Title

Allogeneic and Xenogeneic Transplantation of Adipose-Derived Stem Cells in Immunocompetent Recipients Without Immunosuppressants

Journal

Stem Cells and Development, 21(15)

ISSN

1547-3287

Authors

Lin, Ching-Shwun
Lin, Guiting
Lue, Tom F

Publication Date

2012-10-10

DOI

10.1089/scd.2012.0176

Peer reviewed

STEM CELLS AND DEVELOPMENT
Volume 21, Number 15, 2012

DOI: 10.1089/scd.2012.0176

COMPREHENSIVE REVIEW

Allogeneic and Xenogeneic Transplantation of Adipose-Derived Stem Cells in Immunocompetent Recipients Without Immunosuppressants

Ching-Shwun Lin, Guiting Lin, and Tom F. Lue

Mesenchymal stem cells (MSCs) are well known for their immunomodulatory capabilities. In particular, their immunosuppressive property is believed to permit their allogeneic or even xenogeneic transplantation into immunocompetent recipients without the use of immunosuppressants. Adipose-derived stem cell (ADSC), owing to its ease of isolation from an abundant tissue source, is a promising MSC for the treatment of a wide range of diseases. ADSC has been shown to lack major histocompatibility complex-II expression, and its immunosuppressive effects mediated by prostaglandin E2. Both preclinical and clinical studies have shown that allogeneic transplantation of ADSCs was able to control graft-versus-host disease. In regard to xenotransplantation a total of 27 preclinical studies have been published, with 20 of them performed with the investigators' intent. All 27 studies used ADSCs isolated from humans, possibly due to the wide availability of lipoaspirates. On the other hand, the recipients were mouse in 13 studies, rat in 11, rabbit in 2, and dog in 1. The targeted diseases varied greatly but all showed significant improvements after ADSC xenotransplantation. For clinical application in human medicine, ADSC xenotransplantation offers no obvious advantage over autotransplantation. But in veterinary medicine, xenotransplantation with porcine ADSC is a practical alternative to the costly and inconvenient autotransplantation.

Introduction

First identified in bone marrow, mesenchymal stem cells (MSCs) have now been isolated from most adult tissues including the adipose [1,2]. These cells are multipotent and have been extensively investigated for their therapeutic capabilities in a wide variety of diseases including brain ischemia, cardiac infarction, osteoarthritis (OA), urinary incontinence, and erectile dysfunction [3–5]. While most of these clinical and preclinical trials utilized autologous MSCs, a significant number of studies have examined the feasibility of allogeneic or even xenogeneic MSC transplantation. Since bone marrow MSC (BMSC) is the prototype and also the first MSC type to be investigated for allogeneic and xenogeneic transplantations, it will be briefly discussed in the next section. After that, the rest of this review will focus on adipose-derived stem cell (ADSC). In this review, allogeneic and xenogeneic transplantations are defined respectively as intraspecies and interspecies transplantations in immunocompetent recipients without the use of immunosuppressants. Studies that used immunocompromised recipients and/or immunosuppressants will not be discussed.

Early Allogeneic and Xenogeneic Transplantation Studies with BMSC

In year 2000, Liechty et al. [6] reported that human BMSCs exhibited tissue engraftment and site-specific cell differentiation when transplanted into immunocompetent fetal sheep. These authors concluded that BMSCs might possess immunologic properties that allow their persistence in a xenogeneic environment. In 2001, Devine et al. [7] reported that intravenously injected baboon BMSCs were capable of homing to the bone marrow of allogeneic recipients and persisted for at least 76 days. In the following year, a similar group of researchers reported that baboon BMSCs did not elicit a proliferative response from allogeneic lymphocytes in a mixed lymphocyte reaction [8]. Further, an independent group of researchers reported that human BMSCs were not just noninductive but actually capable of suppressing allogeneic T-lymphocyte proliferation [8].

Because the above-mentioned xenotransplantation experiment was performed with fetal recipients, Saito et al. [9] went a step further to test whether xenotransplantation could succeed in fully immunocompetent recipients. Adult rats were IV injected with mouse BMSCs and, 1 week later,

Knuppe Molecular Urology Laboratory, Department of Urology, School of Medicine, University of California, San Francisco, California.

underwent coronary artery ligation. Twelve weeks later, mouse cells were found to engraft into the bone marrow cavities of rats with or without myocardial infarction while circulating mouse cells were detected only in rats with myocardial infarction. Moreover, mouse cells were found in the infarcted myocardium where they appeared to differentiate into immature cardiac cells or integrate into newly formed blood vessels. Three years later, a similar group of researchers reported again the successful transplantation of mouse BMSCs into infarcted rat heart [10]. However, 2 other studies, both first-authored by Grinnemo, reported unsuccessful transplantation of human BMSCs into infarcted rat heart [11,12]. The discrepancy could perhaps be explained by differences in immunological properties between human and mouse BMSCs. First, murine BMSCs, unlike their human equivalent, lack major histocompatibility complex (MHC) class II expression [13], and second, T-cell inhibition by BMSCs requires cell contact in mice but is mediated by soluble factors in humans [14,15]. In any event, reports of successful xenotransplantation with BMSCs from various species continue to appear frequently; for example, rat BMSC for bone formation in rabbit [16], human BMSC for spinal cord injury in rat [17,18], and human BMSC for bone formation in mice [19].

ADSC as an Ideal MSC for Therapy

MSCs are increasingly believed to reside in the vasculature [2,20–23]; therefore, tissues rich in blood vessels, particularly microvessels (capillaries), are ideal for the isolation of MSCs in large quantities for clinical applications. The adipose tissue is endowed with an extensive capillary network [24] and is one of the rare tissues that can be partially removed from a living person without causing harm. In fact, this partial removal is desired by many patients seeking to improve their image and/or health. In addition, its superficial location makes it easily excisable with virtually no health consequence. As such, unlike bone marrow, the removal of which is not only a health risk but also desired by none, the adipose tissue is routinely abandoned as "medical wastes." As evidence, a worldwide survey published in 2002 shows that between 1994 and 2000 zero death was reported on 66,570 liposuction procedures with a serious adverse event rate of only 0.068% [25]. Further, in the lipoaspirate approximately 2% of nucleated cells can be recovered as MSCs, as compared with 0.002% in the bone marrow aspirate [26]. Thus, while ADSC and BMSC are virtually identical in their therapeutic potential, their difference in clinical applicability is obvious.

