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Overview

Immunomodulation by Mesenchymal Stem Cells in Veterinary Species

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Mesenchymal stem cells (MSC) are adult-derived multipotent stem cells that have been derived from almost every tissue. They are classically defined as spindle-shaped, plastic-adherent cells capable of adipogenic, chondrogenic, and osteogenic differentiation. This capacity for trilineage differentiation has been the foundation for research into the use of MSC to regenerate damaged tissues. Recent studies have shown that MSC interact with cells of the immune system and modulate their function. Although many of the details underlying the mechanisms by which MSC modulate the immune system have been defined for human and rodent (mouse and rat) MSC, much less is known about MSC from other veterinary species. This knowledge gap is particularly important because the clinical use of MSC in veterinary medicine is increasing and far exceeds the use of MSC in human medicine. It is crucial to determine how MSC modulate the immune system for each animal species as well as for MSC derived from any given tissue source. A comparative approach provides a unique translational opportunity to bring novel cell-based therapies to the veterinary market as well as enhance the utility of animal models for human disorders. The current review covers what is currently known about MSC and their immunomodulatory functions in veterinary species, excluding laboratory rodents.

Abbreviations: AT, adipose tissue; BM, Bone marrow; CB, umbilical cord blood; CT, umbilical cord tissue; DC, dendritic cell; IDO, indoleamine 2;3-dioxygenase; MSC, mesenchymal stem cells; PGE_2, prostaglandin E2; VEGF, vascular endothelial growth factor.

Mesenchymal stem cells (MSC, alternatively known as mesenchymal stromal cells) were first reported in the literature in 1968.[39] MSC are thought to be of pericyte origin (cells that line the vasculature)[21,22] and typically are isolated from highly vascular tissues. In humans and mice, MSC have been isolated from fat, placental tissues (placenta, Wharton jelly, umbilical cord, umbilical cord blood), hair follicles, tendon, synovial membrane, periodontal ligament, and every major organ (brain, spleen, liver, kidney, lung, bone marrow, muscle, thymus, pancreas, skin).[23,121] For most current clinical applications, MSC are isolated from adipose tissue (AT), bone marrow (BM), umbilical cord blood (CB), and umbilical cord tissue (CT; Table 1). Both in human and veterinary medicine, MSC promote tissue regeneration and healing via modulation of the immune response, including decreasing the cells and cytokines associated with inflammation and increasing blood flow to promote normal healing rather than scarring.[11,87,99] Clinical trials in human medicine focus on the use of MSC both for their antiinflammatory properties (graft-versus-host disease, irritable bowel syndrome) and their ability to aid in tissue and bone regeneration in combination with growth factors and bone scaffolds (clinicaltrials.gov).[131] For tissue regeneration, the abilities of MSC to differentiate and to secrete mediators and interact with cells of the immune system likely contribute to tissue healing (Figure 1). The current review will not address the specific use of MSC for orthopedic applications and tissue regeneration, although the topic is covered widely in current literature for both human and veterinary medicine.[57,62,90]

Long-term studies in veterinary species have shown no adverse effects with the administration of MSC in a large number of animals.[9,10,53] Smaller, controlled studies on veterinary species have shown few adverse effects, such as minor localized inflammation after MSC administration in vivo.[7,15,17,45,86,92,98] Private companies, educational institutions, and private veterinary clinics (including Tufts University, Cummins School of Veterinary Medicine, University of California Davis School of Veterinary Medicine, VetStem, Celavet, Alamo Pintado Equine Medical Center, and Rood and Riddle Equine Hospital) offer MSC as a clinical treatment for veterinary species. Clinical uses include tendon and cartilage injuries, tendonitis, and osteoarthritis and, to a lesser extent, bone regeneration, spinal cord injuries, and liver disease in both large and small animals.[38,41,113] Even with this broad clinical use, there have been no reports of severe adverse effects secondary to MSC administration in veterinary patients.

