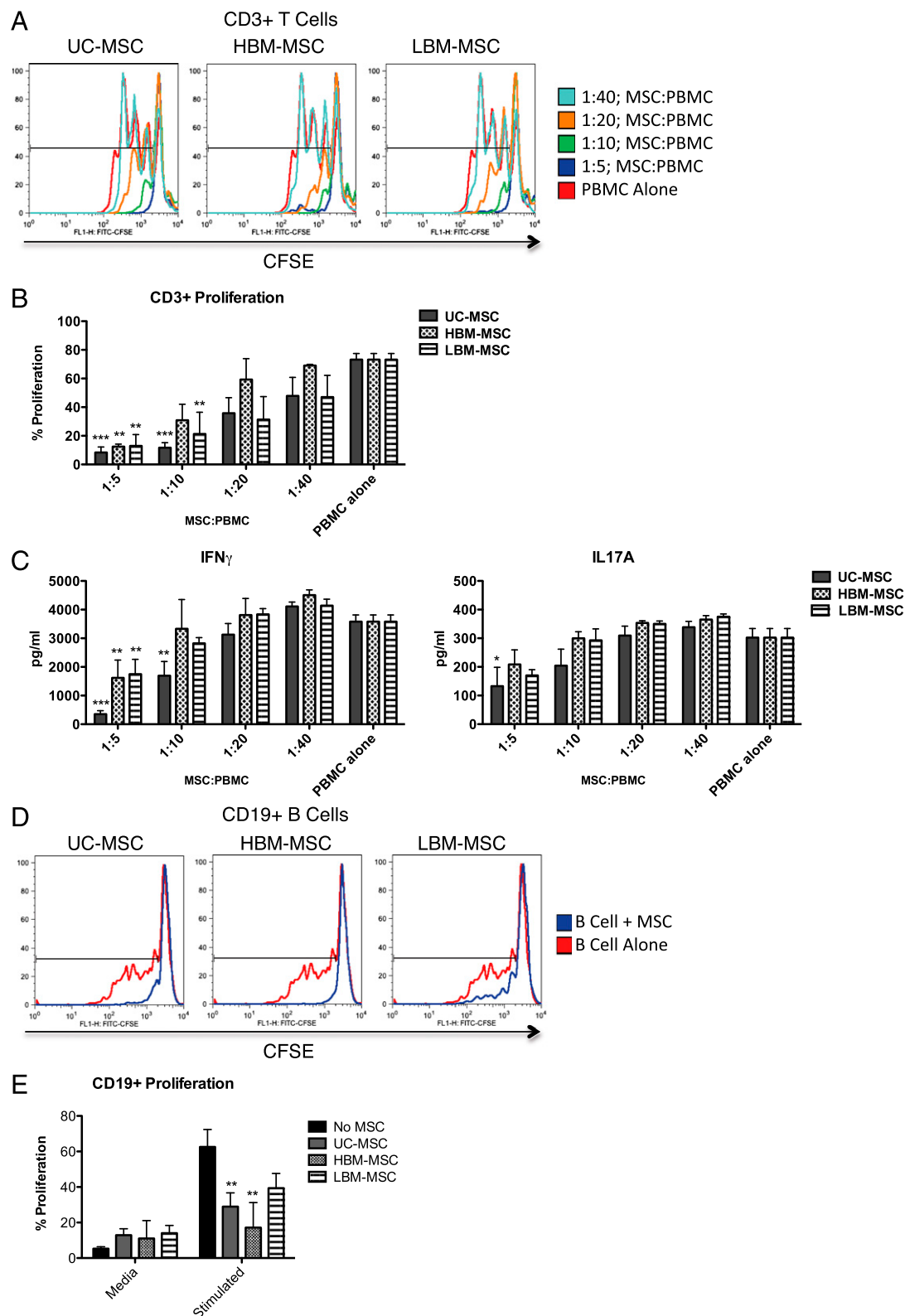
aspects of lupus nephritis were impacted. These differences may reflect known variations in the mechanisms of effect of mouse MSCs (NO mediated) versus hMSCs (IDO mediated) (35, 40).

