Studies of the effect of acemannan on retrovirus infections: clinical stabilization of feline leukemia virus-infected cats

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Feline leukemia is a disease induced by an oncornavirus infection that inevitably causes clinically affected cats to die. It has been estimated that 40% of cats are dead within 4 weeks and 70% within 8 weeks of the onset of clinical symptoms. Acemannan is a complex carbohydrate with both immunostimulatory and direct antiviral properties. Administration of acemannan for 6 weeks intraperitoneally to clinically symptomatic cats significantly improved both the quality of life and the survival rate. Twelve weeks after initiation of treatment, 71% of treated cats were alive and in good health.

Keywords: Feline leukemia; acemannan; immunostimulant; cats; virus disease

Feline leukemia virus (FeLV) is an oncogenic retrovirus that, as the name implies, affects felids of all species. FeLV infections are associated with a variety of disease syndromes in cats. These include lymphoproliferative diseases such as leukemia and lymphosarcoma, cytosuppressive diseases such as immunodeficiency and aplastic anemia,[1] and tissue destructive diseases such as enteritis.[2] FeLV is now the most important severe cause of illness and death in domestic cats. About one third of feline cancer deaths are FeLV related.[3]

Not all cats that become infected with FeLV develop clinical disease. A significant proportion (42%) eliminate the virus, presumably as a result of a successful immune response.[4] This immune response appears to be age related; thus, 100% of newborn kittens exposed to the virus develop disease, but only 15% of exposed cats older than 4 months develop disease.[5] Cats that fail to cast off infection and thus become persistently viremic will eventually develop clinical disease and die. The precise disease syndrome that develops depends in part on the animal's own immune response. Of the clinical manifestations of infection, leukemia is, in fact, a relatively uncommon consequence; only 23% of FeLV-infected cats actually develop a T cell leukemia.[6] Much more commonly, infected cats develop a marrow aplasia reflected by aplastic anemia, leukopenia, or thrombocytopenia. As a result, FeLV-infected cats are profoundly immunosuppressed and suffer from a multitude of secondary immunodeficiency diseases such as pneumonitis, sepsis, skin diseases, and enteritis.[7] Multicentric lymphoma and lymphoma associated with the gastrointestinal tract are also seen.[8] FeLV-affected cats show severe anorexia, cachexia, and progressive weakness.

If a cat succeeds in making antibodies to the feline oncornavirus cell membrane antigen (FOCMA), malignancies will be prevented.[9] As a result, these cats will not develop lymphoma, lymphosarcoma, or leukemia. However, these cats will be unable to prevent development of cytosuppressive forms of FeLV-induced disease, and they will die as a result of aplastic anemia or pancytopenia.

Although only 30% of cats infected with FeLV actually develop clinical disease, such disease is uniformly lethal.[10] There is no effective therapy other than symptomatic treatment. Most affected cats receive antibiotics for bacterial infections and steroids for treatment of myeloid leukemia and for amelioration of thrombocytopenia. Cats with lymphosarcoma may receive steroids or cytotoxic drugs such as cyclophosphamide.

None of these treatments have more than a minimal effect on the animal's quality of life and survivability.[11] Since there is no effective treatment for this disease, the great majority (>90%) of clinically affected cats are euthanized within a few days of diagnosis. It

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has been estimated that 40% are dead within 4 weeks and 70% within 8 weeks of the onset of clinical signs.[12] Consequently, FeLV is the most common infectious cause of premature death of cats in the United States.