MSC Characterization

MSC are defined as highly proliferative, plastic-adherent, fibroblast-like cells capable of osteogenic, chondrogenic, and adipogenic differentiation.[39,101] MSC are further characterized according to their surface protein expression by flow cytometry or immunophenotyping. Full characterization of MSC from veterinary species is hindered by the lack of available species-specific antibodies and reagents. The identification of crossreactive anti-

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Table 1. Tissues from which MSC have been isolated

	Tissue source (reference no.)				
Species	Fat	Bone marrow	Cord blood	Cord tissue	Other
Cat	134	83	56		
Chicken		63			
Cow	138	12	108		
Dog	97	3, 59	78, 119	139	Periodontal ligament[65]
Goat	66	96		4	
Horse	26, 130	37, 40, 123	67	130	Periodontal ligament and gingiva[88]
Nonhuman primate	28, 54	5			
Pig	135	114	70	14, 20, 91	
Rabbit	128	80		32	Fetal liver[93]
Sheep	84	95	42, 55		

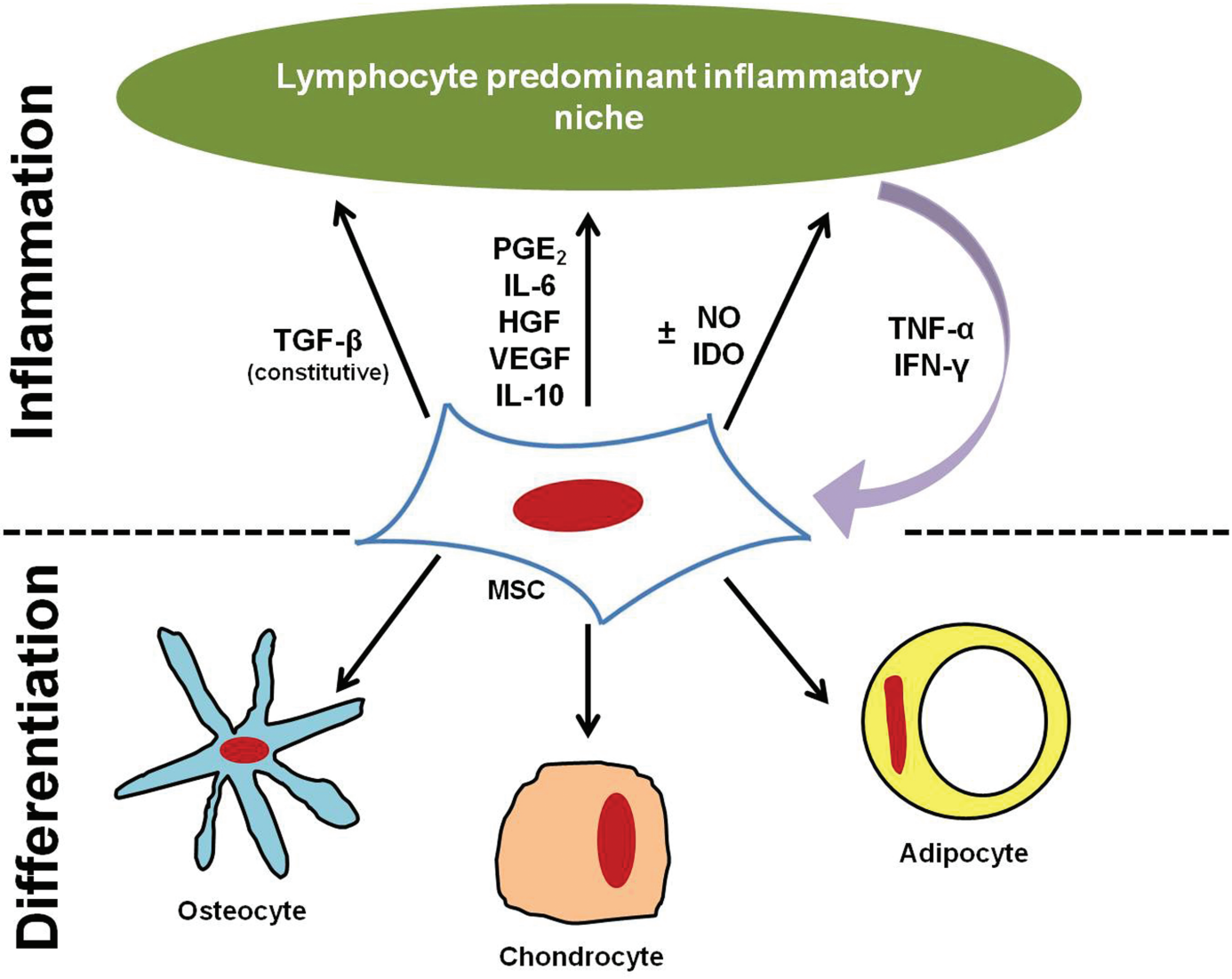

Figure 1. The dual roles of MSC: differentiation and modulation of inflammation.