In research laboratories, the most commonly used procedure for ADSC isolation involves mincing the adipose tissue sample and centrifugation to separate the fatty content from the stromal vascular fraction (SVF) that forms a reddish pellet at the bottom. The SVF can be directly used for therapy or further processed for the isolation of ADSC. In future clinical applications, adipose tissue mincing will undoubtedly be substituted by liposuction, and the SVF isolation handled by all-in-one devices that are now commercially available [5,27,28]. The adoption of these automation procedures has resulted in the high availability of human ADSCs, which could perhaps explain why many preclinical studies chose human ADSCs for transplantation in non-human animals such as rats and mice, thus, intentionally or unintentionally demonstrating ADSC's xenotransplantation potential (Tables 1 and 2).

Evidence for ADSC's Immunomodulatory Capacity: Cell Culture Studies

The first report of ADSC's immunomodulatory and immunosuppressive properties appeared in 2005; specifically, its in vitro experiments showed that ADSC did not provoke alloreactivity and was able to suppress mixed lymphocyte reaction [29]. Moreover, the immunosuppressive effect appeared to require cell–cell contact. However, in 2 separate studies the cell–cell contact requirement was corroborated [30] and disputed [31], respectively. Regardless of this disagreement, the immunosuppressive effect of ADSC has been consistently observed in all subsequent relevant studies [32–36]. Further, in a comparative study, ADSC and BMSC were found to exhibit the same pattern of immunologically relevant surface markers (MHC-I, MHC-II, CD40, and CD40L) [37]. Importantly, both BMSC and ADSC lacked expression of MHC-II, and both did not stimulate allogeneic peripheral blood mononuclear cells. Moreover, these characteristics were retained in both cell types during osteogenic differentiation. As such, it was concluded that allogeneic transplantation of BMSC and ADSC could be employed for tissue engineering [37]. In another study the lack of MHC-II expression in ADSCs was corroborated [31].

While it remains controversial whether cell–cell contact is required for ADSC's immunosuppressive effects [29–31], several studies have demonstrated the importance of soluble factors, among which the most frequently identified being prostaglandin E2 (PGE2) [31,38–41]. Specifically, inhibition of PGE2 by indomethacine effectively abolished ADSC's immunosuppressive effects. In addition, specific inhibition of indoleamine 2, 3 dioxygenase [39] or neutralization of leukemia inhibitory factor [42] has also been shown to abolish ADSC's immunosuppressive effects. Further, ADSC's immunosuppressive activity appears to be mediated through an interleukin-6 (IL-6)-dependent inhibition of dendritic cell differentiation and downregulation of MHC-II, CD40, and CD86 on mature dendritic cells [38]. A subsequent study further showed that ADSC was more potent than BMSC in suppressing dendritic cell differentiation and downregulation of costimulatory molecules on the surface of dendritic cells [43].

Rheumatoid arthritis (RA) is due to a loss in immunological self-tolerance that leads to the activation of autoreactive T cells against joint components. In a 2009 study Gonzalez-Rey et al. [44] found that allogeneic ADSCs were able to suppress the antigen-specific response of T cells from patients with RA. Specifically, ADSC inhibited the proliferative response and the production of inflammatory cytokines by collagen-activated CD4 and CD8 T cells. In addition, ADSC treatment significantly increased the numbers of IL-10-producing T cells and monocytes. ADSC also stimulated the generation of regulatory T cells that can suppress collagen-specific T-cell responses. Together, these findings suggest that allogeneic ADSC transplantation could treat RA by suppression of T-cell and inflammatory responses and by generation and/or activation of antigen-specific regulatory T cells. This dual immunomodulatory effect of suppressing

Table 1. Studies Using Xenogeneic Adipose-Derived Stem Cell Transplantation with Expressed Intent[a]

Publication year/first author	*Recipient species*	*Disease model*	*Cell injection route*	*Cell tracking label/method*	*Cell survival time*	*Histological assessment time point*	*Cell type-specific marker*
2003/Kang [61]	Rat	Cerebral ischemia	Intracerebral	LacZ label	≥14 days	14 and 30 days	MAP2, GFAP
2005/Rodriguez [62]	Mouse	Muscular dystrophy	Intramuscular	FISH	≥180 days	10, 50, 80, and 180 days	Dystrophin
2007/Kim [71]	Rat	Cerebral hemorrhagic stroke	Intravenous	DiO label	≥42 days	42 days	NG2, GFAP, vWF, EBA
2008/Fatar [72]	Rat	Cerebral hemorrhagic stroke	Intravenous	Human mitochondria	UnD at 28 days	28 days	None
2008/Niemeyer [64]	Mouse	Normal	Subcutaneous	FISH, human vimentin	≥8 weeks	4 and 8 weeks	None
2008/Arnalich-Montiel [73]	Rabbit	Corneal injury	Intracorneal	DiI label	>12 weeks	12 weeks	ALDH, keratocan
2009/Kang [65]	Mouse	Type 1 diabetes	Subrenal capsule	Human nuclear antigen	>1y	2 mo and 1y	Insulin
2009/Plaschke [74]	Rat	Oligemia-associated cognitive impairment	Intravenous	BrdU label	UnD at 6 days	6 days	None
2009/Zhu [75]	Rat	Acute myocardial infarction	Vena caudlis	GFP label	28 days	28 days	CD31
2009/Gonzalez [76]	Mouse	Colitis	Intraperitoneal	CFSE	6 days	1, 3, 5, 7 days	None
2009/Gonzalez-Rey [77]	Mouse	Colitis and sepsis	Intraperitoneal	CFSE	6 days	1, 3, 5, 7 days	None
2009/Gonzalez [78]	Mouse	Rheumatoid arthritis	Intraperitoneal or Intraarticular	None	ND	None	None
2010/Li [79]	Mouse	Acute kidney injury	Intravenous	Human nuclear antigen	10 days	3 days, 21 days, and 6 months	Pan-CK
2011/Kim [63]	Dog	Atrial injury	Intravenous	Feridex	4 weeks	4 weeks	α-actin, Cardiac troponin-1, Connexin 43
2011/Keibl [80]	Rat	Bone fracture	Intralesion	None	ND	None	None
20011/Zhou [81]	Mouse	Rheumatoid arthritis	Intravenous	None	ND	None	None
2011/Zhou [82]	Mouse	Autoimmune hearing loss	Intraperitoneal	None	ND	None	None
2012/Kim [83]	Rat	Acute kidney injury	Intravenous	BrdU	7 days	7 days	None
2012/Paul [17]	Rat	Myocardial infarction	Intralesion	FISH	4 weeks	4 weeks	None
2012/Choi [84]	Mouse	Systemic lupus erythematosus	Intravenous biweekly	DiI label	27 weeks	27 weeks	None