Acemannan is a water-soluble, long-chain polydispersed β-(1,4)-linked mannan polymer interspersed with O-acetyl groups. Its molecular weight ranges from 10,000 Da to greater than 1×10^6 Da. Acemannan is a potent immunostimulant clearly able to enhance macrophage release of interleukin-1α, tumor necrosis factor, and prostaglandin E_2.[13] It enhances macrophage phagocytosis and non-specific cytotoxicity.[14] Acemannan also enhances T cell function as demonstrated by the allogeneic response in rodent mixed lymphocyte culture.[14] Other studies have demonstrated that acemannan is an effective adjuvant when administered with antigen and produces morphological changes in lymphoid organs that are indicative of immune stimulation.[15] Acemannan, in addition to its immunostimulatory effect, also has a direct antiviral effect on some viruses. For example, it inhibits growth of HIV, Newcastle disease virus, and influenza virus *in vitro*.[13]

This study was undertaken to determine whether administration of acemannan influences the course of disease in cats infected with FeLV. The specific objective was to determine whether there would be a clinical response in symptomatic FeLV-positive cats treated with acemannan by intraperitoneal (IP) injection under clinical practice situations. A second objective was to determine the safety of acemannan in cats with feline leukemia complex.

Materials and Methods

Study population

The study population included 50 cats that were serologically positive for feline leukemia and that had at least two clinical signs associated with leukemia infection (*i.e.*, anemia, lymphosarcoma, sepsis, leukopenia, feline infectious peritonitis, thrombocytopenia, feline infectious anemia [*Hemobartonella* infection], severe weight loss, anorexia or lymphadenopathy). In addition, FeLV-infected cats that had failed to respond to conventional treatment and had appropriate clinical signs of disease were qualified to enter the study. Thus, all cats in the study were severely ill. These cats were presented by their owners at a large urban veterinary clinic and were otherwise unselected.

Experimental design

Ten cats were to have been randomly assigned to the control group and 40 cats to the treatment group. The investigators, however, amended the protocol and elected to delete the controls because owners insisted that their terminally ill animals be treated with acemannan. No owners would consent to treatment with placebo for fear their pets would die. Six cats failed to complete the study as a result of owner noncompliance. Forty-four cats were therefore included in the final study population. All 44 cats were treated for 6 weeks with acemannan IP once weekly at a dose of 2 mg/kg. The cats were again examined 6 weeks after termination of treatment.

Informed consent

Owners had to agree to have their pets treated with acemannan and to acknowledge this agreement by signing an informed consent form; in this form, they also agreed to return their cats for all assigned visits as required by the protocol.

Acemannan

Acemannan was prepared in kit form containing one 10 mg vial of lyophilized acemannan for injection and one 10 ml vial of normal saline diluent.

Physical examinations

Complete physical evaluations were performed weekly during the 6-week treatment period and then again at week 12. The clinical parameters especially noted included: overall condition and appetite, weight change, body temperature, and the presence of sepsis, lymphosarcoma, or lymphadenopathy. These clinical parameters were scored (*Figure 1*) and used to generate a global clinical score.