Several characteristics of LBM MSCs may factor into their relatively short-term benefits. Previous studies showed early senescence and defective differentiation of LBM MSCs, which may explain a short-term, but not a long-term, benefit (14, 22). Defects in LBM MSC migration, premature death, and increased cytokine production also may play a role in the lack of a full protective effect. Our inability to detect human IDO1 and CFH gene expression in the kidney of LBM MSC-treated mice suggests that these abnormalities may impact *in vivo* efficacy. These findings suggest that the cells never migrated to the diseased kidney, that they migrated to the kidney but died prematurely, or that they are not activated to express IDO and CFH *in vivo*. These findings are consistent with the lack of efficacy of MSCs derived from diseased lupus mice in treating murine lupus and the limited reports of a lack of efficacy of autologous MSC treatment in human lupus (13, 23).

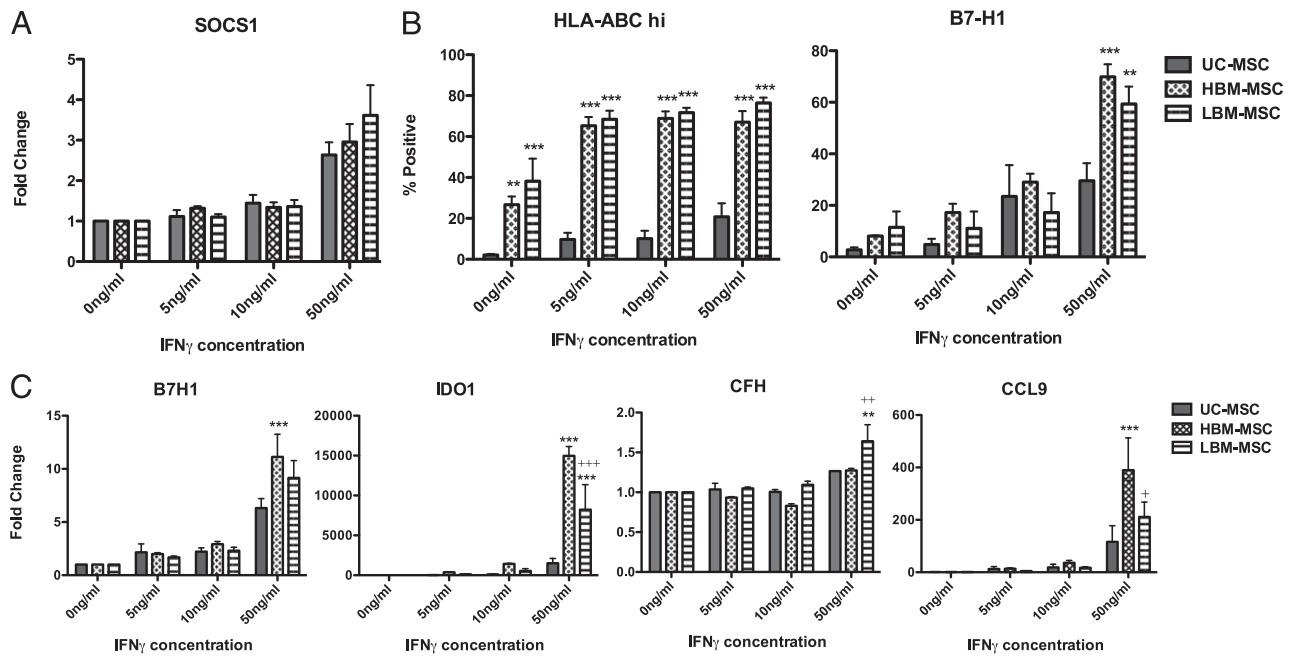
One of the more unexpected findings in this study was that there was consistency in effect of the MSCs depending on their origin. There was very little variation in the effect of the MSCs both *in vitro* and *in vivo*. We used four MSC lines from each source (healthy human BM, LBM, and healthy UC). All of the healthy BM MSC lines and all of the UC MSC lines had similar *in vivo* efficacy and *in vitro* effects. Perhaps most surprising was that all four of the lupus MSC BM-derived lines had similar effects on murine disease and similar effects in *in vitro* assays, despite marked differences in disease activity and medications.