[a]Human ADSCs were used as donor cells in all studies.

ADSCs, adipose-derived stem cell; ALDH, aldehyde-3-dehydrogenase; BrdU, 5-bromo-2-deoxyuridine; CFSE, carboxyfluorescein diacetate succinimidyl ester; DAPI, 4′,6-diamidino-2-phenylindole; EBA, endothelial barrier antigen; FISH, fluorescence in situ hybridization; GFAP, glial fibrillary acidic protein; GFP, green fluorescence protein; MAP2, microtubule-associated protein 2; ND, not determined; NG2, oligodendrocyte precursor marker; UnD, undetectable; vWF, Von Willebrand factor.

Table 2. Studies Using Xenogeneic Adipose-Derived Stem Cell Transplantation Without Expressed Intent[a]

Publication year/ first author	*Recipient species*	*Disease model*	*Cell injection route*	*Cell tracking label/method*	*Cell survival time*	*Histological assessment time point*	*Cell type-specific marker*
2007/Liu [85]	Mouse	Muscular dystrophy	Intramuscular or Intravenous	Human β2-microglobulin	4 weeks	2 and 4 weeks	vWF
2009/Cho [86]	Mouse	Hind limb ischemia	Intramuscular	None	ND	None	None
2010/Yang [87]	Rat	Diabetic retinopathy	Intravenous	Human nuclear antigen	1 week	1 week	Rhodopsin, GFAP
2010/Hwangbo [88]	Rat	Myocardial infarction	Intralesion	DAPI	4 weeks	4 weeks	α-actin, Troponin T, Connexin 43
2010/Jeong [89]	Rat	intervertebral disc degeneration	Intralesion	Human nuclear antigen	2 weeks	2, 4, and 6 weeks	Col-II, Aggrecan
2011/Xuqian [90]	Rabbit	Retina injury	Intralesion	Human nuclear antigen	32 days	32 days	GFAP, Opsin,
2012/Xiao [91]	Mouse	Thrombocytopenia	Intravenous	None	ND	None	None

[a]Human ADSCs were used as donor cells in all studies.
Col-II, collagen-II.

overall T-cell proliferation while promoting the generation of regulatory T cells has also been observed more recently in cocultures of allogeneic ADSC and T cells [45].

Th17 lymphocytes are a subset of CD4+ T cells that produce the proinflammatory cytokine IL-17. These cells have been found to play important roles in the pathogenesis of many autoimmune diseases, including RA and systemic lupus erythematosus (SLE). Thus, it has been proposed that controlling Th17 cells or neutralizing IL-17 may offer therapeutic benefits for these autoimmune diseases [46,47]. In 2011, Lai et al. [48] investigated the effects of allogeneic ADSCs on Th17 cells by coculturing ADSCs with peripheral blood mononuclear cells of SLE patients. The results showed that ADSCs from passage 3 decreased the proportion of Th17 cells and suppressed their production of IL-17; however, ADSCs from passage 8 had the opposite effects. Thus, while allogeneic ADSC may have therapeutic potential toward SLE, their prolonged culturing should be avoided.

The above-mentioned studies suggest that, due to its immunomodulatory capability, ADSC might be suitable for allogeneic transplantation for the treatment of various diseases. However, a recent study showed that, despite being immunosuppressive, ADSCs were susceptible to lysis by allogeneic CD8+ T cells and NK cells [49]. Indeed, in an earlier study these authors also showed that ADSCs induced explosive T-cell proliferation [50]. Thus, whether allogeneic transplantation of ADSC offers therapeutic benefits requires further testing, especially in preclinical and clinical settings.

Immunomodulatory Therapy

In 2006, Yanez et al. [33] reported that allogeneically transplanted ADSCs were able to control experimentally induced graft-versus-host disease (GVHD) in mice. In the same year, Fang et al. [51] reported successful treatment of GVHD in a female patient by using ADSC isolated from an unrelated male donor. In the following year, Fang et al. reported a total of 7 cases of successful GVHD treatment with allogeneic ADSCs in 9 patients [52–54]. Two additional years later Fang et al. published 2 other clinical studies. In one study, a male patient was successfully treated with ADSC isolated from his brother, resulting in the resolution of refractory chronic autoimmune thrombocytopenic purpura [55]. In the other study, 2 patients were successfully treated with ADSCs isolated from unrelated donors, resulting in the resolution of refractory pure red cell aplasia due to major ABO-incompatible hematopoietic stem cell transplantation [56].

Allotransplantation

In a canine spinal cord injury model, injection of allogeneic ADSC into the injured site resulted in significant improvement in both hind limb function and nerve conduction [57]. Histological examination identified expression of neural markers GFAP, Tuj-1, and NF160 in the transplanted cells, suggesting neural differentiation. In another study, allogeneic ADSC seeded on a biomaterial scaffold were found to accelerate spinal fusion in a rat model of lumbar compression fracture [58]. In the recipient rats T-cell priming was undetectable, but significant antibody responses were observed [59]. However, the antibodies were determined to be noncytotoxic and thus not expected to impede the prospective

implementation of allogeneic ADSC for spinal fusion. In an allergic rhinitis mouse model IV injected ADSCs were found to migrate to the nasal mucosa, reduce allergic symptoms, and inhibit eosinophilic inflammation in the nasal mucosa [60]. ADSCs also significantly decreased the allergen-specific IgE level and IgG1/IgG2a ratio, suggesting the inhibition of eosinophilic inflammation was due to a shift from a Th2 immune response to a T-helper response.