Table 2. MSC surface markers, as determined by flow cytometry, RT-PCR, or immunocytochemistry

Species	MSC type		Positive	Negative	Reference
Cat	AT	Reported	CD44, CD90, CD105	CD4, MHCII	134
	BM	Confirmed	MHCI, CD44, CD9	CD4, CD8, CD13, CD14, CD18, CD41/61, CD45, MHCII	83
		Reported	CD90, CD105		134
Chicken	BM	Confirmed	CD44, CD90, CD105	CD45	63
Dog	AT	Confirmed	MHCI, CD44, CD90	MHCII, pan-lymphocyte, CD14, CD45, CD3, CD4, CD8, CD172a, CD11c	61
	BM	Confirmed	CD29, CD90, CD44, CD73, CD106, CD10, CD13	CD34, CD14, CD105, CD3, CD45	60, 77
		Reported	MHCI	MHCII	60
	CT	Reported	CD44, CD29, CD90	CD34, CD45, CD14, CD117	139
	CB	Reported	CD29, CD33, CD44, CD105, CD184	CD4, CD8a, CD10, CD14, CD20, CD24, CD31, CD34, CD38, CD41a, MHCII, CD45, CD49b, CD41/61, CD62p, CD73, CD90, CD133	119
Goat	CT	Reported	CD44	CD34	4
Horse	AT	Confirmed	MHCI, CD29, CD90, CD44	MHCII, CD86, F6B (pan-leukocyte)	16
		Reported	CD73, CD105	CD14, CD34, CD79a, CD45	13, 48, 107
	BM	Confirmed	MHCI, CD29, CD90, CD44	MHCII, CD86, F6B (pan-leukocyte)	16
		Reported	CD73, CD105	CD14, CD34, CD79a, CD45	13, 48, 107
	CT	Confirmed	MHCI, CD29, CD90, CD44, CD105, CD166	MHCII, CD86, F6B (panleukocyte), CD34	15, 16
	CB	Confirmed	MHCI, CD29, CD90, CD44	MHCII, CD86, F6B (panleukocyte)	16, 74
		Reported		CD79a	48
Nonhuman primate	AT	Reported	CD90, MHCI, CD105, CD59, CD106, CD146, CD161, STRO1	CD3, CD4, CD8, CD11b, CD13, CD31, CD164	54
	BM	Reported	CD29, CD90, CD44, MHCI, CD105, CD73, CD166, CD56, CD59, CD106, CD146, CD161, STRO1	CD34, CD14, CD11c, CD45, CD56, CD31, MHCII, CD3, CD4, CD8, CD11b, CD13, CD164	8, 54
Pig	AT	Reported	CD44, CD34		136
	BM	Confirmed	CD90, CD29, CD44, MHCI, CD46	CD172a, CD106, CD56	94, 100
		Reported		CD45, MHCII	50
	CT	Confirmed	CD90, CD44, MHCI	CD31, CD45RA, MHCII	20
	CB	Reported	CD29, CD105, CD49b	CD45, CD133	70
Sheep	BM	Reported	CD44, CD105, CD29, CD166	CD34, CD45, CD14, CD106, CD31, STRO1	85, 95

Markers listed as 'confirmed' were listed as such when the cited study included appropriate validation of antibody crossreactivity (for example through bioassay or Western blots) , documented gene sequences, or published appropriate positive and negative controls. Markers listed as 'reported' either could not be verified, usually because the original report did not include appropriate validation or controls, or have prompted controversy in the literature regarding whether the antibodies crossreact with the species tested.

bodies and the generation of species-specific reagents have been augmented recently by large-scale collaborations (http://www.umass.edu/vetimm/). In addition, several studies have screened antibodies for crossreactivity to MSC from veterinary species.[25,118] Similar to those from humans and mice, MSC from veterinary species express MHC class I but not MHCII or the T-lymphocyte costimulatory molecules CD86 and CD80 (Table 2) .[68,69] Because no single, specific MSC marker has been identified, a panel of antibodies is used. Human MSC are defined as positive for CD105, CD73, and CD90 and negative for CD45, CD34, CD14, CD11b, CD79a, and CD19.[31] Table 2 provides a comprehensive list of antibodies used to identify MSC in veterinary species from different tissue sources.

