Evaluation of the Potential Use of Adipose-Derived Mesenchymal Stromal Cells in the Treatment of Canine Atopic Dermatitis: A Pilot Study*

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CLINICAL RELEVANCE

Stem cells and their potential therapeutic uses in human and veterinary medicine have generated considerable interest. These cells have a number of potentially unique immunologic properties; most notable are their reported regenerative and antiinflammatory capabilities. The aim of this prospective pilot study was to evaluate the efficacy of intravenously administered autogenous adipose-derived mesenchymal stem cells (AD-MSCs) in the treatment of canine atopic dermatitis. AD-MSCs administered intravenously at a dose of 1.3 million cells/kg did not significantly reduce the clinical signs of canine atopic dermatitis or the owner-assessed pruritus level.

INTRODUCTION

Canine atopic dermatitis is the second most common allergic dermatologic disease of dogs, affecting approximately 10% of the canine population.1 Atopic dermatitis is defined as a genetically predisposed inflammatory, pruritic, allergic skin disease with characteristic clinical features associated with IgE antibodies, most commonly directed against environmental allergens.2 Although this disease is common in small animal practice, complete understanding of its pathogenesis has yet to be elucidated. Recently, studies have demonstrated that canine atopic dermatitis is a complex, multifactorial disease involving interactions between skin structure, the immune system, and environmental influences.3,4 This complex physiology and pathology creates a challenging disease to manage.

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Avoidance of environmental allergens is impractical or minimally effective. Atopic dermatitis is often treated with allergen-specific immunotherapy (ASIT) or symptomatically with antiinflammatory, immunosuppressive, or immunomodulatory therapies such as glucocorticoids, cyclosporine A, antihistamines, fatty acid supplementation, and many other topical and systemic drugs and supplements. Although systemic therapy with glucocorticoids and cyclosporine A can be effective, the potential for adverse effects is a concern for both practitioners and pet owners. The success rates for antihistamine and fatty acid therapy are often unsatisfactory. ASIT is used in some patients; however, clinical improvement is often not seen for the first several months of therapy, and many owners are not willing or able to provide this type of therapy. A need for additional effective strategies for controlling atopic dermatitis exists. However, at least some alteration in the immune system appears to play a role in most treatments, including ASIT and cyclosporine A.

A stem cell is characterized by its ability to undergo self-renewal and multilineage differentiation and form terminally differentiated cells. Embryonic stem cells seem to exhibit unlimited differentiation potential but are not generally available in current medical practice or research because of the significant ethical, legal, and political concerns surrounding them. In contrast, nonpluripotent stem cells of adult origin are more readily available and have advantages over their embryonic counterparts, including their immunocompatible nature, better-defined differentiation potential, and broader societal acceptance. Multipotent mesenchymal stromal cells or mesenchymal stem cells (MSCs) are isolated from a variety of adult tissues, including bone marrow stroma and adipose, skin, muscle, and connective tissue. Tissue-specific stem cells vary slightly in phenotype, morphology, proliferation potential, and differentiation capacity. MSCs are currently defined as plastic, adherent, multipotential fibroblast-like cells that express CD73, CD105, and CD90 and are negative for the hematopoietic markers CD14, CD34, and CD45. Although much of the research to date has focused on MSCs derived from bone marrow stroma, research focusing on harvesting and using adipose-derived mesenchymal stem cells (AD-MSCs) has increased. Adipose tissue has been determined to be a rich source of MSCs, providing an abundant and accessible source of adult stem cells. AD-MSCs are easily isolated, with approximately 1 g of adipose tissue yielding approximately $5 \times 10^9$ stem cells, 500 times the number of MSCs harvested from 1 g of bone marrow. AD-MSCs have been demonstrated to be multipotent and have a reliable cell culture behavior.

