Immunosuppressive effects of mesenchymal stem cells on tacrolimus therapy in rat heart transplant model

Authors:

Junshi Doi[1, 2], Yasuhiro Fujimoto[1, 2, 3*], Takumi Teratani[1], Naoya Kasahara[1], Masashi Maeda[1], Tatsuaki Tsuruyama[4], Shinji Uemoto[2], and Eiji Kobayashi[1, 5]

[1] Center for Development of Advanced Technology, Jichi Medical University, Tochigi, Japan

[2] Division of Hepato-Biliary-Pancreatic and Transplant Surgery, Department of Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan

[3] Department of Surgery, Shizuoka Municipal Hospital, Shizuoka, Japan

[4] Department of Pathology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

[5] Department of Organ Fabrication, Keio University School of Medicine, Tokyo, Japan

Email addresses of authors:

Junshi Doi: junshi_d03131979@yahoo.co.jp

Yasuhiro Fujimoto: yfjmt_kyoto@mac.com

Takumi Teratani: teratani@jichi.ac.jp

Naoya Kasahara: umbrella708@gmail.com

Masashi Maeda: masashi.maeda@astellas.com

Tatsuaki Tsuruyama: tsuruyam@kuhp.kyoto-u.ac.jp

Shinji Uemoto: uemoto@kuhp.kyoto-u.ac.jp

Eiji Kobayashi: organfabri@keio.jp

***Corresponding author:**

Yasuhiro Fujimoto, M.D., Ph.D.,

Department of Surgery, Shizuoka Municipal Hospital,

4-27-1 Kita Ando Aoi-ku, Shizuoka City, Japan, 420-8527;

Tel: +81-54-247-6111;

Fax: +81-54-247-6140;

E-mail: yfjmt_kyoto@mac.com

Short title: Immunological interaction between mesenchymal stem cells and tacrolimus

ABSTRACT

Introduction: Mesenchymal stem cells (MSCs) are used in various clinical settings for effective cell therapy by taking advantage of their immunomodulatory, regenerative, and anti-inflammatory properties. We previously investigated the effects of the regenerative and anti-inflammatory properties of MSCs for consideration of their clinical application in organ transplantation. Since immunosuppressant agents are administered in nearly all clinical cases of organ transplantation, clarification of the immunological interaction between MSCs and the calcineurin inhibitor tacrolimus (TAC) is needed.

Aim: Due to potential use of MSCs in organ transplantation, we assessed immunological interactions between TAC and MSCs *in vivo*.

Methods: Adipose tissue-derived MSCs (AT-MSCs) were obtained from wild-type Lewis rats. We then used a rat heart transplantation model to observe the immunosuppressive effects of AT-MSCs, TAC, and those in combination. Following allogeneic heterotopic heart transplantation from ACI rats to Lewis rats, graft survival was assessed in groups treated with AT-MSCs, TAC, both, or neither.

Results: Median survival of the allograft was 14.5 days in the TAC-alone group and 11 days in the MSC+TAC group. Although the difference between those groups was not significant, the survival periods of both were significantly longer as compared to that of the control (median survival =5 days, $P<0.05$) and MSC-alone (median survival =5 days, $P<0.05$) groups.

Conclusions: Administration of AT-MSCs did not decrease the beneficial immunosuppressive effect of TAC on heart graft survival. TAC appears to be compatible and effective for clinical use as an immunosuppressant with AT-MSCs.

Keywords: calcineurin inhibitor, heterotopic heart transplantation model, immunomodulation, immunosuppressant, liver transplantation, mesenchymal stem cells, organ transplantation, tacrolimus.

FOOTNOTES

Abbreviations: AT-MSCs, adipose tissue derived mesenchymal stem cells; CsA, cyclosporine A; HHT, heterotopic heart transplantation; IL, interleukin; LDLT, living donor liver transplantation; LEW, Lewis; MSCs, mesenchymal stem cells; MST, median survival time; TAC, tacrolimus

Grants and financial support: This study was partially funded by Otsuka Pharmaceutical Factory, Inc. (Naruto, Japan, http://www.otsukakj.jp/en/). E.K. was a chief scientific advisor for Otsuka Pharmaceutical Factory, Inc. (2009-2014). This work was not supported by any funding from the National Institutes of Health, Wellcome Trust, or Howard Hughes Medical Institute.

