Donor-derived mesenchymal stem cells are immunogenic in an allogeneic host and stimulate donor graft rejection in a nonmyeloablative setting

Alma J. Nauta, Geert Westerhuis, Alwine B. Kruisselbrink, Ellie G. A. Lurvink, Roel Willemze, and Willem E. Fibbe

Introduction

Human bone marrow stromal cells, also referred to as mesenchymal stem cells (MSCs), are able to differentiate along multiple lineages such as chondrocytes, osteoblasts, adipocytes, myocytes, and astrocytes. MSCs, rare residents in the bone marrow, can be rapidly expanded ex vivo without loss of their multilineage differentiation potential. Because of their ability to migrate to sites of tissue injury, MSCs have emerged as a promising therapeutic modality for tissue regeneration and repair. Several studies in animal models have demonstrated that MSCs are capable of long-term engraftment and in vivo differentiation, and encouraging results have been reported in clinical use.

MSCs are known to secrete a number of cytokines and regulatory molecules implicated in different aspects of hematopoiesis. These characteristics have generated clinical interest to use MSCs to enhance hematopoietic stem cell engraftment. Although animal models provide experimental evidence that MSCs facilitate engraftment, no conclusive evidence has yet been presented in humans. In addition to providing critical growth factors, MSCs display immunosuppressive properties that might facilitate engraftment. In vitro studies with human, baboon, and murine MSCs demonstrated that MSCs suppress the proliferation of T cells induced by alloantigens or mitogens. Furthermore, MSCs have been reported to induce T-cell division arrest, to inhibit the differentiation and maturation of dendritic cells, and to decrease the production of inflammatory cytokines by various immune cell populations. Controversy exists regarding their effect on cytotoxic T-cells and NK cells. Animal studies indicate that, in line with their immunosuppressive capacities in vitro, MSCs also display immunosuppressive capacities in vivo; allogeneic MSCs may prolong skin allograft survival in immunocompetent baboons and may prevent the rejection of allogeneic tumor cells in immunocompetent mice. The mechanisms underlying these effects of MSCs have not been clearly identified. Although conflicting results have been reported, most studies agree that soluble factors are involved. The therapeutic application of the immunosuppressive properties of MSCs has already been exploited in the clinical setting for the treatment of acute graft-versus-host disease after allogeneic stem cell transplantation.

The immunophenotype of MSCs, the low expression of human leukocyte antigen (HLA) class I, and the absence of co-stimulatory molecules, together with the observation that MSCs do not elicit a proliferative response from allogeneic lymphocytes, suggest that MSCs are of inherently low immunogenicity. These properties might open attractive possibilities to use universal donor MSCs for different therapeutic applications.

The aim of this study was to examine whether MSCs display immunosuppressive properties in vivo in murine allogeneic bone marrow transplantation models. The transplantation of host murine MSCs into sublethally conditioned recipients resulted in decreased...
rejection of donor BM cells. In contrast, cotransplantation with allogeneic donor MSCs significantly decreased engraftment of allogeneic BM. Moreover, the infusion of allogeneic MSCs led to the development of a cellular immune response in nonimmunocompromised mice. These results indicate that MSCs can have potent immunosuppressive capacities in vivo, but, importantly, MSCs are not intrinsically immunoprivileged and may trigger immune responses in vivo resulting in graft rejection.

Materials and methods

Mice

Female BALB/c, C57BL/6 (B6), and C3H mice (8-12 weeks old) were obtained from Charles River Laboratories (Maastricht, The Netherlands) and were kept under standard animal housing setting. Congenic C57BL/6-Ly5.1 and BALB/c mice were bred at our own facilities and were used at 8 to 12 weeks of age. Mice used in experiments were sex and age matched.

