MESENCHYMAL STEM CELL THERAPY IN CATS
Current knowledge and future potential

Jessica M Quimby and Dori L Borjesson

Practical relevance: Stem cell therapy is an innovative field of scientific investigation with tremendous potential for clinical application in veterinary medicine. Based on the known desirable immunomodulatory properties of mesenchymal stem cells, this therapy holds promise for the treatment of a variety of inflammatory diseases in cats.

Aims: This review details our current understanding of feline stem cell biology and proposed mechanism of action. Studies performed in feline clinical trials for diseases including gingivostomatitis, chronic enteropathy, asthma and kidney disease are summarized, with the goal of providing an overview of the current status of this treatment modality and its potential for the future.

Emergence of a new therapeutic strategy
Feline mesenchymal stem/stromal cells (MSCs) were first isolated and characterized from bone marrow (bMSCs) in 2002, and later readily expanded from fat, and fetal fluids and amniotic membranes. Currently, adipose-derived MSCs (aMSCs) are most commonly used in clinical applications due to ease of attainment and their superior proliferative ability. Feline MSCs meet the minimal defining criteria for multipotent stem cells as published over a decade ago and ongoing research confirms that they also have similar immunomodulatory potency as more recently defined for human MSCs. In addition to culture-expanded MSCs, regenerative medicine therapies include commercially available patient-side therapies such as platelet-rich plasma, bone marrow concentrate and the stromal vascular fraction (SVF) of fat.

In cats, published data are focused exclusively on the use of culture-expanded MSCs. SVF has the advantage of a fast turnaround time; however, the product is heterogeneous and includes only small numbers of stem cells relative to a culture-expanded population of aMSCs. Thus, in this review, we discuss the biology, immunology and clinical application of culture-expanded MSCs in cats.

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Feline MSC biology and immunology

Like all MSCs, feline MSCs have a fibroblast morphology in culture (Figure 1) and they express CD44 and CD105, and variably express CD90 on their surface.1,2,6 MSCs do not express leukocyte antigens (CD18, CD4) or major histocompatibility complex (MHC) II.1,2,6 Functionally in vitro, feline MSCs undergo trilineage differentiation, inhibit activated lymphocyte proliferation, and secrete a variety of mediators capable of modulating immune cell function.6,7

Feline MSCs are similar to simian MSCs in that 20–50% of feline MSC lines can be infected by a foamy virus.8 Feline foamy virus (FFV) is a member of the Retroviridae family, is present in 20–80% of cats and is not associated with a clinical disorder.3,10 FFV replication in cell lines is associated with syncytial cell formation, decreased proliferation rate and, finally, cell death.11 MSC lines derived from specific pathogen-free (SPF) cats do not appear to be infected with FFV and may be a source of allogeneic MSCs for clinical application. FFV infection of MSC lines may hinder large-scale expansion of autologous MSCs for therapeutic use in feline patients.8

To understand how feline MSCs respond to inflammatory stimuli, they have been incubated with allogeneic peripheral blood mononuclear cells (PBMCs) or stimulated with lectins (concanavalin A), interferon gamma (IFNγ), and/or tumor necrosis factor alpha (TNFα).6,7 In response to these stimuli, MSCs upregulate gene expression of immunomodulatory mediators including indoleamine 2,3-dioxygenase (IDO1), programmed death ligand-1, interleukin (IL)-6, cyclooxygenase 2 and hepatocyte growth factor.7 They also secrete IDO, prostaglandin E2 (PGE2), IL-6, vascular endothelial growth factor, IL-8 and transforming growth factor beta.6,7 The inhibition of lymphocyte proliferation depends on both soluble mediators and direct contact between the aMSCs and lymphocytes; however, the actual ligands and mediators have not been determined.6 In other species, IDO and PGE2 have been implicated in the initiation of lymphocyte cell cycle arrest and/or lymphocyte apoptosis.21–23 Feline aMSCs potently inhibit the pro-inflammatory cytokine TNFα in vitro; however, their interaction with IFNγ is more complicated.8 Feline aMSCs are able to inhibit lymphocyte proliferation and secrete immunomodulatory mediators in the presence of high concentrations of IFNγ.9

