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Alpha Interferon (2b) in Combination with Zidovudine for the Treatment of Presymptomatic Feline Leukemia Virus-Induced Immunodeficiency Syndrome

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The therapeutic efficacies of human recombinant alpha interferon (IFN-α), IFN-α plus zidovudine (AZT), and AZT alone were evaluated in presymptomatic cats with established feline leukemia virus (FeLV)-acquired immunodeficiency syndrome (FAIDS) infection and high levels of persistent antigenemia. Subcutaneous injection of 1.6×10^6 U of human recombinant IFN-α 2b per kg delivered peak concentrations in plasma of 3,600 U/ml at 2 h postadministration with a half-life of elimination of 2.9 h. This dosage of IFN-α could be delivered to cats for up to 12 weeks without significant clinical toxicity. Oral administration of AZT (20 mg/kg three times daily) resulted in peak concentrations in plasma of 3 μg/ml at 2 h with a half-life of elimination of approximately 1.60 h. Treatment of FeLV-FAIDS-infected cats with IFN-α, either alone or in combination with orally administered AZT, resulted in significant decreases in circulating p27 core antigen beginning 2 weeks after the initiation of therapy. AZT alone had no effect on circulating virus antigen. Depending upon whether high (1.6×10^6 U/kg)- or low (1.6×10^4 to 1.6×10^5 U/kg)-dosage IFN-α was used, cats became refractory to therapy 3 or 7 weeks after the beginning of treatment. At these times, IFN-α-treated animals developed antibodies to IFN-α that were neutralizing, specific for human recombinant IFN-α, and dose dependent in magnitude. The results of this study indicate that human recombinant IFN-α is effective in reducing circulating virus antigenic load in cats persistently infected with FeLV-FAIDS. However, the continued efficacy of IFN-α therapy appeared to be limited by the formation of cytokine-specific neutralizing antibodies.

Feline leukemia virus (FeLV)-induced acquired immunodeficiency syndrome (FAIDS) is a frequent cause of death in FeLV-infected cats, 83% of which die within 3.5 years of initial diagnosis, often from opportunistic infections (17). We have characterized a naturally occurring isolate of FeLV (FeLV-FAIDS) which induces persistent viremia and FAIDS without concomitant leukemogenesis when inoculated into specific-pathogen-free cats (19). Most animals infected at 15 weeks of age or more develop a chronic immunodeficiency disease characterized by a prolonged asymptomatic period (6 months to 1 year), progressive depletion of $CD4^+$ and colony-forming T lymphocytes, and suppression of T cell-dependent antibody response; this is followed by persistent diarrhea, cachexia, and opportunistic infections in the terminal stages of the disease (19, 32, 37; E. A. Hoover, S. L. Quackenbush, C. D. Ackley, G. A. Dean, P. R. Donahue, D. Pardi, G. N. Callahan, J. I. Mullins, and M. D. Cooper, XIV Int. Symp. Comp. Res. Leukemia and Related Disorders, p. 84, 1989). Thus, the clinical signs and specific immunological features of FeLV-FAIDS-induced immunodeficiency disease mirror those present in human acquired immunodeficiency syndrome (AIDS) (9).

The pathogenesis of FeLV-FAIDS involves viral replication in the bone marrow and systemic lymphoid tissues, lymphoid follicular hyperplasia, and the onset of antigenemia by 3 weeks postinoculation (19). Preceding the onset of clinical immunodeficiency syndrome, high levels of the major FeLV-FAIDS pathogenic variant virus are detected, principally as unintegrated DNA, in bone marrow, intestines, and systemic lymphoid tissues (32). Studies in which viral chimeras have been used have implicated the envelope gene of the pathogenic variant as encoding lymphocytopathic determinants of FeLV-FAIDS (33). As with FeLV-FAIDS, disease progression in humans with human immunodeficiency virus (HIV) infections may correlate with the expression of more cytopathic variants (7, 14, 41, 47).

Alpha interferon (IFN-α) (2b) has been shown to be a potent inhibitor of both de novo HIV infection in vitro and to prevent the expression of HIV in chronically infected cell lines (18, 36). IFN-α acts on the terminal stages of virus production by preventing assembly and budding of mature virions (36). Likewise, 3′-azido-3′-deoxythymidine triphosphate (AZT) has been shown to bind to reverse transcriptase and potently inhibit the infectivity and cytopathic effect of HIV types 1 and 2 in a variety of cell types (15, 30, 31, 35), although this activity appears to be limited to acutely infected cells, and virus replication in vitro and in vivo continues, despite continuous AZT administration (2, 13, 39, 44). The use of IFN-α alone and in combination with AZT in other mammalian retrovirus infections is limited, but similar to its application for HIV infections, these agents act synergistically to prophylactically prevent murine and feline retrovirus infections (42, 51). Phase I studies in humans have indicated a half-life of IFN-α in plasma of 2 to 3 h following subcutaneous injection, with dose limitations related to pyrexia, lethargy, myalgia, and hematological toxicity (38, 48). Initial clinical trials with IFN-α against HIV-associated Kaposi sarcoma have been encouraging in terms of antiviral and antitumor responses, significant increases in $CD4^+$ cells,

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and decreases in circulating viral antigen in those patients who respond to therapy (4, 10, 23).