Bone marrow transplantation

BALB/c and C57BL/6-Ly5.1 recipients were conditioned with a sublethal 6-Gy or 5-Gy dose of total body irradiation, respectively, 1 day before transplantation. Donor bone marrow (BM) cells from B6 or BALB/c mice were flushed from tibiae, femurs, and humeri using RPMI 1640 (BioWhittaker, Verviers, Belgium) supplemented with 2% heat-inactivated fetal calf serum (FCS; BioWhittaker) and heparin (24 IU/mL; Hospital Pharmacy, Leiden University Medical Center, Leiden, The Netherlands). BM cells were filtered through a sterile nylon mesh, resuspended in sterile phosphate-buffered saline (PBS; Hospital Pharmacy), and injected intravenously with or without murine mesenchymal stem cells from different origins. In vivo depletion of donor CD4+ and CD8+ T cells was accomplished by using 100 μg anti-CD4 mAb (GK1.5) and anti-CD8 mAb 2.43 intraperitoneally on day –5 and day –1.

Culture of murine MSCs

Murine MSCs were obtained from bone marrow aspirates, as described previously,22 and were cultured in α-modified minimum essential medium (α-MEM) supplemented with 10% FCS, 100 IU/mL penicillin, 100 μg/mL streptomycin (Gibco Life Technologies), and 2 mM L-glutamine (BioWhittaker). Before experimental use, MSCs were tested for their ability to differentiate into adipocytes, osteoblasts, and chondrocytes. To induce adipogenic differentiation, MSCs were cultured with 10−8 M dexamethasone and 5 μg/mL insulin, and Oil-red-O was used to detect lipid droplet staining. Osteogenic differentiation was induced by 10−8 M dexamethasone, 10 mM β-glycerophosphate, and 50 μg/mL ascorbic acid. Osteoblasts were identified by Alizarin Red S staining. For chondrocyte differentiation, a pellet culture system was used. Chondrogenic differentiation medium consisted of high-glucose DMEM (Gibco Laboratories, Grand Island, NY) supplemented with 40 μg/mL proline, 50 mg/mL ITS + premix, 100 μg/mL sodium pyruvate, glutamax, 50 μg/mL ascorbate-2-phosphate, 10 ng/mL TGF-β3, 10−7 M dexamethasone, and 500 ng/mL BMP-6. Chondrogenic differentiation was visualized by Toluidine blue staining. Murine MSCs expanded in vitro showed positive surface staining for CD106 and lacked hematopoietic (CD45, CD14) and endothelial (CD31) markers.

Mixed-lymphocyte reactions

Splenocytes were isolated by mechanical dissociation through sterile nylon mesh (100 μM; BD Biosciences, Franklin Lakes, NJ), followed by red blood cell lysis (incubation for 10 minutes at 4°C with NH4Cl (8.4 g/L)/KHCO3 (1 g/L) buffer). Responder splenocytes from BALB/c mice (3 × 105/well in 96-well round-bottom plates) were added to irradiated (30 Gy) stimulator splenocytes in Iscove modified Dulbecco medium (IMDM; BioWhittaker) supplemented with 10% FCS, PS, and 50 μM β-mercaptoethanol. In mitogen proliferative assays, responder splenocytes were incubated with 5 μg/mL concanavalin A (ConA; Sigma). MSCs (60 Gy irradiated) were added in graded doses to the mixed-lymphocyte reaction (MLR). After 5 days of incubation, cells were pulsed with 3H-thymidine (1 μCi [0.037 MBq]/well) for the last 18 hours, harvested, and counted with a Topcount NXT (Canberra Packard, Meriden, CT). Results are expressed in counts per minute and are presented as means (± SD) obtained from triplicate cultures.

