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Pre- and Postexposure Chemoprophylaxis: Evidence that 3′-Azido-3′-Dideoxythymidine Inhibits Feline Leukemia Virus Disease by a Drug-Induced Vaccine Response

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The benefits of postexposure 3′-azido-3′-dideoxythymidine (AZT) prophylaxis following human immunodeficiency virus exposure are unknown. We describe a comprehensive assessment of pre- and postexposure AZT therapy in the feline leukemia virus (FeLV)-cat model for AIDS which included in vitro testing, an in vivo dose-response titration, a postexposure treatment study, plasma drug concentration determinations, and evaluation of the immune response to FeLV. In in vitro studies, AZT prevented FeLV infection of a feline T-lymphoid cell line, giving 50 and 90% inhibition concentrations of 4.6 and 11.1 nM, respectively. In all of the in vivo efficacy studies, AZT was administered by continuous subcutaneous infusion for 28 days. AZT toxicity was excessive at a dosage of 120 mg/kg of body weight per day, causing acute anemia, but AZT was tolerable at 60 mg/kg/day. In preexposure studies, AZT was efficacious in preventing chronic antigenemia at a dosage of ≥15 mg/kg/day, at which plasma AZT concentrations averaged between 0.51 and 0.81 μg/ml (2.13 and 3.03 μM). As a postexposure treatment, at 60 mg/kg/day, AZT prevented chronic FeLV antigenemia when treatment was started up to 96 h post-virus inoculation (p.i.), but not when treatment was started at 192 h p.i. The 4-day period between 96 and 192 h p.i. appears to be critical for establishing chronic viremia. It is presumed that the increase in virus load between 4 and 8 days p.i. was able to overwhelm the immunologic functions responsible for containment of FeLV infection, even though AZT therapy effectively controlled viremia during the treatment period. The antibody response to FeLV varied depending on the time of AZT treatment initiation relative to virus challenge. When AZT treatment was started 48 h before or 8 h after FeLV challenge, antibodies to FeLV were not detected until after AZT treatment was discontinued at 28 days p.i. Following AZT treatment, however, antibody titers rapidly increased at a rate suggestive of a secondary immune response. When AZT treatment was initiated at later time points relative to virus challenge (24, 48, and 96 h p.i.), antibodies to FeLV became detectable during the treatment period. These results indicate that AZT treatment does not completely prevent FeLV infection, even when treatment begins before virus challenge, and that immune sensitization to FeLV proceeds during the prophylactic drug treatment period.

The antiviral agent 3′-azido-3′-deoxythymidine (AZT) effectively blocks the replication cycle of retroviruses (24) by interfering with the transcription of the viral RNA genome into DNA (36, 37). AZT does not inhibit virus entry into the cell (32, 37) or affect posttranscriptional processes (2, 34). Therefore, AZT works prophylactically rather than curatively to prevent the infection of susceptible cells. The double-blind, placebo-controlled trials that led to Food and Drug Administration approval of AZT for the treatment of AIDS demonstrated dramatic reductions in morbidity and a significant increase in life expectancy in patients with AIDS (10). These findings were remarkable considering the substantial viral burdens carried by patients with AIDS. The implication is that new cells continue to be available for infection by human immunodeficiency virus (HIV) even in patients with late-stage disease.

Because AZT has the potential to prevent retrovirus infection, it has been used experimentally as an early postexposure chemoprophylactic treatment for primary HIV infections (3). This form of therapy was originally justified on the basis of studies in animal models: feline leukemia virus (FeLV) in cats (34) and murine leukemia virus (MuLV) in mice (31). The results of the animal studies suggested that pre- or early postexposure AZT therapy effectively prevents retrovirus disease induction. Furthermore, AZT-treated SCID-Hu mice inoculated with HIV have reduced virus loads (23), and macaques inoculated with simian immunodeficiency virus have delayed virus conversion times (20) or become virus positive but do not succumb to acute disease like untreated control animals do (22). Subsequent anecdotal reports of early postexposure AZT therapy in humans infected with HIV, however, failed to confirm protection from HIV infection (8, 18, 19).

