Mesenchymal stem cells efficiently inhibit the proinflammatory properties of 6-sulfo LacNAc dendritic cells

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ABSTRACT

Mesenchymal stem cells emerged as a promising treatment modality for steroid-refractory graft-versus-host disease, which represents a major complication of allogeneic hematopoietic stem cell transplantation. Dendritic cells (DCs) display an extraordinary capacity to induce T-cell responses and play a crucial role in the pathogenesis of graft-versus-host disease. Here, we investigated the impact of mesenchymal stem cells on the proinflammatory capacity of 6-sulfo LacNAc (slan) dendritic cells, representing a major subpopulation of human blood dendritic cells. Mesenchymal stem cells markedly impair maturation of slanDCs and their ability to secrete proinflammatory cytokines, which was dependent on prostaglandin E_2. In contrast, the release of anti-inflammatory IL-10 was improved by mesenchymal stem cells. Furthermore, mesenchymal stem cells efficiently inhibit slanDC-induced proliferation of $CD4^+$ and $CD8^+$ T cells and polarization of naïve $CD4^+$ T lymphocytes into Th1 cells. These results indicate that mesenchymal stem cells significantly impair the high proinflammatory capacity of slanDCs and further substantiate their potential for the treatment of graft-versus-host disease.

Key words: dendritic cells, mesenchymal stem cells, T cells, graft-versus-host disease.

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Introduction

Mesenchymal stem cells (MSCs) represent a population of non-hematopoietic, bone marrow stromal cells, which play a crucial role in supporting hematopoiesis and can differentiate into various mesodermal cell types such as osteocytes, chondrocytes, adipocytes and myocytes.[1-3] Due to their differentiation capability, MSCs emerged as promising candidates for therapeutic applications in tissue engineering. Furthermore, they display immunosuppressive properties that have prompted consideration of their potential use in the treatment of graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HSCT).[3-5] In this context, recent studies have investigated the impact of MSCs on phenotype and function of dendritic cells (DCs), which display an extraordinary capacity to induce T-cell responses[6,7] and may be critical for the initiation and maintainance of GVHD.[8-10] Thus, it has been demonstrated that MSCs inhibit the immunostimulatory capacity of human DCs, which were differentiated from monocytes or hemopoietic stem cells after several days in the presence of various cytokines.[11-14] However, studies exploring the impact of MSCs on native human DCs are limited. Here, we evaluated the influence of MSCs on immunostimulatory properties of 6-sulfo LacNAc (slan) DCs (formerly termed M-$DC8^+$ DCs), representing a proinflammatory subpopulation of human blood DCs.[15-17] SlanDCs are characterized by their selective phenotype (6-sulfo $LacNAc^+$, $CD1c^-$, $CD11c^+$, $CD14^-$, $CD16^+$, $CD45RA^+$, $C5aR^+$), the secretion of large amounts of tumor necrosis factor (TNF)-α and interleukin (IL)-12 and the efficient induction of $CD4^+$ and $CD8^+$ T-cell responses.[15-17] Due to their proinflammatory properties, which are retained after granulocyte-

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colony stimulating factor treatment of peripheral blood stem cell donors,[18] slanDCs may contribute to the pathogenesis of GVHD.

Design and Methods

Generation of human mesenchymal stem cells

The study was approved by the local institutional review board. Bone marrow samples were collected from healthy donors after obtaining informed consent. Isolation and cultivation of MSCs were performed as described.[19] The characteristic phenotype of MSCs ($CD44^+$, $CD73^+$, $CD90^+$, $CD105^+$, $CD166^+$, $CD14^-$, $CD34^-$, $CD45^-$) was determined by flow cytometry. Furthermore, their ability to differentiate into osteocytes and adipocytes was analyzed.

