Lymphocyte T-Cell Immunomodulator (LTCI): Review of the Immunopharmacology of a New Veterinary Biologic

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ABSTRACT

Lymphocyte T-cell immunomodulator (LTCI) has recently become commercially available under a conditional license for the treatment of cats infected with feline leukemia virus (FeLV) and/or feline immunodeficiency virus (FIV). The primary therapeutic effects of LTCI, a protein produced by a thymic stromal epithelial cell line, are mediated through activation of progenitor T helper, CD-4 lymphocytes to mature and produce interleukin-2 and interferon. These cytokines in turn stimulate CD-8 "cytotoxic" T-cells, which attack virus-infected and tumor cells. Immunotherapeutic effects of LTCI have been demonstrated in influenza-infected mice, thrombocytopenic mice, challenge studies in dogs infected with rabies and distemper virus, and FeLV- and FIV-infected cats. A more precise description of the immunotherapeutic potential of LTCI under clinical field conditions must await results of controlled trials in cats, which are currently underway.

INTRODUCTION

On December 15, 2006, the United States Department of Agriculture (USDA) Center for Veterinary Biologics granted a conditional license for "lymphocyte T-cell immunomodulator" (LTCI). Lymphocyte T-cell immunomodulator is "intended as an aid in the treatment of cats infected with feline leukemia virus (FeLV) and/or feline immunodeficiency virus (FIV), and the associated symptoms of lymphocytopenia, opportunistic infection, anemia, granulocytopenia, or thrombocytopenia." This article describes the basic science behind the claims and the immunotherapeutic rationale for LTCI based on published literature, patents, and additional data provided by the manufacturer.

Lymphocyte T-cell immunomodulator represents an immunopharmacologic approach to infectious disease intervention, which is quite different from the traditional pharmaceutical approach. The past several decades have witnessed an expansion of knowledge in immunology, which is being increasingly translated into new therapeutic strategies.[1] Regulation of the immune response is a multifactorial process involving lymphocytes that function to maintain both self tolerance and aggressive defense against microbes as well as homeostasis following successful immunity.[2] The challenge has always been limited understanding on how to precisely modulate the immune system without causing additional harm. These hurdles are quickly being overcome with increased understanding of how immune cells interact, their complex cytokine signals, and a variety of other biochemical and molecular attributes that are being elucidated almost daily.

LTCI: DESCRIPTION AND OVERVIEW

Lymphocyte T-cell Immunomodulator is a

protein produced by stromal epithelial cells of the thymus. Under normal conditions, thymic stromal cells secrete factors that induce thymus-derived, helper lymphocytes (CD-4 T-cells) to mature and produce interleukin (IL)-2 and interferon (IFN). Interleukin-2 and IFN are cytokines that stimulate CD-8 "cytotoxic" T-cells, which attack virus infected cells and tumor cells (Figure 1).

Figure 1. Under viral attack, CD-4 cells fail to mature, fail to produce IL-2 and interferon, and consequently fail to stimulate CD-8 killer cells. This immunosuppression can be overcome by treatment with LTCI.
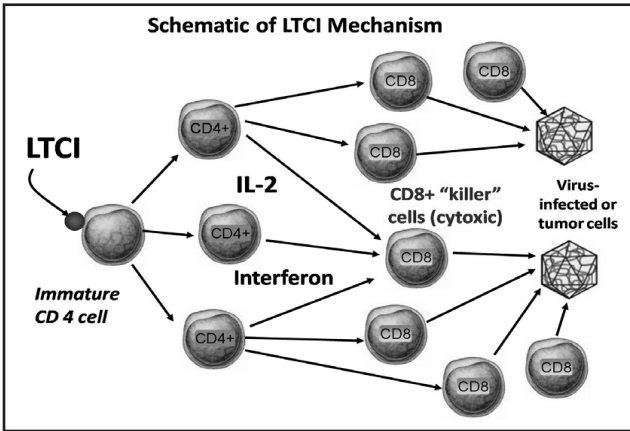
The primary action of LTCI is directed toward T lymphocyte production and activation, resulting in increased production of IL-2 and IFN in physiological amounts and ratios. These cytokines stimulate a cascade of events that enhance or potentiate both cell-mediated immunity as well as antibody-mediated responses.