Competing interests: Eiji Kobayashi was a visiting professor at the Division of Development of Advanced Treatment, Center for Development of Advanced Medical Technology of Jichi Medical University and a special advisor to Otsuka Pharmaceutical Factory Inc. (Naruto, Japan) from 2009 to 2014. Masashi Maeda is a senior researcher at Astellas Pharma Inc. (Ibaraki, Japan) and previously a visiting researcher at Jichi Medical University. There are no patents, products in development, or marketed products in relation to this study to declare. The positions held by Eiji Kobayashi and Masashi Maeda did not alter the authors' adherence to all of the policies of *Medical Research Archives* in regard to sharing data and materials, as described in detail in the Guide for Authors available online. The other authors have no competing financial interests to declare.

1. INTRODUCTION

Liver transplantation is one of the most effective treatments for end-stage liver diseases and, because of a lack of donors, living donor liver transplantation (LDLT) is widely used, though the donors are clearly at some risk (Nadalin et al., 2007). We have explored the potential of use of mesenchymal stem cells (MSCs) as a solution for small-for-size syndrome in LDLT cases caused by graft mismatch in the recipient with the aim of reducing graft size, thus making donations safer. Our previous study demonstrated that MSCs enhance liver regeneration, and also ameliorate and repair hepatic ischemia-reperfusion injury, and we concluded that they are potentially effective for small-for-size syndrome (Kanazawa et al., 2011).

MSCs are multipotent adult stem cells capable of differentiating into osteocytes, adipocytes, osteoblasts, and chondroblasts, as well as other lineage cells. These cells reside in and can be isolated from a variety of tissues and organs, such as bone marrow, adipose tissue, muscle, bone, umbilical cord blood, placenta, spleen, liver, and kidneys, along with others, and secrete various cytokines that promote organ regeneration (Banas et al., 2008; van Poll et al., 2008).

MSCs also have immunomodulatory properties (Marigo & Dazzi, 2011). Clinical application of these cells for solid organ transplantation was pioneered by the Mesenchymal Stem Cells In Solid Organ Transplantation Consortium, which has developed techniques to take advantage of their immunological properties (Dahlke et al., 2009; Franquesa et al., 2013; Hoogduijn et al., 2010).

Presently, understanding of the interaction between basic immunosuppressant agents (e.g., calcineurin inhibitors) and MSCs is incomplete. However, MSC therapy is entering clinical use, thus its impact on patients undergoing therapy with basic immunosuppressive drugs, such as cyclosporine A (CsA) and tacrolimus (TAC), is a critical issue that must be

investigated before clinical application can become common. To the best of our knowledge, no reports have described immunological interactions that occur between TAC and MSCs *in vivo*. Therefore, the aim of this study was to assess the interaction between TAC (a basic calcineurin inhibitor) and MSCs *in vivo* by observing immunosuppression in a rat heterotopic heart transplantation (HHT) model.

2. MATERIALS AND METHODS

2.1. Animals

All experiments were conducted with the approval of the Jichi Medical University Guide for Laboratory Animals (approval number: #1080). Male wild-type Lewis (LEW) rats were purchased from Charles River Breeding Laboratories (Kanagawa, Japan). The rats weighed 230-330 g and were housed in a temperature- and humidity-controlled environment with a 12-hour light/dark cycle, and given access to food (standard laboratory chow) and water ad libitum.

2.2. Isolation and culture of adipose tissue-derived MSCs (AT-MSCs)

An abdominal subcutaneous adipose tissue sample was harvested from a wild-type LEW rat, then adipose tissue-derived MSCs (AT-MSCs) were isolated following mincing, digestion, and centrifugation, as previously described (Iwai et al., 2014). Isolated AT-MSCs were seeded into 10-mL tissue culture dishes (Thermo Scientific, Tokyo, Japan) and cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum. AT-MSCs between the fifth and eighth passage were used for the experiments.