Immunomagnetic isolation of slanDCs and T cells

Blood samples were obtained with informed consent from healthy donors. Immunomagnetic isolation of slanDCs (purity: >90%) from peripheral blood mononuclear cells (PBMCs) was performed as previously described.[15] $CD4^+$ T cells, naïve $CD45RA^+CD4^+$ T cells and $CD8^+$ T lymphocytes were purified from PBMCs by negative depletion using immunomagnetic separation (purity: >90%) according to the manufacturer´s instructions (Miltenyi Biotec, Bergisch-Gladbach, Germany). The culture medium used for all experiments was previously described.[17]

Flow cytometric analysis

Expression analysis of surface or intracytoplasmic molecules of MSCs, slanDCs, $CD4^+$ and $CD8^+$ T cells was performed as described[17] using the following monoclonal antibodies: FITC-conjugated anti-CD3, FITC-conjugated anti-CD4, PE-conjugated anti-CD8, FITC-conjugated anti-CD14, PE-conjugated anti-CD34, FITC-conjugated anti-CD44, FITC-conjugated anti-CD45, PE-conjugated anti-CD45RA, PE-conjugated anti-CD73, PE-conjugated anti-CD83, PE-conjugated anti-CD86, FITC-conjugated anti-CD90, FITC-conjugated anti-CD105, PE-conjugated anti-CD166, FITC-conjugated anti-human leukocyte antigen (HLA)-DR, PE-conjugated anti-intercellular adhesion molecule (ICAM)-1, FITC-conjugated anti-interferon (IFN)-γ, PE-conjugated anti-IL-4, PE-conjugated anti-IL-6 and PE-conjugated isotype-specific anti-mouse antibodies (all BD Biosciences, San José, CA, USA), FITC-conjugated anti-immunoglobulin like transcript (ILT) 3 and FITC-conjugated anti-ILT4 (R&D Systems, Wiesbaden, Germany). Negative controls included labeled isotype-matched irrelevant antibodies (BD Biosciences). M-DC8 hybridoma supernatant was used as described.[17]

Maturation and cytokine production of slanDCs

Freshly isolated slanDCs (2×10^5/well) were cultivated for 12 h in the presence or absence of MSCs (4×10^4/well) and washed. Subsequently, expression levels of the maturation marker CD83, the costimulatory molecule CD86, HLA-DR and the adhesion molecule ICAM-1 at the surface of slanDCs were determined by flow cytometry. In additional experiments, expression levels of ILT3 and ILT4 on slanDCs were analyzed after 24 h by flow cytometry.

To investigate cytokine release, slanDCs were plated in round-bottomed 96-well plates at 2×10^5/well and incubated with MSCs at different MSC-DC ratios (1:2,5; 1:5; 1:10). After 6 h, 1 μg/mL lipopolysaccharide (LPS, Sigma-Aldrich, Taufkirchen, Germany) was added for an additional 18 h to stimulate cytokine release by slanDCs. Supernatants were collected and the concentration of TNF-α, IL-12p70 and IL-10 was determined by ELISA according to the manufacturer's instructions (BD Biosciences). Following our observation that MSCs themselves produce significant amounts of IL-6, we evaluated the impact of MSCs on IL-6 production of slanDCs by flow cytometry. In further experiments, slanDCs and MSCs were cocultured in the presence or absence of the prostaglandin E_2 (PGE_2) inhibitor NS-398 (5 mM, Alexis, San Diego, CA, USA) dissolved in dimethyl sulfoxide (DMSO) or equal amounts of DMSO as a control. PGE_2 concentration was analyzed by ELISA according to the manufacturer´s instructions (R&D Systems). To analyze the contribution of each cell type to the PGE_2 release, slanDCs (1×10^6/well) and MSCs (2×10^5/well) were cocultered in 24-well plates with LPS in the presence or absence of a separating porous membrane (pore size: 0.45 μm; Millipore Cooperation, Bedford, MA, USA). After 24 h, the separated or cocultured slanDCs and MSCs were harvested and washed. Subsequently, slanDCs (2×10^5/well) and MSCs (4×10^4/well) were maintained alone or together for an additional 24 h in round-bottomed 96-well plates. Then, supernatants were collected and PGE_2 concentration was determined by ELISA.

T-cell proliferation and programming

SlanDCs (2×10^5/well) were maintained with or without irradiated MSCs (4×10^4/well). After 6 h, slanDCs were separated from adherent MSCs by resuspension and washed with medium. Flow cytometric analysis revealed that the purity of the obtained slanDCs was >90% and that contaminating MSCs represent less than 5% of the harvested slanDCs.