Xenotransplantation

A total of 27 studies have performed xenotransplantation with ADSCs. Twenty of these studies mentioned the investigators' intent to conduct xenotransplantation while the other 7 did not (Tables 1 and 2). Without exception, all 27 studies used ADSCs isolated from humans, possibly due to the wide availability of lipoaspirates. On the other hand, the recipients were mouse in 13 studies, rat in 11, rabbit in 2, and dog in 1.

The first ADSC xenotransplantation study was published in 2003, in which intracerebral transplantation of human ADSCs was found to improve neurological functions in a cerebral ischemic rat model [61]. Interestingly, despite being a xenogeneic transplantation, the investigators observed no evidence of inflammation or rejection. They thus offered several explanations including the brain being partially immunoprivileged and ADSCs being lacking MHC-II expression. Two years later, another study conducted a more detailed examination of ADSC's potential for xenogeneic transplantation [62]. First, in cell culture experiments human ADSCs were shown not to induce proliferation of murine splenocytes; then, in animal transplantation experiments human ADSCs were found not to cause murine CD3 lymphocyte infiltration. This study's main purpose, however, was to show that ADSC had the potential for treating muscular dystrophy. Specifically, transplantation of human ADSCs into a murine model of muscular dystrophy resulted in high-level expression of human dystrophin and long-term engraftment of the transplanted cells. In a more recent study of using human ADSCs to treat experimentally induced atrial injury in dogs, intravenous administration of human ADSCs caused virtually no changes in the composition of peripheral blood lymphocytes of the recipient dogs, thus indicating immunocompatibility [63].

The feasibility of using ADSC (and BMSC) for xenogeneic transplantation was specifically investigated in a 2008 study [64]. Undifferentiated and osteogenically differentiated ADSCs (and BMSCs) were subcutaneously injected into immunocompetent mice and then tracked at 4 and 8 weeks postinjection. The results show that undifferentiated ADSCs/BMSCs survived for at least 8 weeks while osteogenically differentiated ADSCs/BMSCs were eliminated at 4 weeks. The authors thus concluded that undifferentiated ADSCs/BMSCs were suitable for xenogeneic transplantation. However, in another study differentiated insulin-producing cells from human ADSCs were found to survive for a remarkable period of 1 year following subrenal capsule transplantation into streptozotocin-induced diabetic mice [65]. Thus, it appears that under certain conditions both undifferentiated and differentiated ADSCs could be used for xenotransplantation.

Prospective Clinical Application of Allogeneic and Xenogeneic ADSCs

Liposuction is a well-established clinical practice in human medicine, and processing the liposuction material into SVF can be done expeditiously with commercially available devices [5,27,28]. Therefore, the future application of ADSCs in human medicine will be conducted mostly, if not exclusively, in an autologous fashion. On the other hand, liposuction is not a standard procedure in veterinary medicine, and the manual isolation of SVF or ADSC from each dog or cat is an impractical proposition for most veterinary clinics. Thus, the allogeneic or xenogeneic application of ADSCs in veterinary medicine is worthy of consideration.

In the mainstream media and in the Internet there have been thousands of claims about ADSC's "miraculous" therapeutic efficacy in treating animal diseases, especially canine OA. In the scientific literature, there have been 3 studies that used autologous ADSCs to treat canine OA [66–68], and the results all indicated ADSC's efficacy in ameliorating OA symptoms. However, the adoption of this novel OA treatment into veterinarian practice faces many challenges, including (1) most veterinary clinics lack the equipment and expertise for ADSC isolation, (2) excision of adipose tissue causes donor site morbidity, (3) individually made ADSC isolation is costly and time-consuming, and (4) at least 2 veterinarian appointments are needed for adipose tissue procurement and ADSC injection. However, these obstacles can be overcome if the therapeutic ADSCs are from an allogeneic or xenogeneic source. For example, canine and porcine ADSCs can be prepared in commercial-scale quantities, portions of which are stored in liquid nitrogen or further propagated. Upon receiving an order from a vet clinic, the cells can be shipped in a syringe via an express courier; and upon its arrival, the cell preparation can be injected by the veterinarian into the diseased joint of a patient dog. Thus, there is no need for the veterinary clinic to purchase expensive equipment or hire cell-isolation technicians. The demand on the veterinarian is minimal as well.

From an immunological point of view, allotransplantation is perhaps a better choice than xenotransplantation. However, from an ethical point of view, the harvest of canine tissues for commercial purpose is definitely less acceptable than the harvest of porcine tissues. Thus, xenotransplantation of porcine ADSC for veterinary uses is expected to have a better chance to succeed. In addition, the practice of porcine organ transplantation in humans has been extensively investigated with the establishment of strict guidelines [69]. Thus, the breeding of donor pigs and the harvest of their adipose tissue can follow these established guidelines. It should be further pointed out that, in more than 2 centuries of investigation there has been no documentation of transfer of viruses from donor pig tissues to recipient humans [70]. Thus, it is reasonable to expect that porcine ADSC will be safe for transplantation into dogs and cats. If so, xenotransplantation of porcine ADSC should provide great therapeutic benefits to our best friends.

Author Disclosure Statement

No competing financial interests exist.