2.3. Immunosuppression by TAC with AT-MSCs in an HHT model

Abdominal HHT was used to test the immunomodulatory effect of AT-MSCs, as rat HHT models are considered to be the most sensitive for testing immunosuppressive drugs (Kobayashi, 2012; Uchida et al., 1999). In contrast, various factors influence rat orthotopic

liver transplantation models, making them relatively inconvenient for immunosuppressant screening (Kobayashi, 1998). Thus, we used an allogeneic HHT technique in the present study. ACI rats (male, 8-9 weeks old, 180-210 g) and inbred LEW rats (male, 7-8 weeks old, 230-290 g) (both purchased from Charles River Breeding Laboratories) were used as donors and recipients, respectively. All LEW recipient rats were assigned to one of four groups: the control group, which received daily intramuscular administrations of 1 mL normal saline for 14 days from the day of heart transplantation and no transplantation of AT-MSCs; the TAC-alone group, which received daily intramuscular administrations of 0.03 mg/kg TAC in normal saline for 14 days from the day of heart transplantation and no transplantation of AT-MSCs; the MSC-alone group, which received daily intramuscular administrations of 1 mL of normal saline for 14 days from the day of heart transplantation as well as transplantation of 2×10^6 AT-MSCs via the penile vein; and the MSC+TAC group, which received daily intramuscular administrations of 0.03 mg/kg TAC for 14 days from the day of heart transplantation as well as transplantation of 2×10^6 AT-MSCs via the penile vein. In the applicable groups, transplantation of AT-MSCs was performed twice, just after heart transplantation (postoperative Day 0) and on postoperative Day 3.

HHT was performed as previously described (Ono & Lindsey, 1969). Following the surgical procedure, abdomens of recipient rats were palpated daily for signs of rejection. Graft survival was defined as the interval between heart transplantation (postoperative Day 0) and day of rejection, which was determined by lack of palpable cardiac contractions of the allogeneic rat heart graft.

2.4. Statistical Analysis

Survival analysis of the allografts was conducted using the Kaplan-Meier method, while statistical comparisons of the results were conducted with a log-rank test. A P value of 0.05 was considered to

indicate statistical significance.

3. RESULTS

3.1. Immunomodulatory effect of AT-MSCs

The median survival of the allografts was 5 days in both the control and MSC-alone groups, with no significant difference between them (Figure 1, Table 1). Transplantation of AT-MSCs alone did not provide adequate immunosuppression to significantly prolong allograft survival.

Table 1. Effects of injection of adipose tissue-derived mesenchymal stem cells (AT-MSCs) on rejection of allogeneic rat heart grafts in presence of low-dose tacrolimus (TAC).

Group	TAC	AT-MSCs	n	Survival of each rat in group (days)	MST (days)
Control	-	-	8	5, 5, 5, 5, 5, 5, 6, 6	5
TAC alone	+	-	5	7, 9, 13, 16, 17, 20 *	14.5
MSC alone	-	+	5	4, 5, 5, 5, 6	5
MSC+TAC	+	+	5	9, 9, 11, 20, 23 *	11

Allograft survival in all four groups is shown. Grafted hearts from ACI rats were implanted into the abdomens of Lewis rats. In groups with AT-MSC administration, 2×10^6 AT-MSCs were systemically injected twice (Day 0, Day 3) via the penile vein. In groups with TAC administration, TAC (0.03 mg/kg) was administered intramuscularly daily for 14 days from the day of transplantation onward. Allograft survival in both the TAC and MSC+TAC groups was significantly longer as compared to the control and MSC-alone groups (*$P<0.05$ vs. control group or MSC-alone group).