Given the differential efficacy of the MSCs, we next sought to determine whether an *in vitro* assay exists that could predict *in vivo* efficacy. The obvious goal of this research is to treat human lupus patients with MSCs in a controlled trial. It is important that we select for this trial the most effective MSC origin, which we hopefully could identify using *in vitro* assays prior to infusion.





**FIGURE 5.** T cell and B cell proliferation in CFSE-dilution assays cocultured with different sources of MSCs and IFN- $\gamma$  production of T cell–proliferation assay. **(A)** Representative plots of CD3 $^{+}$  T cell proliferation in CFSE-dilution assay cocultured with each source of MSC. **(B)** Proliferation of CD3 $^{+}$  T cells incubated alone ( $n = 5$ ) or with varying ratios of UC MSCs ( $n = 5$ ), HBM MSCs ( $n = 4$ ), or LBM MSCs ( $n = 5$ ). Cultures were stimulated with 1  $\mu$ g/ml of anti-human CD3/CD28 Abs. **(C)** Supernatants were collected from PBMC coculture assay, and IFN- $\gamma$  and IL-17A cytokine levels were analyzed by ELISA. **(D)** Representative plots of CD19 $^{+}$  B cell proliferation in CFSE-dilution assay cocultured with each source of MSC. **(E)** Proliferation of CD19 $^{+}$  B cells incubated alone ( $n = 4$ ) or at a 1:1 ratio with UC MSCs ( $n = 5$ ), HBM MSCs ( $n = 4$ ), or LBM MSCs ( $n = 5$ ). Cultures were stimulated with 2.5  $\mu$ g/ml CpG, 1  $\mu$ g/ml, 2.5  $\mu$ g/ml F(ab') $_2$  anti-human IgM/IgA/IgG, and 1000 U/ml IL-2. Data are mean ( $\pm$  SEM) percentage of proliferating cells or pg/ml IFN- $\gamma$ . \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ , two-way ANOVA with Bonferroni posttest.



**FIGURE 6.** Flow cytometry and quantitative PCR analysis of MSC markers after in vitro IFN- $\gamma$  licensing. UC MSC ( $n = 4$ ), HBM MSC ( $n = 4$ ), and LBM MSC ( $n = 4$ ) were cultured with 0, 5, 10, or 50 ng/ml of IFN- $\gamma$ . **(A)** SOCS1 expression relative to GAPDH in IFN- $\gamma$ -licensed MSCs. **(B)** Percentage of HLA-ABC<sup>+</sup> and B7-H1<sup>+</sup> MSCs cultured with various concentrations of IFN- $\gamma$ . **(C)** IDO1, B7-H1, CFH, and CCL9 expression relative to GAPDH in IFN- $\gamma$ -licensed MSCs ( $n = 3$  for each MSC source). Data are mean  $\pm$  SEM. The asterisk (\*) denotes statistical comparison between UC-MSC and other MSCs, and the plus sign (+) denotes statistical comparison between HBM-MSC and other MSCs.  $^+p \leq 0.05$ ,  $^{++}p \leq 0.01$ ,  $^{+++}p \leq 0.001$ .

Thus, we performed a number of immune assays, including standard assays used now for assessment of MSCs for human treatment, as well as measures to differentiate effective (UC MSC/HBM MSC) from ineffective (LBM MSC) derivations. Suppression of T cell proliferation is the most widely used assay to determine immune competence of MSCs. We found that all of the MSC lines suppressed T cell proliferation in a dose-dependent fashion, although UC MSCs were more effective in suppressing IFN- $\gamma$  production. Thus, the standard assay does not allow identification of in vivo inefficacy. Our results also showed that healthy UC MSCs and HBM MSCs significantly suppressed B cell proliferation; LBM MSCs had an effect but it did not reach statistical significance. We observed more significant differences between BM-derived MSCs and UC-derived MSCs than between lupus and control MSCs.