Trilineage differentiation has been demonstrated for MSC from many species and tissues, including cow BM- and CB-MSC;[12,108] dog AT-, BM-, CB-, and CT-MSC,[59,97,119,139] goat AT- and BM-MSC;[96,112] chicken BM-MSC;[63] horse AT- BM-, CT-, and CB-MSC;[37,67,107,130] sheep AT-, BM-, and CB-MSC;[55,84,85,95] rabbit BM- and fetal-liver–

MSC;[80,93] pig AT- and BM-MSC;[114,135] and nonhuman primate BM- and AT-MSC.[8,54]

Immunomodulatory Properties of MSC

A deeper understanding of the mechanisms by which MSC derived from veterinary species modulate inflammation and contribute to healing will benefit both humans and animals. Many veterinary species serve as models for human diseases for which cellular therapy is currently being investigated (for example, pigs for cardiovascular disease, goats for orthopedic lesions[96,120]). In addition, MSC therapy increasingly is used as a mainstay for a variety of companion animal disorders including tendon, bone, and cartilage injuries in horses and arthritis in dogs.[9,123] MSC have been shown to interact with CD4 and CD8 lymphocytes and, once activated in the presence of pro-inflammatory mediators, secrete mediators that downregulate inflammation.[122]

Lymphocyte proliferation. MSC derived from all tissue sources have potent immunomodulatory capabilities in vitro. Autologous and allogeneic MSC are nonimmunogenic, and completely unmatched MSC do not induce leukocyte proliferation in the absence of activation in vitro.[16,95,103] MSC are also antiinflammatory. The ability of MSC to inhibit the proliferation of stimulated T lymphocytes in vitro has been well described for MSC from nonhuman primates,[5,8] dogs,[61,65,77] chickens,[63] rabbits,[80,93] pigs,[20,103] sheep,[95] and horses.[16,99] Lymphocyte proliferation in vitro is maximally inhibited at a MSC:lymphocyte ratio of 1:1, 1:5 or 1:10.[5,16,61,63,80,81] One proposed mechanism for this inhibition of lymphocyte proliferation is MSC-induced T-cell–cycle arrest in G0, which is thought to be regulated at a molecular level by decreases in lymphocytic cyclin D levels.[44,65] MSC decrease the expression of activation markers (CD25, CD38, and CD69) on T cells, preventing their activation and proliferation.[47,76] Pretreatment of MSC with IFNγ, a mediator largely present in inflammatory environments, further enhances the ability of MSC to decrease lymphocyte proliferation.[80,103] Furthermore, in one in vitro experiment, xenogenic pig BM-MSC did not stimulate human lymphocyte proliferation; rather, they dose-dependently inhibited lymphocyte proliferation after stimulation.[81]

Both cell–cell contact and soluble factors are thought to play a role in MSC-induced inhibition of lymphocyte proliferation. Toll-like receptors, intracellular adhesion molecule 1, and vascular cell adhesion molecule 1 on the surface of MSC and FAS-ligand–dependent interactions are thought to play a part in cell–cell mediated immunosuppression, although this contribution has not been examined in veterinary species.[2,27,79,111,127] MSC inhibit lymphocyte proliferation even in transwell assays where MSC are physically separated from lymphocytes, supporting the idea that MSC produce soluble factors involved in immunomodulatory activity.[61,77,89] In addition, preconditioned media taken from cultures of activated MSC, defined as those MSC exposed to proinflammatory mediators, inhibits lymphocyte proliferation.[61]