The potent in vivo prophylactic antiviral activity of IFN-α against FeLV-FAIDS and Rauscher murine leukemia viruses (3, 42, 51) as well as the reported activity of IFN-α A/D against feline herpesvirus replication (50) prompted us to study the effect of IFN-α alone and in combination with AZT in treating persistent viremia in the FeLV-FAIDS model. In the study reported here, we first investigated the pharmacokinetics of IFN-α administered subcutaneously and then studied its therapeutic efficacy, either alone or in combination with AZT, in cats that were persistently infected with FeLV-FAIDS. Our results demonstrate the potential usefulness of IFN-α to significantly decrease the circulating FeLV antigenemia. The potential long-term use of this human recombinant cytokine in cats was limited, however, due to the induction of specific antibodies which completely neutralized its continued effectiveness.

MATERIALS AND METHODS

Animals. All animals used in this study were from a breeding colony of cesarean-derived specific-pathogen-free cats maintained at Colorado State University. These animals were age-matched, 8 months of age at the time of inoculation, and free of infection and immunity to horizontally transmitted feline viruses. Cats derived from this colony have been used previously to characterize the pathogenicity of FeLV-FAIDS (19, 32, 33).

Virus inoculation. The virus inoculum used in this study consisted of the molecularly cloned, pathogenic, replication-defective FeLV-FAIDS clone 61C, which was rescued by cotransfection with its replication-competent counterpart FeLV-FAIDS clone 61E (33). The molecular clones used therefore represented the essential retroviral genomes contained within the highly infectious and pathogenic tissue origin field isolate of FeLV-FAIDS (19). FeLV-FAIDS 61E/C was produced from AH927 feline fibroblasts that were cotransfected with clones 61C and 61E, which are expressed in approximately equal representations (33). The infectious inoculum used in vivo contained 6×10^5 focus-forming units and was injected intraperitoneally. After inoculation with FeLV-FAIDS, cats were tested weekly for the presence of circulating p27. The cats used in this study were persistently viremic for at least 12 weeks before undergoing experimental therapy.

Antiviral compounds. AZT was provided by Burroughs Wellcome Co., Research Triangle Park, N.C., through the Developmental Therapeutics Branch, AIDS Program, National Institute of Allergy and Infectious Diseases, Bethesda, Md., under the auspices of Margaret I. Johnston. Human recombinant IFN-α (2b) (2×10^8 U/mg) was provided by Schering Plough Research Inc., Bloomfield, N.J., by Jerome Schwartz. IFN-α (2b) is highly purified, has been shown to be free of contamination by *Escherichia coli* proteins, and has not been shown to elicit anti-*E. coli* antibody in patients given IFN-α parenterally (J. Schwartz, personal communication).

In vivo treatment protocol. Cats were randomly divided into five treatment groups (group 1, AZT only, $n = 6$; group 2, AZT plus IFN-α, $n = 6$; group 3, IFN-α only, 10^6 U/kg, $n = 3$; group 4, IFN-α only, 10^5 U/kg, $n = 9$; group 5, IFN-α only, 10^4 U/kg, $n = 3$). All treatment regimens were begun when cats were persistently viremic for at least 12 weeks. Cats were treated by oral administration of AZT (20 mg/kg) given three times daily. This dosage was reduced to 10 mg/kg three times daily from days 28 to 49 and was then increased to 20 mg/kg three times daily until the end of the treatment period (77 days). IFN-α pharmacokinetic studies were performed in two cats during the first 24 h prior to inoculation with FeLV-FAIDS.

IFN-α was administered concurrently with AZT at a dosage of 1.6×10^5 U/kg. Depending on the treatment group, IFN-α given alone was injected subcutaneously once daily at dosages that ranged from 1.6×10^6 to 1.6×10^4 U/kg for the entire 77-day treatment period.

Hematologic parameters were monitored weekly in all cats for the duration of the study.