References

1. da Silva Meirelles L, PC Chagastelles and NB Nardi. (2006). Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J Cell Sci 119:2204–2213.
2. Lin CS, ZC Xin, CH Deng, H Ning, G Lin and TF Lue. (2010). Defining adipose tissue-derived stem cells in tissue and in culture. Histol Histopathol 25:807–815.
3. Lin CS, ZC Xin, Z Wang, C Deng, YC Huang, G Lin and TF Lue. (2012). Stem cell therapy for erectile dysfunction: a critical review. Stem Cells Dev 21:343–351.
4. Lin CS and TF Lue. (2012). Adipose-derived stem cells: therapy through paracrine actions. In: *Stem Cells and Cancer Stem Cells*. Hayat MA, ed. Springer, New York, pp 203–216.
5. Lin CS and TF Lue. (2012). Stem cell therapy for stress urinary incontinence: a critical review. Stem Cells Dev 21: 834–843.
6. Liechty KW, TC MacKenzie, AF Shaaban, A Radu, AM Moseley, R Deans, DR Marshak and AW Flake. (2000). Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after *in utero* transplantation in sheep. Nat Med 6:1282–1286.
7. Devine SM, AM Bartholomew, N Mahmud, M Nelson, S Patil, W Hardy, C Sturgeon, T Hewett, T Chung, et al. (2001). Mesenchymal stem cells are capable of homing to the bone marrow of non-human primates following systemic infusion. Exp Hematol 29:244–255.
8. Bartholomew A, C Sturgeon, M Siatskas, K Ferrer, K McIntosh, S Patil, W Hardy, S Devine, D Ucker, et al. (2002). Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. Exp Hematol 30:42–48.
9. Saito T, JQ Kuang, B Bittira, A Al-Khaldi and RC Chiu. (2002). Xenotransplant cardiac chimera: immune tolerance of adult stem cells. Ann Thorac Surg 74:19–24; discussion 24.
10. MacDonald DJ, J Luo, T Saito, M Duong, PL Bernier, RC Chiu and D Shum-Tim. (2005). Persistence of marrow stromal cells implanted into acutely infarcted myocardium: observations in a xenotransplant model. J Thorac Cardiovasc Surg 130:1114–1121.
11. Grinnemo KH, A Mansson, G Dellgren, D Klingberg, E Wardell, V Drvota, C Tammik, J Holgersson, O Ringden, C Sylven and K Le Blanc. (2004). Xenoreactivity and engraftment of human mesenchymal stem cells transplanted into infarcted rat myocardium. J Thorac Cardiovasc Surg 127: 1293–1300.
12. Grinnemo KH, A Mansson-Broberg, K Leblanc, M Corbascio, E Wardell, AJ Siddiqui, X Hao, C Sylven and G Dellgren. (2006). Human mesenchymal stem cells do not differentiate into cardiomyocytes in a cardiac ischemic xenomodel. Ann Med 38:144–153.
13. Krampera M, S Glennie, J Dyson, D Scott, R Laylor, E Simpson and F Dazzi. (2003). Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. Blood 101:3722–3729.
14. Di Nicola M, C Carlo-Stella, M Magni, M Milanesi, PD Longoni, P Matteucci, S Grisanti and AM Gianni. (2002). Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood 99:3838–3843.
15. Tse WT, JD Pendleton, WM Beyer, MC Egalka and EC Guinan. (2003). Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. Transplantation 75:389–397.
16. Kim HJ, JB Park, JK Lee, EY Park, EA Park, KD Riew and SK Rhee. (2008). Transplanted xenogenic bone marrow stem cells survive and generate new bone formation in the posterolateral lumbar spine of non-immunosuppressed rabbits. Eur Spine J 17:1515–1521.
17. Paul A, S Srivastava, G Chen, D Shum-Tim and S Prakash. (2012). Functional assessment of adipose stem cells for xenotransplantation using myocardial infarction immunocompetent models: comparison with bone marrow stem cells. Cell Biochem Biophys [Epub ahead of print]; DOI: 10.1007/s12013-011-9323-0.
18. Pal R, C Gopinath, NM Rao, P Banerjee, V Krishnamoorthy, NK Venkataramana and S Totey. (2010). Functional recovery after transplantation of bone marrow-derived human mesenchymal stromal cells in a rat model of spinal cord injury. Cytotherapy 12:792–806.
19. Guan M, W Yao, R Liu, KS Lam, J Nolta, J Jia, B Panganiban, L Meng, P Zhou, et al. (2012). Directing mesenchymal stem cells to bone to augment bone formation and increase bone mass. Nat Med 18:456–462.
20. Lin G, M Garcia, H Ning, L Banie, YL Guo, TF Lue and CS Lin. (2008). Defining stem and progenitor cells within adipose tissue. Stem Cells Dev 17:1053–1063.
21. da Silva Meirelles L, TT Sand, RJ Harman, DP Lennon and AI Caplan. (2009). MSC frequency correlates with blood vessel density in equine adipose tissue. Tissue Eng Part A 15:221–229.
22. Ergun S, D Tilki and D Klein. (2011). Vascular wall as a reservoir for different types of stem and progenitor cells. Antioxid Redox Signal 15:981–995.
23. Corselli M, CW Chen, B Sun, S Yap, JP Rubin and B Peault. (2012). The tunica adventitia of human arteries and veins as a source of mesenchymal stem cells. Stem Cells Dev 21:1299–1308.
24. Crandall DL, GJ Hausman and JG Kral. (1997). A review of the microcirculation of adipose tissue: anatomic, metabolic, and angiogenic perspectives. Microcirculation 4: 211–232.
25. Housman TS, N Lawrence, BG Mellen, MN George, JS Filippo, KA Cerveny, M DeMarco, SR Feldman and AB Fleischer. (2002). The safety of liposuction: results of a national survey. Dermatol Surg 28:971–978.
26. Strem BM, KC Hicok, M Zhu, I Wulur, Z Alfonso, RE Schreiber, JK Fraser and MH Hedrick. (2005). Multipotential differentiation of adipose tissue-derived stem cells. Keio J Med 54:132–141.
27. Guven S, M Karagianni, M Schwalbe, S Schreiner, J Farhadi, S Bula, K Bieback, I Martin and A Scherberich. (2012). Validation of an automated procedure to isolate human adipose tissue-derived cells by using the Sepax® technology. Tissue Eng Part C Methods. [Epub ahead of print]; DOI: 10.1089/ten.tec.2011.0617.
28. Lin K, Y Matsubara, Y Masuda, K Togashi, T Ohno, T Tamura, Y Toyoshima, K Sugimachi, M Toyoda, H Marc and A Douglas. (2008). Characterization of adipose tissue-derived cells isolated with the Celution system. Cytotherapy 10:417–426.
29. Puissant B, C Barreau, P Bourin, C Clavel, J Corre, C Bousquet, C Taureau, B Cousin, M Abbal, et al. (2005). Immunomodulatory effect of human adipose tissue-derived adult stem cells: comparison with bone marrow mesenchymal stem cells. Br J Haematol 129:118–129.
30. Wolbank S, A Peterbauer, M Fahrner, S Hennerbichler, M van Griensven, G Stadler, H Redl and C Gabriel. (2007). Dose-dependent immunomodulatory effect of human stem cells from amniotic membrane: a comparison with human