MSC interactions with other immune cells have been studied widely in both humans and rodents, although this research has not yet been broadly extended to MSC from veterinary species. Human MSC decrease proliferation of both $CD4^+$ and $CD8^+$ T cells, cause a shift toward a Th2 phenotype, and inhibit Th17 differentiation and function.[1,43] Human and rodent MSC modulate dendritic cell (DC) development from monocytes and impair DC function. Impaired DC function includes modulation in MHCII and T-cell costimulatory molecule expression, downregulation of cytokine production, and prevention of DC homing to lymph nodes.[35,43,137] The downstream effect of these changes includes limitation of the ability of DC to stimulate a T cell response. Similar to their effect on T lymphocytes, human MSC inhibit B-cell proliferation in a dose-dependent manner, blocking progression of the cell cycle.[46,125,137]

Mediator production. Soluble immunosuppressive factors demonstrated to be produced or expressed by MSC from veterinary species include TGFβ1,[8,16,61,77,80,103] hepatocyte growth factor,[61] PGE_2,[16,61,77] indoleamine 2,3-dioxygenase (IDO),[61] nitric oxide (NO),[16,63] vascular endothelial growth factor,[8,77] and IL6[8,16,61] (Table 3). Mediator production by MSC in veterinary species has not been tested exhaustively, and the mediators reported in Table 3 are those that have been published in the literature. A mediator's absence from the list does not imply that MSC do not produce it but rather that its production has not yet been determined. Some mediators are produced constitutively, whereas others are secreted after MSC activation by cytokines or mediators found in inflammatory environments. Mediators produced by MSC downregulate inflammation and stimulate angiogenesis.[33,64,136]

The particular mediators produced by MSC can vary by species and by tissue source. Horse MSC differentially produce mediators depending on MSC tissue source: equine MSC derived from hemic sources (BM- and CB-MSC) produce NO, whereas those MSC derived from solid tissues (CT- and AT-MSC) do not.[16] Whether these differences in mediator production in vitro confer any functional differences in vivo is unknown, although anecdotal evidence suggests decreased healing time for equine tendon lesions after the injection of equine CB-MSC compared with other tissue-derived MSC. We speculate that the production of NO by horse CB-MSC causes increased angiogenesis in vivo. Species-associated variability in the production of NO and IDO by MSC has been described. Human MSC produce high levels of IDO and do not produce NO, whereas mouse MSC produce high levels of NO and do not produce IDO.[109] Human and mouse MSC are reported to induce T regulatory cells ($CD4^+CD25^+$ $FoxP3^+$), a population of T cells that suppress the immune response and promote T-cell tolerance to antigens.[29,36] This induction, or preferential production, of T-regulatory cells is dictated by the presence of TGFβ1 and IL10 in the microenvironment. Research is ongoing with regard to constitutive compared with induced production of TGFβ1 by human MSC.[47,105]

TGFβ1 production by veterinary species varies by species, tissue type, and MSC activation status. Dog BM-MSC, pig BM-MSC, and horse AT-, BM-, CT-, and CB-MSC do not increase TGFβ1 production after exposure to activated lymphocytes.[16,77,103] Dog BM-MSC produce TGFβ1 at levels insufficient to inhibit lymphocyte proliferation.[77] Conversely, dog AT-MSC increase TGFβ1 production after exposure to lymphocytes, and rabbit BM-MSC increase their TGFβ1 production after IFNγ pretreatment in vitro.[61,80] To date, no studies in veterinary species have demonstrated that MSC production of TGFβ1 shifts T lymphocytes to a T-regulatory phenotype.

The role of specific mediators on lymphocyte proliferation or function can be evaluated via chemical blockade of individual mediators and measurement of corresponding lymphocyte proliferation in a MSC–lymphocyte coculture (mixed lymphocyte reaction). Although not yet documented in veterinary species, blocking TGFβ1 produced by human BM-MSC results in de-

Table 3. Mediator production by MSC in veterinary species

Species	MSC type	Mediators		Reference
		ELISA	Gene expression	
Chicken	BM	NO[a]		63
Dog	AT	TGFβ, HGF, PGE_2, IDO	TGFβ, IL6, IL8, VEGF, HGF, COX2	61
	BM	TGFβ, VEGF, PGE_2		77
Horse	AT, CT	PGE_2, IL6, TGFβ		16
	BM, CB	PGE2, IL6, TGFβ, NO[a]		16
Nonhuman primate	BM		IL6, VEGF, TGFβ, HGF	8
Pig	BM	TGFβ, IL10		103
Rabbit	BM	IL10, TGFβ		80