Determination of IFN-α in plasma. Pharmacokinetic analysis was accomplished by serial blood sampling over a 24-h period after subcutaneous administration of IFN-α at 1.6×10^6 U/kg. Posttreatment plasma samples were analyzed by using a vesicular stomatitis virus (VSV) inhibition microassay. Briefly, Crandell feline kidney cells (3×10^4 cells per well) were plated for 24 h in 96-well plates (25860; Corning Glass Works, Corning, N.Y.). Monolayers were then washed with phosphate-buffered saline before twofold dilutions of test sera (100 μl in triplicate wells) were applied in parallel with IFN-α at dilutions ranging from 25,000 to 39 U/ml. After 24 h, the monolayers were again washed with phosphate-buffered saline and challenged with 100 50% tissue culture infective doses of VSV (Indiana strain) in a 200-μl total volume. The virus control wells were monitored for complete cytopathic effect, and the assay was stopped at 18 to 24 h postinoculation using a crystal violet-Formalin dye solution, as outlined by Rubinstein et al. (40). The monolayers were then air-dried, the dye was eluted with an ethylene glycol monomethyl ether solution (Sigma Chemical Co., St. Louis, Mo.), and the A_{590} was read using a modification of a procedure described by Armstrong (1). Percent dye uptake of the IFN-α standards were calculated, and test values (in units per milliliter) were determined from the standard curve. The pharmacokinetic curve was then analyzed on NONLIN, a Fortran program for obtaining polyexponential parameter estimates, as outlined by Metzler et al. (29).

Detection and quantitation of circulating FeLV p27 antigen. FeLV p27 antigen was measured by enzyme-linked immunosorbent assay using monoclonal antibodies (p27 A2 and B3) developed by Lutz et al. (28) and supplied by Niels C. Pedersen, University of California, Davis. Antigen-binding antibody at 2 μg/ml was used to coat 96-well microdilution plates, 50 μl of test serum was added to each well, and 50 μl of a second, horseradish peroxidase-conjugated FeLV p27 monoclonal antibody was added and incubated for 30 min. The plates were then rinsed and blot dried, and 50 μl of 3,3′,5,5′-tetramethylbenzidine in the presence of a peroxidase substrate solution (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) was added per well. After 10 min, the reaction was read and the A_{650} was determined. Test well reactions were considered positive if an absorbance value of greater than 0.100 was obtained. Background readings were obtained with FeLV-negative, specific-pathogen-free cat serum.

Serial twofold dilutions of affinity column-purified p27 (purified with monoclonal antibody A2, cyanogen bromide-activated Sepharose 4B [Pharmacia, Uppsala, Sweden]) in 2% bovine serum albumin–0.1% Triton X-100–TEN (0.05 M Tris, 0.001 M EDTA, 0.15 M NaCl [pH 7.4]) buffer were reacted in the aforementioned antigen-binding antibody assay and developed with 3,3′,5,5′-tetramethylbenzidine. A standard curve was delineated, and sample values (in nano-

grams per milliliter) were derived from this curve. Background readings were 30 ng/ml.

Detection and quantification of IFN-α neutralizing antibody. An antibody-capture enzyme-linked immunosorbent assay was developed to detect IFNα-specific antibody in cat serum. Antibody-binding antigen at 75 ng/ml was used to coat 96-well microdilution plates; serial dilutions of heat-inactivated (56°C for 30 min) sera (duplicate wells) were incubated at room temperature for 60 min. The plates were then washed and reacted with goat anti-cat immunoglobulin G (IgG; heavy and light chains) conjugated with horseradish peroxidase (Organon Teknika Corp., Durham, N.C.) for 60 min at room temperature. The plates were then washed and blot dried, and 50 μl of *o*-phenylenediamine in the presence of 30% hydrogen peroxide was added to each well. The reaction was stopped with 50 μl of 2.5 N H_2SO_4, and the A_{490} was determined. Twofold dilutions of monoclonal antibody to IFN-α (Accurate Chemical and Scientific Corp., Westbury, N.Y.) were run in parallel with test serum, and the neutralizing activity of unknown test serum (neutralizing units per milliliter) was determined from this standard curve.

Specific neutralizing activity was determined by modifications of the feline interferon microassay. Briefly, CrFK monolayers were prepared as described above for the standard VSV inhibition assay, and after 24 h twofold dilutions of interferon ranging from 10,000 to 175 U/ml were plated in 50 μl of medium. Heat-inactivated (56°C for 30 min) test serum was added at a 1:20 dilution and incubated with IFN-α overnight at 37°C. The plates were then washed, and test wells (samples were run in duplicate) were challenged with 100 50% tissue culture infective doses of VSV.