Amplification Effect

It is important to note that CD-4 cells are affected at an early stage, building a cascade that multiplies several orders of magnitude. Treatment of immunosuppressed individuals with IL-2 or IFN has similar effects. However, these cytokines must be administered at frequent intervals in high doses to achieve therapeutic effects at the tissue level.

Why Is This Important for Veterinary Medicine?

In cats, as in other species, the thymus is the major target organ for replication of viruses.

Severe thymus atrophy was detected in kittens infected with feline leukemia virus (FeLV).[3] In cats infected with panleukopenia virus, involution of the thymus and marked depletion of lymphocytes occurs early in the disease.[4] Lymphoid tissue from cats with feline infectious peritonitis (FIP) show T- and B-cell depletion often including massive thymic involution or atrophy.[5] In young cats infected with FIV, antiviral (zidovidene) therapy reduces the viral load but does not prevent thymic involution.[6] Thus there appears to be a firm rationale for thymus-derived immunotherapeutic factors such as LTCI. In theory, restoration of thymic-derived substances is a rational immunotherapeutic approach to counteracting the deleterious effects of viruses or other infectious agents.

EARLY STUDIES: THE THYMUS CONNECTION

In order to understand LTCI, it is useful to review the thymus gland, thymus-derived cells (T-cells), and the history of the development of LTCI.

Thymus Gland

Prior to 1960, the thymus gland was thought to be of little importance. In adult animals, the thymus is almost non-existent because it atrophies as animals reach adulthood. It was observed, however, that when pre-adolescent animals are thymectomized, they experience a variety of maladies including increased incidence of infection, failure to grow, neuromuscular disorders, cancer, etc, collectively known as "wasting disease."[7] The greater susceptibility to infection was attributable to a marked decrease in peripheral blood lymphocytes in thymectomized animals. Importantly, it was also demonstrated that thymus-derived lymphocytes (T-cells) were also important regulators of bone marrow-derived, antibody-producing lymphocytes (B-cells).[8]

By the late 1960s, it had been dem-

onstrated that cells or regulatory factors extracted from the thymus gland could prevent many of the manifestations of wasting disease.[9,10] This suggested that the thymus produces substances important in the development of immunity and the logic of extracting factors from thymus glands in much the same manner that insulin was prepared from the pancreas for therapeutic use in diabetes. The difficulty was that the thymus is a very small gland and produces very small quantities of the factors of interest. However, thymic peptides were identified and tested clinically in humans for treatment of some immunodeficiencies, malignancies, and infections such as hepatitis B, with mixed results.[11,12]

Thymus Epithelial Cell Line

Thymus tissue is composed of at least 3 components: fibroblastic, macrophage, and epithelial, all of which are likely involved in the differential events in the thymus.[13] In order to delineate the functions of each component, it was necessary to develop cloned pure cell lines. A major technical obstacle was overcome by 1983 when cloned cell lines of thymic origin were characterized as epithelium.[14] This accomplishment facilitated characterization of the immunological functions of the epithelial component of the thymus.

IMMUNOPHARMACOLOGY OF LTCI

Incubation of immature T-cells with supernatants of cloned thymic epithelial cells and of other cell lines that produce IL-2 greatly enhanced the cytotoxic response to allogenic major histocompatibility complex antigens.[14] These early results suggested that thymic epithelial supernatants promote the response of a helper CD-4 cell population. Results of subsequent studies demonstrated that the factors had no direct effect on CD-8 cytotoxic T-cells, but rather stimulated CD-4 helper cells, which in turn stimulated the cytotoxic response.