HGF, hepatocyte growth factor; IDO, indoleamine 2;3-dioxygenase; IL, interleukin; PGE_2, prostaglandin E2; TGF, transforming growth factor; VEGF, vascular endothelial growth factor
[a]NO measured by using Greiss reagent.

creased production of T-regulatory cells[36] and a significant reversal in the inhibition of T cell proliferation,[30,47] indicating that TGFβ1 has an important role in MSC function. Blocking PGE_2[61,77] or IDO[61] produced by dog BM- or AT-MSC restores lymphocyte proliferation, indicating that both mediators have functional roles in modulating the MSC–lymphocyte interaction. In addition, according to our experience, PGE_2 appears to be the primary mediator responsible for inhibition of lymphocyte proliferation by horse AT-, BM-, CT-, and CB-MSC because blocking PGE_2 restores lymphocyte proliferation.

MSC immunomodulatory function is stimulated by proinflammatory mediators, namely IFNγ and TNFα[34,103] (Figure 1). MSC derived from IFNγ-receptor knock-out mice are unable to inhibit a lymphocyte proliferative response.[110] At baseline, neither unstimulated lymphocytes nor MSC produce these proinflammatory mediators.[16,103] However, activated lymphocytes secrete IFNγ and TNFα and stimulate MSC. Dog AT-MSC decrease TNFα production and increase IFNγ production by lymphocytes,[61] whereas horse BM-, AT-, CB-, and CT-MSC decrease both TNFα and IFNγ.[16,99] Pig BM-MSC decrease IFNγ and IL2 production by lymphocytes. Whether the decrease in these mediators is related to a decrease in the total number of lymphocytes present in culture or due to a functional shift in lymphocyte phenotype and the induction of T-regulatory cells is unknown.

The relevance of these mediators in vivo is largely unknown in veterinary medicine. Few studies have measured mediators in fluids or tissues, and both the kinetics of inflammation and the redundant functions of many mediators make interpretation of mediator concentrations at isolated time points difficult. In an equine model of osteoarthritis, PGE_2 levels were significantly decreased in synovial fluid after BM-MSC treatment compared with those in untreated affected joints.[40] The authors attributed this difference to the decrease in swelling and inflammation noted 1 week after MSC administration.[40] Human MSC exposed in vitro to joint fluids taken from patients with osteoarthritis and rheumatoid arthritis upregulated their mRNA expression of IL6 and IDO and suppressed lymphocyte proliferation.

There is still some disparity in the literature regarding species-specific identification of inflammatory mediators that are produced by or inhibited by MSC. Variability in results could stem from the methodology by which researchers measure cytokine production; test kits and reagents are not always specific for veterinary species and have been adapted from human or lab animal methodologies. Large-scale collaborations and increased communication through the veterinary stem cell research community will enable all researchers to ensure they are accessing the most appropriate tools for their research.

MSC In Vivo

In vitro compared with in vivo results. MSC safety and function has been studied in vivo in healthy animals, in patients with naturally occurring disease, and in animals serving as models of human diseases. Although extensive in vitro studies have shown that MSC are effective at decreasing inflammation, in vivo results are variable. Factors that contribute to variable therapeutic outcomes include natural variation in disease processes and lack of standards for MSC dose, route of administration, tissue source, or measured endpoints. Table 4 outlines the species, MSC dose, MSC source, and route of administration for the in vivo studies discussed in the current review.

An example of a strong correlation between in vitro and in vivo studies was a skin graft model in baboons. Skin grafts from MHC-mismatched donors were transplanted to adult baboons, after which a single BM-MSC dose was administered intravenously. Grafts in baboons that received MSC infusions were rejected more slowly (11 d) than were those in animals not treated with MSC (7 d).[5] The delay in graft rejection attributed to MSC was comparable to the alleviation of graft rejection by antigraft rejection pharmaceuticals currently on the market.[5] The authors of the study postulated that the MSC were sufficient to subdue the lymphocyte response but insufficient to inhibit recruitment of inflammatory cells to the graft.[5]