mesenchymal stem cells from adipose tissue. Tissue Eng 13:1173–1183.
31. Cui L, S Yin, W Liu, N Li, W Zhang and Y Cao. (2007). Expanded adipose-derived stem cells suppress mixed lymphocyte reaction by secretion of prostaglandin E2. Tissue Eng 13:1185–1195.
32. McIntosh K, S Zvonic, S Garrett, JB Mitchell, ZE Floyd, L Hammill, A Kloster, Y Di Halvorsen, JP Ting, et al. (2006). The immunogenicity of human adipose-derived cells: temporal changes in vitro. Stem Cells 24:1246–1253.
33. Yanez R, ML Lamana, J Garcia-Castro, I Colmenero, M Ramirez and JA Bueren. (2006). Adipose tissue-derived mesenchymal stem cells have in vivo immunosuppressive properties applicable for the control of the graft-versus-host disease. Stem Cells 24:2582–2591.
34. Keyser KA, KE Beagles and HP Kiem. (2007). Comparison of mesenchymal stem cells from different tissues to suppress T-cell activation. Cell Transplant 16:555–562.
35. Yoo KH, IK Jang, MW Lee, HE Kim, MS Yang, Y Eom, JE Lee, YJ Kim, SK Yang, et al. (2009). Comparison of immunomodulatory properties of mesenchymal stem cells derived from adult human tissues. Cell Immunol 259:150–156.
36. DelaRosa O, E Lombardo, A Beraza, P Mancheno-Corvo, C Ramirez, R Menta, L Rico, E Camarillo, L Garcia, et al. (2009). Requirement of IFN-gamma-mediated indoleamine 2,3-dioxygenase expression in the modulation of lymphocyte proliferation by human adipose-derived stem cells. Tissue Eng Part A 15:2795–2806.
37. Niemeyer P, M Kornacker, A Mehlhorn, A Seckinger, J Vohrer, H Schmal, P Kasten, V Eckstein, NP Sudkamp and U Krause. (2007). Comparison of immunological properties of bone marrow stromal cells and adipose tissue-derived stem cells before and after osteogenic differentiation in vitro. Tissue Eng 13:111–121.
38. Djouad F, LM Charbonnier, C Bouffi, P Louis-Plence, C Bony, F Apparailly, C Cantos, C Jorgensen and D Noel. (2007). Mesenchymal stem cells inhibit the differentiation of dendritic cells through an interleukin-6-dependent mechanism. Stem Cells 25:2025–2032.
39. Kang JW, KS Kang, HC Koo, JR Park, EW Choi and YH Park. (2008). Soluble factors-mediated immunomodulatory effects of canine adipose tissue-derived mesenchymal stem cells. Stem Cells Dev 17:681–693.
40. Najar M, G Raicevic, HI Boufker, H Fayyad Kazan, C De Bruyn, N Meuleman, D Bron, M Toungouz and L Lagneaux. (2010). Mesenchymal stromal cells use PGE2 to modulate activation and proliferation of lymphocyte subsets: Combined comparison of adipose tissue, Wharton's Jelly and bone marrow sources. Cell Immunol 264:171–179.
41. Yanez R, A Oviedo, M Aldea, JA Bueren and ML Lamana. (2010). Prostaglandin E2 plays a key role in the immunosuppressive properties of adipose and bone marrow tissue-derived mesenchymal stromal cells. Exp Cell Res 316: 3109–3123.
42. Najar M, G Raicevic, HI Boufker, H Fayyad-Kazan, C De Bruyn, N Meuleman, D Bron, M Toungouz and L Lagneaux. (2010). Adipose-tissue-derived and Wharton's jelly-derived mesenchymal stromal cells suppress lymphocyte responses by secreting leukemia inhibitory factor. Tissue Eng Part A 16:3537–3546.
43. Ivanova-Todorova E, I Bochev, M Mourdjeva, R Dimitrov, D Bukarev, S Kyurkchiev, P Tivchev, I Altunkova and DS Kyurkchiev. (2009). Adipose tissue-derived mesenchymal stem cells are more potent suppressors of dendritic cells differentiation compared to bone marrow-derived mesenchymal stem cells. Immunol Lett 126:37–42.
44. Gonzalez-Rey E, MA Gonzalez, N Varela, F O'Valle, P Hernandez-Cortes, L Rico, D Buscher and M Delgado. (2010). Human adipose-derived mesenchymal stem cells reduce inflammatory and T cell responses and induce regulatory T cells in vitro in rheumatoid arthritis. Ann Rheum Dis 69:241–248.
45. Kuo YR, CC Chen, S Goto, IT Lee, CW Huang, CC Tsai, CT Wang and CL Chen. (2011). Modulation of immune response and T-cell regulation by donor adipose-derived stem cells in a rodent hind-limb allotransplant model. Plast Reconstr Surg 128:661e–672e.
46. Lubberts E. (2008). IL-17/Th17 targeting: on the road to prevent chronic destructive arthritis? Cytokine 41:84–91.
47. Pernis AB. (2009). Th17 cells in rheumatoid arthritis and systemic lupus erythematosus. J Intern Med 265:644–652.
48. Lai K, K Zeng, F Zeng, J Wei and G Tan. (2011). Allogeneic adipose-derived stem cells suppress Th17 lymphocytes in patients with active lupus in vitro. Acta Biochim Biophys Sin (Shanghai) 43:805–812.
49. Crop MJ, SS Korevaar, R de Kuiper, JN Ijzermans, NM van Besouw, CC Baan, W Weimar and MJ Hoogduijn. (2011). Human mesenchymal stem cells are susceptible to lysis by CD8+ T-cells and NK cells. Cell Transplant 20:1547–1559.
50. Crop MJ, CC Baan, SS Korevaar, JN Ijzermans, W Weimar and MJ Hoogduijn. (2010). Human adipose tissue-derived mesenchymal stem cells induce explosive T-cell proliferation. Stem Cells Dev 19:1843–1853.
51. Fang B, YP Song, LM Liao, Q Han and RC Zhao. (2006). Treatment of severe therapy-resistant acute graft-versus-host disease with human adipose tissue-derived mesenchymal stem cells. Bone Marrow Transplant 38:389–390.
52. Fang B, Y Song, RC Zhao, Q Han and Q Lin. (2007). Using human adipose tissue-derived mesenchymal stem cells as salvage therapy for hepatic graft-versus-host disease resembling acute hepatitis. Transplant Proc 39:1710–1713.
53. Fang B, Y Song, Q Lin, Y Zhang, Y Cao, RC Zhao and Y Ma. (2007). Human adipose tissue-derived mesenchymal stromal cells as salvage therapy for treatment of severe refractory acute graft-vs.-host disease in two children. Pediatr Transplant 11:814–817.
54. Fang B, Y Song, L Liao, Y Zhang and RC Zhao. (2007). Favorable response to human adipose tissue-derived mesenchymal stem cells in steroid-refractory acute graft-versus-host disease. Transplant Proc 39:3358–3362.
55. Fang B, YP Song, N Li, J Li, Q Han and RC Zhao. (2009). Resolution of refractory chronic autoimmune thrombocytopenic purpura following mesenchymal stem cell transplantation: a case report. Transplant Proc 41:1827–1830.
56. Fang B, Y Song, N Li, J Li, Q Han and RC Zhao. (2009). Mesenchymal stem cells for the treatment of refractory pure red cell aplasia after major ABO-incompatible hematopoietic stem cell transplantation. Ann Hematol 88:261–266.
57. Ryu HH, JH Lim, YE Byeon, JR Park, MS Seo, YW Lee, WH Kim, KS Kang and OK Kweon. (2009). Functional recovery and neural differentiation after transplantation of allogenic adipose-derived stem cells in a canine model of acute spinal cord injury. J Vet Sci 10:273–284.
58. Lopez MJ, KR McIntosh, ND Spencer, JN Borneman, R Horswell, P Anderson, G Yu, L Gaschen and JM Gimble. (2009). Acceleration of spinal fusion using syngeneic and allogeneic adult adipose derived stem cells in a rat model. J Orthop Res 27:366–373.