Immunoblot analysis of IFN-α antibody. Sequential serum samples were obtained from cats that were treated with AZT, IFN-α at 1.6×10^6 U/kg in combination with AZT, and IFN-α given alone at 1.6×10^5 U/kg (the treatment protocol was described above). Purified recombinant IFN-α (12 μg) was fractionated by electrophoresis with a 10% polyacrylamide slab gel in the presence of sodium dodecyl sulfate. The protein bands on the gel were transferred to a polyvinyldifluoride membrane (Immobilon; IPVHOOO10; Millipore Corp., Bedford, Mass.) for 90 min. After transfer, the membrane was blocked overnight using 10% non-fat dried milk (Blotto) in 0.01 M phosphate-buffered saline (pH 7.4). This sheet was then cut into strips, and each strip was washed three times with Western immunoblot diluent and wash buffer composed of 0.01 M phosphate-buffer saline, 0.5% Tween 80, 0.35 M NaCl, and 0.001 M EDTA · $2H_2O$ (pH 7.2) (WDWB). The strips were then incubated with a 1:100 dilution of cat serum in 10% Blotto-WDWB for 1 h at room temperature. The strips were then washed three times in WDWB and reacted with a 1:500 dilution of goat anti-cat IgG (heavy and light chains) horseradish peroxidase (3203-0081; Organon Teknika Corp.) that was diluted in 10% Blotto-WDWB for 60 min. After the strips were washed, they were incubated with 1 ml of 3,3',5,5'-tetramethylbenzidine (Kirkegaard Perry Laboratories), which was used as the substrate. This color reaction was then stopped with distilled water.

Statistical analysis. Significant differences in the mean levels of p27 in serum as well as neutralizing antibody titers were determined by Student's *t* test. *P* values less than 0.05 were considered significant.

RESULTS

IFN-α pharmacokinetics in plasma. Subcutaneous administration of IFN-α to two cats at a dose of 1.6×10^6 U/kg resulted in levels of 3,600 U/ml in plasma at 2 h postadministration and a plasma elimination half-life of 2.89 h (Fig. 1). Levels in plasma greater than 1,000 U/ml could be maintained in cats for up to 8 h postadministration. The average area under the curve was approximately 1.1×10^4 U · h/ml. No IFN-α was detectable in plasma at 12 h postadministration.

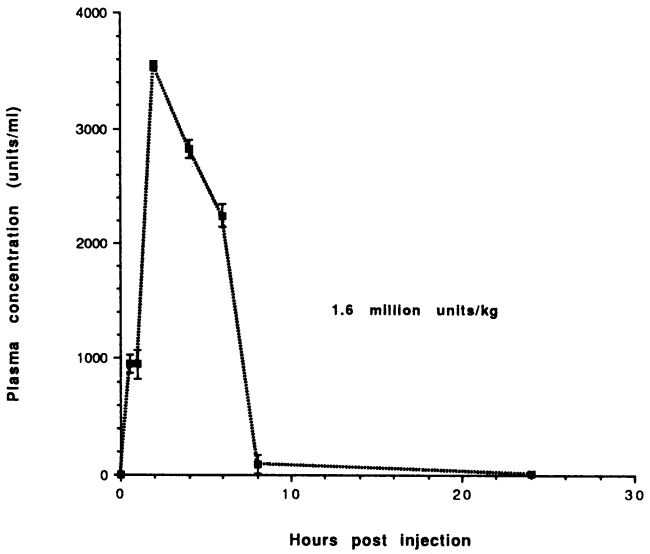

FIG. 1. Pharmacokinetics of IFN-α. Analysis of plasma from cats ($n = 2$) after subcutaneous administration of 1.6×10^6 U of IFN-α per kg. Values derived from a VSV inhibition assay represent the means of triplicate wells for each time point. Standard error bars represent the standard error of the mean.

Treatment of cats with persistent FeLV-FAIDS infection. The cats included in this study were monitored weekly for circulating p27 and were persistently viremic for at least 12 weeks prior to the start of treatment. The relative effectiveness of IFN-α alone (10^5 U/kg), IFN-α at the same dosage in combination with AZT, or AZT alone is compared in Fig. 2. IFN-α, with or without concurrent administration of AZT, was capable of rapidly reducing the level of circulating p27 within the first 14 days of treatment. These levels remained markedly depressed until day 42, when circulating p27 in serum increased, despite continued treatment with IFN-α. Except for one time point at day 63, those cats treated with combination therapy displayed p27 levels statistically similar to those in cats that received IFN-α alone throughout the entire treatment period. AZT alone failed to significantly reduce circulating p27 levels in treated animals.