Table 4. MSC sources, dose ranges, and routes of administration for in vivo studies

Species	MSC type	Dose	Route of administration	Reference
Dog	BM	1.1-1.8 × 10^6/kg	intravenous	77
	BM	1 × 10^6/kg	intravenous	89
Horse	CT	1 × 10^6	intradermal	15
	CT	7.5 × 10^6	intraarticular	17
	BM	5.6-15 × 10^6	intraarticular	40
Nonhuman primate	BM	20 × 10^6/kg	intravenous	5
	BM	4.3-6.4 × 10^8/kg	intraportal	8
	BM	3.4-6.5 × 10^6/kg	intravenous	8
Pig	BM	15-18 × 10^6	subcutaneous	103
	BM	10-40 × 10^6	subpericardial	103
	BM	1 × 10^7/dose	intravenous	72
	BM	1 × 10^7/dose	intravenous	71

Other examples illustrate poor correlation between in vitro and in vivo studies. In vitro, pig BM-MSC decreased lymphocyte proliferation and inhibited production of pro-inflammatory mediators.[103] In vivo, allogeneic pig BM-MSC elicited both a cellular and humoral response when injected either subcutaneously or intracardiac.[103] Antidonor alloantibodies (IgM or IgG) were detected after a single or multiple subcutaneous doses of BM-MSC, although cytolytic activity was not detected after single doses of MSC.[103] Intracardiac injection of single or multiple doses of BM-MSC elicited both alloantibody production and cytolytic activity of donor MSC;[103] Limitations to the study included low sample number (n = 2 for subcutaneous, n = 3 for intracardiac) and variable numbers of injected MSC (range, 1.5 × 10^7 to 1.20 × 10^8 MSC). The results of the cited study suggest that allogeneic BM-MSC in this pig model are not anti-inflammatory and may not escape immune surveillance. The findings of this study[103] are notable as a general exception to a body of literature in veterinary and human medicine suggesting that allogeneic MSC are well-tolerated and highlight the need for standardized methods of measuring the immune response to MSC both in vivo and in vitro.

Studies comparing autologous (self) and allogeneic (non-self) MSC are prominent in veterinary medicine, with a focus on healing of orthopedic injuries.[49,58,102] A few studies in veterinary medicine have focused on the safety and efficacy of autologous and allogeneic MSC with regard to their immunomodulatory properties. Our study comparing intradermal injection of autologous and allogeneic equine CT-MSC revealed that 2 rounds of intradermal injections failed to induce a significant cell-mediated response, as measured in vivo by wheal formation and induration and ex vivo by histopathology on biopsied tissue and by mixed lymphocyte reactions.[15] Wheal formation and induration indicated no difference between MSC injection and control (saline) injections.[15] Results from mixed lymphocyte reactions indicated that neither autologous or allogeneic MSC stimulated nor suppressed baseline T-cell proliferation, even after multiple MSC injections. Taken together, the results indicate that CT-MSC could be administered in vivo multiple times without eliciting a cellular immune response.[15] This in vivo study parallels the in vitro findings comparing immunomodulation by horse AT-, BM-, CT-, and CB-derived MSC.[16] A similar study showed no difference in inflammatory response to horse autologous compared with allogeneic MSC after intraarticular administration.[17]

Graft-versus-host disease and T regulatory cells. Clinical trials are underway to determine the efficacy of MSC in treating humans with graft-versus-host disease.[52,73,75] However, results of studies using MSC to treat graft-versus-host disease in veterinary species have been mixed. Two studies in dogs showed no difference in the rate of graft rejection between dogs that received MSC after dog leukocyte antigen-identical bone marrow transplants compared with those that did not receive MSC treatment, even though in vitro data demonstrated diminished leukocyte proliferation in the presence of MSC.[77,89] Two other studies found that pigs given composite tissue allografts and BM-MSC had prolonged graft survival when compared with animals that did not receive BM-MSC treatment.[71,72] In the cited study,[72] pigs given cyclosporine along with irradiation had marked evidence of graft-versus-host disease, whereas pigs given cyclosporine, irradiation, and MSC had no evidence of graft-versus-host disease and the least rejection of transplanted tissue.[72] In a second set of experiments, T-cell phenotypes were investigated in peripheral blood and graft tissue.[71] The authors found a significant increase in the percentages of $CD4^+CD25^+$ and $CD4^+FoxP3^+$ T cells (T regulatory cells) in both the blood and graft when pigs were given cyclosporine, irradiation, and MSC compared with pigs that did not receive MSC, indicating that T-regulatory cells were induced both globally and locally.[71]