59. McIntosh KR, MJ Lopez, JN Borneman, ND Spencer, PA Anderson and JM Gimble. (2009). Immunogenicity of allogeneic adipose-derived stem cells in a rat spinal fusion model. Tissue Eng Part A 15:2677–2686.
60. Cho KS, HK Park, HY Park, JS Jung, SG Jeon, YK Kim and HJ Roh. (2009). IFATS collection: immunomodulatory effects of adipose tissue-derived stem cells in an allergic rhinitis mouse model. Stem Cells 27:259–265.
61. Kang SK, DH Lee, YC Bae, HK Kim, SY Baik and JS Jung. (2003). Improvement of neurological deficits by intracerebral transplantation of human adipose tissue-derived stromal cells after cerebral ischemia in rats. Exp Neurol 183:355–366.
62. Rodriguez AM, D Pisani, CA Dechesne, C Turc-Carel, JY Kurzenne, B Wdziekonski, A Villageois, C Bagnis, JP Breittmayer, et al. (2005). Transplantation of a multipotent cell population from human adipose tissue induces dystrophin expression in the immunocompetent mdx mouse. J Exp Med 201:1397–1405.
63. Kim U, DG Shin, JS Park, YJ Kim, SI Park, YM Moon and KS Jeong. (2011). Homing of adipose-derived stem cells to radiofrequency catheter ablated canine atrium and differentiation into cardiomyocyte-like cells. Int J Cardiol 146: 371–378.
64. Niemeyer P, J Vohrer, H Schmal, P Kasten, J Fellenberg, NP Suedkamp and AT Mehlhorn. (2008). Survival of human mesenchymal stromal cells from bone marrow and adipose tissue after xenogenic transplantation in immunocompetent mice. Cytotherapy 10:784–795.
65. Kang HM, J Kim, S Park, H Kim, KS Kim, EJ Lee, SI Seo, SG Kang, JE Lee and H Lim. (2009). Insulin-secreting cells from human eyelid-derived stem cells alleviate type I diabetes in immunocompetent mice. Stem Cells 27:1999–2008.
66. Black LL, J Gaynor, C Adams, S Dhupa, AE Sams, R Taylor, S Harman, DA Gingerich and R Harman. (2008). Effect of intraarticular injection of autologous adipose-derived mesenchymal stem and regenerative cells on clinical signs of chronic osteoarthritis of the elbow joint in dogs. Vet Ther 9:192–200.
67. Black LL, J Gaynor, D Gahring, C Adams, D Aron, S Harman, DA Gingerich and R Harman. (2007). Effect of adipose-derived mesenchymal stem and regenerative cells on lameness in dogs with chronic osteoarthritis of the coxofemoral joints: a randomized, double-blinded, multicenter, controlled trial. Vet Ther 8:272–284.
68. Guercio A, P Di Marco, S Casella, V Cannella, L Russotto, G Purpari, S Di Bella and G Piccione. (2012). Production of canine mesenchymal stem cells from adipose tissue and their application in dogs with chronic osteoarthritis of the humeroradial joints. Cell Biol Int 36:189–194.
69. Schuurman HJ. (2008). Regulatory aspects of pig-to-human islet transplantation. Xenotransplantation 15:116–120.
70. Cooper DK. (2012). A brief history of cross-species organ transplantation. Proc (Bayl Univ Med Cent) 25:49–57.
71. Kim JM, ST Lee, K Chu, KH Jung, EC Song, SJ Kim, DI Sinn, JH Kim, DK Park, et al. (2007). Systemic transplantation of human adipose stem cells attenuated cerebral inflammation and degeneration in a hemorrhagic stroke model. Brain Res 1183:43–50.
72. Fatar M, M Stroick, M Griebe, I Marwedel, S Kern, K Bieback, FL Giesel, C Zechmann, S Kreisel, et al. (2008). Lipoaspirate-derived adult mesenchymal stem cells improve functional outcome during intracerebral hemorrhage by proliferation of endogenous progenitor cells stem cells in intracerebral hemorrhages. Neurosci Lett 443:174–178.
73. Arnalich-Montiel F, S Pastor, A Blazquez-Martinez, J Fernandez-Delgado, M Nistal, JL Alio and MP De Miguel. (2008). Adipose-derived stem cells are a source for cell therapy of the corneal stroma. Stem Cells 26:570–579.
74. Plaschke K. (2009). Human adult mesenchymal stem cells improve rat spatial cognitive function after systemic hemorrhagic shock. Behav Brain Res 201:332–337.
75. Zhu XY, XZ Zhang, L Xu, XY Zhong, Q Ding and YX Chen. (2009). Transplantation of adipose-derived stem cells overexpressing hHGF into cardiac tissue. Biochem Biophys Res Commun 379:1084–1090.
76. Gonzalez MA, E Gonzalez-Rey, L Rico, D Buscher and M Delgado. (2009). Adipose-derived mesenchymal stem cells alleviate experimental colitis by inhibiting inflammatory and autoimmune responses. Gastroenterology 136:978–989.
77. Gonzalez-Rey E, P Anderson, MA Gonzalez, L Rico, D Buscher and M Delgado. (2009). Human adult stem cells derived from adipose tissue protect against experimental colitis and sepsis. Gut 58:929–939.
78. Gonzalez MA, E Gonzalez-Rey, L Rico, D Buscher and M Delgado. (2009). Treatment of experimental arthritis by inducing immune tolerance with human adipose-derived mesenchymal stem cells. Arthritis Rheum 60:1006–1019.
79. Li K, Q Han, X Yan, L Liao and RC Zhao. (2010). Not a process of simple vicariousness, the differentiation of human adipose-derived mesenchymal stem cells to renal tubular epithelial cells plays an important role in acute kidney injury repairing. Stem Cells Dev 19:1267–1275.
80. Keibl C, A Fugl, G Zanoni, S Tangl, S Wolbank, H Redl and M van Griensven. (2011). Human adipose derived stem cells reduce callus volume upon BMP-2 administration in bone regeneration. Injury 42:814–820.
81. Zhou B, J Yuan, Y Zhou, M Ghawji, Jr., YP Deng, AJ Lee, U Nair, AH Kang, DD Brand and TJ Yoo. (2011). Administering human adipose-derived mesenchymal stem cells to prevent and treat experimental arthritis. Clin Immunol 141:328–337.
82. Zhou Y, J Yuan, B Zhou, AJ Lee, M Ghawji, Jr. and TJ Yoo. (2011). The therapeutic efficacy of human adipose tissue-derived mesenchymal stem cells on experimental autoimmune hearing loss in mice. Immunology 133:133–140.
83. Kim JH, DJ Park, JC Yun, MH Jung, HD Yeo, HJ Kim, DW Kim, JI Yang, GW Lee, et al. (2012). Human adipose tissue-derived mesenchymal stem cells protect kidneys from cisplatin nephrotoxicity in rats. Am J Physiol Renal Physiol 302:F1141–F1150.
84. Choi EW, IS Shin, SY Park, JH Park, JS Kim, EJ Yoon, SK Kang, JC Ra and SH 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.
85. Liu Y, X Yan, Z Sun, B Chen, Q Han, J Li and RC Zhao. (2007). Flk-1+ adipose-derived mesenchymal stem cells differentiate into skeletal muscle satellite cells and ameliorate muscular dystrophy in mdx mice. Stem Cells Dev 16:695–706.
86. Cho HH, YJ Kim, JT Kim, JS Song, KK Shin, YC Bae and JS Jung. (2009). The role of chemokines in proangiogenic action induced by human adipose tissue-derived mesenchymal stem cells in the murine model of hindlimb ischemia. Cell Physiol Biochem 24:511–518.
87. Yang Z, K Li, X Yan, F Dong and C Zhao. (2010). Amelioration of diabetic retinopathy by engrafted human adipose-derived