To investigate T-cell proliferation, slanDCs (1×10^4 cells/well) were incubated with allogeneic $CD4^+$ or $CD8^+$ T cells (1×10^5 cells/well) for four days in round-bottomed 96-well plates. ^{3}H-thymidine (1 μCi, Hartmann Analytic, Braunschweig, Germany) was added to each well for the last 18 h of culture. Cells were harvested and incorporation was determined in a beta counter (Wallac, Freiburg, Germany).

To analyze T-cell programming, MSC-preincubated slanDCs (1×10^4 cells/well) were cocultured with allogeneic naïve T cells (1×10^5 cells/well) in the presence of LPS for eight days. Thereafter, T cells were stimulated with 10 ng/mL phorbol myristate acetate (PMA, Sigma-Aldrich) and 1 μg/mL ionomycin (Sigma-Aldrich) for 4 h and evaluated for IFN-γ and IL-4 production.

Statistical analysis

Student's *t* test was performed to evaluate the significance of the results. Values of $p<0.05$ were considered as significant.

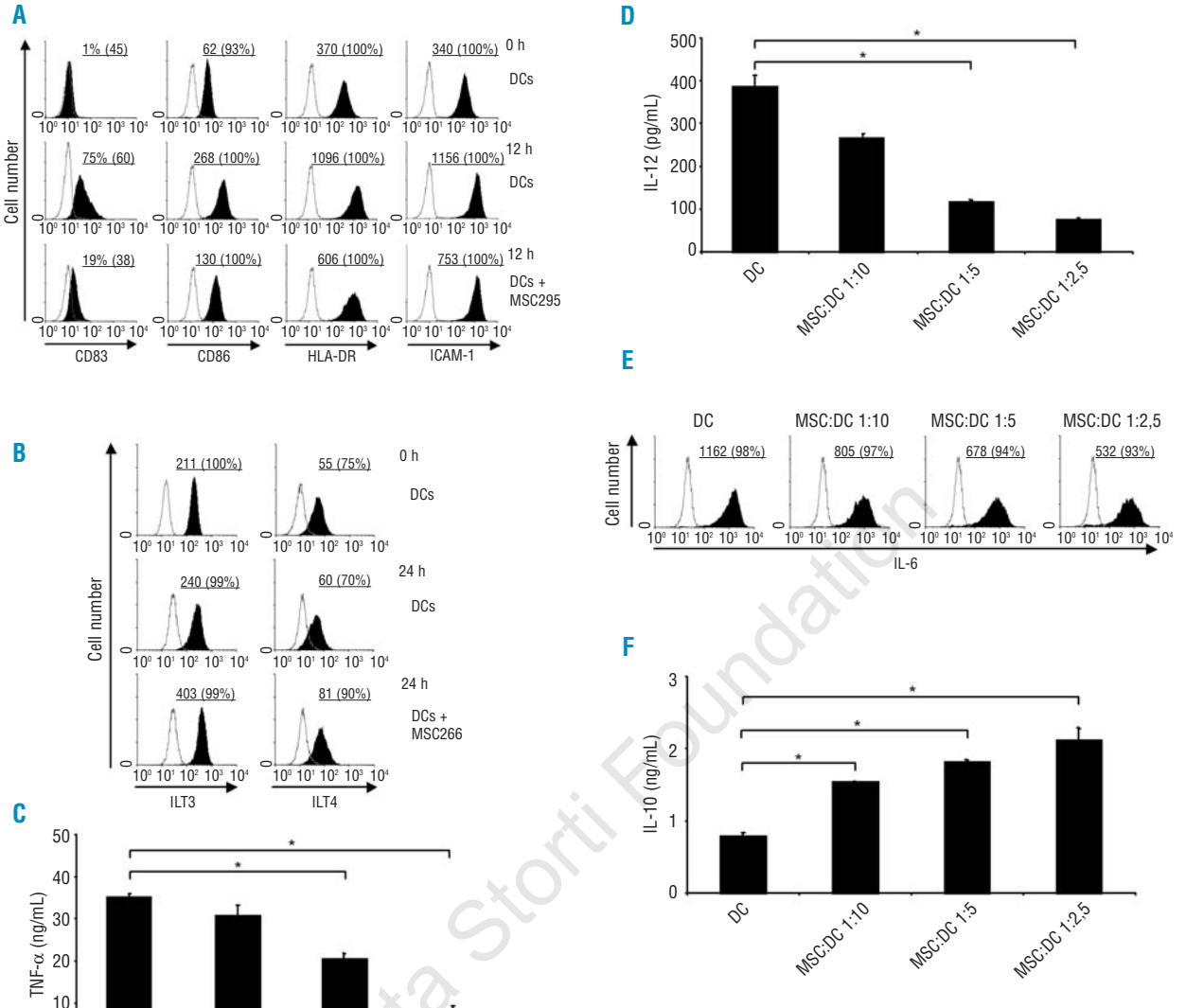

Figure 1. Impact of Mesenchymal stem cells (MSCs) on maturation and cytokine production of slanDCs. (A) SlanDCs were maintained in the presence or absence of MSCs for 12 h. Subsequently, expression levels of CD83, CD86, HLA-DR and ICAM-1 at the surface of slanDCs were determined by flow cytometry. The results of one representative MSC line out of three performed with similar results are depicted. Values represent the MFI and percentage of cells staining positive for each surface molecule (filled) compared to the respective isotype control (empty). (B) SlanDCs were coincubated with or without MSCs for 24 h. Then, expression levels of ILT3 and ILT4 on slanDCs were analyzed by flow cytometry. The results of one representative MSC line out of three performed with similar results are demonstrated. Values represent the MFI and percentage of cells staining positive for each surface molecule (filled) compared to the respective isotype control (empty). (C-F) SlanDCs were cultivated with MSCs at different MSC-DC ratios (1:2,5, 1:5, 1:10). After 6 h, DCs were stimulated with LPS for an additional 18 h. Supernatants were collected and concentration of TNF-α, IL-12 and IL-10 was analyzed by ELISA. Intracellular IL-6 expression of slanDCs was determined by flow cytometry. The results of one representative MSC line out of three different MSC lines performed with similar results are demonstrated. (C, D, F) Values represent the mean ± SE of triplicate samples. Asterisks indicate a statistically significant difference. (E) Values represent the MFI and percentage of cells staining positive for IL-6 (filled) compared to the respective isotype control (empty).