These findings led to the tentative hypothesis that thymus epithelium produces factors that stimulate thymocytes to produce IL-2. This was demonstrated further by the finding that the cytotoxic response to incubation of T cells with thymic factors was completely blocked by antibody directed against the IL-2 receptor 7D4 or 3C7.[15] By contrast, incubation of T cells with antibodies that do not block cytokine receptors had no effect.

Further Purification

Crude thymic cell culture supernatants were subsequently purified by anion and cation exchange chromatography, which yielded a substantially homogeneous glycoprotein with an isoelectric point of 6.5. This newly identified factor appears to have comprehensive and executive level regulatory effects. The USDA termed the factor "lymphocyte T-cell immunomodulator" (LTCI).

In addition to stimulating IL-2, LTCI was found, in many assays and different cell lines, to stimulate production of several other cytokines including IFN-γ and IL-10. It was important, therefore, to distinguish LTCI biologically and biochemically from cytokines themselves. Results of a variety of immunoassays have shown LTCI to be distinct from IL-1, IL-2, IL-3, IL-4, IL-6, IL-7, and granulocyte colony-stimulating factor (G-CSF). Furthermore, preliminary sequence data show no homology with any known molecule.

Interleukin-2 production by the Jurkat cell line, a well-accepted IL-2 assay, was chosen as the potency test for LTCI (Figure 2).

Suppression of Viral (HIV) Replication, Induction of Apoptosis

Based on the foregoing results, it appeared that LTCI might be a candidate immunotherapeutic for immune deficient conditions. Acquired immunodeficiency syndrome (AIDS) in humans was an obvious target disease, for which FIV in cats is a model. However, all known immune modulators such as IL-2, G-CSF, etc, were known to potentiate replication of human immunodeficiency virus (HIV) in vitro. Therefore, LTCI samples were submitted to the AIDS testing laboratory at National Institutes of Health

Figure 2. Log phase Jurkat cells were incubated with LTCI for 48 hours. IL-2 was determined by bioassay after a 4-hour additional incubation with CTLL-2 indicator cells in triplicate wells. The colorimetric reaction was read at 450 nm.
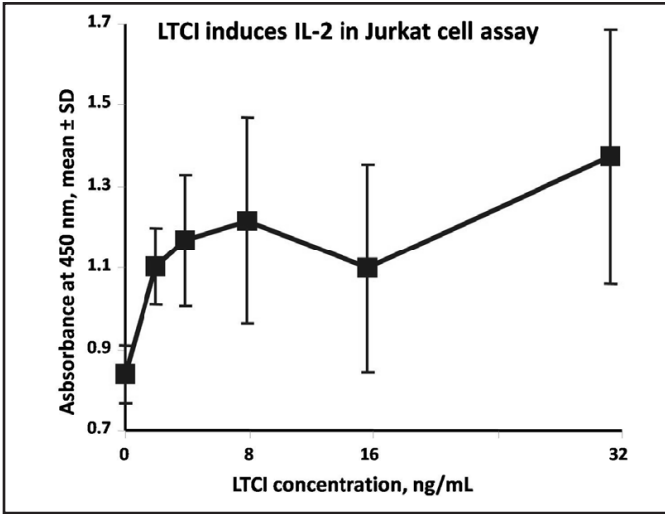
Figure 3. LTCI reduced virus load in HIV-infected peripheral lymphocytes, as measured by p24 antigen. In this assay, traditional chemokines enhance virus production.
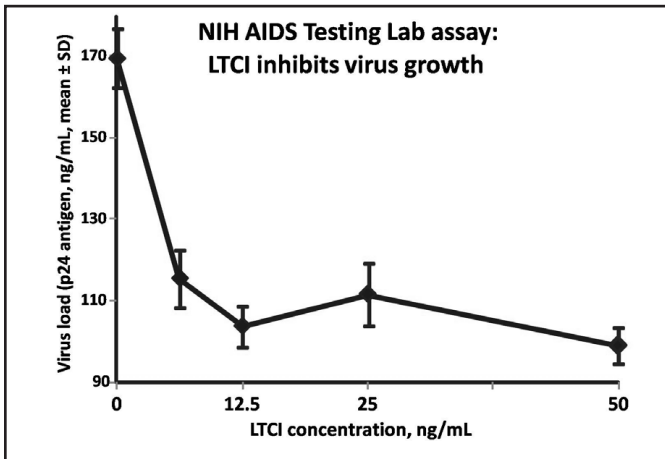
(NIH) to test its effect on virus replication and cell viability. In this standard NIH assay, all chemokines tested induced increased production of HIV by virally infected peripheral blood lymphocytes. In contrast, LTCI, which is not a traditional cytokine, had a suppressive effect on virus production (Figure 3). This effect occurred too quickly to be accounted for by cell-mediated killing. When cell viability data were examined, there was a correlation between cell killing and the number of HIV-infected cells in culture. These results suggested, and other data confirmed, that LTCI directly induces programmed cell death (apoptosis) of virally infected cells.