Chimerism analysis

Blood was collected at various time points after bone marrow transplantation (BMT), and red blood cells were removed. Multilineage chimerism was determined by flow cytometry (FACScalibur) using fluorescein isothiocyanate (FITC)–conjugated anti-H–2Kb (clone AF6-88.5) or phycoerythrin (PE)–conjugated anti-CD45.1 mAbs (clone A20) specific for the donor and PE-conjugated anti–H–2Kb (clone SFI-1.1) or FITC-conjugated anti-CD45.2 mAbs (clone 104) specific for the recipient in combination with allophycocyanin (APC)–conjugated anti-CD3e (clone 145-2C11), anti-B220 (clone RA3-6B2), and anti–GR-1 (clone RB6-8C5) mAbs. All antibodies were obtained from PharMingen (Alphen aan de Rijn, The Netherlands). Chimerism was expressed by percentage donor granulocytes, and engraftment was considered positive if at least 10% of recipient granulocytes had the donor BM Ly5 or H-2 phenotype.

Immunologic analyses

Specificity of the induced tolerance was determined by performing MLR and cell-mediated lympholysis (CML) assays. MLRs were performed by obtaining spleen cells from recipient BALB/c and B6-Ly5.1 mice 4 months after transplantation and then were used as responders (3 × 105/well in round-bottom 96-well plates) against γ-irradiated (30 Gy) BALB/c, B6, or DBA splenocytes stimulated for 48 hours with 50 μg/mL LPS. After 5 days of incubation, 3H-thymidine (1 μCi [0.037 MBq]/well) was added overnight, and thymidine incorporation was measured. CML assays were performed by incubating recipient spleen cells (3 × 105/well in 24-well plates) with irradiated D1 cells (105/well). After 6 days of culture, effector cells were collected and incubated for 4 hours with 105 chromium 51 (51Cr)–labeled target cells expressing H-2Kb (EL4) or H-2Kd (p815) MHC molecules. Specific lysis was defined as 100 × (experimental release – spontaneous release)/(detergent release – spontaneous release).

In vivo immunization of mice

MSCs derived from different origins were injected (0.5 × 106 cells) intravenously into BALB/c (MSCs from B6 and BALB/c) or B6 (MSCs from BALB/c and B6) mice. Four weeks later, in vivo cytototoxicity was determined as described previously.22 In brief, allogeneic and syngeneic splenocytes (105/mL in PBS) were differentially labeled for 10 minutes at 37°C with 0.5 or 0.5 μM carboxy fluorescein diacetate succinimidyl ester (CFDA-SE; Molecular Probes, Leiden, The Netherlands), respectively. Labeled cells (10 × 106) were injected intravenously in a 1:1 ratio, and after various time points the ratio of carboxy fluorescein succinimidyl ester (CFSE)1/CFSE2 donor cells was determined by flow cytometry.

Statistical analysis

Statistical analysis was performed by Student 2-tailed unpaired t test or 2-way analysis of variance (ANOVA) using GraphPadPRISM (GraphPad Software, San Diego, CA). Differences were considered statistically significant when P was below .05.

Results

MSCs inhibit the proliferation of T cells

To confirm the in vitro immunomodulatory effect of murine MSCs, graded doses of BALB/c or B6 MSCs were added to mixed lymphocyte cultures of BALB/c and B6 splenocytes. Murine
MSCs inhibited the proliferation of allogeneic responder splenocytes in a dose-dependent manner (Figure 1A). Similarly, murine MSCs inhibited the proliferation of responder splenocytes induced by ConA (Figure 1B), whereas murine MSCs themselves did not elicit a proliferative response of allogeneic splenocytes (Figure 1C).

Optimization of BM engraftment in sublethally irradiated recipient mice

Initial experiments were performed to determine the dose of sublethal irradiation that resulted in graft rejection in 50% of recipient mice. Engraftment of donor cells in peripheral blood was examined by flow cytometry at various time points after transplantation. All BALB/c recipients conditioned with 7 Gy showed long-term multilineage chimerism, whereas after the exposure to 6.5 Gy or 6 Gy, only 80% or 30%, respectively, of the BALB/c mice showed long-term multilineage engraftment (Table 1). In the multiple minor mismatched transplantsations, long-term chimeras were observed in all recipients exposed to 6 Gy, 50% of the recipients exposed to 5 Gy, and in none of the recipients exposed to 4 Gy (Table 1).