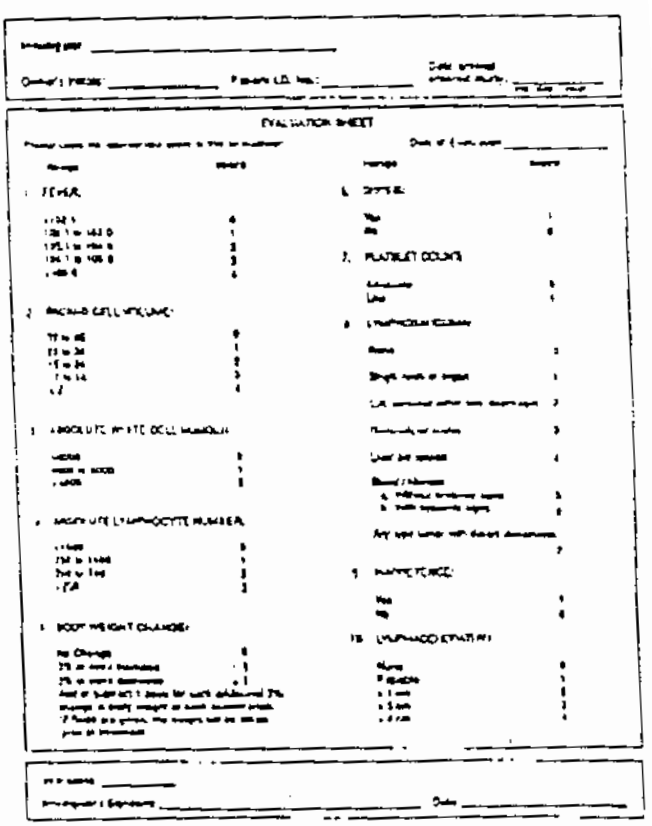

Figure 1. Evaluation sheet

Adverse event data were obtained as the study progressed and by checklist as a summary at the end of the study. Effectiveness was assessed by a clinical scoring system. Body weight at entry into the study was the starting point for determining body weight change. All other scores were determined from laboratory data and by clinical examination and observation.

Hematology and serology

Hematological examination included complete red cell counts, hematocrit, hemoglobin, differential white cell, and estimated platelet counts. Serological tests for FeLV antigenemia were performed by a national veterinary reference laboratory. The test employed was a commercial ELIZA test (CITE® Idexx).

Clinical pathology

Blood chemistry values measured included glucose, BUN, uric acid, alkaline phosphatase, inorganic phosphorus, SGOT, creatinine, CPK, albumin, total bilirubin, total protein, calcium, LDH, SGPT, and cholesterol. Laboratory values were obtained at the beginning of the study and then again at weeks 6 and 12 (*Figure 1*). Study parameters consisted of history, exam, complete blood count, and a biochemical panel.

Statistical analysis

Data were analyzed by means of a Wilcoxon signed-ranks test. In addition, data were examined using the Friedman and Kepner-Robinson analyses of variance.

Results

Survival

At the end of the 12-week study, 29 acemannan-treated cats were known to be alive. Two of the original 44 were lost to follow-up, and one other died of an unrelated cause, giving a 71% survival rate for those cats that completed the study (29/41).

Of 15 cats that died of FeLV-related disease, five died from malignancies or marrow aplasia within 9 days of entering the study. These cats can be considered to have been terminal—beyond rescue by any available therapy. Seven other cats died during the 12-week study, and three died within 4 weeks of completing the study. Analysis of 11 historical controls at the same clinic indicated that nine cats died or were euthanized within 2 months, and one other was dead within 5 months of being diagnosed with FeLV.

Two months after completion of the study, a telephone survey of owners was conducted. Attempts were made to contact owners of all surviving cats. Although the owners of five cats had moved and could not be located, owners of 22 cats were interviewed. One stated that his cat had died 4 weeks after the 12-week follow-up, but the remaining 21 cats were reported alive and well (*Table 1*).

Table 1. Survival at time of owner follow-up

Clinical status		No. of cats
Known survivors		21
Weeks after beginning treatment	No. of cats still alive	
35–39	3	
30–34	8	
25–29	6	
20–24	4	
Dead		15
Non-compliant		3
Lost to follow-up		5
Total		44

All owners of surviving cats reported that they were pleased with the results of treatment, stating that their cats had returned to their normal state of activity and were healthy, happy pets (up to 39 weeks after entry into the study). The owner of the cat that died 4 weeks after the study (10 weeks after the final acemannan injection) said that he was encouraged by his cat's having shown improvement during treatment, but that his pet began to deteriorate after the 12-week follow-up.

Clinical status

The clinical entry global mean score for the test cats was 4.65 and for the historical controls was 4.857. The mean score for treated animals at week 6 was 3.071, whereas the mean score at 12 weeks was 2.500. This score was derived from both subjective and objective analysis (*Figure 1*). The change was not statistically significant for the entire test group. If, however, the age of the cats was taken into account, it was found that overall clinical scores were significantly improved ($P = .049$) for cats under 4 years of age.