IN VIVO DATA

Results of in vivo studies support the in vitro data and basic mechanisms that translate into biological properties of LTCI, including antiviral and hematopoietic effects. Treatment effects were studied in laboratory animal models, including cats with experimentally induced FIV infections.

Murine Influenza Model

Influenza is an immunosuppressive virus against which protection and recovery depends on both humoral antibody- and cell-mediated immunity. In experimentally infected mice, LTCI treatment enhanced the primary antibody response 8-fold. However, the cell-mediated response to influenza is critical for recovery from infection. In order to test the effect of LTCI on cell-mediated immunity, lymphocytes from influenza-infected, LTCI treated mice were tested for cytotoxic killer cell response. The killing activity as measured by target cell lysis was enhanced at least 9-fold in lymphocytes from LTCI-treated mice (Figure 4).

Platelet Recovery

Based on the coincidental finding of increased platelets in severely thrombocytopenic, virus-infected cats, platelet recovery studies were conducted in a chemotherapy (carboplatin)-induced thrombocytopenic model in mice. In carboplatin-treated mice, LTCI treatment resulted in significantly increased platelet counts as illustrated in Figure 5.

Figure 4. Killing activity of lymphocytes from influenza-infected mice with or without LTCI treatment as measured by target cell lysis.
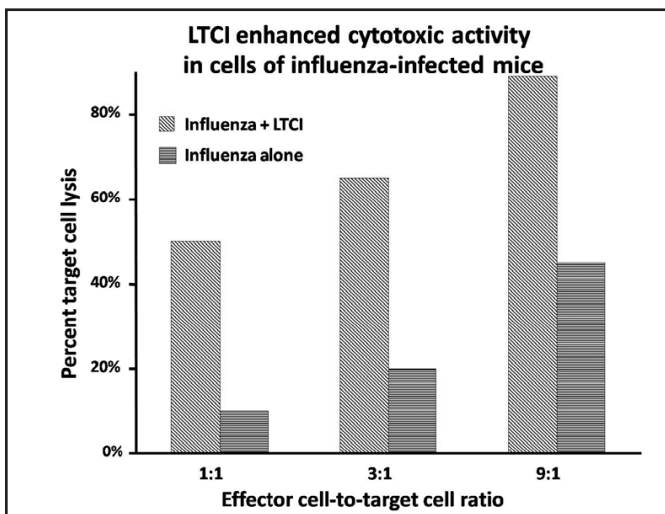
Figure 5. Platelet counts in chemotherapy (carboplatin)-induced thrombocytopenic mice.
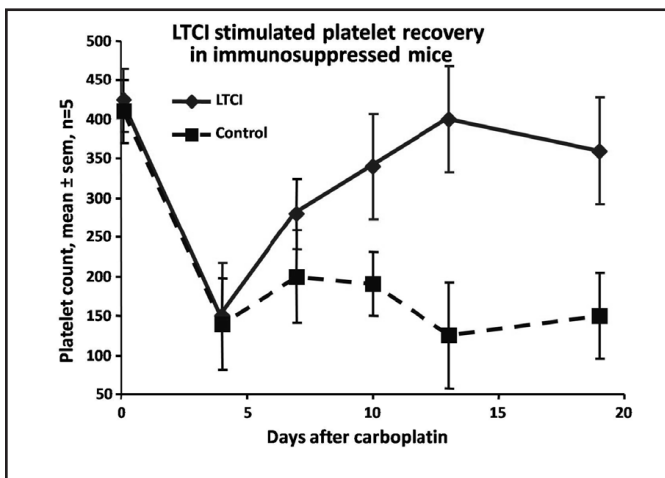
APPLICATIONS IN VETERINARY MEDICINE