The dose-dependent response of cats to IFN-α is illustrated in Fig. 3. At day 14, those cats that received high-dosage IFN-α exhibited a more pronounced decline in p27 compared with that in cats administered either an intermediate or low dose of IFN-α, although this difference was not statistically significant ($P > 0.15$). In two of three cats in the high-dose group, circulating p27 levels were reduced below background levels; however, by day 21 all cats within this group rapidly became refractory to continued treatment with high-dose IFN-α. This refractoriness was dose dependent, in that cats that received 10^5 or 10^4 U/kg continued to respond to treatment after day 21 and did not become completely refractory to therapy with IFN-α until after day 42 (Fig. 3). In this regard, treatment with an intermediate dose of IFN-α (10^5 U/kg) produced a significantly greater effect than that produced in the other treatment groups, in that p27 levels

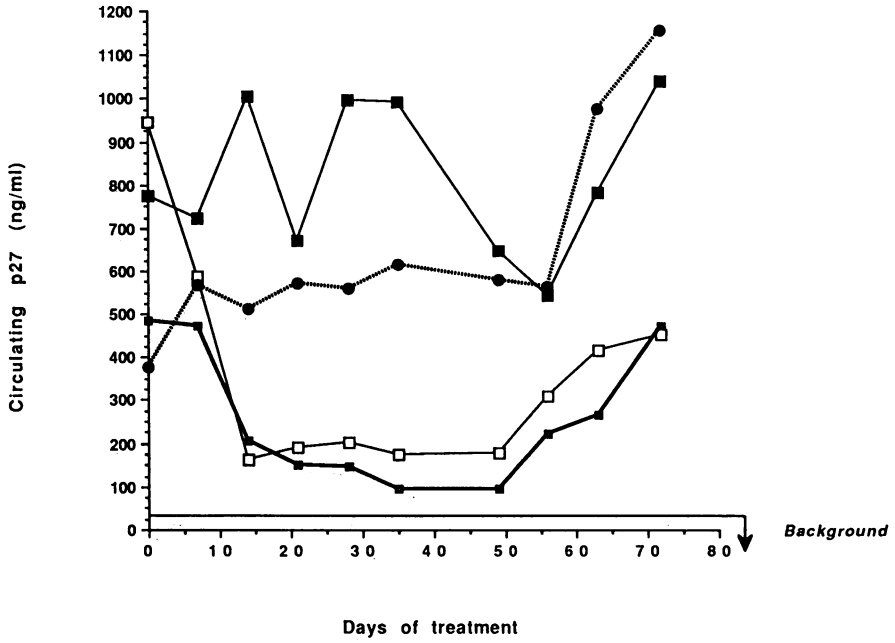

FIG. 2. Circulating p27 antigen levels in cats treated with IFN-α in combination with AZT. Lines are representative of weekly enzyme-linked immunosorbent assay results for each treatment group. Serum samples were assayed in triplicate. Symbols: ■, AZT (20 mg/kg; $n = 6$); □, AZT plus IFN-α (100,000 U/kg; $n = 6$); ■, IFN-α (100,000 U/kg; $n = 9$); ●, placebo ($n = 6$).

were significantly lower at days 49 ($P = 0.0001$) and 63 ($P < 0.0006$) relative to the levels in cats treated with 10^4 U/kg before rising to levels comparable to those in cats given either the high- or the low-dose IFN-α treatment.

Neutralizing antibody response to IFN-α. Regardless of the dose, the development of neutralizing antibody correlated with the onset of refractoriness to treatment with IFN-α. Figure 4 illustrates high levels of IFN-α antibody formation by day 21 in cats treated with 10^6 U/kg compared with significantly lower levels of antibody ($P < 0.002$) in cats treated with 10^5 U/kg. At the point at which both of these treatment groups become refractory to therapy (day 21 versus day 42), the comparative antibody titers in serum were 1:120 versus 1:56, respectively, which was a signifi-