Abbreviations: MST, median survival time

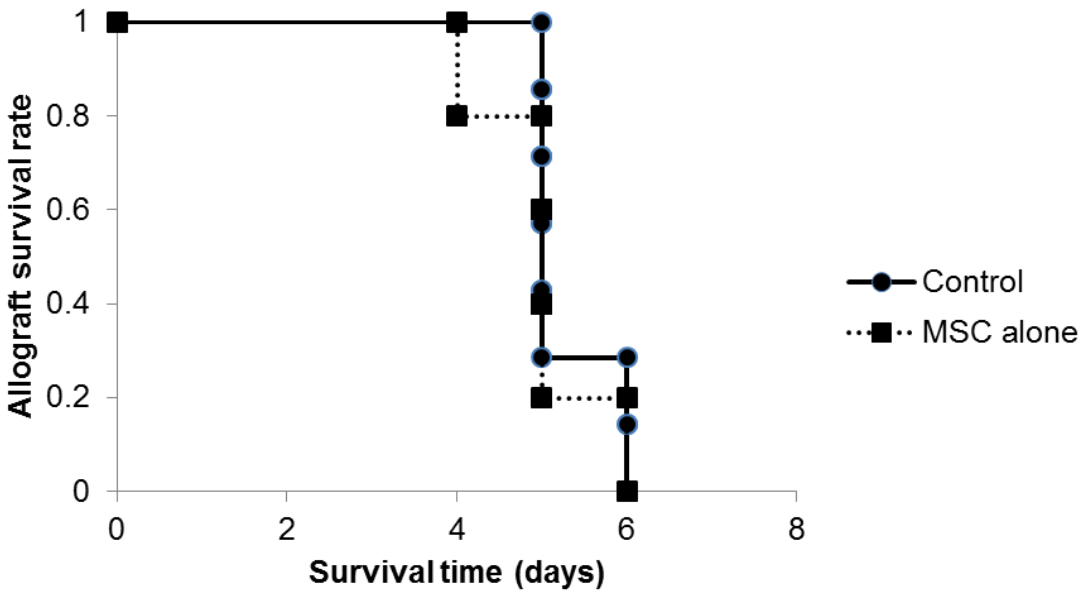

Figure 1. Kaplan-Meier survival plots for allograft hearts in the Control and MSC-alone groups. There was no significant difference between these groups.

3.2. Immunosuppression by TAC not reduced by AT-MSCs in HHT model

The median survival of the allografts was 14.5 days in the TAC-alone and 11 days in the MSC+TAC group. Although the difference between these 2 groups was not significant, survival in each was significantly longer as compared to that in the control (median survival =5 days, $P<0.05$) and MSC-alone (median survival =5 days, $P<0.05$) group (Figure 2, Table 1). AT-MSCs did not prolong allograft heart survival or accelerate graft rejection.

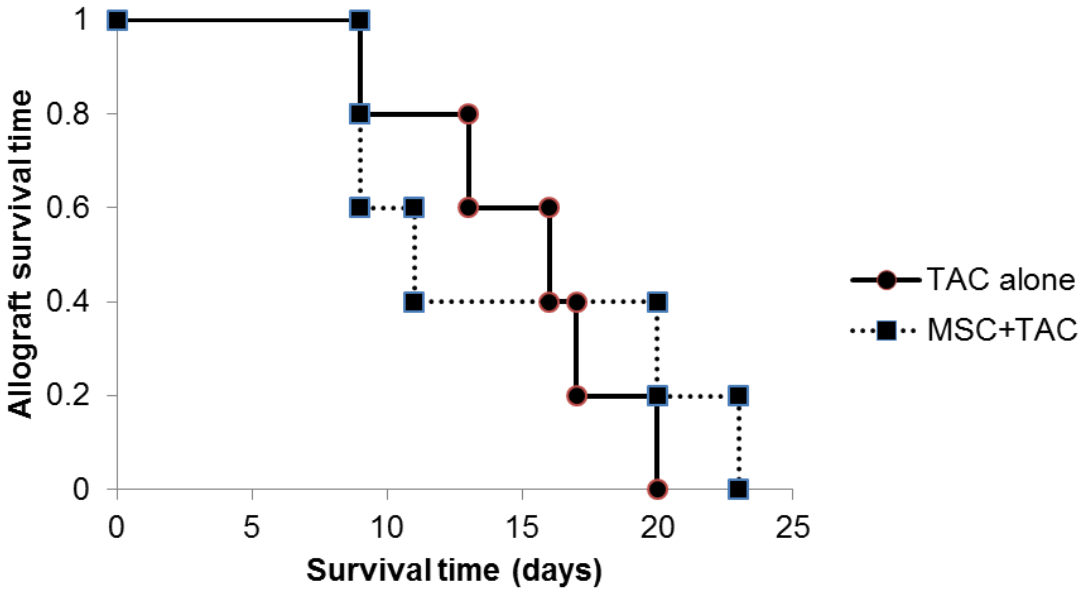

Figure 2. Kaplan-Meier survival plots for allograft hearts in the TAC-alone and MSC+TAC groups. There was no significant difference between these groups.

4. DISCUSSION

TAC exerts a potent immunosuppressive effect by inhibiting calcineurin, and blocking the transcription and production of interleukin (IL)-2, which are key steps in T-cell activation (Kino et al., 1987; Liu et al., 1991; Vicari-Christensen, Repper, Basile, & Young, 2009). In addition, recent findings suggest that dendritic cells may be a target of TAC (Y. H. Lee et al., 2007; Y. R. Lee et al., 2005). The emergence of TAC has greatly improved the results of organ transplantation and is widely used (Henry, 1999).