Table 1. Effect of irradiation dose on the level of chimerism

<table>
<thead>
<tr>
<th>Donor → recipient, irradiation dose, Gy</th>
<th>B6 donor chimerism (%)</th>
<th>Donor chimerism in chimeric mice 12-16 weeks after Tx, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GR-1</td>
<td>B220</td>
</tr>
<tr>
<td>B6 → BALB/c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>3 of 10 (30)</td>
<td>47.6 ± 47.9</td>
</tr>
<tr>
<td>6.5</td>
<td>4 of 5 (80)</td>
<td>99.6 ± 0.2</td>
</tr>
<tr>
<td>7</td>
<td>5 of 5 (100)</td>
<td>99.9 ± 0.5</td>
</tr>
<tr>
<td>BALB/b → B6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0 of 6 (0)</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>3 of 6 (50)</td>
<td>85.6 ± 11.4</td>
</tr>
<tr>
<td>6</td>
<td>5 of 5 (100)</td>
<td>89.3 ± 22.2</td>
</tr>
</tbody>
</table>

BALB/c mice underwent irradiation with 6, 6.5, or 7 Gy (day 1) and underwent transplantation with 10 × 10^6 B6-depleted BM cells (day 0). B6 recipient mice were exposed to 4, 5, or 6 Gy (day 1) and underwent transplantation with 10 × 10^6 BALB/b BM cells (day 0). Donor chimerism was determined by flow cytometry 12 to 16 weeks after transplantation.

Tx indicates transplantation; ND, not determined.

Recipient of syngeneic MSCs are tolerant of donor and host antigens

To examine whether chimerism was associated with tolerance to donor and host antigens, MLR and cytotoxic T-cell responses were assessed against B6 (donor) and BALB/c (host) antigens. Splenocytes from chimeric BALB/c recipients of B6 BM and BALB/c MSCs did not generate proliferative responses to B6 (D1 cells or BALB/c splenocytes) or BALB/c stimulators in mixed lymphocyte responses (Figure 3A; left). In contrast, BALB/c recipients of B6 BM cells alone that rejected their grafts exhibited a high proliferative response to donor B6 antigens (Figure 3A; right). Moreover, splenocytes from chimeric recipients failed to induce CTLs against targets of H-2b haplotype (RMA) or H-2d haplotype (p815), whereas nonchimeric recipients generated allogeneic donor CTLs (Figure 3B).

Allogeneic MSCs decrease engraftment after allogeneic BMT

Previous studies have suggested that MSCs are immunoprivileged and that the immunosuppressive effect of MSCs on T cells appeared to be independent of MHC matching between MSCs and T cells. To examine whether MSCs had to be MHC matched to recipient mice to exert their graft-facilitating effect, recipient mice underwent transplantation with allogeneic BM in the presence or absence of syngeneic or allogeneic donor MSCs. As shown in Figure 4, none of the recipient B6 mice that received transplanted multiple minor antigen–mismatched BALB/b BM and BALB/b MSCs experienced engraftment, whereas 44% of recipient mice did so after the administration of
BALB/c BM cells alone (P < .05). In contrast, the addition of syngeneic MSCs increased the proportion of engraftment in mice (82%), as presented in Figure 2A.

The reduced engraftment with allogeneic donor MSCs was also observed in the major HLA mismatch transplantation model (B6 BM transplanted into BALB/c recipients) (13% engraftment vs 50% with syngeneic MSCs) (Figure 4B). To examine whether a single dose of allogeneic MSCs could hamper the engraftment of allogeneic BM cells, BALB/c recipients were irradiated with a higher dose (6.5 Gy) to allow a higher rate of engrafted recipient mice after the administration of B6 BM cells only. Under these conditions, 80% of recipient mice experienced engraftment. In contrast, only 22% of recipient BALB/c mice did so after the administration of B6 BM cells and a single dose of B6 MSCs (Figure 4C). Furthermore, infusion of a single dose of allogeneic MSCs in the multiple minor mismatch transplantation model also significantly reduced the number of chimeric mice (0% vs 38% in the presence of allogeneic MSCs). In contrast, no difference in the number of chimeric mice was observed after infusion of a single dose of syngeneic MSCs in both models at the time of BM transplantation (data not shown).