MSCs have been extensively characterized by their ability to differentiate, with clinical therapeutic applications for repair and regeneration of damaged tissues. Currently, more than 60 registered, ongoing clinical trials are assessing the efficacy of MSCs in treating various human cardiovascular diseases and inflammatory disease or evaluating their use in orthopedic and organ transplantation settings. Veterinary medicine has only begun to evaluate these cells and their therapeutic potential. Autologous AD-MSC therapy for veterinary medicine has been available since 2003 and has been used commercially in more than 2,500 horses and more than 500 dogs. No systemic adverse events have been reported in dogs. To date, most research has focused on the regenerative capabilities of MSCs. Recently, however, the focus of research has dramatically shifted from MSCs’ roles in tissue engineering toward demonstrating and charac-
terizing their immunomodulatory capacities.\textsuperscript{33,41,42} In canine studies, autogenous AD-MSCs have demonstrated benefit in treating degenerative inflammatory diseases, including osteoarthritis, and their value is believed to come from a mechanism other than their regenerative effects.\textsuperscript{35,39} These findings are consistent with discoveries of the antiinflammatory properties of MSCs. MSCs exhibit potent inhibitory activity on lymphocyte proliferation\textsuperscript{21,34,43–45} and share characteristics with CD4\textsuperscript{+} CD25\textsuperscript{+} T regulatory (T\textsubscript{reg}) cells, including the release of inhibitory cytokines and expression of inhibitory molecules.\textsuperscript{44} Their antiinflammatory nature gives MSCs potential for success in the treatment of various inflammatory diseases, and several studies assessing their use in treatment of inflammatory and autoimmune disease have begun.\textsuperscript{41,46–50} MSCs have demonstrated success in the treatment of perianal fistulas in human patients with Crohn’s disease,\textsuperscript{20,50} nonhealing wounds,\textsuperscript{20,51} severe refractory graft-versus-host disease,\textsuperscript{52,53} multiple sclerosis,\textsuperscript{47,54} and arthritis.\textsuperscript{49,55} MSCs have exhibited a potent in vivo inhibitory activity on lymphocyte proliferation in a non-cognate–dependent fashion (i.e., it does not require the expression of major histocompatibility complex).\textsuperscript{45} This inhibition of T lymphocyte proliferation results in the decreased production of Th1 cytokines.\textsuperscript{44} MSCs also inhibit B lymphocyte proliferation.\textsuperscript{44} Additional MSC-mediated effects that inhibit immune function include alteration of the maturation of antigen-presenting cells, suppression of differentiation and functions of dendritic cells, and alteration of the cytokine secretion profiles of naïve dendritic cells and natural killer cells.\textsuperscript{21,41,56}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{visual_pruritus_scale.png}
\caption{The visual pruritus scale provided to owners to indicate their dog’s level of pruritus over the previous 24 hours.}
\end{figure}
Although the exact pathogenesis of canine atopic dermatitis remains elusive, specific aspects of its immunologic mechanisms have been described. Atopic dermatitis is most commonly associated with IgE antibodies to environmental allergens.\textsuperscript{1,2,57} However, numerous other factors play a role in the development of clinical signs, including defective epidermal barrier function, processing of allergens by epidermal Langerhans cells, polarization of T-lymphocyte cytokine responses, increased degranulation of cutaneous mast cells, and increased susceptibility to secondary bacterial and yeast infections.\textsuperscript{58} No previous study has been published investigating the effects of AD-MSCs on canine cytokine profiles in vivo.

Based on its antiinflammatory properties and immunomodulatory capabilities described in dogs, MSC therapy could theoretically ameliorate the clinical signs of atopic dermatitis. It has also been demonstrated that murine MSCs exert antiproliferative activity on antigen-specific responses.\textsuperscript{44} Human and murine MSCs can induce the generation of CD4\textsuperscript{+} CD25\textsuperscript{+} T\textsubscript{reg} lymphocytes in both in vitro and in vivo settings.\textsuperscript{44} Given the similarity of canine MSCs to human and murine MSCs, it may be speculated that MSCs may also promote generation of T\textsubscript{reg} cells during the treatment of atopic dermatitis. In both human and canine studies, the production of T\textsubscript{reg} cells and IL-10 has been demonstrated to be an essential event in the successful treatment of atopic dermatitis using ASIT.\textsuperscript{16,59}

To date, no clinical trials evaluating the potential therapeutic effect of MSCs on canine dermatologic inflammatory disease in vivo have been published. The aim of this pilot study was to evaluate the potential use of intravenously administered autogenous AD-MSCs for the treatment of canine atopic dermatitis.