Results and Discussion

Mesenchymal stem cells inhibit maturation and modulate cytokine secretion of slanDCs

To get novel insights into the impact of MSCs on immunostimulatory properties of slanDCs, we evaluated whether MSCs influence maturation and cytokine production of this blood DC subset. We found that MSCs efficiently reduce the percentage of slanDCs expressing the maturation marker CD83 and the cell surface density of CD86, HLA-DR and ICAM-1 (Figure 1A) indicating that MSCs inhibit the spontaneous maturation of this DC subset. This MSC-mediated effect has also been described in previous studies. Thus, it has been reported that MSCs impair the differentiation of human monocytes and hemopoietic stem cells into DCs.[12-14,20] Furthermore, it has been demonstrated that MSCs inhibit the differentiation of $CD11c^+$ myeloid DCs.[21] In further experiments, we investigated whether MSCs modulate the expression levels of the inhibitory molecules ILT3 and ILT4, which were shown to be upregulated at the surface of tolerogenic DCs.[22] As shown in Figure 1B, MSCs increased the cell surface density of ILT3 and ILT4 on slanDCs.

Proinflammatory cytokines such as TNF-α play a critical role in the induction and maintainance of GVHD.[23] Therefore, we investigated the impact of MSCs on cytokine release of activated slanDCs, which produce large amounts of TNF-α, IL-6 and IL-12.[16,17] Interestingly, MSCs profoundly impaired the capacity of LPS-activated slanDCs to secrete TNF-α, IL-6 and IL-12 (Figure 1C-E). In contrast, the production of anti-inflammatory IL-10 by slanDCs was markedly enhanced by MSCs (Figure 1F). MSCs did not secrete significant amounts of IL-10 under these conditions as determined by flow cytometry (*data not shown*). Furthermore, we found that MSCs are not able to induce IL-10 secretion by slanDCs in the absence of LPS (*data not shown*). These results support recent studies demonstrating that MSCs markedly inhibit the production of TNF-α and IL-12 by monocyte-derived DCs.[11,12] In addition, it has been reported that MSCs impair TNF-α release by $CD1c^+$ myeloid DCs and enhance IL-10 secretion by plasmacytoid DCs.[24] These findings reveal that MSCs direct native human DCs toward a tolerogenic phenotype.