Further analysis of the clinical scores indicate that there was a progressive improvement in subjective clinical scores in treated animals. Each animal's appetite significantly improved ($P = .0022$) by 12 weeks. Incidence of sepsis was significantly reduced ($P = .0329$) in treated animals at 6 and 12 weeks, suggesting that the animals had an enhanced immunological function.

Hematology and serology

When hematological parameters were analyzed, a consistent trend was clearly apparent. There was an improvement in major blood parameters during the 6 weeks the cats were under treatment. Median hematocrit values rose from 30.85 to 32.20, hemoglobin rose from 9.80 to 10.95, red cell counts rose from 6.99 to 7.13 $\times$ 10^6/mm^3, while total leukocyte and lymphocyte counts declined (12.95 to 12.15 $\times$ 10^3/mm^3 and

1998 to 1862, respectively). Although these hematological changes were uniform, they were not statistically significant. Hemoglobin levels were significantly improved ($P = 0.0469$) at 12 weeks, while eosinophil levels climbed significantly ($P = 0.018$) between 6 and 12 weeks.

All animals showed FeLV antigenemia at the beginning of the study. One cat converted to viremia negative following 6 weeks of treatment but had reverted to seropositive by 12 weeks.

Clinical pathology

Clinical pathology scores showed statistically significant increases for albumin ($P = .043$) and total serum protein ($P = .041$) between weeks 6 and 12. Alkaline phosphatase levels in cats younger than 4 years of age were significantly increased between weeks 0 and 6 ($P = .052$), but the values remained within the normal range.

Discussion

The results of this study show clearly that acemannan-treated cats lived significantly longer than the historical controls. In addition, enhanced quality of life of these animals was reflected in their improved health and increased activity. The increase in serum proteins was associated with the improved nutritional status of all cats, and lack of sepsis was indicative of their well being. Owner satisfaction with the therapy was very high.

In this trial, a placebo-treated control group of cats was not employed because owners would not agree to participate if they were not assured that their terminally ill cats would receive the drug. Study of historical controls in the same clinic indicated that 10 out of 11 infected cats not included in the trial died within 64 days of diagnosis. Nine of the 10 animals were euthanized. One died as a result of anemia and a probable lymphosarcoma. Unfortunately, the prognosis of FeLV-infected cats is so poor that euthanasia is considered to be an appropriate response to a diagnosis of the disease. For this reason, it is difficult to determine what the survival rate of these cats would have been had they been allowed to live.

In an attempt to enlarge this control group, an analysis of 46 recent admissions to the Texas A&M College of Veterinary Medicine Small Animal Clinic was made. All 46 cats had clinical signs of FeLV disease and would have been eligible for entrance to this study. Of these 46 animals, 40 were euthanized or died within 5 days of a diagnosis of feline leukemia, five more were dead by 10 days, and only one animal was known to have survived for as long as 5 months.

Perhaps the most reliable figures in this respect come from Povey, who in 1976 reported that 40% of cats with clinical feline leukemia were dead by 4 weeks while 70% were dead by 8 weeks following onset of clinical symptoms.[12] Tompkins and Cummins reported that although 22% of cats diagnosed as suffering from feline leukemia eventually recover clinically, "the great majority" were euthanized within a week.[16] On this basis, it is clear that acemannan enhanced survival of treated cats (*Figure 2*).

The reason for the clinical improvement seen in treated cats cannot be traced directly to any one hematological or clinical parameter. Hematocrit, hemoglobin, and RBC counts all increased slightly, suggesting that the bone marrow function was improved. The known antiviral affect of acemannan as well as its immunostimulatory properties may have played a role in reducing virus burden in these animals.

