

Stem Cell Therapy in Feline Chronic Enteropathy: In Practice – Clinical Application

Craig Webb, PhD, DVM, DACVIM

Introduction

Feline Chronic Enteropathies appear to result from a disruption of the normal gastrointestinal mucosal immunity and a loss of tolerance to intestinal antigens. Intestinal inflammation is a common characteristic of these diseases and therapy is aimed at modulating the gastrointestinal immune response and reducing antigenic stimulation. Dietary manipulation and glucocorticoids are the mainstays of therapy for feline chronic enteropathies such as inflammatory bowel disease (IBD), but treatment is challenging and often unsatisfactory.

Mesenchymal stem cells (MSC) have been shown to alter immune responses and reduce inflammation through direct interactions with T-cells, NK cells, neutrophils and dendritic cells, and by increasing regulatory T cells (Tregs). MSC can be generated from adult tissues and do not induce a clinical immune response when delivered to allogeneic recipients. In mouse models of acute colitis, administration of a single injection of adipose-derived MSC has been shown to ameliorate clinical and microscopic signs of colitis, reduce systemic and mucosal pro-inflammatory cytokine production, increase IL-10 secretion, and induce regulatory Tregs in mesenteric lymph nodes. Experimental and clinical evidence shows that MSC therapy is safe and induces long-lasting remission in many patients with active severe Crohn's disease that is otherwise refractory to standard treatments. Adipose-derived MSC are currently being used in phase III clinical trials for inflammatory bowel disease in humans.

In Vitro

The immunomodulatory properties of MSC have been extensively characterized *in vitro*. It would appear prior activation by the inflammatory cytokines IFN- γ , TNF- α , and IL-1 β is required. This activation results in the MSC production of prostaglandin E2 (PGE2), an immune suppressant that inhibits Tcell mitogenesis and IL-2 production, induces a T helper (Th2) response, and stimulates IL-10 production by macrophages. Activated MSCs also produce IL-6 and HLA-G5, which inhibits the differentiation of monocytes to dendritic cells and suppresses T cell proliferation and cytotoxicity. Perhaps of most relevance, activated MSC decrease the production of inflammatory cytokines IL-12, IFN- γ , and TNF- α by dendritic cells and promote the generation of CD4+CD25+FoxP3+ T regulatory cells (Tregs) and the anti-inflammatory cytokine IL-10. MSC appear to exert their immunomodulatory effects through both direct contact and the production of soluble factors.

Mouse Models

A murine model of IBD (Crohn's disease) is induced by the administration of trinitrobenzene sulfonic acid. Mice are then treated with human adipose-derived mesenchymal stem cells (hASCs). Both the clinical (weight-loss, diarrhea, survival) and histopathological severity of the disease were significantly reduced by this treatment. The therapeutic effect appears to be mediated by an hASC-induced down regulation of the Th1 immune and inflammatory response. Inflammatory cytokine production, including TNF- α , IFN- γ , IL-6, IL-1 β , and IL-12 was decreased while levels of the anti-inflammatory cytokine, IL-10, was increased in IBD mice following hASC treatment. Consistent with the hASC cell surface receptor profile, there appeared to be a specific homing of the administered stem cells to inflamed and lymphoid tissue; colonic inflammation led to colonic recruitment of administered stem cells.

The cellular make-up of colonic tissue was shifted from Th1 differentiation and activation to proliferation of IL-10 producing T cells by hASC administration, increasing the number of CD4+CD25+Foxp3+ T cells and down-regulating the Th1 inflammatory response. Similar results were seen with hASC treatment in a dextran sulfate sodium-induced IBD murine model.

Human Models

Twelve patients with Crohn's disease refractory to standard therapy received hematopoietic stem cell transplantation (HSCT) and all 12 achieved remission (CDAI < 150), with no serious or lasting complications. A large percentage of these patients remained in remission and without the need for medication up to 5 years after treatment. In a separate study, 3 patients with severe refractory Crohn's disease received autologous HSCT. Stem cell therapy resulted in long-term remission in all 3 patients, with 1 patient able to discontinue immunosuppressive medications for several years. HSCT therapy requires immune ablation of the patient prior to stem cell transplantation, a significant deterrent to the use of this particular strategy in the veterinary patient population.