\section*{MATERIALS AND METHODS}

\subsection*{Study Participants}

Five client-owned dogs with naturally occurring, nonseasonal atopic dermatitis were included. Cases were enrolled during a 2-month period (April and May 2009). All dogs participated in the study with the owners’ informed consent. All procedures used in this study were approved by the Animal Dermatology Clinic (Tustin).

\subsection*{Inclusion Criteria}

Each patient was more than 1 year of age. Inclusion criteria of a minimum body weight of 2.3 kg (5 lb) and a body condition score of five or greater on a nine-point scale were established to ensure adequate adipose tissue for harvesting. All dogs were judged healthy on physical examination aside from cutaneous disease. Before enrollment, routine serum chemistry and hematology evaluations were performed to ensure overall systemic health. The diagnosis of atopic dermatitis was based on suggestive history, compatible clinical signs, and exclusion of other pruritic skin disease and met the criteria established by Willemsse and Prélaud.\textsuperscript{60-62} The diagnosis was supported by

\begin{table}
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\begin{tabular}{|l|l|l|l|l|}
\hline
Dog & Breed & Sex & Age (years) & Weight (kg) \\
\hline
1 & Labrador retriever & MN & 2 & 35.5 \\
2 & Staffordshire bull terrier & FS & 1 & 21.8 \\
3 & Chihuahua & MN & 2.5 & 2.8 \\
4 & English bulldog & FI & 3.5 & 16.1 \\
5 & Labrador retriever & FS & 12 & 27 \\
\hline
\end{tabular}
\caption{Signalment of Dogs Enrolled}
\end{table}

\textit{FI} = female, intact; \textit{FS} = female, spayed; \textit{MN} = male, neutered.
positive reactions to intradermal testing using environmental allergens (Greer Veterinary Allergy Products, Greer Laboratories, Lenoir, NC) and/or allergen-specific IgE serology (Liquid Gold Allergy Laboratory, Veterinary Allergy Reference Laboratory, Pasadena, CA). Cutaneous adverse food reactions were ruled out by failure to show any improvement when fed a novel protein and carbohydrate elimination diet for a minimum of 8 weeks or any worsening on provocative diet challenge. Additionally, no changes were made to the dogs’ diet formulations for 60 days before day 0 of the study or during the study. The owners agreed to follow a set schedule of veterinary appointments and to observe their dogs for any abnormal behaviors or adverse effects for the entire study period.

Exclusion Criteria

Dogs with clinical evidence of ectoparasite infestation, a concurrent diagnosis of unregulated or uncontrolled flea allergy dermatitis, or clinical evidence of active bacterial or fungal infections were excluded. Other exclusion criteria were the ongoing use of topical, oral, or injectable antiinflammatory or immunosuppressive medications, including antihistamines, glucocorticoids, cyclosporine, and NSAIDs. For dogs being weaned from these medications, the following withdrawal times were instituted: 8 weeks for topical, oral, or injectable glucocorticoids; 4 weeks for oral cyclosporine; and 2 weeks for oral antihistamines.

Concurrent Medications

Concurrent medications were permitted, provided that the clinical signs of atopic dermatitis were stable. ASIT was maintained if it had been employed for at least 1 year, other inclusion criteria were met, and no changes were made to the ASIT protocol during the study period. All dogs were maintained with a flea control program that included the topical antiparasiticides fipronil/S-methoprene (Frontline Plus, Merial) or imidacloprid/permethrin (K9 Advantix, Bayer HealthCare), applied at least monthly. Dogs with a history of recurrent superficial bacterial pyoderma or Malassezia dermatitis were enrolled if they had no clinical evidence of pyoderma and had not received antimicrobials in the 4 weeks before the trial. Additionally, if a dog had commenced a course of antimicrobials 2 weeks before stem cell admin-

<table>
<thead>
<tr>
<th>TABLE 2. Summary of Concurrent Treatments Given to Dogs</th>
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<tbody>
<tr>
<td><strong>TREATMENT WITH SYSTEMIC ANTIBIOTICS</strong></td>
</tr>
<tr>
<td>No history of bacterial pyoderma or Malassezia dermatitis</td>
</tr>
<tr>
<td>Antimicrobials</td>
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<tr>
<td>No antimicrobials</td>
</tr>
</tbody>
</table>

ASIT = allergen-specific immunotherapy.
istration and its level of pruritus was not altered, these medications would be maintained throughout the trial. If superficial bacterial pyoderma and/or *Malassezia* dermatitis developed during the course of the study, treatment with appropriate antimicrobials was initiated. If the secondary infections remained controlled on treatment and no evidence of active infection was detected on cytology, the patient’s scores would be included in the study. Routine bathing with HyLyt shampoo (TEVA Animal Health) once a month was permitted.