The transcriptome of three feline aMSC lines has been defined and functional analysis of the most highly expressed genes revealed processes relating to: 1) the regulation of apoptosis; 2) cell adhesion; 3) response to oxidative stress; and 4) regulation of cell differentiation.8 Together these data suggest that feline MSCs are highly similar in their functional and, notably, immunomodulatory capacities to MSCs of other species.
In developing MSC clinical trials, there are a number of factors that need to be defined and standardized, including cell type (allogeneic or autologous), cell formulation (fresh or frozen, with or without additives), cell dose, route of cell administration and number of doses given. In cats, clinical trials have been performed with both autologous and allogeneic MSCs, with varying efficacy.

**Cell type** Data from clinical trials in cats with FCGS suggest that autologous MSCs may be more effective and result in more rapid clinical cure than the administration of allogeneic MSCs. However, these data represent relatively low numbers of cats (seven cats for each group) and no attempts were made to optimize allogeneic cell efficacy (ie, increasing cell dose or number of doses). As described for most species, MSC numbers may decline with age, leading to an increased lag time to culture expand these cells for therapeutic purposes in geriatric cats. Concurrent disease states, such as uremia, may have an effect on the therapeutic capability of autologous MSCs, as previous studies have demonstrated that MSCs obtained from uremic rats exhibit reduced proliferation in culture, premature senescence and decreased capacity to induce angiogenesis.

For allogeneic cell use, cells derived from tissues from young, ‘donor-qualified’ (full infectious disease testing) or SPF cats will be necessary for US Food and Drug Administration-compliant clinical trials.

### Clinical applications of MSCs in feline disease

Given the desirable immunomodulatory properties of MSCs, this therapy has potential for treatment of a variety of inflammatory diseases. In cats, MSCs have been evaluated as therapies for a number of inflammatory, degenerative and immune-mediated diseases including feline chronic gingivostomatitis (FCGS), acute and chronic kidney disease (CKD), enteropathies and asthma. An overview of clinical studies is provided in Table 1. MSCs could also be explored for feline osteoarthritis and cardiomyopathy, among other diseases. Additionally, many of these naturally occurring diseases have translational potential for similar diseases in people, including oral inflammatory diseases, chronic pancreatitis, idiopathic cystitis, CKD, inflammatory bowel disease, osteoarthritis, cardiomyopathy and asthma.