On the other hand, several *in vitro* and *in vivo* studies have demonstrated that MSCs have tissue regenerative and immunomodulatory properties (Banas et al., 2008; Bartholomew et al., 2002; Di Nicola et al., 2002; Kanazawa et al., 2011; Marigo & Dazzi, 2011; Prockop, Kota, Bazhanov, & Reger, 2010; Sakaida et al., 2004; van Poll et al., 2008). Briefly, the regenerative effects of MSCs to ameliorate inflammation, repair damaged tissue, and promote tissue regeneration are achieved mainly by secretion of cytokines,

growth factors, and differentiation factors, rather than by undergoing differentiation and replacing targeted damaged cells themselves. Soluble factors secreted by MSCs include IL-6, transforming growth factor-β, and prostaglandin E2, as well as hepatocyte growth factor, epidermal growth factor, fibroblast growth factor, platelet-derived growth factor, vascular endothelial growth factor, and insulin-like growth factor (Banas et al., 2008; Wang, Chen, Cao, & Shi, 2014). Furthermore, MSCs exert immunomodulatory effects by suppressing the activation and function of diverse immune cells, such as macrophages, neutrophils, natural killer cells, dendritic cells, T lymphocytes and B lymphocytes. Also, in addition to inhibiting proliferation of T lymphocytes in general, immunomodulation mediated by MSCs is specifically linked to regulatory T cells, and induction of those cells is directly or indirectly enhanced by MSCs (Akiyama et al., 2012; Deng et al., 2014; Luz-Crawford et al., 2013). During this process, several factors secreted, which include transforming growth factor-β, indoleamine 2,3-dioxygenase, nitric oxide, prostaglandin E2, IL-1 receptor antagonist and IL-10, and cell-cell contact by MSCs play an important role (Burr, Dazzi, & Garden, 2013; Ge et al., 2010; Ghannam, Bouffi, Djouad, Jorgensen, & Noël, 2010).

The mechanism by which MSCs exert their tissue regenerative or immunomodulatory effects must be elucidated prior to clinical application. Additionally, similar attention should be paid to the type of immunosuppressants that can be used concurrently with MSCs in the clinical setting of organ transplantation, because MSCs may interfere with certain drugs. Several studies of immunological interactions between immunosuppressants and MSCs have been presented (Buron et al., 2009; Eggenhofer et al., 2011; Hoogduijn et al., 2008). Here, we used a rat HHT model with a high level of immune response, which showed strong rejection characteristics because of the combination

of rat strains used (ACI to LEW). We speculated that administration of AT-MSCs did not prolong allograft heart survival in the MSC-alone group, because those cells alone did not exert an immunosuppressive effect in this strong rejection model. On the other hand, our HTT model showed sensitive detection of the decline in immunosuppressive state caused by interactions between TAC and AT-MSCs. Thus, allograft heart survival in the MSC+TAC group was the same as that in the TAC group, indicating that administration of AT-MSCs did not weaken the immunosuppressive effect of TAC. These findings are in contrast with those of previous reports showing that MSCs antagonize the immunosuppressive effects of TAC *in vitro*, while they are consistent with another report that showed a possibly harmless interaction between MSCs and TAC in regard to immunosuppressive effects in a porcine model (Buron et al., 2009; Hoogduijn et al., 2008; Poncelet et al., 2008). A possible explanation for our results is that the immunosuppressive effects of MSCs and TAC did not negatively affect each other *in vivo*, because MSCs exert immunomodulatory effects by downregulating the IL-2 receptor and inhibiting production of IL-2, which are components of the calcineurin pathway (Bartholomew et al., 2002; Le Blanc et al., 2004; Rasmusson, Ringdén, Sundberg, & Le Blanc, 2005). However, it is difficult to compare our results with those from other MSC-related studies noted above, partly because the mechanism by which MSCs exert their immunomodulatory effect differs according to the type of lymphocyte reaction, and also because the immunomodulatory functions of MSCs are highly plastic according to their immune and inflammatory microenvironments (Rasmusson et al., 2005). In other words, the immunomodulatory behavior of MSCs may be altered depending on how or how strongly the immune system is activated, or what types of inflammatory processes occur around the MSCs (Eggenhofer et al.,