It is now apparent that preexposure or early postexposure AZT chemoprophylaxis does not uniformly prevent primary retrovirus infection (1, 8, 18–20, 22, 23, 25, 28, 31, 34). What may be important in the early stage of infection is the antiviral immune response which controls the spread of infection. In this regard, results of additional work with AZT-treated MuLV-infected mice supported the hypothesis that the high degree of protection observed in that model was due to a drug-induced vaccine effect in which prophylactic AZT therapy suppressed, but did not prevent, early virus

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expression and thus permitted the equivalent of an attenuated vaccinating infection to proceed (30).

This report gives a comprehensive evaluation of the efficacy of AZT in pre- and postexposure therapeutic regimens in FeLV-inoculated cats. The drug-mediated protection against FeLV in cats was compatible with the drug-induced vaccine effect reported for the MuLV model.

MATERIALS AND METHODS

Reagents. AZT was provided by the AIDS Research and Reference Reagent Program, National Institute of Allergy and Infectious Diseases, Bethesda, Md. For both in vitro and in vivo studies, AZT was dissolved in pyrogen-free normal saline adjusted to pH 7.4 with 1 M NaOH.

ELISA for FeLV antigen. An antigen-capture enzyme-linked immunosorbent assay (ELISA) for FeLV p27 protein (VIRACHEK FeLV ELISA kit; Synbiotics Corp., San Diego, Calif.) was used for in vitro assays to measure virus infectivity and to measure FeLV antigenemia in plasma samples. In virus inhibition assays in vitro, cell pellets were suspended in 200 μl of extraction buffer (0.05 M NaCl, 0.02 M Tris hydrochloride [pH 7.4], 10% aprotinin, 0.5% Nonidet P-40, 0.5% sodium deoxycholate) before testing by ELISA. Plasma samples were tested as described in the kit instructions. Color changes were measured in an ELISA reader (Molecular Devices, Menlo Park, Calif.), and the optical densities were recorded.

In vitro virus inhibition assay. The antiviral activity of AZT against FeLV was tested in the feline T-lymphoid cell line 3201 provided by William Hardy, Jr., Memorial Sloan Kettering Cancer Center, New York, N.Y. Ten million cells were added to various concentrations of AZT in complete medium (1:1 mixture of RPMI 1640–Lebovitz-15 adjusted to 15% heat-inactivated fetal bovine serum, 2% L-glutamine, 1% penicillin-streptomycin) to achieve final concentrations of 0, 1.5, 6, 23, 94, and 374 nM AZT. The cell suspensions were incubated at 37°C in 5% CO_2 for 18 h. Following AZT equilibration, pelleted cells were treated with 0.3 mg of DEAE-dextran (Sigma Chemical Co., St. Louis, Mo.) for 30 min and were then suspended to 3×10^6 cells per ml in complete medium containing AZT at appropriate concentrations. A 0.25-ml sample of each cell suspension plus AZT was plated in six-well culture dishes (Costar, Cambridge, Mass.) with 1 ml of virus suspension (3.8×10^3 focus-forming units per ml) diluted 10^{-1}, and the cultures were incubated for 2 h at 37°C in 5% CO_2 for virus adsorption. Control wells containing virus-infected cells and uninfected cells in AZT-free medium were also plated. An additional 3 ml of AZT-medium was added to each well, and plates were incubated at 37°C with 5% CO_2 for 5 days. Cells and culture fluids were harvested on day 5 and were tested for virus antigen by an antigen-capture ELISA for FeLV p27 protein. AZT was previously shown to be cytotoxic at concentrations greater than 37 μM (unpublished data). The 50% and 90% inhibition concentrations (IC_{50} and IC_{90}, respectively) were determined by using the median effect equation (4).

Cats. The specific-pathogen-free cats used in the studies reported here were from the specific-pathogen-free breeding colony maintained in the Department of Veterinary Pathobiology, The Ohio State University, Columbus.

Hematology. Blood profiles, cell counts, and differentials were obtained by the Clinical Laboratory of The Ohio State University School of Veterinary Medicine.

Fluorescent antibody to FeLV. Antibody to FeLV was measured in an indirect membrane immunofluorescence assay in which the target was a live feline lymphoma cell line (FL-74) (35) that chronically produces FeLV and that expresses FeLV antigen at the cell membrane, as described previously (9).