Lastly, we used IFN- $\gamma$  licensing to examine markers that are indicative of MSC function. Prior studies suggested that treating MSCs with IFN- $\gamma$  may enhance in vivo efficacy and that these assays are predictive of in vivo efficacy (34, 35, 41). Therefore, we were interested in whether IFN- $\gamma$  licensing differentiated lupus versus control MSCs and whether treatment with IFN- $\gamma$  could enhance the immunosuppressive abilities of lupus MSCs. A previous study examined the effects of CD8<sup>+</sup> T cell-produced IFN- $\gamma$  on UC MSCs and LBM MSCs and found defective IDO production by LBM MSCs (35). Conversely, we observed increases in B7-H1 and IDO1 suppressive gene expression in both healthy and lupus BM-derived MSCs, similar to the findings by another group that examined only BM-derived MSCs (34). We also saw increased CFH expression in LBM MSCs. Our in vitro results show that MSCs from various sources, including lupus patients, are able to suppress T and B cells to some capacity. Furthermore, IFN- $\gamma$  licensing of LBM MSCs revealed their ability to upregulate markers that are indicative of suppression, suggesting that standard assays of MSC function are not likely to identify or explain the inability of LBM MSCs to maintain inhibition of disease in

lupus-prone mice. These in vitro findings suggest that the failure of LBM MSCs to impact lupus disease to the same extent as healthy MSCs is likely due to defects in migration, premature death, or an inability to maintain suppressive functions in an excessively inflammatory environment. Whether the defective immunosuppressive function in lupus-derived MSCs is intrinsic or a result of disease remains unanswered. Our prior reports suggest that it is an effect of disease, because MSCs derived from predisease lupus-prone mice are as effective as control MSCs, whereas MSCs from mice after disease onset are not effective (13). The follow-up question of whether lupus-derived MSCs can be “rescued” remains to be answered.

In conclusion, our study demonstrated that, like murine LBM MSCs, human LBM MSCs are not as effective as UC MSCs and HBM MSCs in ameliorating disease in lupus-prone mice. Moreover, in vitro assessments of immunomodulatory functions detected a reduced capacity of LBM MSCs to inhibit IFN- $\gamma$  production and CD19<sup>+</sup> B cell proliferation, although inhibition of CD3<sup>+</sup> proliferation and IFN- $\gamma$ -licensing results were indicative of immune activity by LBM MSCs. Together, the overall effectiveness of LBM MSCs in vitro, other than B cell inhibition, suggest that they are effective in cell-to-cell inhibition, indicating that their ineffectiveness in vivo is due to other functional defects. These defects of LBM MSCs may lie in their migration to inflamed tissues or their inability to persist and function during systemic inflammation. Studies regarding chemotactic and migratory properties of LBM MSCs in vivo are yet to be completed, but they may provide insight into the defects uncovered in this study. Additionally, future studies regarding LBM MSCs’ responsiveness to IFN- $\gamma$  stimulation may prove to be useful in enhancing the properties of these cells both in vitro and in vivo. Although these studies showed that LBM MSCs are not yet a suitable source of MSCs for cell therapy in lupus, it is important to continue to define differences in MSCs, because it appears that donors and source of origin impact their function. Finally, these studies involved the infusion

of hMSCs into mice. It is possible that LBM MSCs are effective when given to patients; however, our studies in mice and humans and the limited in vivo studies in patients suggest that allogeneic MSCs are more suitable for human trials.

## Acknowledgments

We thank Dr. Osama Naga (Medical University of South Carolina) for technical assistance with the experiments.

## Disclosures

The authors have no financial conflicts of interest.