**Adipose Tissue Collection**

Approximately 2 g of subcutaneous adipose tissue was collected from the interscapular region by surgical excision. The site was clipped and cleaned with 2% chlorhexidine acetate scrub (Nolvasan Surgical Scrub, Pfizer Animal Health) and 70% isopropyl alcohol rinse. A local anesthetic block with 2% lidocaine HCl (1 mL SC) was performed. A 2-cm surgical incision was made, and approximately 1.5 cm³ of subcutaneous adipose tissue was resected using a scalpel or surgical scissors. The adipose tissue was weighed with a gram scale to ensure that an appropriate sample was obtained. The incision was closed with two subcutaneous horizontal interrupted ligatures and three cruciate sutures using 3-0 PDS (Poly-Dox Sutures, Phoenix Pharmaceuticals, St. Joseph, MO) violet monofilament (polydioxanone) suture. The adipose tissue was placed into a labeled sterile tube containing 15 mL of phosphate buffered saline (PBS). The sample tube was placed in a temperature-controlled transport box filled with a frozen cold pack and shipped by overnight express for processing (RN L Biostar, Germantown, Maryland). Isolation and culture of AD-MSCs for injection took approximately 4 to 6 weeks. Each patient received approximately 1.3 million cells/kg via a single intravenous injection over a period of 10 minutes through a 20-gauge catheter in the cephalic vein. The administration was performed as an outpatient visit, and each dog was monitored at the clinic for 1 hour after injection.

**Isolation and Culture of Canine AD-MSCs**

Cells were isolated using previously described modified methods. Briefly, the adipose tissue was extensively washed with PBS, minced, then washed again several times to remove debris and blood. The tissue underwent enzymatic digestion with 1 mg/mL collagenase I (Invitrogen, Carlsbad, CA) at 37°C for 60 minutes under gentle agitation. The resultant mixture was filtered through a 100-µm cell strainer, and the infranatant was centrifuged at 1500 rpm for 5 minutes. The cell
pellet was resuspended in RNL K Cell Medium (RNL Bio Co. Ltd, Seoul, Korea; RKCM) containing 5% fetal bovine serum (FBS), and centrifugation was repeated at 1500 rpm for 5 minutes. The cells from the pellet stromal vascular fraction were suspended and cultured in RKCM overnight at 37°C in 5% CO₂ air. The following day, the cells were evaluated for cell attachment, morphology, and viability via a trypan blue exclusion method. At this point, the stromal vascular fraction consisted of a heterogeneous cell mixture including AD-MSCs, hematopoietic stem cells, fibroblasts, pericytes, and endothelial cells. After 24 hours, nonadherent cells were removed via PBS washing. The culture medium was changed to RKCM containing 5% FBS to further promote attachment and proliferation of the MSCs. For the next 4 to 5 days, the cell cultures were maintained until confluence was achieved. The plasticity of the AD-MSCs was assessed after lineage induction. The cells were then cultured and expanded in RKCM containing 5% FBS until the appropriate number of cells (approximately 1 x 10⁶ cells/kg) was achieved. With the initial 2 g of adipose tissue, this took an average of 3 to 4 weeks per dog. The cells were washed and suspended with PBS in a 10-mL solution placed into two sterile 6-mL syringes, which were shipped overnight at a controlled temperature to the clinic for administration. Using similar methodology, the success rate of harvest of MSCs from canine subcutaneous fat samples is 100%, consistent with reported MSC isolation from other mammals, including rabbits, mice, horses, pigs, and humans.⁶³–⁶⁸