When focusing on the underlying mechanisms, we found that the interaction between MSCs and slanDCs induced a strong increase of PGE_2 secretion, which was almost completely abrogated by NS-398 (Figure 2A). To investigate into the contribution of each cell type to the observed PGE_2 release, slanDCs were cocultered with MSCs in the presence or absence of a separating porous membrane. Thereafter, the separated or coincubated slanDCs and MSCs were harvested, washed and cultured for additional 24 h. As demonstrated in Figure 2B, MSCs represent the main producers of PGE_2 under these conditions. Following this observation, the relevance of PGE_2 for MSC-mediated impairment of TNF-α and IL-12 release by slanDCs was investigated. Notably, inhibition of PGE_2 secretion by NS-398 resulted in a significant improvement of TNF-α and IL-12 production by LPS-activated slanDCs (Figure 2C and D) indicating that this MSC-mediated immunomodulatory effect is critically dependent on PGE_2. This finding is in line with a previ-

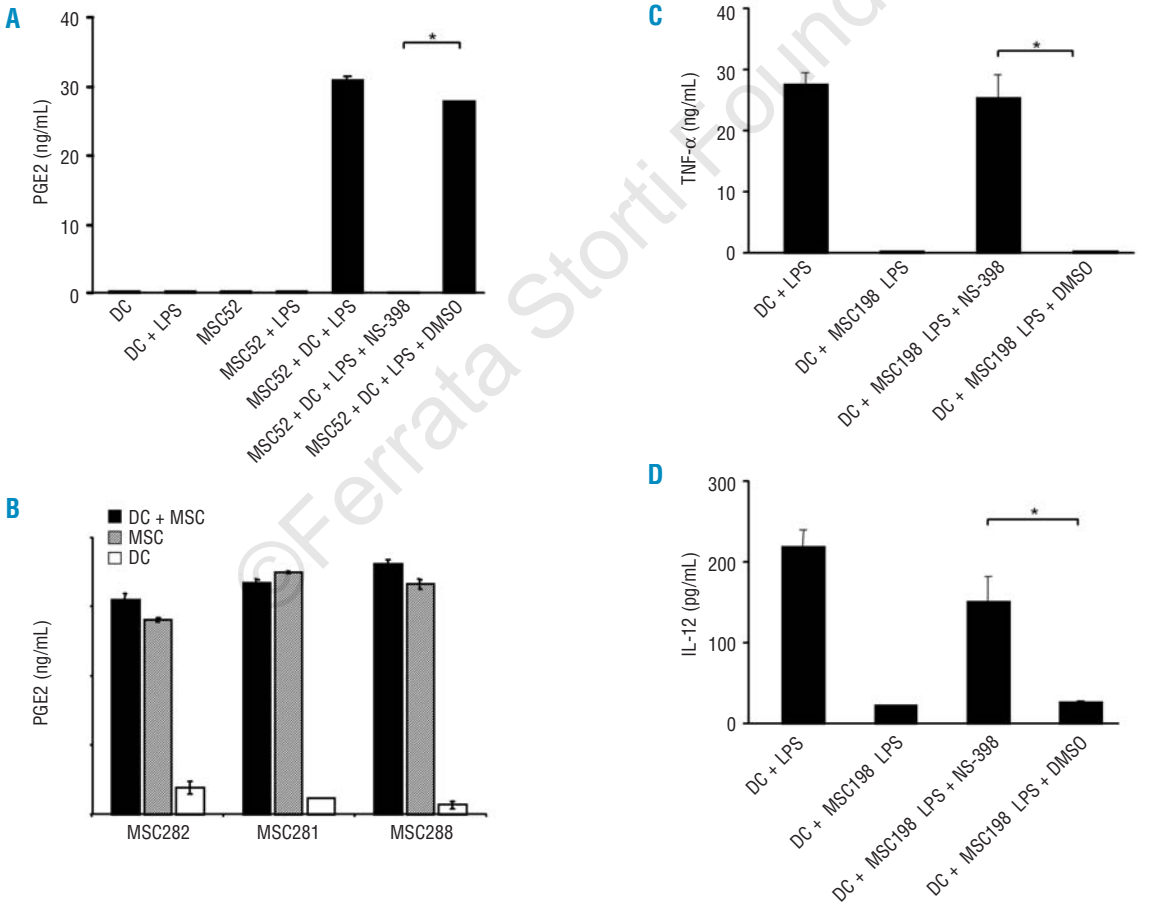

Figure 2. MSC-mediated impairment of TNF-α and IL-12 production by slanDCs is critically dependent on PGE_2. (A) MSCs were cultivated with or without slanDCs in the presence or absence of NS-398. After 24 h, supernatants were collected and concentration of PGE_2 was determined. (B) SlanDCs and MSCs were cocultered in the presence or absence of a separating porous membrane. After 24 h, the separated or coincubated slanDCs and MSCs were harvested, washed and cultured for an additional 24 h. Then, supernatants were collected and PGE_2 concentration was measured. (C,D) MSCs were cultivated with slanDCs in the presence or absence of NS-398. After 24 h, supernatants