To examine the effect of third-party MSCs on the engraftment of allogeneic BM, recipient BALB/c mice underwent transplantation with allogeneic B6 BM cells with or without B6 or C3H MSCs. Engraftment level was not affected by cotransplantation with third-party MSCs (63% in the absence and presence of C3H MSCs), whereas the infusion of B6 MSCs decreased the number of chimeric mice (25% vs 63% in the absence of MSCs) (Figure 4D). These results suggest that the infusion of syngeneic, allogeneic, and third-party MSCs have contrasting effects, whereas syngeneic MSCs can promote long-term chimerism, allogeneic MSCs appear to hamper the engraftment of allogeneic BM, and third-party MSCs seem to have a neutral effect on engraftment.

Allogeneic MSCs are immunogenic

The decreased engraftment of allogeneic BM observed after the injection of allogeneic MSCs suggests that MSCs may trigger an alloresponse. To examine whether the infusion of allogeneic MSCs can induce T-cell responses, naive immunocompetent B6 recipient mice were infused with PBS, 0.5 × 10^6 B6 MSCs, 0.5 × 10^6 BALB/c MSCs, or 0.5 × 10^6 BALB/b SP cells, and, 4 weeks later, an in vivo cytotoxicity assay was performed. B6 mice that had received BALB/c SP cells eliminated almost all CFSE-labeled BALB/c splenocytes within 1 day, indicating that the infusion of 0.5 × 10^6 was sufficient to trigger a memory T-cell response (Figure 5A-B). The elimination kinetics of allogeneic BALB/c splenocytes in B6 that received syngeneic B6 MSCs resembled that of PBS-treated mice (60% survival after 3 days). In contrast, B6 mice treated with BALB/b MSCs eliminated almost all CFSE-labeled allogeneic BALB/c cells within 3 days (Figure 5A-B), indicating that the infusion of allogeneic MSCs can trigger a memory T-cell response.

Next, the immunogenicity of major MHC-mismatched MSCs was examined. Although NK cells and T cells play a role in the elimination of allogeneic cells, treatment with B6 MSCs or C3H resulted in decreased survival of CFSE-labeled allogeneic B6 SP cells (55% and 9% after 3 hours and 1 day, respectively) or C3H SP cells (54% and 11% after 3 hours and 1 day, respectively) compared with treatment with BALB/c MSCs (84% and 18% after 3 hours and 1 day, respectively), suggesting the involvement of memory T cells (Figure 5C-D).

Discussion

MSCs have gained interest for their use in regenerative medicine because of their ability to differentiate into several mesenchymal...
tissues and their ability to be easily expanded ex vivo. Further clinical interest has been gained by the observations that MSCs may be immunoprivileged and may display immunosuppressive properties. Although considerable amounts of in vitro data support the nonimmunogenicity and immunomodulatory effects of MSCs, relatively little information is available on the immunogenicity and immunomodulatory capacities of MSCs in vivo. In the present study, we examined whether MSCs display immunosuppressive properties in murine allogeneic bone marrow transplantation models. The infusion of host MSCs into sublethally irradiated recipients promoted engraftment of allogeneic BM that was associated with tolerance to donor and recipient antigens. Conversely, cotransplantation of allogeneic donor MSCs hampered allogeneic BM engraftment, associated with a strong proliferative response of host spleen cells against donor cells. Moreover, reduced survival of allogeneic splenocytes was observed in naive immunocompetent mice after the injection of allogeneic MSCs, showing that MSCs had triggered the onset of a memory T-cell response. These data strongly suggest that MSCs are not intrinsically immunoprivileged.