#### Table 1. MSCs could also be explored for feline osteoarthritis and cardiomyopathy, among other diseases. Additionally, many of these naturally occurring diseases have translational potential for similar diseases in people, including oral inflammatory diseases, chronic pancreatitis, idiopathic cystitis, CKD, inflammatory bowel disease, osteoarthritis, cardiomyopathy and asthma.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Study type</th>
<th>MSC source</th>
<th>Interventions</th>
<th>Outcome</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Gingivostomatitis</td>
<td>Open-label, baseline-controlled clinical trial</td>
<td>Autologous adipose-derived</td>
<td>Two doses IV aMSCs 30 days apart (7 cats)</td>
<td>Complete remission (3 cats), substantial improvement (2 cats), no response (2 cats)</td>
<td>Arzi et al18</td>
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<td>Arzi et al9</td>
</tr>
<tr>
<td>Acute experimental asthma</td>
<td>Randomized, placebo-controlled study</td>
<td>Allogeneic adipose-derived</td>
<td>Five doses IV aMSCs (4 cats), with 14, 14, 14, 30 and 30 days between each respective dose, or placebo (2 cats)</td>
<td>Decrease in airway eosinophilia, diminished airway hyper-responsiveness, decreased airway remodeling</td>
<td>Trzil et al31</td>
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<tr>
<td>Chronic experimental asthma</td>
<td>Randomized, placebo-controlled study</td>
<td>Allogeneic adipose-derived</td>
<td>Six doses IV aMSCs (5 cats) 14 days apart or placebo (4 cats)</td>
<td>Decreased airway remodeling</td>
<td>Trzil et al10</td>
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<tr>
<td>Chronic enteropathy</td>
<td>Owner-blinded, randomized, placebo-controlled clinical trial</td>
<td>Allogeneic adipose-derived</td>
<td>Two doses IV aMSCs 14 days apart (7 cats) or placebo (4 cats)</td>
<td>Improved clinical signs in 5/7 aMSC-treated cats and 0/4 placebo cats</td>
<td>Webb and Webb29</td>
</tr>
<tr>
<td>CKD</td>
<td>Open-label, baseline-controlled clinical trial</td>
<td>Autologous adipose-derived or bone marrow-derived</td>
<td>Single unilateral intrarenal injection of aMSCs (3 cats) or bMSCs (3 cats)</td>
<td>Mild decrease in serum creatinine and increase in GFR in 2 cats that received aMSCs</td>
<td>Quimby et al24</td>
</tr>
<tr>
<td>CKD</td>
<td>Three open-label, baseline-controlled, pilot clinical trials</td>
<td>Allogeneic adipose-derived</td>
<td>Three doses IV aMSCs 14 days apart (16 cats)</td>
<td>Mild decrease in serum creatinine in 7/15 cats (1 cat excluded due to medication non-compliance) and increase in GFR in 4/16 cats. Side effects included vomiting and increased respiratory rate</td>
<td>Quimby et al25</td>
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<td>CKD</td>
<td>Randomized, placebo-controlled clinical trial</td>
<td>Allogeneic adipose-derived</td>
<td>Three doses IV aMSCs (4 cats) 14 days apart or placebo (3 cats)</td>
<td>No significant improvement in creatinine or GFR</td>
<td>Quimby et al26</td>
</tr>
<tr>
<td>Ischemic AKI</td>
<td>Randomized, placebo-controlled study</td>
<td>Allogeneic adipose-derived or bone marrow-derived</td>
<td>Single dose IV aMSCs (5 cats), bMSCs (5 cats) or fibroblasts (5 cats) 1 h after unilateral ischemia</td>
<td>No difference in percentage of cats with AKI</td>
<td>Rosselli et al37</td>
</tr>
<tr>
<td>CKD</td>
<td>Open-label, baseline-controlled clinical trial</td>
<td>Allogeneic amnion-derived</td>
<td>Two doses IV amnion-derived MSCs 21 days apart (9 cats)</td>
<td>Mild decrease in serum creatinine filtration rate; AKI = acute kidney injury; CKD = chronic kidney disease</td>
<td>Vidane et al28</td>
</tr>
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**IV = intravenous; MSC = mesenchymal stem cell; aMSCs = adipose-derived MSCs; bMSCs = bone marrow-derived MSCs; GFR = glomerular filtration rate; AKI = acute kidney injury; CKD = chronic kidney disease.**
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- **Cell formulation** Both fresh and frozen/thawed aliquots of MSCs have been used in clinical trials. To date, there are no studies that have directly compared the efficacy of fresh (initial culture or culture revived) vs frozen/thawed cells; however, all studies that have reported efficacy in naturally occurring disease have used fresh cells for administration.18,28,29,35
- **Cell dose** MSC dose has ranged from 1 million cells/cat to 25 million cells/cat (range <1 million cells/kg to >5 million cells/kg).18 Effective cell doses for enteropathy and FCGS were 2–5 million cells/kg.18,29 To determine if higher doses of MSCs could safely be given to cats, one of the authors (DB) administered 50 million MSCs to SPF cats intravenously with no adverse effects (unpublished data).
- **Route of administration** MSCs have primarily been administered intravenously.18,26,29,31 However, other routes of administration, including intraperitoneal,38 intrarenal28 and retroperitoneal,39 have been described and are relatively safe. The ideal route of MSC administration will need to be determined for each disease individually and will increasingly be based on safety, feasibility, mechanism of action (ie, whether the cells act locally or systemically to modify disease) and cell tracking studies.