The clinical relevance of LTCI treatment was further explored in a series of experiments in vaccine trials in dogs and also preliminary clinical studies in FIV- and FeLV-infected cats.

Rabies Virus Challenge Model in Dogs

In dogs vaccinated with a killed rabies virus vaccine plus LTCI, antibody response was enhanced at least 5-fold compared with titers in vaccinated dogs using conventional adjuvant (Figure 6).[15] Moreover, in the standard NIH rabies challenge model in mice, LTCI treatment resulted in significantly enhanced survival (data not shown).

Distemper Virus Challenge Model in Dogs

Treatment with LTCI improved survival in live virus-vaccinated dogs challenged with virulent distemper virus compared to vaccinated dogs given conventional adjuvant (alum) or non-vaccinates (Figure 7).[15]

## FELINE STUDIES

FIV Controlled Study #1

Eleven FIV-infected cats, confirmed to be viremic and FIV antibody-positive, were randomly assigned into control (n = 5) and treated (n = 6). Many, but not all, cats were lymphocytopenic at baseline. These cats were administered either saline or LTCI injections at 0, 14, and 28 days. Blood samples for hematology determinations were taken weekly, and clinical observations were made daily for 5 weeks. Significant increases in lymphocyte counts relative to controls were detected in the LTCI treatment group. Clinically, more rapid recovery from respiratory infections was observed in LTCI-treated cats. Finally, examination of blood and bone marrow confirmed that LTCI-treated cats had improved viremic status and bone marrow cellularity compared with untreated controls.

FIV Controlled Study #2

In a separate study, a group of 11 chronically FIV-infected cats (2+ years old) were randomized into control (n = 5) and treatment (n = 6) groups and given 3 weekly injections of either saline or LTCI. These cats were all in the latent stage of the disease both clinically and by laboratory measurement. Feline immunodeficiency virus load

Figure 6. Anti-rabies antibody titers in dogs immunized with killed rabies vaccine with or without LTCI.
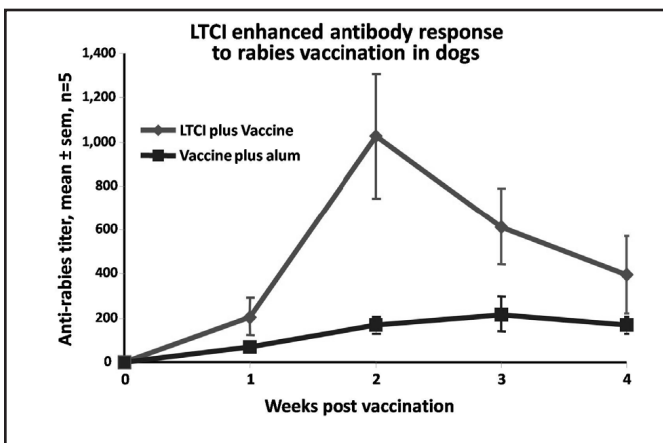
Table 1. LTCI increased lymphocytes in FeLV- or FIV-infected cats (mean ± SEM, n = 22).