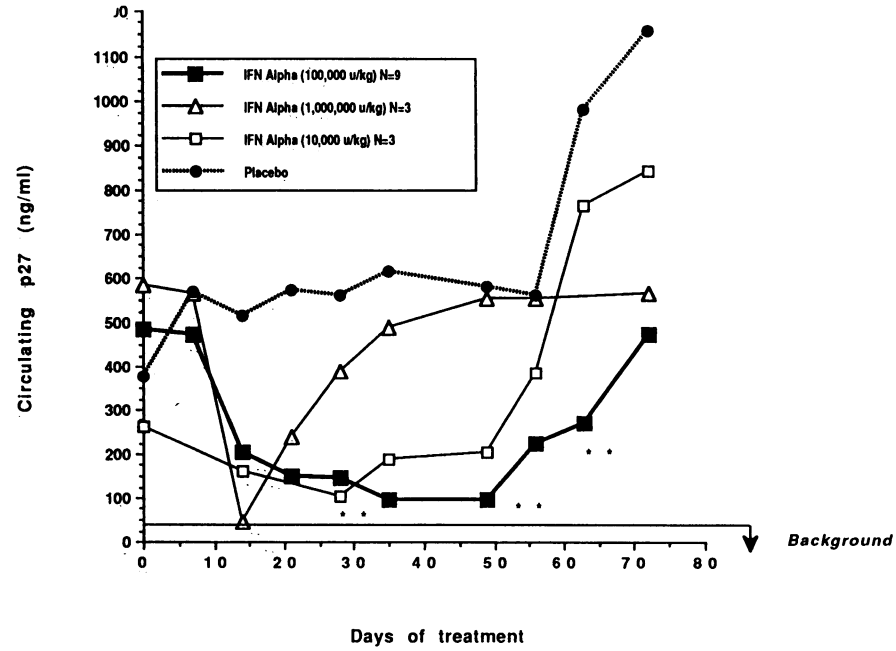

FIG. 3. Circulating p27 antigen levels in cats treated with dosages of IFN-α ranging from 10^4 to 10^6 U/kg. Lines are representative of weekly enzyme-linked immunosorbent assay results for each treatment group. Serum samples were assayed in triplicate, and asterisks represent a statistically significant difference ($P < 0.05$) between treatment groups, as described in the text.

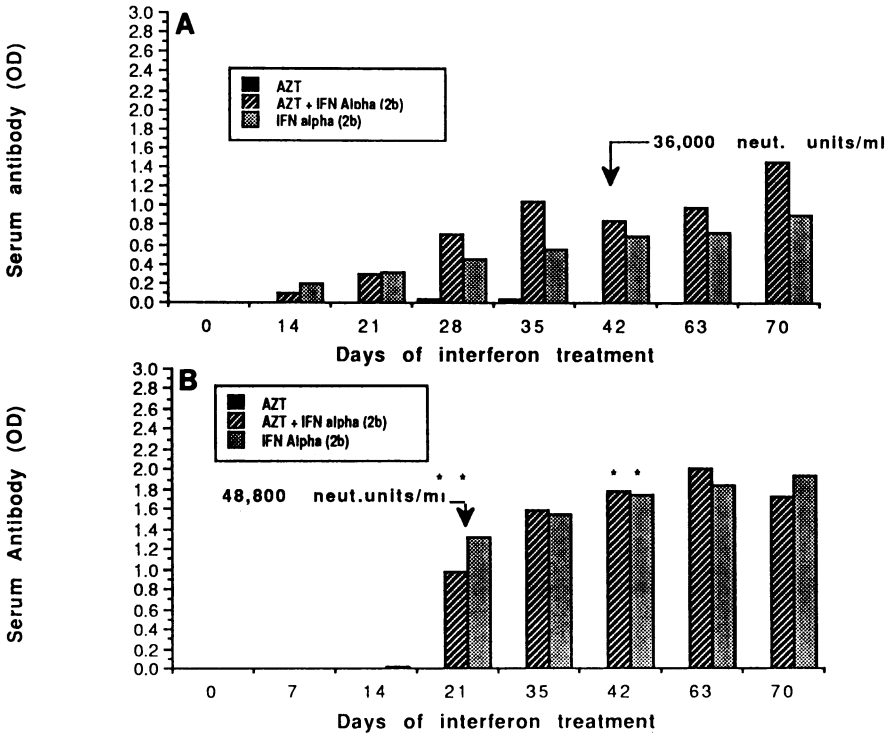

FIG. 4. Neutralizing antibody response to IFN-α in cats treated with intermediate (A) or high (B) doses of IFN-α. Serum samples were screened at 1:20 (duplicate wells) for individual cats, and bars represent averages of all cats per group. Asterisks indicate a statistically significant difference between treatment groups at days 21 and 42 of IFN-α treatment. OD, Optical density.

cantly higher antibody response ($P = 0.002$) in cats that received high-dose IFN-α. By standardizing this assay with monoclonal antibody to IFN-α, these feline serum titers represent 48,880 neutralizing U/ml at day 21 for cats given the high-dose treatment compared with 36,000 neutralizing U/ml at day 42 for cats given the intermediate-dose treatment (10^5 U/kg).

The specificity of this antibody response is illustrated by an immunoblot (Fig. 5) that compared the responses in sera from cats treated with low-dose IFN-α, high-dose IFN-α in combination with AZT, and AZT alone and virus-infected control animals. The intensity of the antibody response increased throughout the treatment period and demonstrated a dose-dependent pattern in the duration of response (Fig. 5). Preliminary data, using neutralization and immunoblotting, indicated that the antibody produced by cats treated with high- or low-dose IFN-α is specific for human recombinant IFN-α and does not react with feline recombinant IFN-α (data not shown).