**Experimental Design**

Dogs that met the study criteria were enrolled. Each dog received a single intravenous injection of autogenous AD-MSCs. Dogs were evaluated for clinical lesions on the collection and administration days and 2 to 3 weeks, 6 to 8 weeks, and 10 to 12 weeks after administration. The extent and severity of lesions were recorded by the same investigator using CADESI-03 (Canine Atopic Dermatitis Extent and Severity Index) value scoring and a visual pruritus scale (VPS). The CADESI-03 is a validated assessment of four clinical lesions (erythema, excoriations, lichenification, and self-induced alopecia) at 62 anatomical sites using scores from 0 (normal) to 5 (most severe), yielding a final score of 0 to 1240.⁶⁹,⁷⁰

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**TABLE 3. Visual Pruritus Scale and CADESI-03 Scores for Each Patient**

<table>
<thead>
<tr>
<th>Dog</th>
<th>Adipose collection</th>
<th>AD-MSC injection</th>
<th>Recheck evaluations (weeks)</th>
<th>CADESI-03 Score</th>
<th>Recheck evaluations (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td>2–3</td>
<td>6–8</td>
<td>10–12</td>
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<tr>
<td>1</td>
<td>6.5</td>
<td>2.8</td>
<td>0.5</td>
<td>0.1</td>
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<tr>
<td>2</td>
<td>9.9</td>
<td>9.9</td>
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<td>6.1</td>
<td>3.4</td>
<td>5.2</td>
<td>6.9</td>
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<tr>
<td>4</td>
<td>8</td>
<td>8.7</td>
<td>6</td>
<td>9.5</td>
<td>*</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>4.5</td>
<td>2.2</td>
<td>2.7</td>
<td>2.5</td>
</tr>
</tbody>
</table>

CADESI = Canine Atopic Dermatitis Extent and Severity Index.

*Case was lost to follow up.
The VPS used by the owners to assess pruritus was a vertical 10-cm visual analog scale containing features of severity and behavior71(Figure 1). The owners were asked to grade their dog’s pruritus level during the preceding 24 hours by placing a short horizontal mark in accordance with their perception. After the owners marked the vertical line, a transparent numeric scale was superimposed over the line, allowing determination of a score ranging from 0 (no pruritus, normal dog) to 10 (extremely severe pruritus), with a precision to one decimal place. Because long-term management of canine atopic dermatitis often requires control of additional associated or secondary dermatoses, including pyoderma and bacterial or Malassezia overgrowth, the presence of these infections was monitored with cytology at each visit. For this study, cytologic findings were defined as the following: bacterial overgrowth (>5 bacteria/oil-immersion field [oif]), superficial bacterial pyoderma (detection of intracellular bacteria and inflammatory cells), and Malassezia overgrowth (>2 yeast/oif).1,72

Statistics
All analyses were performed using SAS V 9.2 (Cary, NC). A paired t test was used to compare VPS and CADESI-03 scores before injection; 2 to 3, 6 to 8, and 10 to 12 weeks after injection; and at final postinjection assessments (6 to 8 weeks or 10 to 12 weeks). All hypothesis tests were two-sided, and the significance level was α = 0.05.

RESULTS
Five dogs—two Labrador retrievers, one Chihuahua, one English bulldog, and one Staffordshire bull terrier—were enrolled in the study (Table 1). The dogs’ ages ranged from 16 months to 12 years, with a median age of 3 years. Weights ranged from 2.8 to 35.5 kg. The primary end points for this study were the effects on degree of pruritus and lesion scores. Two dogs (#1 and #4) were lost to follow up at the third recheck appointment 8 weeks after administration; therefore, the results from the final recheck were not included in statistical analysis. At 12 weeks, the owner of dog 1 was contacted by telephone and described minimal pruritus or clinical signs. Dog 4 had become increasingly pruritic, according to the owner, who had followed up with the referring veterinarian to resume glucocorticoid therapy used before study enrollment.