2011; Wang et al., 2014). Therefore, differences between *in vitro* and *in vivo* studies should be carefully considered when discussing the behavior of MSCs. Our results show that TAC has distinct properties from CsA in terms of its immunosuppressive interaction with MSCs, which may be comparable to those presented by Inoue et al., who used a similar rat HHT model and demonstrated that MSCs promote allograft rejection, thus interfering with the immunosuppressive effect of CsA when co-administered (Inoue et al., 2006). However, the mechanism of the differences between TAC and CsA remains unknown. TAC and CsA each bind to immunophilins, such as FK binding protein and cyclophilin, respectively, thus inhibiting the activity of calcineurin and production of IL-2 in T cells. TAC and CsA show no other distinct differences in their pharmacodynamic actions except for the type of immunophilins they bind, and no clear or rational explanation for this difference in immunosuppressive interaction between those and MSCs has been established (Hemenway & Heitman, 1999). Further investigation is needed to elucidate the interaction between MSCs and various immunosuppressants.

In conclusion, we assessed the immunological interaction between TAC and AT-MSCs *in vivo*. We found that AT-MSCs do not interfere with the immunosuppressive effect of TAC, thus it may be an optimal candidate immunosuppressant for concurrent use with MSCs in clinical trials and practice.

REFERENCES

Akiyama, K., Chen, C., Wang, D., Xu, X., Qu, C., Yamaza, T., . . . Shi, S. (2012). Mesenchymal-stem-cell-induced immunoregulation involves FAS-ligand-/FAS-mediated T cell apoptosis. *Cell Stem Cell, 10*(5), 544-555. doi:10.1016/j.stem.2012.03.007

Banas, A., Teratani, T., Yamamoto, Y., Tokuhara, M., Takeshita, F., Osaki, M., . . . Ochiya, T. (2008). IFATS collection: in vivo therapeutic potential of human adipose tissue mesenchymal stem cells after transplantation into mice with liver injury. *Stem Cells, 26*(10), 2705-2712. doi:10.1634/stemcells.2008-0034

Bartholomew, A., Sturgeon, C., Siatskas, M., Ferrer, K., McIntosh, K., Patil, S., . . . Hoffman, R. (2002). Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. *Exp Hematol, 30*(1), 42-48.

Buron, F., Perrin, H., Malcus, C., Héquet, O., Thaunat, O., Kholopp-Sarda, M. N., . . . Morelon, E. (2009). Human mesenchymal stem cells and immunosuppressive drug interactions in allogeneic responses: an in vitro study using human cells. *Transplant Proc, 41*(8), 3347-3352. doi:10.1016/j.transproceed.2009.08.030

Burr, S. P., Dazzi, F., & Garden, O. A. (2013). Mesenchymal stromal cells and regulatory T cells: the Yin and Yang of peripheral tolerance? *Immunol Cell Biol, 91*(1), 12-18. doi:10.1038/icb.2012.60

Dahlke, M. H., Hoogduijn, M., Eggenhofer, E., Popp, F. C., Renner, P., Slowik, P., . . . Group, M. S. (2009). Toward MSC in solid organ transplantation: 2008 position paper of the MISOT study group. *Transplantation, 88*(5), 614-619. doi:10.1097/TP.0b013e3181b4425a

Deng, Y., Yi, S., Wang, G., Cheng, J., Zhang, Y., Chen, W., . . . Yang, Y. (2014). Umbilical cord-derived mesenchymal stem cells instruct dendritic cells to acquire tolerogenic phenotypes through the IL-6-mediated upregulation of SOCS1. *Stem Cells Dev, 23*(17), 2080-2092. doi:10.1089/scd.2013.0559

Di Nicola, M., Carlo-Stella, C., Magni, M.,

Milanesi, M., Longoni, P. D., Matteucci, P., . . . Gianni, A. M. (2002). Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood, 99*(10), 3838-3843.