	Baseline	4 Weeks	*P* Value
Total leuko-cytes × 10³/μL	15.49 ± 2.11	14.4 ± 1.6	NS
Lymphocyte %	22.8% ± 3.3%	28.5% ± 2.3%	0.041
Lymphocyte count per μL	2,751 ± 318	3,742 ± 408	0.004
Red blood cells × 10⁶/μL	6.68 ± 0.45	6.95 ± 0.39	NS
Clinical score*	8.7 ± 0.8	4.8 ± 1.0	0.005*

NS = not significant.
*Non-parametric signed rank test.

Table 2. LCTI increased lymphocytes and red blood cells (RBCs) in FeLV- or FIV-infected cats (n = 22).

Parameter		Baseline	After Treatment	
	n	Count	Count	% Change
Leukopenia (<3.9)	3	3.5 ± 0.27	5.7 ± 1.23	61.3%
Leukocytosis (>16)	10	24.3 ± 2.4	18.8 ± 2.4	-22.5%
Lymphopenia (<1,300)	4	992 ± 151	2,376 ± 638	139.6%
Anemia (RBCs <5.3)	5	3.7 ± 0.84	4.8 ± 0.81	29.2%

was determined before and 28 days after the start of LTCI treatment by quantitative polymerase chain reaction (PCR). Viral load was reduced in 4 of 6 cats treated with LTCI, by an average of 46% from baseline. In contrast, only 1 of 5 cats showed a reduction in FIV viral load as determined by PCR.

Field Efficacy Study in Naturally Infected Cats

The objective of this clinical field study was to determine the effects of biweekly treatments of LTCI in cats with confirmed FeLV or FIV infections. Blood samples were collected from each cat at baseline for complete blood count and confirmation of FIV or FeLV infection. Clinical signs of gingivitis, diarrhea, rhinitis, weight loss, fever, anorexia, lymphadenopathy, conjunctivitis, abscesses, and skin lesions were monitored according to a weighted numerical scoring system. Injections of LTCI were given subcutaneously after the initial work-up and every 2 weeks thereafter. Complete blood counts and clinical score evaluations were repeated after 3 injections. Adverse events were monitored throughout the study. A total of 23 FIV- or FeLV-infected cats entered the study of which 22 qualified and completed as scheduled. No significant adverse reactions attributable to LTCI treatment were detected. Treatment with LTCI resulted in improvements in both clinical and hematological parameters as summarized in Table 1.
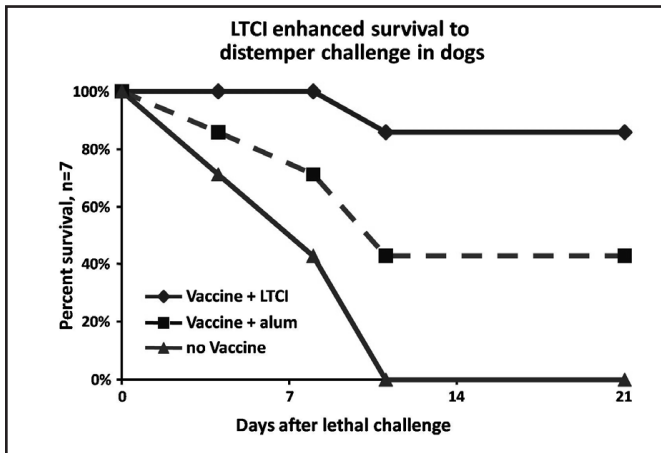
The overall percentage improvement in hematology and clinical findings in this field study in FIV- or FeLV-infected cats are summarized in Figure 8.

In this population of 22 cats, only 4 presented with lymphocytopenia, 3 with leukopenia, and 5 with anemia, all of which improved markedly after LTCI treatment

(Table 2). Interestingly, 10 cats (45%) presented with leukocytosis, which improved in most cats, but none had lymphocytosis at baseline. These results suggest that in FIV- and FeLV-infected cats, hematology parameters are highly variable and that clinical findings must also be relied on to assess treatment effects.