T-regulatory cells increase after MSC infusion in a primate model of allogeneic islet cell engraftment. Nonhuman primates that received islet cells and BM-MSC had significantly enhanced islet function 1 month after transplantation, compared with those that had not received MSC. Rejection episodes in the animals that had not received MSC were reversed with additional infusions of allogeneic BM-MSC.[8] The presence of T regulatory cells in peripheral blood was increased after episodes of rejection and additional MSC infusion when compared with levels before MSC infusion.[8] Graft dysfunction was noted as T-regulatory cells decreased in peripheral blood.[8]

Benefits and Risks

Traditional drug therapy to downregulate the immune response (for example, cyclosporine, steroids) is associated with several undesirable side effects, including increased risk of secondary infections and other 'bystander' effects that limit the long-term use of these drugs. Although the associated research is in its infancy, modifying the immune response by using cellular therapy may reduce these bystander effects. The cells may be modified by the niche into which they are injected, with less prolonged immune suppression. MSC may be given as an adjunct therapy and thus lower the effective dose of immunosuppressive drugs. Several studies in humans and rodents have linked favorable outcomes in solid-organ transplant recipients with combined use of MSC and traditional immunosuppressive therapeutics.[82,126] The dose and method of administration of cellular therapies can easily be adapted to suit the needs of the patient.

As with any new drug or procedure, risks related to the use of MSC as a clinical product are continually assessed and analyzed.[51,104] Risks include the potential for tumor formation or inappropriate MSC differentiation, for transmission of infectious disease through contaminated product, and of trauma at the site of administration.[51] Disease transmission via MSC has not been described in the literature, although in human medicine, stem cell donors are screened rigorously for infectious disease.[115,124] Extensive screening of veterinary donors for infectious disease will be a necessary component of allogeneic MSC product use. MSC products are cultured routinely for microbial contamination prior to administration. Conceptually, given their ability for trilineage differentiation and their immunosuppressive features, MSC could either form tumors or impair tumor surveillance and thus increase the risks of tumor formation. Tumor promotion or formation after MSC administration has not been studied specifically in veterinary medicine, however it has been evaluated widely in rodents and human medicine.[6,18,106,132] Most experimental data suggest that MSC do not form tumors in vivo. In addition, tumors in humans or veterinary patients that have received MSC have not been reported (many of our horse patients have been monitored for at least 6 y). Malignant transformation of MSC in vitro has been reported, although several studies have been retracted after the discovery that these MSC cell cultures were contaminated with cancer cell lines.[24,117,129]

Areas of Future Study

Several published reports have indicated that although MSC are largely anti-inflammatory, they also may have the capacity to function as antigen-presenting cells and further an inflammatory response.[19,116] Human and mouse BM-MSC, exposed to low levels of IFNγ in vitro, showed increased MHCII expression and decreased ability to inhibit lymphocyte proliferation.[19,116] It has been proposed that MSC are binary in nature, with the capability of sensing their environment through toll-like receptors (or yet other unidentified receptors) and responding as either pro-inflammatory antigen-presenting cells or as anti-inflammatory cells.[133] This binary action may explain some of the discrepant results from in vivo studies and furthers the need for study of MSC in diverse species and disease conditions.

MSC from veterinary species have been well studied with regard to their ability to decrease lymphocyte proliferation and produce immunomodulatory mediators in vitro. However, many questions remain to be answered. For example, we do not know how MSC interact with and regulate B cells, natural killer cells, DCs, or neutrophils; whether MSC from veterinary species alter lymphocyte phenotype, induce the development of T regulatory cells in vitro as well as in vivo; or whether MSC are anti-inflammatory in vivo in all species. Additional studies undertaken by researchers and collaborative working groups, as well as information sharing though professional organizations such as the North American Veterinary Regenerative Medicine Association, will play a crucial role in answering many of these questions.

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