## References

- Klyushnchenkova, E., J. D. Mosca, V. Zernetkina, M. K. Majumdar, K. J. Beggs, D. W. Simonetti, R. J. Deans, and K. R. McIntosh. 2005. T cell responses to allogeneic human mesenchymal stem cells: immunogenicity, tolerance, and suppression. *J. Biomed. Sci.* 12: 47–57.
- Corcione, A., F. Benvenuto, E. Ferretti, D. Giunti, V. Cappiello, F. Cazzanti, M. Riso, F. Gualandi, G. L. Mancardi, V. Pistoia, and A. Uccelli. 2006. Human mesenchymal stem cells modulate B-cell functions. *Blood* 107: 367–372.
- Sotiropoulou, P. A., S. A. Perez, A. D. Gritzapis, C. N. Baxevanis, and M. Papamichail. 2006. Interactions between human mesenchymal stem cells and natural killer cells. *Stem Cells* 24: 74–85.
- Beyth, S., Z. Borovsky, D. Mevorach, M. Liebergall, Z. Gazit, H. Aslan, E. Galun, and J. Rachmilewitz. 2005. Human mesenchymal stem cells alter antigen-presenting cell maturation and induce T-cell unresponsiveness. *Blood* 105: 2214–2219.
- Ren, G., L. Zhang, X. Zhao, G. Xu, Y. Zhang, A. I. Roberts, R. C. Zhao, and Y. Shi. 2008. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell* 2: 141–150.
- Prevosto, C., M. Zancolli, P. Canevali, M. R. Zocchi, and A. Poggi. 2007. Generation of CD4+ or CD8+ regulatory T cells upon mesenchymal stem cell-lymphocyte interaction. *Haematologica* 92: 881–888.
- English, K., F. P. Barry, and B. P. Mahon. 2008. Murine mesenchymal stem cells suppress dendritic cell migration, maturation and antigen presentation. *Immunol. Lett.* 115: 50–58.
- Wang, J., L. Liao, and J. Tan. 2011. Mesenchymal-stem-cell-based experimental and clinical trials: current status and open questions. *Expert Opin. Biol. Ther.* 11: 893–909.
- Tsokos, G. C. 2011. Systemic lupus erythematosus. *N. Engl. J. Med.* 365: 2110–2121.
- Nowling, T. K., and G. S. Gilkeson. 2011. Mechanisms of tissue injury in lupus nephritis. *Arthritis Res. Ther.* 13: 250–259.
- Lo, M. S., and G. C. Tsokos. 2012. Treatment of systemic lupus erythematosus: new advances in targeted therapy. *Ann. N. Y. Acad. Sci.* 1247: 138–152.
- Lichtman, E. I., S. M. Helfgott, and M. A. Krieger. 2012. Emerging therapies for systemic lupus erythematosus—focus on targeting interferon- $\alpha$ . *Clin. Immunol.* 143: 210–221.
- Gu, F., I. Molano, P. Ruiz, L. Sun, and G. S. Gilkeson. 2012. Differential effect of allogeneic versus syngeneic mesenchymal stem cell transplantation in MRL/lpr and (NZB/NZW)F1 mice. *Clin. Immunol.* 145: 142–152.
- Sun, L., K. Akiyama, H. Zhang, T. Yamaza, Y. Hou, S. Zhao, T. Xu, A. Le, and S. Shi. 2009. Mesenchymal stem cell transplantation reverses multiorgan dysfunction in systemic lupus erythematosus mice and humans. *Stem Cells* 27: 1421–1432.
- Ji, S., Q. Guo, Y. Han, G. Tan, Y. Luo, and F. Zeng. 2012. Mesenchymal stem cell transplantation inhibits abnormal activation of Akt/GSK3 $\beta$  signaling pathway in T cells from systemic lupus erythematosus mice. *Cell. Physiol. Biochem.* 29: 705–712.
- Gu, Z., K. Akiyama, X. Ma, H. Zhang, X. Feng, G. Yao, Y. Hou, L. Lu, G. S. Gilkeson, R. M. Silver, et al. 2010. Transplantation of umbilical cord mesenchymal stem cells alleviates lupus nephritis in MRL/lpr mice. *Lupus* 19: 1502–1514.