Thus, a 3-dose regimen of LTCI resulted in improved clinical signs in FIV- or FeLV-infected cats. In lymphocytopenic and anemic cats, which were surprisingly rare in this population, lymphocyte and erythrocyte counts also improved markedly.

Figure 7. Percent survival of dogs vaccinated with a live distemper vaccine plus either LTCI or conventional adjuvant (alum) or not vaccinated (controls), then challenged with virulent distemper virus.

Figure 8. Percentage of overall improvement in hematology and clinical parameters in field cases of FIV- or FeLV-infected cats treated with LTCI.
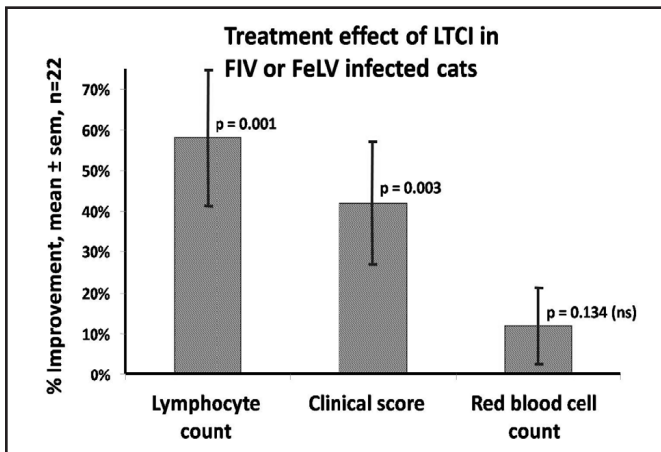
DISCUSSION

Lymphocyte T-cell immunomodulator is a selective, committed stem cell promoter, whose primary therapeutic effects are mediated through the activation and control of progenitor CD-4 helper lymphocytes to mature and produce cytokines including IL-2 and IFN. The availability of LTCI represents a new strategy for managing infectious disease through intervention at the cellular level. Although treatment with cytokines themselves (IL-2 or IFN) is an immunologically rational approach, neither cytokine was effective by itself in FeLV-induced immunodeficiency syndrome.[16] Treatment with orally administered recombinant or natural IFN-α met with little success in experimentally induced FeLV,[17] whereas recombinant feline IFN-ω, which is now commercially available, was more promising in symptomatic FeLV/FIV co-infected cats.[18] In contrast to treatment with cytokines themselves, LTCI represents an upstream approach, resulting in endogenous production of these same regulatory cytokines, potentially resulting in a more natural intervention.

The results of immunopharmacologic investigations and of preliminary clinical studies in cats are compelling. However, more experience under clinical field conditions in infected cats and other species is needed to further define the therapeutic relevance of LTCI. Clinical field studies in FeLV- or FIV-infected cats are complicated by the facts that the diseases differ from each other, cats present in different stages of the disease, and the subjects may or may not be showing clinical signs or hematological abnormalities at the time of examination.

In summary, the immunopharmacologic rationale for the role of LTCI as an Immunomodulator includes the following in vitro and in vivo evidence:

- LTCI induces immature T-cells to mature and produce IL-2, IFN-γ, and other cytokines
- In turn, IL-2 and IFN stimulate CD-8, cytotoxic, killer T-cell effects
- In a murine influenza model, LTCI treatment enhances both antibody- and cell-mediated, cytotoxic responses
- In a murine thrombocytopenic model, LTCI stimulates recovery of platelet counts
- LTCI suppresses virus (HIV) replication and induces programmed cell death (apoptosis)
- In canine rabies and distemper challenge models, LTCI enhances both antibody response and survival
- In experimentally induced FIV in cats, LTCI improved lymphocyte and erythrocyte counts, viremic status, and bone marrow cellularity
- In field cases of FIV or FeLV, LTCI treatment improved clinical signs and hematological parameters

A more precise understanding of the immunotherapeutic and clinical potential of LTCI must await results of well-controlled clinical studies in cats, which are required under the terms of the conditional license. Obtaining additional clinical experience is now possible with the commercial availability of LTCI.

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