were collected and concentration of TNF-α and IL-12 was determined. The results of one representative MSC line out of (A) eight or (B,C,D) three different MSC lines performed with similar results are demonstrated. Values represent the mean ± SE of triplicate samples. Asterisks indicate a statistically significant difference.

ous study demonstrating the contribution of PGE_2 to an impaired TNF-α release by activated DCs.[24] Furthermore, recent reports documented that also other soluble factors such as IL-6 and macrophage-colony stimulating factor as well as cell-to-cell contact play a role in the MSC-mediated inhibition of differentiation and cytokine production of monocyte- or hemopoietic stem cell-derived DCs.[12-14,25]

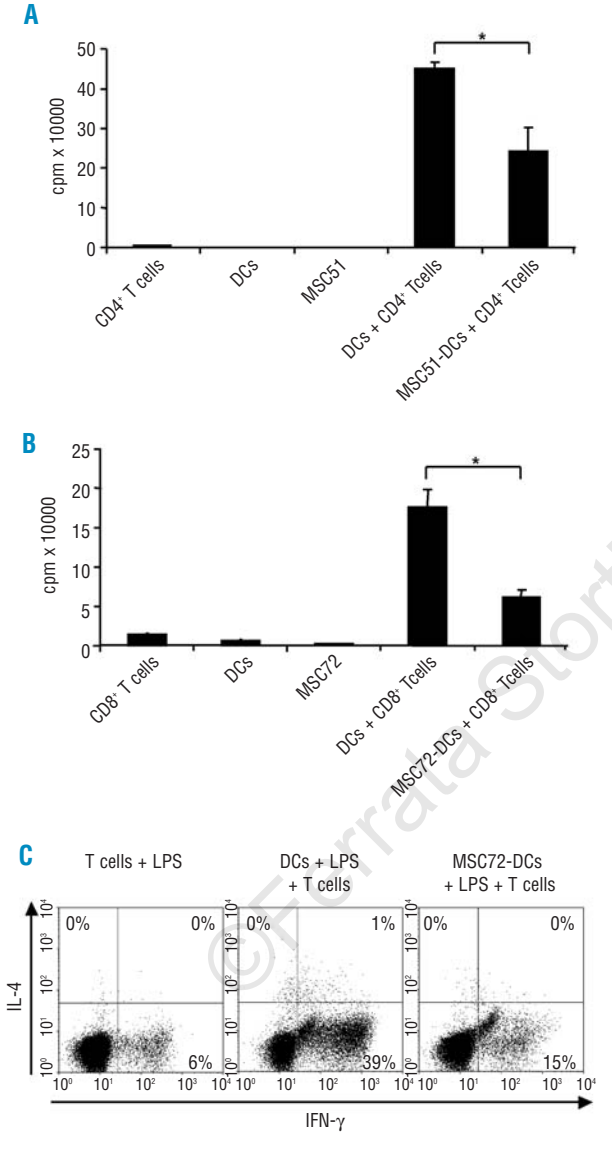

Figure 3. Influence of mesenchymal stem cells (MSCs) on slanDC-mediated proliferation and programming of T cells. SlanDCs were maintained for 6 h in the presence of MSCs. Subsequently, DCs were separated from MSCs and cocultured with allogeneic (A) $CD4^+$ T cells or (B) $CD8^+$ T cells. After four days, ^{3}H-thymidine incorporation was determined. The results of one representative MSC line out of three different MSC lines performed with similar results are depicted. Asterisks indicate a statistically significant difference. (C) SlanDCs were cultured for 6 h with MSCs. Thereafter, DCs were separated from MSCs and coincubated with allogeneic naïve T cells in the presence of LPS for eight days. Subsequently, T cells were stimulated with PMA and ionomycin and the percentage of IFN-γ- or IL-4-producing $CD4^+$ T cells was determined. The results of one representative MSC line out of three different MSC lines performed with similar results are shown.

Mesenchymal stem cells impair slanDC-induced T-cell proliferation and programming

Based on the finding that $CD4^+$ and $CD8^+$ T cells play a pivotal role in the pathogenesis of GVHD,[23] we evaluated the influence of MSCs on slanDC-mediated T-cell proliferation. Therefore, slanDCs were maintained in the presence or absence of irradiated MSCs for 6 h and subsequently separated from adherent MSCs by resuspension. The purity of the obtained slanDCs was >90%. Contaminating MSCs represent less than 5% of the harvested slanDCs. Then, slanDCs were incubated with allogeneic $CD4^+$ or $CD8^+$ T cells. We found that MSCs significantly reduced the capacity of slanDCs to induce proliferation of allogeneic $CD4^+$ and $CD8^+$ T cells (Figure 3A and B). To investigate whether 5% contaminating MSCs are able to directly inhibit T-cell proliferation, MSCs (5×10^2 cells/well) were incubated with the T cells (1×10^5 cells/well) in the presence of PHA. We observed that the amount of contaminating MSCs did not influence the proliferation of stimulated T cells (*data not shown*).

Following our observation that LPS-activated slanDCs direct the polarization of naïve $CD4^+$ T lymphocytes into Th1 cells,[17] which promote GVHD by secreting cytokines such as IFN-γ and TNF-α,[23] we determined the impact of MSCs on this functional property of slanDCs. As depicted in Figure 3C, MSCs profoundly inhibited the capacity of LPS-activated slanDCs to direct the differentiation of naïve $CD4^+$ T cells into IFN-γ-producing Th1 cells. These results reveal that MSCs significantly impair slanDC-induced T-cell proliferation and polarization of naïve $CD4^+$ T lymphocytes into Th1 cells and that these effects remain when MSCs are removed from the culture. In summary, these results provide evidence that MSCs significantly impair the immunostimulatory capacity of the highly proinflammatory slanDCs, which may contribute to the pathogenesis of GVHD.[18] This immunomodulatory effect of MSCs makes them an even more attractive approach for the prevention and treatment of GVHD after allogeneic HSCT. This therapeutic strategy is supported by recent clinical trials demonstrating that the administration of MSCs induced clinical responses in therapy-resistant acute GVHD patients.[26,27] Since slanDCs have been found in inflamed tissues of rheumatoid arthritis and psoriasis vulgaris,[17] our results may also have implications for the potential use of MSCs in the treatment of autoimmune diseases.

Authorship and Disclosures

RW performed research and analyzed the data; DW performed research; MB analyzed the data and revised the manuscript; SZ performed research; KS, MPB, UP, GE, EPR analyzed the data and revised the manuscript; MS designed research, analyzed the data and wrote the manuscript.

The authors reported no potential conflicts of interest.