**Feline chronic gingivostomatitis**

FCGS is a severe, painful, inflammatory oral disease that is often refractory to treatment and is estimated to affect 0.7–12% of cats presenting to veterinary practices.40–42 Clinical signs reflect oral discomfort, including inappetence, reduced grooming, weight loss and hypersalivation. FCGS can be debilitating and may result in euthanasia of severely affected cats. There is no cure and the current standard of care consists of full or near-full mouth tooth extraction and lifelong therapy with antibiotics, steroids and analgesics.42 The pathogenesis of FCGS is complex and multifactorial, loss of self-tolerance to antigens, alterations in innate immunity or underlying viral disease.43,44 Histologically, lesions in cats are characterized by lymphocyte-rich inflammation with a predominance of effector T cells and B cells.40

Given the predominance of T cell activation in FCGS and the absence of a curative therapy, aMSCs have been utilized to treat FCGS in two clinical trials because of their potent ability to downregulate T cell activation. In both clinical trials, FCGS cats that were refractory to full mouth tooth extraction were enrolled. In addition, these cats had no known concurrent diseases (eg, feline immunodeficiency virus/feline leukemia virus negative).18,35 In each trial, seven FCGS cats received two intravenous (IV) injections of $2 \times 10^7$ aMSCs 3–4 weeks apart. In the first trial, the seven cats received autologous aMSCs;18 in the second trial, the seven cats received unmatched, allogeneic aMSCs from SPF donor cats.35 The outcome of these published studies, as well as other clinical trials, is summarized in the box below.

Clinical trials are ongoing and have expanded to include a multicenter investigation with a crossover design to ensure that control cats are recruited. Ongoing work includes: 1) pursuing mechanism of action and biomarker studies (focused on immunomodulation, and ensuing mechanism of action and biomarker studies);6 2) refining clinical trial parameters to increase the therapeutic efficacy of aMSC infusion; 3) determining whether aMSCs can be used prior to full mouth tooth extraction; and 4) enrolling cats with concurrent diseases to determine efficacy in a broader range of diseased cats.

**FCGS clinical trials – what to conclude**

Overall, 35 cats with naturally occurring, refractory FCGS have been treated to date through clinical trials run at UC Davis. There have been no significant adverse events associated with aMSC infusion. The response to aMSC therapy has been remarkable (Figure 2).

Response rate for substantial improvement or complete cure after aMSC therapy was 64% (9/14 cats) for the two published studies described above, and the overall response rate is closer to 72% (unpublished data). No cats have regressed or relapsed after therapy – so therapy, if efficacious, appears to be curative (some cats are >4 years out since aMSC infusion). Clinical cure is associated with histopathologic resolution of T and B cell inflammation. The data to date suggest that autologous therapy may be slightly more effective, especially for the most severely affected cats, and that improvement/cure occurs more quickly after autologous cell infusion than after allogeneic cell infusion.18,35

![Figure 2](Image)
Asthma

Feline asthma is a component of feline lower respiratory disease that is characterized by airway eosinophilia, airway hyper-responsiveness and remodeling. Clinical management to target inflammation and open airways consists of oral or inhaled corticosteroid and bronchodilator therapy. However, these therapies are associated with side effects and are typically lifelong, and may not result in a therapeutic response or prevent progression of disease.

The application of MSC therapy to this disease process would be ideal given the immunomodulatory capabilities demonstrated by MSCs and their passage through the lung when administered intravenously. In rodent models, improvement in airway eosinophilia, airway hyper-responsiveness and remodeling has been demonstrated following MSC therapy.30 Two placebo-controlled pilot studies have evaluated aMSC therapy in cats in an experimental model of feline asthma; both involved sensitization to Bermuda grass allergen, which results in development of an asthmatic phenotype.