Patients with refractory Crohn's disease were treated with bone marrow derived mesenchymal stem cells. Two IV injections, $1-2 \times 10^6$ cells/kg, 7 days apart, were administered. The Crohn's disease activity index (CDAI) was monitored and colonoscopies were performed prior to treatment and 6 weeks following stem cell administration.

Three of 10 patients showed significant clinical improvement (a decrease in the CDAI) while in 3 other patients the disease progressed and required surgical intervention. Importantly, there were no serious adverse events associated with stem cell injections, supporting the safety and feasibility of this treatment modality.

Fifty patients with either ulcerative colitis or Crohn's disease received IV injection of bone-marrow derived MSC (1.5×10^8 cells). A significant decrease in clinical indices of disease and tissue inflammation was seen in treated patients compared to patients who did not receive MCS therapy. Clinical remission was achieved in 40 of patients and the use of corticosteroids was discontinued in 34 patients, while MCS treatment was deemed ineffective in 10 patients.

Stem cell therapy has been used extensively and effectively to ameliorated the pain, diarrhea, hemorrhage, inflammation and fistulization seen in cases of Crohn's disease refractory to established therapies, other fistulizing bowel diseases, and radiation-induced colitis. To date, over 100 human patients with these disorders have taken part in MCS clinical trials and adverse side-effects appear virtually non-existent, even with repeated treatments. The positive response in these patients is attributed to a modulation of the inflammatory lymphocyte subsets from effector T cells to regulatory T cells, stimulation of angiogenesis, and decreasing fibrosis. Administered MCS appear to favor the proliferation of endogenous tissue stem cells through the release of paracrine trophic factors.

Feline Chronic Enteropathies

The most commonly diagnosed Feline Chronic Enteropathy is inflammatory bowel disease. IBD in cats is not subdivided into ulcerative colitis and Crohn's disease, as IBD is in human patients. The cytokine profile in cats with IBD compared to cats with non-IBD GI disease shows an increase in both immunomodulatory cytokines IL-10 and TNF- β as well as the proinflammatory cytokines IL-6, IL-18, TNF- α , and IL-12p40. In a separate study the proinflammatory cytokines IL-1, IL-8, and IL-12 were increased in cats with IBD. Clearly there is significant immune dysregulation in feline IBD, and although the cytokine profile is complex and incompletely understood, it appears consistent with a Th1 response, as seen in humans with Crohn's disease. The trophic properties along with the anti-inflammatory and immunomodulatory effects of MSC administration make it a theoretically beneficial therapeutic modality for the treatment of feline IBD. The early success reported in animal models and clinical trials with human patients suffering from Crohn's disease further suggest that the use of MSC therapy in feline IBD warrants further investigation. Our laboratory has shown that feline adipose-derived MSC (fMSC) can be generated in large quantities to allow for clinical use, and that these fMSC are plastic-adherent, spindle-shaped cells that possess tri-lineage differentiation capabilities and suppress T-cell proliferation in vitro. Allogeneic fMSC have been safely and repeatedly administered to healthy and diseased cats with no notable side effects. We completed a Proof-of-Concept study (JFMS 2014) where we showed, in a placebo-controlled, double-blinded study design, that fMSC therapy was safe, and resulted in marked improvement in clinical signs of chronic enteropathy for 5/7 of the cats that received the treatment. The cats, the clients, and the specific results will be presented.

What's All This Have To Do With My Clients And Their Cats?

Adipose-derived feline mesenchymal stem cells ARE NOT embryonic stem cells, and so a significant barrier to their use (those based on philosophical, religious, and ethical beliefs) has been removed. Any client with a keyboard can quickly immerse themselves in the internet enthusiasm for the "silver bullet" potential of stem cell therapy – and then they come to see you! As summed up by Dr. Dori Borjesson, (Cyranoski 2013), many veterinarians offer stem cell therapies to satisfy demanding customers, so "Clinicians are sucked into giving treatment" even in the absence of research to support such treatment.