Eggenhofer, E., Renner, P., Soeder, Y., Popp, F. C., Hoogduijn, M. J., Geissler, E. K., . . . Dahlke, M. H. (2011). Features of synergism between mesenchymal stem cells and immunosuppressive drugs in a murine heart transplantation model. *Transpl Immunol, 25*(2-3), 141-147. doi:10.1016/j.trim.2011.06.002

Franquesa, M., Hoogduijn, M. J., Reinders, M. E., Eggenhofer, E., Engela, A. U., Mensah, F. K., . . . Group, M. S. (2013). Mesenchymal Stem Cells in Solid Organ Transplantation (MiSOT) Fourth Meeting: lessons learned from first clinical trials. *Transplantation, 96*(3), 234-238. doi:10.1097/TP.0b013e318298f9fa

Ge, W., Jiang, J., Arp, J., Liu, W., Garcia, B., & Wang, H. (2010). Regulatory T-cell generation and kidney allograft tolerance induced by mesenchymal stem cells associated with indoleamine 2,3-dioxygenase expression. *Transplantation, 90*(12), 1312-1320. doi:10.1097/TP.0b013e3181fed001

Ghannam, S., Bouffi, C., Djouad, F., Jorgensen, C., & Noël, D. (2010). Immunosuppression by mesenchymal stem cells: mechanisms and clinical applications. *Stem Cell Res Ther, 1*(1), 2. doi:10.1186/scrt2

Hemenway, C. S., & Heitman, J. (1999). Calcineurin. Structure, function, and inhibition. *Cell Biochem Biophys, 30*(1), 115-151. doi:10.1007/BF02737887

Henry, M. L. (1999). Cyclosporine and tacrolimus (FK506): a comparison of efficacy and safety profiles. *Clin Transplant, 13*(3), 209-220.

Hoogduijn, M. J., Crop, M. J., Korevaar, S. S., Peeters, A. M., Eijken, M., Maat, L. P., . . . Baan, C. C. (2008). Susceptibility of human mesenchymal stem cells to tacrolimus, mycophenolic acid, and rapamycin. *Transplantation, 86*(9), 1283-1291. doi:10.1097/TP.0b013e31818aa536

Hoogduijn, M. J., Popp, F. C., Grohnert, A., Crop, M. J., van Rhijn, M., Rowshani, A. T., . . . Group, M. S. (2010). Advancement of

mesenchymal stem cell therapy in solid organ transplantation (MISOT). *Transplantation, 90*(2), 124-126. doi:10.1097/TP.0b013e3181ea4240

Inoue, S., Popp, F. C., Koehl, G. E., Piso, P., Schlitt, H. J., Geissler, E. K., & Dahlke, M. H. (2006). Immunomodulatory effects of mesenchymal stem cells in a rat organ transplant model. *Transplantation, 81*(11), 1589-1595. doi:10.1097/01.tp.0000209919.90630.7b

Iwai, S., Sakonju, I., Okano, S., Teratani, T., Kasahara, N., Yokote, S., . . . Kobayash, E. (2014). Impact of ex vivo administration of mesenchymal stem cells on the function of kidney grafts from cardiac death donors in rat. *Transplant Proc, 46*(5), 1578-1584. doi:10.1016/j.transproceed.2013.12.068

Kanazawa, H., Fujimoto, Y., Teratani, T., Iwasaki, J., Kasahara, N., Negishi, K., . . . Kobayashi, E. (2011). Bone marrow-derived mesenchymal stem cells ameliorate hepatic ischemia reperfusion injury in a rat model. *PLoS One, 6*(4), e19195. doi:10.1371/journal.pone.0019195

Kino, T., Hatanaka, H., Miyata, S., Inamura, N., Nishiyama, M., Yajima, T., . . . Aoki, H. (1987). FK-506, a novel immunosuppressant isolated from a Streptomyces. II. Immunosuppressive effect of FK-506 in vitro. *J Antibiot (Tokyo), 40*(9), 1256-1265.

Kobayashi, E. (1998). *Orthotopic liver transplantation in the rat: survival relationship between different strain combinations* (W. Timmermann, H.-J. Gassel, K. Ulrichs, R. Zhong, & A. Thiede Eds. Organtransplantation in Rats and Mice: Microsurgical Techniques and Immunological Principles ed.). Berlin: Springer.

Kobayashi, E. (2012). *Translational research by use of microsurgical technologies: Muster the art and learn the science.* Paper presented at the 4th Congress of Microsurgery, Timisoara, Romania.

Le Blanc, K., Rasmusson, I., Götherström, C., Seidel, C., Sundberg, B., Sundin, M., . . . Ringdén, O. (2004). Mesenchymal stem cells inhibit the expression of CD25 (interleukin-2 receptor) and CD38 on phytohaemagglutinin-activated lymphocytes. *Scand J Immunol, 60*(3), 307-315. doi:10.1111/j.0300-

9475.2004.01483.x

Lee, Y. H., Lee, Y. R., Im, S. A., Park, S. I., Kim, K. H., Gerelchuluun, T., . . . Lee, C. K. (2007). Calcineurin inhibitors block MHC-restricted antigen presentation in vivo. *J Immunol, 179*(9), 5711-5716.