Concurrent Medications
The concurrent medications that the dogs received during the study, other than monthly flea control, are listed in Table 2. The treat-
ment protocol was altered in only one dog (#2) at the first recheck and continued until the completion of the study (weeks 2 through 10). Dog 2 required systemic antibacterial treatment (cephalexin, Karalex Pharma, LLC, Woodcliff Lake, NJ) for superficial bacterial pyoderma that resolved as demonstrated by regular cytology assessment. Two of the five dogs (#1 and #4) were receiving antimicrobials before enrollment; these drugs were continued until the dogs exited the study. Dog 1 was receiving oral ketoconazole (USPharmcopeia) for 2 weeks before AD-MSC administration and continued treatment for an additional 8 weeks until the study exit. Dog 4 had been treated with cephalexin (Karalex Pharma, LLC) for 4 weeks before AD-MSC administration and continued therapy for 7 weeks.

### Adverse Effects

No adverse effects were observed in relation to the intravenous administration of AD-MSCs. No adverse effects were reported by the owners during the weeks after injection. One complication occurred secondary to the collection of the adipose tissue: dog 2 developed a seroma at the incision site. The fluid was drained, and a Penrose drain was placed to prevent recurrence. No bacterial or fungal growth was detected with culture of the aspirated fluid. The area was completely healed after 2 weeks, with no recurrence or subsequent development of adverse effects.

### Visual Pruritus Scale

There was no significant difference between the dogs’ initial VPS scores (mean initial score: 7.14). The VPS scores from the administration of the AD-MSCs through the following recheck examinations are depicted in Figure 2 and Table 3. Mean VPS scores decreased by 2, 1, 0.5, and 0.7 at 2 to 3 weeks, 6 to 8 weeks, 10 to 12 weeks, and final assessment, respectively. There was no significant improvement in mean VPS scores at 6 to 8 weeks ($P = .1444$), 10 to 12 weeks ($P = .5784$), or final assessment ($P = .3842$). At 2 to 3 weeks after injection there was a significant reduction in VPS scores ($P = .0170$). Only one dog demonstrated a sustained reduction in VPS score for two recheck examinations compared with baseline after the treatment injection.

### CADESI-03 Scores

The CADESI-03 scores throughout the course of the study are depicted in Figure 3 and Table 3. Mean CADESI-03 scores decreased by 12 at 2 to 3 weeks postinjection. Mean CADESI-03 scores increased by 57, 277, and 142 at 6 to 8 weeks, 10 to 12 weeks, and final assessment, respectively. There was no significant improvement in mean CADESI-03 scores at 2 to 3 weeks ($P = .7671$), 6 to 8 weeks ($P = .4730$), 10 to 12 weeks ($P = .1832$), or final assessment ($P = .2783$).

### DISCUSSION

Although several treatment options for canine atopic dermatitis are available, no single therapy is completely successful in eliminating the associated clinical signs, and no fast-acting treatment modality without potentially serious adverse effects and a high cost exists. Some dogs remain notably pruritic even with multimodal treatment protocols. In this open, non-controlled, nonblinded pilot clinical trial, the use of autogenous AD-MSCs at 1.3 million cells/kg delivered intravenously was well tolerated but did not demonstrate a significant reduction in the clinical signs associated with canine atopic dermatitis.

Adipose tissue has been demonstrated to be a viable source of MSCs in numerous species, including dogs and humans. MSCs in dogs and humans have characteristics, morphology,
and plasticity similar to those of human AD-MSCs. MSCs expanded from adipose tissue are considered at least equivalent and potentially superior to those from bone marrow in terms of differentiation ability, angiogenesis, and immunomodulatory effects. The adherent cells isolated from canine adipose tissue can be defined as multipotent MSCs with the ability to differentiate into at least four mesodermal lineages. There is some debate about whether repeated culturing of harvested AD-MSCs may alter the potency of these cells. In the current study, the cells were cultured to achieve a larger number of cells from a small amount of harvested issue. Previous canine studies collected a larger amount of adipose tissue (at least 23 g), which allowed for a quicker return from processing for administration. The extraction of 23 g of adipose tissue is a more invasive procedure than the extraction of 2 g, and as the efficacy of AD-MSCs for canine atopic dermatitis had yet to be researched, this type of harvesting procedure was not used in the current study. Repeated culturing, up to four passages, has also demonstrated a reduction in other cell populations mixed with the MSCs, specifically endothelial cells. However, it is also important to note that prolonged cloning for more than 4 months has been shown to result in malignant transformation of human AD-MSCs. Regardless of differences in isolation, culture procedures, and time in passage, the reported immunophenotypes of these cells are relatively consistent. Future studies evaluating the use of direct harvested AD-MSCs should be considered to ensure that in vivo properties are similar among the various methods of processing.