It appears that currently there are 2 veterinary companies vying for your stem cell business; Vet-Stem (www.vet-stem.com) which offers Vet-Stem® Regenerative Cell Therapy® and MediVet America, LLC, (www.medivet-america.com) which offers an in-house kit. In either case, the majority of these commercial treatments involve patients with orthopedic and musculoskeletal problems: chronic osteoarthritis, soft tissue injuries of the joints, tendons and ligaments, and fractures, although feline gingivitis, kidney disease, IBD, and pulmonary fibrosis are also reported as targets. Neither website provides any references or cites any research on the use of their product in cats with chronic enteropathies, including IBD.

In both cases the process begins with the harvesting of adipose tissue from the patient to be treated (autologous treatment). Vet-Stem has you ship that adipose tissue to their facility for processing, the company returns the injection-ready product (Vet-Stem® Regenerative Cell Therapy®) within 24 hours, at a cost of approximately \$2,000 - \$3,500, and with the requirement that the veterinarian has completed the company's accreditation course. MediVet America provides a kit for the in-house processing of adipose tissue, producing an injection-ready product in approximately 4 hours, at cost of about \$1,800. Both companies claim to have serviced thousands of pets, although neither provides a specific number for the cats that have received treatment.

MediVet America states that “Adult stem cells are highly concentrated in the fat tissue. At this concentration, it is no longer necessary to culture the stem cells to acquire the necessary cell numbers to make a healing impact. The stem cells are contained within a pool of cells in the fat termed the Stromal Vascular Fraction (SVF). The SVF may impart anti-inflammatory effects, add bioactive peptides, and contribute to reformation and architectural organization. These are benefits lost once stem cells are cultured.” The company provides an enzyme system to break down the adipose tissue and a filter and antibiotic wash for sterility of the resultant stromal vascular fraction. A key step appears to be the LED light activation of proliferation, differentiation, and induction prior to the reintroduction into the patient.

MediVet claims that “we have seen positive clinical improvement in 95% of the arthritic cases performed nationwide.” Vet-Stem processes the adipose tissue within their own facility and returns injection-ready Vet-Stem Regenerative Cells (VSRC™) within 24 hours, “a functionally diverse cell population able to communicate with other cells in their local environment.” Bob Harman, Vet-Stem, Inc. CEO is quoted as saying there is “an 80% success rate in improvement of quality of life”. Again, there are no references or cited research on the use of this therapy in cats with chronic enteropathies, including IBD. The website states that Vet-Stem is currently evaluating the use of stem cells for the treatment of IBD, feline CKD, liver disease, immune-mediated diseases, and heart disease. Their website states that cancer, systemic infection, neurologic disorders (including spinal cord injuries), uncontrolled diabetes mellitus, and any organ disease disqualifies a pet for Vet-Stem therapy.

Conclusion

- Stem cell therapy is not currently regulated by the FDA.
- “Stem cell therapy” is actually the injection of a heterogeneous population of cells, including mesenchymal stem cells, endothelial progenitor cells, fibroblasts, haematopoietic and immune cells, and others.
- In addition to our study of chronic enteropathy in cats, a search of PubMed for studies on MSC therapy in clinical cases of feline diseases produces two pilot studies looking at their use in cats with CKD – there is much that remains to be done!
- Stem cells have become the latest in a long line of therapies in veterinary medicine where our use is fast and far out-pacing our understanding.
- Proceed with optimism and hope, but significant contemplation and caution.

Suggested Reading

Trepanier L. Idiopathic inflammatory bowel disease in cats. Rational treatment selection. *J Fel Med Surg* 11: 32-38, 2009.

Burns K. Stem cells in theory & practice. *J Am Vet Assoc* 238: 396-399, 2011.

Cyranoski, D. Stem cells boom in vet clinics. *Nature* 496: 148-149, 2013.

Lopez, MJ. Stem cell therapies: reality in the making. *Clinician's Brief* June: 69-71, 2013.

Quimby, JM et al. Evaluation of intrarenal mesenchymal stem cell injection for treatment of chronic kidney disease in cats: a pilot study. *J Fel Med Surg* 13: 418-426, 2011.

Webb TL, Webb CB. Stem cell therapy in cats with chronic enteropathy: a proof-of-concept study. *J Fel Med Surg* 2014. [Epub ahead of print]

Quimby JM, Dow SW. Novel treatment strategies for feline chronic kidney disease: A critical look at the potential of mesenchymal stem cell therapy. *Vet J* 204: 241-246, 2015

NOTES:
