Lymphocyte T-Cell Immunomodulator (LTCI), a Potent Immune Modulating Biologic, Exhibits Safety and Efficacy in the Pain Management of Dogs with Moderate-Severe Osteoarthritis.

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ABSTRACT

Canine and human populations in the U. S. experience the same incidence of osteoarthritis, and both suffer similar problems associated with treatment and control. Treating pain with nonsteroidal anti-inflammatory drugs (NSAIDs) can be effective, but the potential for significant cardiovascular, gastrointestinal, and renal problems are well-documented. Lymphocyte T-Cell Immunomodulator (LTCI) was proven to be a relatively fast-acting therapy for reduction of pain in dogs with osteoarthritis (OA). In this study, those treated with LTCI vs placebo-treated animals demonstrated increased function and mobility as well as noteworthy decreased lymphocyte counts, which strongly supports the use of LTCI in veterinary practice to serve as an alternative to high risk-to-benefit ratios of corticosteroids and NSAIDs.

INTRODUCTION

It is an interesting coincidence that both the canine and human populations in the U.S. experience the same incidence of osteoarthritis, 20%,1,2 and unfortunately, both suffer similar problems associated with the treatment and control of the inflammation responsible for pain, especially in the elderly of both species.

Treating pain with nonsteroidal anti-inflammatory drugs (NSAIDs) can be effective, but constant monitoring of blood values is required, and the potential for significant cardiovascular, gastrointestinal, and renal problems are well-documented.3

NSAIDs accomplish pain reduction by either selective or non-selective blocking of COX-1 and/or COX-2 cyclooxygenase enzymes. The COX-1 isoform synthesizes the prostaglandins responsible for vasodilation. COX-2 produces prostaglandins that maintain diuresis and natriuresis and may

induce hypertension. Hypertension monitoring is underutilized in practice, and with many cases going undetected, it presents an additional, often overlooked risk potential in the use of NSAIDs for veterinary patients. Science is looking carefully for data to support the validity and viability of nutraceuticals in the treatment of joint discomfort associated with arthritis.

Nutraceuticals are widely used to relieve joint discomfort associated with arthritis. As these products become more widely accepted as adjuncts to conventional treatment therapies, science is looking carefully for data to support: the validity and viability of their anti-inflammatory activities; their ability to decrease the risk of certain cancers and cardiovascular disease; their production of macrophages, B and T cells and Natural Killer Cells; and the mediation of humoral and cell-mediated immunity.[4]

In 2008 the National Institutes of Health (NIH) published the results of the first large-scale, multicenter clinical trial in the U.S., the Glucosamine/chondroitin Arthritis Intervention Trial (GAIT), which tested pain reduction by glucosamine and chondroitin in the treatment of human knee arthritis. In conclusion, it stated that, "…the rate of response to glucosamine and chondroitin, either alone or in combination, was not significantly higher than the rate of response to placebo," and "...there were no significant differences between the placebo and the G, C or combined-treatment groups."[5]

Patients who suffer from joint disease and arthritis and the health care professionals involved in treating them continue to search for a safe, scientifically-proven and efficacious response to the problems.

But in a promising double-blinded, placebo-controlled clinical trial, Lymphocyte T-Cell Immunomodulator (LTCI) was proven to be a relatively fast-acting therapy for reduction of pain in dogs with osteoarthritis (OA).

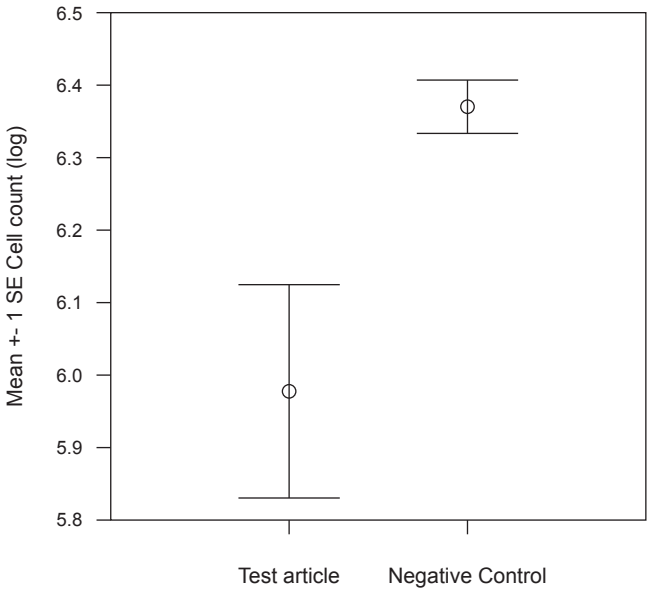
BACKGROUND

Lymphocyte T-Cell Immunomodulator (LTCI) has been studied in the laboratory over the last 29 years and clinically for the past 10 years for its potent immune modulating effects. It has demonstrated greatly enhanced cytolytic T-cell responses to antigens through a mechanism of action that increases the number of newly formed CD-4 positive regulatory T-Cells that produce interleukin-2 (IL-2), and enhances CD-4 function. In vitro data exhibits amplification of CD-4 cell function and is a possible explanation for its profound in vivo effects. The need for only microgram dosing likely explains the lack of side effects, both at the injection site and in long-term symptomatic observations.

Initial research by S-Cell Biosciences, Inc., parent company of T-Cyte Therapeutics, Inc., was directed toward people, where analogous cells from a bovine source for use in the enhancement of immune responses to viruses were derived. In dogs immunized

	STUDY DAY										
Event	-14-0	0	3	6	9	12	15	18	21	24	28
Owner Consent Form	•										
Case History	•										
Force Plate Analysis	•										•
Radiographs (if needed)	•										
Physical Exam	•										•
Body Weight	•	•					•				•
Blood Work	•										•
Eligibility Assessment	•	•									
Dose with LTCI / Placebo		•	•	•	•	•	•	•	•	•	•

Figure 1.



with killed rabies vaccine plus LTCI vs. vaccine plus aluminum hydroxide, pre-clinical human studies demonstrated a 5-fold increase in neutralizing antibody response rabies virus.

A follow-up NIH mouse challenge study indicated that LTCI could double the potency of standard rabies vaccine. In a further Smith Kline Animal Health canine immunization and challenge study, a comparison was made between standard distemper vaccine and LTCI: 86% of the recipients of the vaccine + LTCI responded, but only 43% of the dogs immunized with vaccine alone were responsive. All unvaccinated controls died, but there were no adverse effects observed in any dogs receiving LTCI.

Subsequently, a Solvay Animal Health feline study indicated that LTCI + killed FIV vaccine induced 42.9% protection. The vaccine alone provided no protection from viremia; there was a 50% protection induced by Freund's complete adjuvant. The studies suggested that LTCI could be effective as an immunotherapeutic treatment for immuno-compromised hosts.

Immunopathologic studies indicated that FIV and FeLV diseases are similar to the hu-man immunodeficiency virus (HIV) disease. Therefore, FIV and/or FeLV-infected cats were chosen as pre-clinical models for hu-man AIDS.

After an initial mild, transient leucopenia, fever and lymphadenopathy, remission occurred for 4-5 years. The viruses infected CD-4+ helper cells, causing a drop in white blood cell (WBC) count and led to increased susceptibility to opportunistic infections. A field study of natural infection was performed, and data was collected from 23 infected cats. Enrollment requirements were single infection and at least two of the common symptoms of FIV/FeLV infections. The enrolled patients were chroni-cally, seriously ill when presented at the clinic by the owners. Several were brought in for euthanasia. FIV/FeLV diagnoses were confirmed by laboratory antibody tests (IDDEX).

Most of the cats were lymphopenic, either by differential or absolute lymphocyte determination. Many of the cats were also anemic, some severely (hematocrits 8-12%). Two cats were also granulocytopenic. As observed in the experimentally infected cat study, cats improved significantly within 72 hours, as reported by the owners and confirmed by the practitioners at the second visit 2 weeks later. One µg of LTCI was given subcutaneously on days 0, 14 and 28. Data accumulated from 23 animals--lymphocyte levels from blood drawn on days 0 and 28-- were compared, and the efficacy of LTCI therapy was determined based upon absolute lymphocyte counts as well as improvement in clinical symptoms.

Post-treatment lymphocyte counts increased by an average of 38%. As a result, T-Cyte Therapeutics was granted a conditional license by the USDA for LTCI as "an aid in the treatment of FeLV and/or FIV disease and associated symptoms…".

LTCI enhances the immune status of FIV/FeLV-infected cats, corresponding to significant improvement in the signs and symptoms observed by clinicians. After 5 1/2 years of in-field use, the therapeutic effect

Table 1. *Force Plate Analysis LTCI Treatment*

	Average, S.D. Pre Rx	Average, S.D. Post Rx	P Value	↑ Function ↓ Function
#1	25.5 ± 2.1	35.5 ± 7.2	< 0.01	↑
#2	3.6 ± 7.7	8.2 ± 5.0	< 0.01	↑
#5	4.6 ± 0.8	8.4 ± 0.6	< 0.01	↑
#6	20.8 ± 0.3	28.5 ± 06	< 0.01	↑
#7	12.2 ± 8.5	13.6 ± 2.9	< 0.01	↑
#9	25.4 ± 4.0	46.6 ± 1.6	< 0.01	↑
#12	16.3 ± 0.5	21.5 ± 2.2	< 0.01	↑
#14	32.0 ± 1.4	30.2 ± 1.4	< 0.01	↓
#15	64.9 ± 1.2	67.1 ± 3.1	< 0.01	↑
#17	28.6 ± 1.7	30.9 ± 5.3	< 0.01	↑
#20	12.1 ± 1.7	15.9 ± 0.3	< 0.01	↑
#22	1.1 ± 0.3	3.3 ± 1.2	< 0.01	↑

appears to be long-lasting, and no noteworthy adverse effects have been observed.

Worldwide concern regarding a new influenza pandemic caused by an H5N1 avian influenza arose in 2006 after reported deaths in Southeast Asia. Given LTCI's profound immune enhancing properties, T-Cyte's parent company, S-Cell Biosciences, Inc., contracted the study of LTCI in ferrets experimentally infected with a laboratory strain of H5N1 influenza virus. (Ferrets are the preferred animal model for influenza studies due to their infection pathology and immune responses that simulate those in humans.) Of concern to investigators was that the vigorous enhancement of the immune response could exacerbate the immunopathology of the infection, especially the cell-mediated immune component: Profound lung congestion and possible death could ensue through the influx of lymphocytes into the respiratory tract in response to virus.

Interestingly, the 1918 worldwide flu pandemic resulted in more young people dying because of more robust immune responses: The sudden influx of lymphocytes into their lungs caused a "cytokine storm" and the resultant release of myriad chemoattractants and lymphokines.

Contrary to this, the LTCI treatment led to a sizeable reduction in upper respiratory cell counts, as determined in nasal wash fluid (Figure 1). The results were both statistically and clinically significant, since the immunopathology of influenza is a result of the influx of lymphocytes into the respiratory tract.

This striking finding encouraged the testing the efficacy of LTCI in arthritis, another disease where the influx of lymphocytes causes the immunopathology. In collagen-induced arthritis in rodents[6], foreign collagen suspended in complete Freund's adjuvant was injected into the tail base. Over a 4-week period, the antigen traveled to the distal lymph nodes and footpads. Infiltration of lymphocytes reacting to the foreign collagen caused footpad swelling and damage to the surrounding tissue. This mouse model is the "gold standard" for animal testing in the pre-clinical evaluation of compounds used to treat human rheumatoid arthritis (RA), where it has been long known that CD-4 lymphocyte abnormalities exist[7].

In a study conducted by T-Cyte using the collgen-induced arthritis model, mice were innoculated at the tail base on day 0. On day 30, all mice exhibited a greater than 80% increase

in footpad thickness (Figure 2). Beginning on day 30, and every 3 days thereafter for 4 weeks, the mice were injected subcutaneously with either LTCI or a sterile diluent. Within 72 hours of the initial treatment, a marked reduction in footpad thickness was observed in mice receiving the LTCI. The swelling continued to subside until the experiment was terminated.

Based upon the positive results in this RA model, T-Cyte proceeded to determine if treatment with LTCI would result in a simi-larly profound reduction in the lymphocyte-induced immunopathology of canine arthri-tis. The current best practices in treating a condition affecting 20% of the adult canine population includes the use of non-steroidal anti-inflammatory drugs (NSAIDS), which can be especially problematic in elderly patients. There has been a long prevailing belief that OA is a 'wear and tear' disease.

In 2005[8], observations that lymphocyte subset abnormalities in RA and OA were identical strongly suggested that a similar immunological etiology existed in both joint-related diseases. It has been recently demonstrated that synovial fluid from OA and RA human patients contains the same abnormal CD-4 subsets[9].

In 2011 it was reported that dogs with stifle synovitis and cranial cruciate ligament rupture had an increase in CD-4 lymphocytes in the affected joint, in direct correlation with radiographic evidence of arthritis[10].

In light of the above data, T-Cyte proposed and conducted a double-blinded, placebo-controlled study of 24 dogs to determine whether LTCI would have a positive effect on OA in dogs clinically manifesting arthritic disease.

A modification of the rodent model treatment schedule was administered: Subcutaneous injections were given every 3 days for 2 weeks, then twice a week for two weeks for a total of 10 injections. Each dog was evaluated both prior to the study and at the end of its regimen.

The primary determinant for the LTCI osteoarthritis study was whether there was a clinically significant improvement in the functioning of the dog's affected limb(s). Results were based upon the objective measurement of relative pressure exerted by the affected limb(s) on days 0 and 28 using a TekScan MatScan11 force plate to determine pressure values as a measure of joint pain. Four-hundred data points were captured for each force plate measurement.

Table 2. *Force Plate Pacebo Analysis*

	Average, S.D. Pre Rx	Average, S.D. Post Rx	P Value	↑ Function ↓ Function
#3	2.5 ± 1.3	6.5 ± 1.8	< 0.01	↑
#4	17.0 ± 4.0	10.0 ± 1.1	< 0.01	↓
#8	Had a torn ACL therefore exluded			
#10	7.4 ± 2.6	5.9 ± 4.6	< 0.01	↓
#11	29.1 ± 0.4	32.2 ± 0.8	< 0.01	↓
#13	19.9 ± 0.6	11.6 ± 1.7	< 0.01	↓
#16	34.8 ± 3.9	18.5 ± 3.3	< 0.01	↓
#18	21.7 ± 3.0	10.1 ± 1.2	< 0.01	↓
#19	37.0 ± 2.0	3.5 ± 0.9	< 0.01	↓
#21	6.3 ± 0.6	13.8 ± 3.0	< 0.01	↑
#23	42.8 ± 6.3	24.4 ± 3.8	< 0.01	↓
#24	17.3 ± 1.2	14.0 ± 1.0	< 0.01	↓

Table 3. *Effect of LTCI Treatment on Absolute Lymphocyte Counts*

LTCI Treated

	Absolute lymphcytes Before LTCI treatment	Absolute lymphcytes After LTCI treatment	Lymphcyte Change
	2431	2752	321
	2160	2000	-160
	2871	1700	-1171
	4736	1886	-2950
	4248	3627	-621
	924	954	30
	1972	2268	294
	1890	1750	-140
	785	1571	797
	4158	3350	-808
	2816	2320	-596
	3672	3956	284
Average	2721.916667	2345.3333333	-393.3333333
Standard Deveation	1279.933483	906.51203	979.958706

Placebo

	Absolute lymphcytes Before LTCI treatment	Absolute lymphcytes After LTCI treatment	Lymphcyte Change
	1372	1470	398
	910	832	-78
	1482	1702	220
	1157	1792	635
	3050	3100	50
	1674	1582	-92
	3150	3525	375
	1738	1670	-58
	2814	2814	0
	2247	2232	-15
	1551	1742	191
	928	798	127
Average	1839.166667	1938.25	146.0833333
Standard Deveation	793.8284488	840.9125049	227.1997352

P Value for difference between LTCI and Placebo	0.04377735

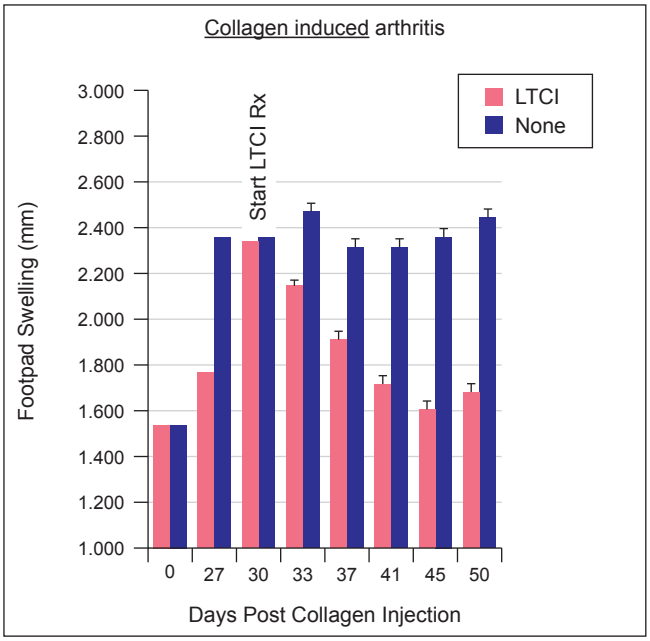
Force plate technology has long been validated in human medical practice to assess the clinical improvement of physically impaired individuals undergoing therapy for a variety of orthopedic conditions12, and more recently in veterinary medicine to assess lameness in horses[13].

A surprising secondary finding of this clinical study involved the measurement of blood lymphocyte counts: There is a correlation between lymphocytosis in the blood and an abnormal influx of lymphocytes into tissues in acute viral infections and immune-mediated diseases[14]. In the above-referenced ferret influenza study, elevated blood lymphocytes were initially observed upon infection with virus and then normalized upon resolution of the infection. Thus a hypothesis was put forth that LTCI might cause a similar effect in immune-mediated diseases such as osteoarthritis, based upon its significant immunomodulatory activity.

RESULTS

The stance pressure of the affected leg of each enrolled dog was measured by the MatScan force plate prior to the first treat-ment and then again at the end of the 30-day study period. The instrument simultaneously measured the static force exerted by each limb; values were expressed as a percentage of total pressure. Four-hundred data point measurements were captured over an 8-second period (Figure 3). Data were summarized for the LTCI-treated dogs (Table 1) and for placebo dogs (Table 2). As indicated in Table 1, 11 of 12 treatment dogs