The graft-promoting effect induced by the infusion of syngeneic MSCs is in accordance with results obtained from studies conducted in animal models. The mechanisms underlying these properties of MSCs are still unclear. However, 2 hypotheses have been proposed, the first related to the immunomodulatory properties of MSCs. The immunosuppressive effects of MSCs have been generally ascribed to the inhibitory effect on T cells, but recently it was also shown that MSCs have inhibitory effects on other immune cells in vitro, including dendritic cells. In the major MHC mismatch transplantation, T cells and NK cells can contribute to the
matopoietic recovery after coinfusion of autolo- 
gous-blood stem cells and culture-expanded mar-
drow mesenchymal stem cells in advanced breast 
cancer patients receiving high-dose chemo-


8. Almeida-Porada G, Porada CD, Tran N, Zanjani 
ED. Cotransplantation of human stromal cell pro-
genitors into preimmune fetal sheep results in 
early appearance of human donor cells in circula-
tion and boosts cell levels in bone marrow at later 
time points after transplantation. Blood. 2000;95: 
3620-3627.

Mesenchymal stem cells promote engraftment of 
human umbilical cord blood-derived CD34(+) 
cells in NOD/SCID mice. Exp Hematol. 2002;30: 
870-878.

Bone marrow mesenchymal stem cells induce 
division arrest anergy of activated T cells. Blood. 

man bone marrow stromal cells suppress T-lym-
phocyte proliferation induced by cellular or non-
specific mitogenic stimuli. Blood. 2002;99:3838-
3843.

marrow mesenchymal stem cells inhibit the re-


Bone marrow mesenchymal stem cells induce 
division arrest anergy of activated T cells. Blood. 

chymal stem cells inhibit differentiation and func-

15. Aggarwal S, Pittenger MF. Human mesenchymal 
stem cells modulate allogeneic immune cell re-

16. Potian JA, Aviv H, Ponzio NM, Harrison JS, 
Rameshwar P. Veto-like activity of mesenchymal 
References

1. Pittenger MF, Mackay AM, Beck SC, et al. Multi-
lineage potential of adult human mesenchymal 

2. Wu GD, Nolta JA, Jin YS, et al. Migration of mes-
enchymal stem cells to heart allografts during 
chronic rejection. Transplantation. 2003;75:679-
685.

3. Pereira RF, O’Hara MD, Laptev AV, et al. Marrow 
stromal cells as a source of progenitor cells for 
nhematopoietic tissues in transgenic mice with a 
phenotype of osteogenesis imperfecta. Proc 

allogeneic bone marrow-derived mesenchymal 
stromal cells engraft and stimulate growth in children 
with osteogenesis imperfecta: implications for cell 
therapy of bone. Proc Natl Acad Sci U S A. 2002; 
99:8932-8937.

enchymal stem cell treatment for infection of 
metachromatic leukodystrophy (MLD) and Hurler 
syndrome (MPS-IH). Bone Marrow Transplant. 

6. Koc ON, Gerson SL, Cooper BW, et al. Rapid he-
matoimmune rejection of allografts. However, predominantly T cells and not NK 
cells play a role in the rejection of multiple minor antigen-matched allografts, suggesting that the putative immunomodu-
latory effect of syngeneic MSCs that promotes engraftment is 
mediated by the suppression of host T cells. Although the 
mechanism underlying the MSC-mediated suppression of T-cell 
activation in vitro is not completely understood and is surrounded 
by controversy, most studies agree that soluble factors are involved. 
The graft-promoting effect by systemic administration of MSCs 
also suggests that MSCs might exert immunoregulatory effects 
through the secretion of soluble immunoregulatory mediators. 
MSCs can produce transforming growth factor β, prostaglandin 
E2, indoleamine 2,3-dioxygenase (IDO), which can exert 
immunosuppressive effects on T-cell responses.