mesenchymal stem cells in streptozotocin diabetic rats. Graefes Arch Clin Exp Ophthalmol 248:1415–1422.
88. Hwangbo S, J Kim, S Her, H Cho and J Lee. (2010). Therapeutic potential of human adipose stem cells in a rat myocardial infarction model. Yonsei Med J 51:69–76.
89. Jeong JH, JH Lee, ES Jin, JK Min, SR Jeon and KH Choi. (2010). Regeneration of intervertebral discs in a rat disc degeneration model by implanted adipose-tissue-derived stromal cells. Acta Neurochir (Wien) 152:1771–1777.
90. Xuqian W, L Kanghua, Y Weihong, Y Xi, D Rongping, H Qin, D Fangtian and R Chunhua Zhao. (2011). Intraocular transplantation of human adipose-derived mesenchymal stem cells in a rabbit model of experimental retinal holes. Ophthalmic Res 46:199–207.
91. Xiao J, C Zhang, Y Zhang, X Zhang, J Zhao, J Liang, X Zhong and Y Chen. (2012). Transplantation of adipose-derived mesenchymal stem cells into a murine model of passive chronic immune thrombocytopenia. Transfusion [Epub ahead of print]; DOI: 10.1111/j.1537-2995.2012.03642.x.

Address correspondence to:
Dr. Ching-Shwun Lin
Knuppe Molecular Urology Laboratory
Department of Urology
School of Medicine
University of California
San Francisco, CA 94143-0738

E-mail: clin@urology.ucsf.edu

Received for publication April 3, 2012
Accepted after revision May 23, 2012
Prepublished on Liebert Instant Online May 23, 2012