- Youd, M., C. Blickarz, L. Woodworth, T. Touzjian, A. Edling, J. Tedstone, M. Ruzek, R. Tubo, J. Kaplan, and T. Lodie. 2010. Allogeneic mesenchymal stem cells do not protect NZB $\times$ NZW F1 mice from developing lupus disease. *Clin. Exp. Immunol.* 161: 176–186.
- Schena, F., C. Gambini, A. Gregorio, M. Mosconi, D. Reverberi, M. Gattorno, S. Casazza, A. Uccelli, L. Moretta, A. Martini, and E. Traggiai. 2010. Interferon- $\gamma$ -dependent inhibition of B cell activation by bone marrow-derived mesenchymal stem cells in a murine model of systemic lupus erythematosus. *Arthritis Rheum.* 62: 2776–2786.
- Chang, J.-W., S.-P. Hung, H.-H. Wu, W.-M. Wu, A.-H. Yang, H.-L. Tsai, L.-Y. Yang, and O. K. Lee. 2011. Therapeutic effects of umbilical cord blood-derived mesenchymal stem cell transplantation in experimental lupus nephritis. *Cell Transplant.* 20: 245–257.
- Choi, E. W., I. S. Shin, S. Y. Park, J. H. Park, J. S. Kim, E. J. Yoon, S. K. Kang, J. C. Ra, and S. H. Hong. 2012. Reversal of serologic, immunologic, and histologic dysfunction in mice with systemic lupus erythematosus by long-term serial adipose tissue-derived mesenchymal stem cell transplantation. *Arthritis Rheum.* 64: 243–253.
- Wang, D., H. Zhang, J. Liang, X. Li, X. Feng, H. Wang, B. Hua, B. Liu, L. Lu, G. S. Gilkeson, et al. 2012. Allogeneic mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus: 4 years experience. *Cell Transplant.* 10.3727/096368912X658719.
- Sun, L., Y. H. Zhang, X. B. Feng, Y. Y. Hou, L. W. Lu, and L. M. Fan. 2007. Abnormality of bone marrow-derived mesenchymal stem cells in patients with systemic lupus erythematosus. *Lupus* 16: 121–128.
- Carrión, F., E. Nova, C. Ruiz, F. Díaz, C. Inostroza, D. Rojo, G. Mönckeberg, and F. E. Figueroa. 2010. Autologous mesenchymal stem cell treatment increased T regulatory cells with no effect on disease activity in two systemic lupus erythematosus patients. *Lupus* 19: 317–322.
- Sun, L., D. Wang, J. Liang, H. Zhang, X. Feng, H. Wang, B. Hua, B. Liu, S. Ye, X. Hu, et al. 2010. Umbilical cord mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus. *Arthritis Rheum.* 62: 2467–2475.
- Dominici, M., K. Le Blanc, I. Mueller, I. Slaper-Cortenbach, F. Marini, D. Krause, R. Deans, A. Keating, D. Prockop, and E. Horwitz. 2006. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8: 315–317.
- Pittenger, M. F., A. M. Mackay, S. C. Beck, R. K. Jaiswal, R. Douglas, J. D. Mosca, M. A. Moorman, D. W. Simonetti, S. Craig, and D. R. Marshak. 1999. Multilineage potential of adult human mesenchymal stem cells. *Science* 284: 143–147.
- Yang, J., Y. Chu, X. Yang, D. Gao, L. Zhu, X. Yang, L. Wan, and M. Li. 2009. Th17 and natural Treg cell population dynamics in systemic lupus erythematosus. *Arthritis Rheum.* 60: 1472–1483.
- Menke, J., W. A. Rabacal, K. T. Byrne, Y. Iwata, M. M. Schwartz, E. R. Stanley, A. Schwarting, and V. R. Kelley. 2009. Circulating CSF-1 promotes monocyte and macrophage phenotypes that enhance lupus nephritis. *J. Am. Soc. Nephrol.* 20: 2581–2592.