Tolerance of treatment. At 3 weeks after the start of treatment, the total erythrocyte counts of cats that received AZT or AZT in combination with IFN-α declined to 60% of the values in placebo-treated control cats, but they remained within the lower range of normal limits during the entire treatment period. Although these erythrocyte counts continued to decline to 48% of the control values by 10 weeks postadministration of AZT, total erythrocyte counts recovered to levels comparable to control values within 14 days after treatment was withdrawn. IFN-α by itself had no effect on the total erythrocyte count. Only transient increases were noted in total leukocyte counts in animals that received either IFN-α or AZT plus IFN-α, due primarily to increases in neutrophils and eosinophils. However, total leukocyte counts remained within normal limits during the entire treatment period. As has been noted in previous studies (51), those animals that received IFN-α therapy became transiently anorexic and lost weight during the first 14 days of treatment. These symptoms dissipated by day 21, and weight gain was evident throughout the remainder of the study.

DISCUSSION

Previous studies in our laboratory have demonstrated that IFN-α in combination with AZT is synergistic in inhibiting de novo FeLV-FAIDS infection in vitro and in vivo (51). These and other studies indicating that human IFN-α might be efficacious against de novo FeLV infection and other feline viruses in vitro and in vivo (8, 21, 50) prompted us to study the capacity of IFN-α, with and without concomitant administration of AZT, to reverse the course of early, presymptomatic persistent FeLV-FAIDS infection.

The pharmacokinetics of IFN-α delivered subcutaneously to cats (half-life, 2.9 h) were similar to those recorded for humans, monkeys, and dogs, in which the elimination half-life ranged from 1.8 to 4.8 h (38, 48). In contrast to other mammalian retrovirus studies in which IFN-α has been used to inhibit de novo virus infection (3, 8, 42), results of this study demonstrate that IFN-α has a significant, dose-dependent antiviral effect on persistent antigenemia. High-dose IFN-α (10^6 U/kg) rapidly reduced the antigenic load to background levels within 14 days, whereas 10^5 or 10^4 U/kg produced a more gradual effect which persisted for a fourfold greater period of time. This dose-dependent antiviral response indicated a possible threshold effect in cats that was associated with the relative efficiency of IFN-α in controlling FeLV-FAIDS antigenemia. In the only other study of IFN-α

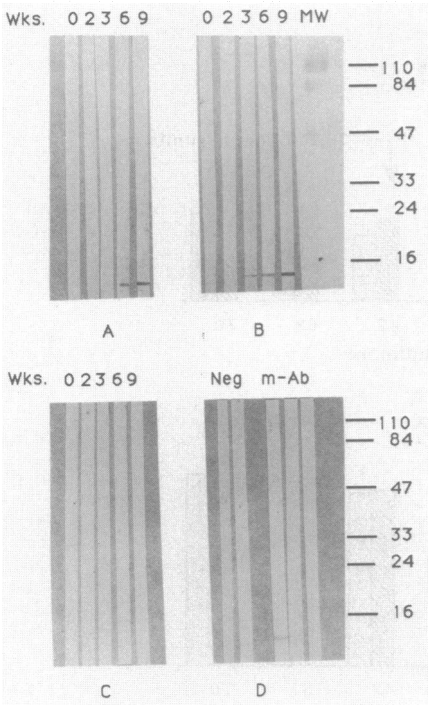

FIG. 5. Immunoblot analysis of sequential serum samples for antibodies to IFN-α. IFN-α (12 μg) was fractionated by 10% polyacrylamide gel electrophoresis and reacted with serum derived from cats treated with IFN-α at 10^5 U/kg (A), IFN-α at 10^6 U/kg in combination with AZT (B), and AZT alone (C) and placebo-treated control (Neg) cats and monoclonal antibody (m-Ab) to IFN-α at 1:500, 1:1,000, and 1:5,000 (D). Molecular weights (MW) on the right are in thousands.

administration to FeLV-challenged cats, Cummins et al. (8) reported that very low dose IFN-α, given orally, could not prevent the development of persistent viremia, although clinical disease appeared to be delayed. Likewise, in this regard, only extremely high dose IFN-α, approaching borderline toxicity, could induce a curative effect on retrovirus-induced murine AKR leukemia (3).