References

1. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143-7.
2. Chamberlain G, Fox J, Ashton B, Middleton J. Mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells 2007; 25:2739-49.
3. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nat Rev Immunol 2008; 8:726-36.
4. Nauta AJ, Fibbe WE. Immunomodulatory properties of mesenchymal stromal cells. Blood 2007; 110:3499-506.
5. Le Blanc K, Ringdén O. Mesenchymal stem cells: properties and role in clinical bone marrow transplantation. Curr Opin Immunol 2006:18:586-91.
6. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, et al. Immunobiology of dendritic cells. Annu Rev Immunol 2000;18:767-811.
7. Steinman RM, Banchereau J. Taking dendritic cells into medicine. Nature 2007;449:419-26.
8. Shlomchik WD, Couzens MS, Tang CB, McNiff J, Robert ME, Liu J, et al. Prevention of graft-versus-host disease by inactivation of host antigen-presenting cells. Science 1999;285: 412-5.
9. Matte CC, Liu J, Cormier J, Anderson BE, Athanasiadis I, Jain D, et al. Donor APCs are required for maximal GvHD but not for GvL. Nat Med 2004;10:987-92.
10. Merad M, Collin M, Bromberg J. Dendritic cell homeostasis and trafficking in transplantation. Trends Immunol 2007;28:353-9.
11. Beyth S, Borovsky Z, Mevorach D, Liebergall M, Gazit Z, Aslan H, et al. Human mesenchymal stem cells alter antigen-presenting cell maturation and induce T-cell unresponsiveness. Blood 2005;105:2214-9.
12. Jiang XX, Zhang Y, Liu B, Zhang SX, Wu Y, Yu XD, et al. Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. Blood 2005; 105:4120-6.
13. Nauta AJ, Kruisselbrink AB, Lurvink E, Willemze R, Fibbe WE. Mesenchymal stem cells inhibit generation and function of both CD34+-derived and monocyte-derived dendritic cells. J Immunol 2006;177: 2080-7.
14. Li YP, Paczesny S, Lauret E, Poirault S, Bordigoni P, Mekhloufi F, et al. Human mesenchymal stem cells license adult CD34+ hemopoietic progenitor cells to differentiate into regulatory dendritic cells through activation of the Notch pathway. J Immunol 2008;180:1598-608.
15. Schäkel K, Mayer E, Federle C, Schmitz M, Riethmüller G, Rieber EP. A novel dendritic cell population in human blood: one-step immunomagnetic isolation by a specific mAb (M-DC8) and in vitro priming of cytotoxic T lymphocytes. Eur J Immunol 1998;28:4084-93.
16. Schäkel K, Kannagi R, Kniep B, Goto Y, Mitsuoka C, Zwirner J, et al. 6-Sulfo LacNAc, a novel carbohydrate modification of PSGL-1, defines an inflammatory type of human dendritic cells. Immunity 2002;17:289-301.
17. Schäkel K, von Kietzell M, Hänsel A, Ebling A, Schulze L, Haase M, et al. Human 6-sulfo LacNAc-expressing dendritic cells are principal producers of early interleukin-12 and are controlled by erythrocytes. Immunity 2006;24:767-77.
18. Baumeister SH, Holig K, Bornhäuser M, Meurer M, Rieber EP, Schäkel K. G-CSF mobilizes slanDCs (6-sulfo LacNAc+ dendritic cells) with a high proinflammatory capacity. Blood 2007;110:3078-81.
19. Oswald J, Boxberger S, Jorgensen B, Feldmann S, Ehninger G, Bornhäuser M, et al. Mesenchymal stem cells can be differentiated into endothelial cells in vitro. Stem Cells 2004;22:377-84.
20. Zhang W, Ge W, Li C, You S, Liao L, Han Q, et al. Effects of mesenchymal stem cells on differentiation, maturation, and function of human monocyte-derived dendritic cells. Stem Cells Dev 2004;13:263-71.
21. Maccario R, Podestà M, Moretta A, Cometa A, Comoli P, Montagna D, et al. Interaction of human mesenchymal stem cells with cells involved in alloantigen-specific immune response favors the differentiation of CD4+ T-cell subsets expressing a regulatory/suppressive phenotype. Haematologica 2005;90: 516-25.
22. Manavalan JS, Rossi PC, Vlad G, Piazza F, Yarilina A, Cortesini R, et al. High expression of ILT3 and ILT4 is a general feature of tolerogenic dendritic cells. Transplant Immunol 2003;11:245-58.
23. Welniak LA, Blazar BR, Murphy WJ. Immunobiology of allogeneic hematopoietic stem cell transplantation. Annu Rev Immunol 2007;25: 139-70.
24. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 2005;105:1815-22.
25. Djouad F, Charbonnier LM, Bouffi C, Louis-Plence P, Bony C, Apparailly F, et al. Mesenchymal stem cells inhibit the differentiation of dendritic cells through an interleukin-6-dependent mechanism. Stem Cells 2007;25:2025-32.
26. Le Blanc K, Rasmussen I, Sundberg B, Götherström C, Hassan M, Uzunel M, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 2004; 363:1439-41.
27. Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet 2008;371: 1579-86.