Another possibility involves the concept of providing stromal 
support of donor hematopoietic stem cells that may enhance 
hematopoiesis after transplantation. Although there is minimal 
evidence that donor stromal cells can engraft after systemic 
admission in the bone marrow of recipients, experimental 
evidence indicates that MSCs can enhance the engraftment of 
hematopoietic stem cells in nonobese diabetic–severe compro-

mised immunodeficient (NOD-SCID) mice and fetal sheep.8,9 The 
mechanism of enhancement is not understood and may not require 
homeing of MSCs to BM. Support could be mediated by hematopoietic 
growth factors produced by MSCs, including stem cell factor 
(SCF), macrophage–colony-stimulating factor (M-CSF), granulo-
cyte macrophage–colony-stimulating factor (GM-CSF), interleu-
in-6 (IL-6), and IL-11.27,28 

In the present study, the infusion of allogeneic MSCs can prime 
allogeneic T cells in naive immunocompetent mice, indicating that 
MSCs are immunogenic under these conditions. Several studies 
have described that fetal calf serum present in culture media can 
influence immune responses against cultured cells.29,30 However, it 
is not very likely that the infusion of allogeneic cultured MSCs 
triggers an FCS response because CFSE-labeled allogeneic spleen 
cells that had not been exposed to FCS were specifically elimi-
nated. Furthermore, no memory response was observed after 
the infusion of syngeneic cultured MSCs. In support of these 
observations are recent findings demonstrating that allogeneic MSCs 
can be immunogenic in immunocompetent animals.31,32 Injection of 
adult MSCs in rat myocardium was associated with significant 
infiltration primarily of macrophages and with rejection, whereas 
persistent engraftment of adult human MSCs was observed in 
immunologic-incompetent rats. Furthermore, peripheral blood lym-
phocytes of rats injected with adult MSCs proliferated after 
restimulation with adult MSCs in vitro, suggesting cellular 
immunization. Moreover, another study32 demonstrated that implanted 
mouse MSCs were rejected in allogeneic recipient mice and that 
splenocytes of mice implanted with allogeneic MSCs were 
avivated upon restimulation with allogeneic MSCs in vitro. The 
immune response elicited by the infusion of allogeneic donor 
MSCs may enhance the response of the host against donor 
antigens, resulting in increased rejection of allogeneic donor 
BM. In contrast, though the infusion of third-party MSCs induces a 
memory T-cell response, it does not affect the engraftment of 
unrelated allogeneic BM. Moreover, the immune response pro-

voked by the infusion of third-party MSCs may result in rapid 
rejection of the MSCs. Therefore, no engraftment-enhancing 
effects are observed after cotransplantation with third-party MSCs.

In conclusion, our results show that MSCs are capable of 
modulating immune responses in vivo and that these responses are 
affected by MHC antigen matching between donors and recipients. 
In addition, MSCs are not intrinsically immunoprivileged and are 
capable of inducing a memory T-cell response after injection in 
vivo in immunocompetent hosts, in striking contrast to the general 
notion that MSCs are nonimmunogenic. After cotransplantation in 
an immunocompromised host that has received sublethal irradiation, 
allogeneic donor MSCs still induce an allogeneic response 
that results in graft rejection. These results suggest that the 
immunogenic properties of allogeneic MSCs are not fully 
prevented by the conditioning regimen. Although it is still unclear 
whether the immunogenicity of allogeneic MSCs is preserved after 
a full myeloablative conditioning regimen, the possibility that 
allogeneic or third-party MSCs are immunogenic should be taken 
into account in designing clinical studies in the setting of allogeneic 
stem cell transplantation.

Acknowledgments

We thank Darwin Prockop (Tulane University Health Sciences 
Center, New Orleans, LA) for providing valuable reagents.


Donor-derived mesenchymal stem cells are immunogenic in an allogeneic host and stimulate donor graft rejection in a nonmyeloablative setting

Alma J. Nauta, Geert Westerhuis, Alwine B. Kruisselbrink, Ellie G. A. Lurvink, Roel Willemze and Willem E. Fibbe