This study was designed to evaluate the clinical effects of AD-MSC therapy on atopic dermatitis. In a small number of dogs, the administered dose of AD-MSCs did not have a significant effect. The lack of clinical significance in the current study may be due to the fact that most of the cases enrolled demonstrated a long history of poor response to multiple therapies. The owners who sought to have their pets enrolled were often frustrated by the minimal effectiveness of current therapeutic options. The five patients included in this pilot study were unusually difficult to control even though some had low CADESI-03 or VPS scores because of concurrent therapy.

The small study size limits the ability to determine the merit of this treatment option. In previous studies evaluating canine atopic dermatitis treatment with ASIT, approximately 50% to 100% of patients responded to therapy (i.e., showed a reduction in clinical signs). In the current study, two dogs developed a trend toward sustained reduction in VPS; however, these results were not statistically significant. One dog demonstrated a mild reduction in CADESI-03 score. A larger number of cases and/or administration of a higher dose of AD-MSCs may have demonstrated a significant reduction in clinical signs.

An additional limitation on the use of AD-MSCs in practice is the high cost and invasiveness of collection as well as isolation of these cells. The cost of processing and cloning AD-MSCs is approximately $1200 per patient.

Adipose tissue has been demonstrated to be a viable source of MSCs in numerous species, including dogs and humans.
at the time of publication. This cost, added to that for harvesting, is prohibitive for most pet owners. When compared with traditional intradermal testing and induction of ASIT, AD-MSC therapy may be four times more expensive.

For all but one patient, a significant reduction in VPS score was seen at the first recheck after the AD-MSC injection. The CADESI-03 score did not demonstrate a significant decrease, but the mean scores were reduced at the 2- to 3-week postinjection interval. These findings may imply a transient response to AD-MSC injection. Additional injections on a regular basis may be required to propagate immunomodulatory effects and thereby reduce clinical signs. Conversely, this was a nonblinded study, and the initial improvement may simply point to owner or investigator bias or placebo effect. Although a blinded, controlled trial is preferred to evaluate therapeutic outcome, the purpose of the current study was to determine if there is a significant response in dogs to justify a larger controlled trial. Additional blinded studies are required to determine if the initial improvement was genuinely due to the treatment.

Studies evaluating the in vivo systemic effects of AD-MSCs in regard to modulation of immune function have not previously been performed; therefore, the dose and route of administration used in the current study may not have been sufficient to reach the desired therapeutic effect. The dose used in the current study was based primarily on the work of RNLBiostar and extrapolated from previous canine, human, and murine studies. A higher dose, in addition to repeated administration of MSCs, may be required to produce a higher degree of modification of immune function and create a more notable clinical response. The route of administration may also be questioned, as previous studies evaluated localized disease treated with local injections. Local therapy via intraarticular injections of AD-MSCs resulted in improvement of osteoarthritis in dogs. However, because atopic dermatitis is a systemic disease, intravenous administration was preferred. MSCs migrate preferentially to sites of injury and inflammation, where they promote functional recovery of tissue as well as a decrease in inflammation. Systemic delivery of these cells has been described in many studies, which demonstrated the homing capacity of MSCs as well as their residence in tissue for extended periods of time.

This open, noncontrolled, nonblinded pilot clinical trial has provided limited information because of its small sample size and the case selection. Future studies would benefit from larger case numbers, a blinded placebo control group, enrollment of less refractory or chronically affected patients, and possible changes in dose or processing protocol. Additionally, studies need to be pursued to determine the exact nature of AD-MSCs in vitro and in vivo and what cytokine or immunomodulatory properties they possess in canine patients.

**CONCLUSION**

The results of this pilot study indicate that systemic administration of 1.3 million cells/kg AD-MSCs appears safe but ineffective as a management option for canine atopic dermatitis. The efficacy of the treatment was assessed based on the response of each patient compared with its own baseline score before administration. Future studies assessing the use of AD-MSCs in atopic dermatitis should likely evaluate other dose regimens or processing procedures.

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