Asthma clinical trials – what to conclude

**Acute induced asthma** Cats receiving aMSCs experienced a decrease in airway eosinophilia and diminished airway hyper-responsiveness at day 133 compared with placebo. Airway remodeling, as represented by lung attenuation and bronchial wall thickening scores assessed via CT imaging, was significantly decreased in aMSC-treated cats at 9 months (Figure 3).31

**Chronic induced asthma** In this chronic model, cats that received aMSCs did not experience a decrease in airway eosinophilia and diminished airway hyper-responsiveness compared with placebo. However, significantly reduced lung attenuation and bronchial wall thickening scores, assessed via CT imaging, were seen at 8 months, once again indicating a positive effect on airway remodeling.

aMSC therapy has been shown to have a positive effect on airway remodeling in two placebo-controlled pilot studies using experimental models of feline asthma.

The first study involved administration of allogeneic aMSCs to cats in which asthma had been induced a relatively short time period before treatment.31 A total of five IV injections of allogeneic aMSCs were administered (four cats) or saline placebo (two cats). Due to difficulties with expansion of cells to obtain adequate quantities for the study, the number of cells administered varied between $2 \times 10^6$ and $1 \times 10^7$ per cat; initially aMSCs were from cryopreservation and subsequently they were cultured fresh from cryopreserved adipose.

The second study assessed the effects of aMSC infusion on cats that had chronic experimentally induced asthma (induced 9 months prior to study initiation).30 Cats were randomized to receive a total of six IV injections of fresh allogeneic aMSCs cultured from cryopreserved adipose (five cats) at a dose of $3.6 \times 10^6$ to $2.5 \times 10^7$ per cat (average $1.4 \times 10^7$ per cat) or saline placebo (four cats).

The results of these two clinical trials are described in the box below.
Chronic enteropathy

Chronic enteropathies such as inflammatory bowel disease are common in cats and likely result from alterations in gastrointestinal mucosal immunity and loss of tolerance to intestinal antigens. Clinical management is typically lifelong and includes dietary therapy and corticosteroid administration. Patient and/or owner compliance can be a concern with chronic therapies and not all cats experience a treatment response. Therefore, alternative approaches are needed.

The efficacy of allogeneic aMSCs for the treatment of cats with clinical signs of chronic enteropathy (diarrhea and/or vomiting for >3 months) was assessed in a randomized, single-blinded, placebo-controlled clinical trial. Two IV injections of 2 x 10^6/kg aMSCs fresh cultured from cryopreserved adipose (seven cats) or saline placebo (four cats) were administered 2 weeks apart and cats were followed for 1–2 months. Owners answered a questionnaire at the beginning and end of the study about medications, supplements, diet, appetite and clinical signs, including quantification of the frequency and consistency of diarrhea, and the presence and frequency of vomiting. A Texas A&M gastrointestinal panel (feline pancreatic lipase immunoreactivity, feline trypsin-like immunoreactivity, folate and cobalamin) was performed at the beginning of the study and 2 weeks after the second injection. No other changes in diet or medications were allowed during the study period. Results of the trial are reported in the box below.

Therapy with aMSCs has appeared well tolerated and potentially effective for palliation of chronic enteropathy in cats, and additional study is warranted.

Kidney disease

A series of pilot studies assessing the safety and efficacy of administration of MSCs for treatment of cats with CKD has been conducted.