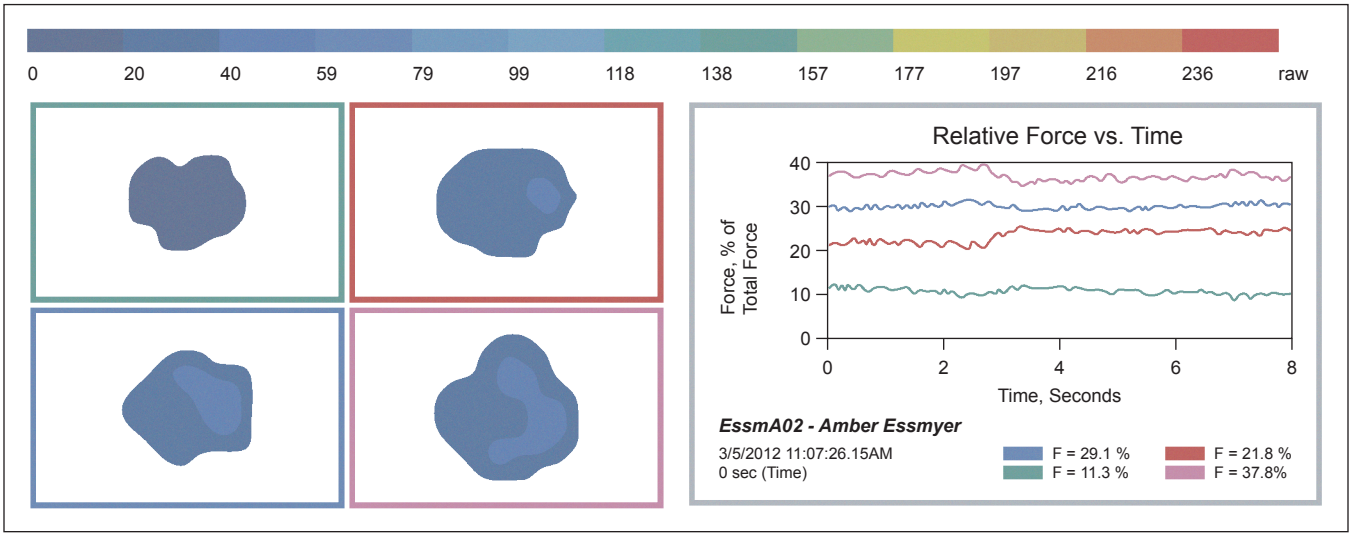
Figure 2.



experienced a significant (P<0.01) increase in functional pressure exerted by the affected limb. In contrast, only three placebo dogs exhibited increased function (Table 2). The remaining 8 placebo dogs had substantial functional decreases. One dog (# 8) was excluded from the study because of a ruptured anterior cruciate ligament.

According to both the Student's t-test and the Fisher Exact Probability statistic, the results of the LTCI group vs. the placebo group were highly significant for each dog within each group (P<0.01). Furthermore, using analysis of variance (ANOVA), the difference between the LTCI-treated group and the placebo group

Figure 2.



was significant (P<0.045).

In addition to functional pressure measurements, each dog had a complete blood count (CBC) and differential performed upon entry. At the completion of the trial, a second CBC and differential was performed, and many of the dogs exhibited the elevated lymphocyte count associated with acute infection or immunemediated disease.

LTCI as a modulator of immune reactivity effectively reduced the influx of lymphocytes into the upper respiratory tract in acute influenza infection in ferrets. It was anticipated that lymphocyte counts would be similarly attenuated in immune-mediated canine osteoarthritis: On average, LTCI-treated dogs exhibited a greater than 10% decrease in absolute lymphocyte counts compared to placebo dogs (Table 3). In contrast, the placebo group experienced a 5% increase in absolute lymphocyte counts. According to Student's t-test, the average change in lymphocyte counts between the LTCI and placebo groups was a statistically significant P<0.04.

In dogs diagnosed with moderate to severe OA, those treated with LTCI vs. placebo-treated animals demonstrated increased function and mobility as well as noteworthy decreased lymphocyte counts. This strongly supports the use of LTCI in veterinary practice to serve as an alternative to high risk-to-benefit ratios of corticosteroids and NSAIDs as a safe, efficacious choice for veterinary patients.

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