The importance of significant reductions in circulating antigenic load in animals with retrovirus infections is severalfold. Studies by Liu et al. (26, 27) suggest that a reduction in circulating FeLV antigenemia may be associated with a reversal of hypocomplementemia and a return of endogenous IFN and virus-specific neutralizing antibody production to normal levels, leading to the eventual reversal of viremia. In HIV-induced immunodeficiency disease in humans, a positive response to IFN-α treatment has been correlated with the level of pretreatment antigenemia (13), while progression to clinical AIDS is 20-fold greater in males who are seropositive and antigenemic (12). Likewise, reductions in the levels of circulating p24 induced by IFN-α have been associated with a resurgence in circulating $CD4^+$ cells and an antitumor response to HIV-associated Kaposi sarcoma (10, 23). In this regard, high levels of circulating p24 and the subsequent formation of HIV-specific circulating immune complexes have been associated with late-stage progression to clinical disease and a decline in virus-specific antibody formation in patients with AIDS (5, 24). Thus, early control of retroviral antigenemia may influence subsequent immunological function and the progression to clinical disease.

AZT alone or combined with IFN-α in cats persistently infected with FeLV-FAIDS did not have an appreciably greater effect than that of cytokine treatment alone. This is in direct contrast to studies of prophylactic therapy which demonstrated that combined treatment with AZT and IFN-α is most effective in blocking de novo FeLV-FAIDS infection (51). Similarly, although circulating levels of p24 in human patients have been significantly reduced by delivery of AZT alone (4, 11, 13, 34, 43, 45), these patients often become refractory to therapy (2, 39) and a loss of susceptibility to AZT has been shown in sequential HIV isolates during treatment (22, 25). In the present study, because no response was noted from the start of therapy with AZT alone, it is unlikely that the lack of therapeutic response, as measured by decrements in antigenemia, is due to the emergence of AZT-resistant variants. In assaying the inhibitory activity of AZT against several isolates of FeLV in vitro, we have uniformly found that antiviral activity is rapidly diminished if one delays initiation of AZT treatment for 48 h postinfection (unpublished data). Thus, the lack of response to AZT alone noted in the present study may be a reflection of virus burden due to the considerably greater magnitude of antigenemia induced by FeLV-FAIDS versus that induced by HIV.

Although IFN-α treatment was effective in reducing antigenemia in cats, a limitation to continuing effective therapy was the development of neutralizing antibody to human recombinant IFN-α, a response which was dose dependent in intensity and duration. While treatment with 10^6 U/kg reduced antigen levels to below background in two of three cats, the antiviral responses of animals treated with an intermediate dosage of IFN-α (10^5 U/kg) were significantly longer in duration (7 weeks) and intensity compared with the responses of animals treated with either 10^6 and 10^4 U/kg. To our knowledge, this dose-dependent neutralizing antibody response has not been reported in other animal models. Resistance to treatment has been correlated with the development of neutralizing antibodies to human recombinant IFN-α (both 2a and 2b) in human patients treated for neoplastic disease, although dose dependency of the antibody response was not as clearly established in those studies (16, 20, 46, 49). Patients treated with IFN-α for hairy cell leukemia developed significant titers as early as 16 weeks after the start of treatment, with a median of 7 months postadministration (46). By comparison, cats developed significant antibody titers between 3 and 7 weeks postadministration. Unlike what was observed in cats, conflicting reports exist as to the clinical significance of these IFN-α antibody titers in humans. Up to 20% of patients treated with IFN-α for chronic myelogenous leukemia developed neutralizing antibody and no longer responded to therapy (49), while no significant association was seen in patients treated for hairy cell leukemia, renal cell carcinoma, or AIDS-associated Kaposi sarcoma (20). In light of recent evidence demonstrating antibody formation to endogenously released IFN during persistent virus infection (6), an important consideration would be whether these patients also made cross-reacting antibody that neutralized endogenously released IFN-α. Preliminary evidence associated with the studies reported here indicates that even cats with extremely high titers of IFN-α do not have antibodies which cross-react with or neutralize feline IFN-α (unpublished data).

Our results indicate that IFN-α therapy alone, at least for a limited period of time, should prove valuable in greatly reducing FeLV-FAIDS antigenic load in persistently viremic animals. In this regard, IFN-α may serve to partially restore

the paralyzed immune system in these antigenemic animals, thus creating a time frame in which to reconstitute cell-mediated immunity and permit a response to reduced levels of viral antigen. Results of our preliminary studies (unpublished data) suggest that activated lymphoid cells, in combination with concentrations of IFN-α which only minimally affect virus replication, significantly reduce virus replication in vitro. To this end, we are exploring cytokine treatment in combination with the adoptive transfer of immune cells in an attempt to reverse persistent FeLV-FAIDS infection in vivo.

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