The first MSC study in cats with CKD was designed to assess the safety and feasibility of autologous intrarenal MSC therapy. Six cats (two healthy, four with CKD) received a single unilateral intrarenal injection of autologous bMSCs or aMSCs via ultrasound guidance. Two IRIS stage 3 CKD cats that received aMSCs experienced modest improvement in glomerular filtration rate (GFR) and a mild decrease in serum creatinine concentration. Intrarenal injection of MSCs did not induce immediate or longer term adverse effects but it was concluded that the number of sedations and interventions required to implement this approach made it unattractive for clinical application. In the course of conducting this study it was also determined that expanding sufficient numbers of autologous MSCs in culture from elderly diseased patients was very difficult and time-consuming. A more recent study in which one healthy cat received an intrarenal injection of amniotic-derived allogeneic MSCs documented hematuria and significant stress as a result of the procedure, and this study also determined the technique not to be clinically feasible.

The feasibility of IV administration of allogeneic aMSCs to cats with CKD has also been investigated. Stable CKD cats with no concurrent illness were enrolled in a series of pilot studies and received every 2 weeks an IV infusion of allogeneic aMSCs collected and cryopreserved from healthy young SPF cats. Six cats in pilot 1 received 2 x 10^6 cryopreserved aMSCs per infusion, five cats in pilot 2 received 4 x 10^6 cryopreserved aMSCs per infusion, and five cats in pilot 3 received 4 x 10^6 aMSCs cultured from cryopreserved adipose. Cats in pilot 1 had few adverse effects from the aMSC infusions and there was a statistically significant decrease in serum creatinine concentrations during the study period. However, the degree of decrease was judged not to represent a clinically relevant improvement.

Notably, adverse effects of aMSC infusion were observed in the majority of cats enrolled in the second pilot study following treatment with MSCs taken directly from cryopreservation. Vomiting occurred in 2/5 cats during infusion, and increased respiratory rate and effort was noted in 4/5 cats. In contrast, cats in pilot study 3 that received aMSCs cultured from cryopreserved adipose did not experience any adverse side effects. Serum creatinine concentrations, urinary cytokines and GFR did not change significantly in cats in
these latter two studies. Based on the accumulated results of the three pilot studies, it appeared that use of higher doses of aMSCs taken directly from cryopreservation was the source of the treatment-related adverse effects. The most likely explanation for this reaction is an instant blood-mediated inflammatory reaction, which results in clumping of the cells as they contact the blood and potential subsequent micropulmonary thromboembolism.46

A placebo-controlled, blinded, one-way, crossover clinical study assessing the efficacy of allogeneic MSCs expanded from cryopreserved adipose with repeated administrations has also been performed.26 Four cats were randomized to receive $2 \times 10^6$ aMSCs/kg intravenously at 2, 4 and 6 weeks and three cats were randomized to receive saline placebo. While administration of aMSCs was not associated with adverse effects, significant improvement in renal function (as determined by serum creatinine and GFR by nuclear scintigraphy) was not observed in the weeks following administration.

The IV administration of allogeneic MSCs derived from amniotic membrane has been assessed in nine cats with CKD that received two injections of $2 \times 10^6$ MSCs 21 days apart.28 One cat experienced vomiting during the first administration, but otherwise the MSCs were well tolerated. A statistically significant but mild decrease in serum creatinine was seen, with stable body weight over the course of the study. Mild improvements in proteinuria and urine specific gravity were also seen. However, studies with a control group are necessary to determine if changes are attributable to MSC therapy or normal variation in values.

Application of aMSCs for acute kidney injury (AKI) in an ischemic kidney model has also been investigated.27 Adult research cats underwent unilateral renal ischemia for 60 mins. One hour after reperfusion, $4 \times 10^6$ of one of the following cell types were administered via jugular catheter: aMSCs (five cats), bMSCs (five cats) or fibroblasts (five cats). Three historical control cats that had previously undergone ischemia as part of this model were used for comparison. No difference in the percentage of cats that developed AKI and no difference in urine specific gravity, proteinuria, GFR or histopathology were noted as a result of MSC administration in this model.27

At this time, MSC therapy for feline CKD should be considered an experimental and unproven therapy.
References


Mesenchymal stem cell therapy in cats