Lee, Y. R., Yang, I. H., Lee, Y. H., Im, S. A., Song, S., Li, H., . . . Lee, C. K. (2005). Cyclosporin A and tacrolimus, but not rapamycin, inhibit MHC-restricted antigen presentation pathways in dendritic cells. *Blood, 105*(10), 3951-3955. doi:10.1182/blood-2004-10-3927

Liu, J., Farmer, J. D., Lane, W. S., Friedman, J., Weissman, I., & Schreiber, S. L. (1991). Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. *Cell, 66*(4), 807-815.

Luz-Crawford, P., Kurte, M., Bravo-Alegría, J., Contreras, R., Nova-Lamperti, E., Tejedor, G., . . . Carrión, F. (2013). Mesenchymal stem cells generate a CD4+CD25+Foxp3+ regulatory T cell population during the differentiation process of Th1 and Th17 cells. *Stem Cell Res Ther, 4*(3), 65. doi:10.1186/scrt216

Marigo, I., & Dazzi, F. (2011). The immunomodulatory properties of mesenchymal stem cells. *Semin Immunopathol, 33*(6), 593-602. doi:10.1007/s00281-011-0267-7

Nadalin, S., Malagò, M., Radtke, A., Erim, Y., Saner, F., Valentin-Gamazo, C., . . . Broelsch, C. E. (2007). Current trends in live liver donation. *Transpl Int, 20*(4), 312-330. doi:10.1111/j.1432-2277.2006.00424.x

Ono, K., & Lindsey, E. S. (1969). Improved technique of heart transplantation in rats. *J Thorac Cardiovasc Surg, 57*(2), 225-229.

Poncelet, A. J., Nizet, Y., Vercruysse, J., Hiel, A. L., Saliez, A., & Gianello, P. (2008). Inhibition of humoral response to allogeneic porcine mesenchymal stem cell with 12 days of tacrolimus. *Transplantation, 86*(11), 1586-1595. doi:10.1097/TP.0b013e31818bd96f

Prockop, D. J., Kota, D. J., Bazhanov, N., & Reger, R. L. (2010). Evolving paradigms for repair of tissues by adult stem/progenitor cells (MSCs). *J Cell Mol Med, 14*(9), 2190-2199. doi:10.1111/j.1582-4934.2010.01151.x

Rasmusson, I., Ringdén, O., Sundberg, B.,

& Le Blanc, K. (2005). Mesenchymal stem cells inhibit lymphocyte proliferation by mitogens and alloantigens by different mechanisms. *Exp Cell Res, 305*(1), 33-41. doi:10.1016/j.yexcr.2004.12.013

Sakaida, I., Terai, S., Yamamoto, N., Aoyama, K., Ishikawa, T., Nishina, H., & Okita, K. (2004). Transplantation of bone marrow cells reduces CCl4-induced liver fibrosis in mice. *Hepatology, 40*(6), 1304-1311. doi:10.1002/hep.20452

Uchida, H., Kobayashi, E., Matsuda, K., Mizuta, K., Sugimoto, K., Kawarasaki, H., . . . Fujimura, A. (1999). Chronopharmacology for deoxyspergualin: toxicity and efficacy in the rat. *Transplantation, 67*(9), 1269-1274.

van Poll, D., Parekkadan, B., Cho, C. H., Berthiaume, F., Nahmias, Y., Tilles, A. W., & Yarmush, M. L. (2008). Mesenchymal stem cell-derived molecules directly modulate hepatocellular death and regeneration in vitro and in vivo. *Hepatology, 47*(5), 1634-1643. doi:10.1002/hep.22236

Vicari-Christensen, M., Repper, S., Basile, S., & Young, D. (2009). Tacrolimus: review of pharmacokinetics, pharmacodynamics, and pharmacogenetics to facilitate practitioners' understanding and offer strategies for educating patients and promoting adherence. *Prog Transplant, 19*(3), 277-284.

Wang, Y., Chen, X., Cao, W., & Shi, Y. (2014). Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. *Nat Immunol, 15*(11), 1009-1016. doi:10.1038/ni.3002