Mannans probably have at least three mechanisms of antiviral activity *in vivo*. First, they are potent interferon inducers.[17–19] A mannan derived from *Candida albicans* for example, induced high levels of interferon activity two hours after intravenous administration. Second, acemannan has potent antiviral activity against HIV, Newcastle disease virus, and influenza. This has been attributed to interference with viral glycosylation.[20] Third, mannans are known to be potent immunostimulants.[21,22] Acemannan enhanced the ability of mouse T cells to respond to mitogenic stimuli, probably as a result of promoting the release of helper factors from macrophages.[13] Acemannan has been demonstrated to induce the production of interleukin-1 and tumor necrosis factor from macrophages in mice.[14] Other mannans have been demonstrated to stimulate cytotoxic activities of macrophages, natural killer cells, and cytotoxic T cells. They have been shown to promote interleukin-1 production by macrophages and to enhance antibody production.[23]

Currently, available therapy for feline leukemia is unsatisfactory. Cytotoxic agents that have been em-

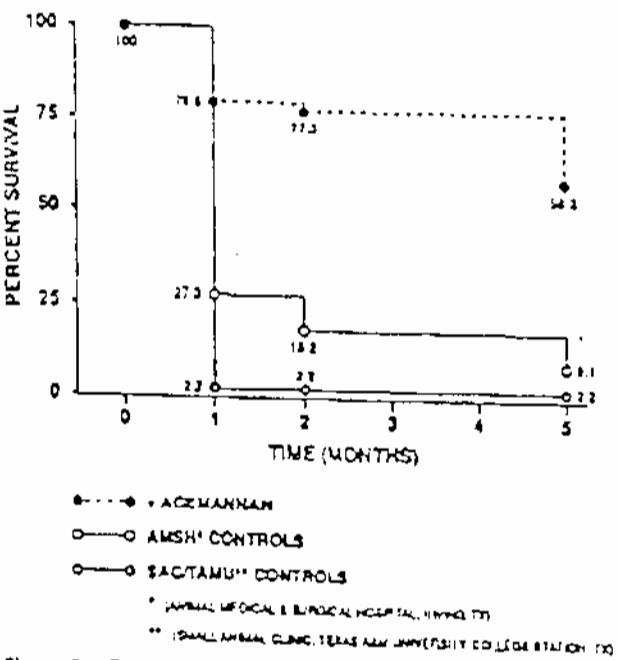

Figure 2. Survival curves for acemannan treated cats together with two groups of historical controls.

ployed include methotrexate and cyclophosphamide as well as corticosteroids such as prednisolone and betamethazone. The use of multiple cytotoxic drugs in these cats has not been successful in inducing more than temporary remission.[11]

The published literature, however, suggests that immunostimulants may be of significant benefit in treating clinically ill FeLV-infected cats. In a recent publication, it was reported that inactivated *Propionobacterium acnes* (ImmunoRegulin®) was of benefit in the treatment of clinical FeLV.[24] The mechanism of action of killed *Propionobacterium acnes* is unclear although it also probably acts as a potent stimulator of interleukin-1 production. Staphylococcal protein A has also been used to treat small numbers of FeLV-infected cats. It appears to act as a bone marrow stimulant and reduce viral burden as well as increase chemiluminescent responses in affected cats.[25] Staphylococcal protein A acts to remove immune complexes and is also an interferon inducer. It may be that interferon plays a critical role in some aspects of resistance to FeLV. A bovine β-interferon appeared to have a beneficial effect on FeLV-associated nonregenerative anemia and caused significant clinical improvement.[16] However, only four cats were treated in this study.

In addition to their direct and indirect antiviral effects, mannans are well recognized as having bone marrow stimulating activity.[26] Thus, mannans possess a combination of antiviral, immunostimulating, and bone marrow stimulating activities that are perfectly tailored to the treatment of FeLV. The results of only 6 weeks of treatment must be considered dramatic when measured against the usually poor prognosis of this disease. Undoubtedly, a much longer treatment period would have produced even more dramatic results although the prognosis for acemannan-treated FeLV-diseased cats obviously depends upon the initial clinical state of the cat. It is clear from the results of this study that additional studies on the use of acemannan for the treatment of FeLV are warranted. The significant improvement in viability as well as the overall health of the treated cats suggests that acemannan is an effective treatment of FeLV infection.

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