- Singh, R. G., S. Usha, S. S. Rathore, S. K. Behura, and N. K. Singh. 2012. Urinary MCP-1 as diagnostic and prognostic marker in patients with lupus nephritis flare. *Lupus* 21: 1214–1218.
- Chan, R. W.-Y., L.-S. Tam, E. K.-M. Li, F. M.-M. Lai, K.-M. Chow, K.-B. Lai, P. K.-T. Li, and C.-C. Szeto. 2003. Inflammatory cytokine gene expression in the urinary sediment of patients with lupus nephritis. *Arthritis Rheum.* 48: 1326–1331.
- Guo, Z., C. Zheng, Z. Chen, D. Gu, W. Du, J. Ge, Z. Han, and R. Yang. 2009. Fetal BM-derived mesenchymal stem cells promote the expansion of human Th17 cells, but inhibit the production of Th1 cells. *Eur. J. Immunol.* 39: 2840–2849.
- Traggiai, E., S. Volpi, F. Schena, M. Gattorno, F. Ferlito, L. Moretta, and A. Martini. 2008. Bone marrow-derived mesenchymal stem cells induce both polyclonal expansion and differentiation of B cells isolated from healthy donors and systemic lupus erythematosus patients. *Stem Cells* 26: 562–569.
- Ren, G., J. Su, L. Zhang, X. Zhao, W. Ling, A. L'huillier, J. Zhang, Y. Lu, A. I. Roberts, W. Ji, et al. 2009. Species variation in the mechanisms of mesenchymal stem cell-mediated immunosuppression. *Stem Cells* 27: 1954–1962.
- Chinnadurai, R., I. B. Copland, S. R. Patel, and J. Galipeau. 2014. IDO-independent suppression of T cell effector function by IFN- $\gamma$ -licensed human mesenchymal stromal cells. *J. Immunol.* 192: 1491–1501.
- Wang, D., X. Feng, L. Lu, J. E. Konkel, H. Zhang, Z. Chen, X. Li, X. Gao, L. Lu, S. Shi, et al. 2014. A CD8 T cell-indoleamine 2,3-dioxygenase axis is required for mesenchymal stem cell suppression of human systemic lupus erythematosus. *Arthritis Rheumatol.* 66: 2234–2245.10.1002/art.38674.
- Ni, X., Y. Q. Jia, W. T. Meng, L. Zhong, and Y. Zeng. 2009. Expression of B7-H1 molecule on human bone marrow mesenchymal stem cells and its effects on T lymphocyte proliferation. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* 17: 990–993.
- Meisel, R., A. Zibert, M. Laryea, U. Göbel, W. Däubener, and D. Dilloo. 2004. Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. *Blood* 103: 4619–4621.
- Ryan, J. M., F. Barry, J. M. Murphy, and B. P. Mahon. 2007. Interferon- $\gamma$  does not break, but promotes the immunosuppressive capacity of adult human mesenchymal stem cells. *Clin. Exp. Immunol.* 149: 353–363.
- Ho, A., L. S. Magder, S. G. Barr, and M. Petri. 2001. Decreases in anti-double-stranded DNA levels are associated with concurrent flares in patients with systemic lupus erythematosus. *Arthritis Rheum.* 44: 2342–2349.
- Sato, K., K. Ozaki, I. Oh, A. Meguro, K. Hatanaka, T. Nagai, K. Muroi, and K. Ozawa. 2007. Nitric oxide plays a critical role in suppression of T-cell proliferation by mesenchymal stem cells. *Blood* 109: 228–234.
- Polchert, D., J. Sobinsky, G. Douglas, M. Kidd, A. Moadsiri, E. Reina, K. Genrich, S. Mehrotra, S. Setty, B. Smith, and A. Bartholomew. 2008. IFN- $\gamma$  activation of mesenchymal stem cells for treatment and prevention of graft versus host disease. *Eur. J. Immunol.* 38: 1745–1755.