Determination of plasma AZT concentrations. Plasma AZT concentrations were determined by high-pressure liquid chromatographic (HPLC) analysis by using a Hewlett-Packard model HP1090 binary pumping system equipped with a diode-array detector. Plasma samples of 200 μl collected from individual animals were filtered through Amicon Cetrifree micropartition filters (Amicon Division, W. R. Grace & Co., Danvers, Mass.) to remove plasma proteins. The filtrate was injected onto a Hewlett-Packard Hypersil MDS C8 column (100 by 2.1 mm) with a mobile phase consisting of a mixture of 95% 0.02 M potassium phosphate buffer adjusted to pH 6.0 and 5% acetonitrile. The flow rate was 1 ml/min and the run time was 5.5 min. AZT was detected at 266 nm with peak elution at 3.2 min. External standards were used to prepare a calibration table for concentration determinations. The procedure was sensitive to a concentration of approximately 0.4 μg/ml.

Challenge FeLV. The FeLV used as challenge virus was pooled plasma from FeLV-viremic specific-pathogen-free cats experimentally infected with Rickard FeLV. Heparinized blood from seven cats was collected on ice, pooled, and centrifuged. The plasma was distributed into 0.5-ml aliquots and frozen at −85°C until it was used. The pooled plasma contained 4×10^3 focus-forming units per ml, as determined on sarcoma-positive, leukemia-negative 81c cells (11). The challenge inoculum titrated to induce 100% chronic viremia was 0.1 ml given intravenously. The 50% inhibitory dose was approximately 0.01 ml when the dose was given by the intravenous route.

Pre- and postchallenge treatment with AZT. AZT was administered by continuous subcutaneous infusion to 8- to 10-week-old cats as described previously (27, 33). AZT infusion began at −48, 8, 24, 48, 96, or 192 h postchallenge, depending on the study protocol. Dosage rates of 120, 60, 30, 15, or 1 mg/kg of body weight per day during drug infusion were calculated on the basis of body weight at the beginning of the study (time zero). No adjustments were made for weight gain during the treatment period. Cats were challenged by intravenous injection with 0.1 ml of the pooled plasma from FeLV-viremic cats. Cats were monitored daily. Blood samples were collected once a week and were analyzed for concentrations of AZT in plasma, FeLV antigenemia by ELISA, FeLV antibody by immunofluorescence, hematology, and blood biochemical profiles. Drug toxicity was assessed on the basis of hematologic changes.

Statistical analysis. The significance of efficacy studies was determined by a two-tailed chi-square analysis with the Fisher exact test.

RESULTS

AZT prophylaxis titration in vitro. The IC_{50} and IC_{90} for AZT inhibition of FeLV expression by 3201 cells in vitro were 4.6 and 11.1 nM, respectively (Fig. 1).

AZT prophylaxis titration in vivo. AZT was titrated in vivo in cats to determine its maximal tolerated dose and minimal effective dose. AZT was administered by continuous subcutaneous infusion at dosages of 120, 60, 30, 15, 1, or 0 mg/kg/day to individual groups of cats (Table 1). Drug infusion began 48 h before virus challenge and was continued for 28 days unless toxicity was encountered.

Acute anemia developed in cats given 120 mg/kg/day

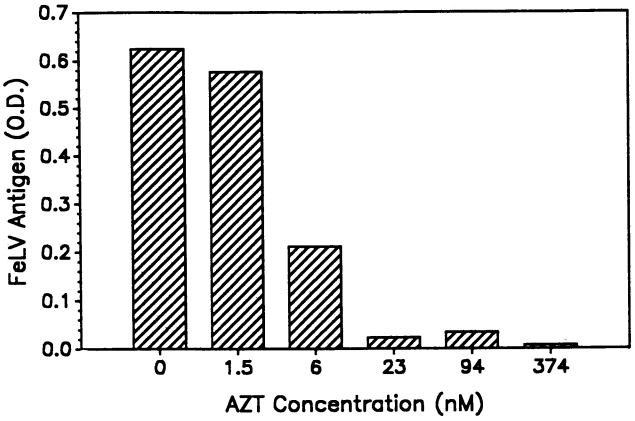

FIG. 1. Inhibitory effect of AZT on FeLV infection of 3201 (feline T-lymphoid) cells in vitro as determined by antigen-capture ELISA. The 3201 cells were incubated with the indicated concentration of AZT for 18 h prior to the addition of virus. Cells were collected at day 5 postinfection, extracted, and assayed for FeLV antigen. The optical densities (O.D.) for the ELISA are plotted against AZT concentrations. The IC_{50} and IC_{90} were 5.6 and 11.1 ± 3.6 nM, respectively, as determined by using the median effect equation (4).

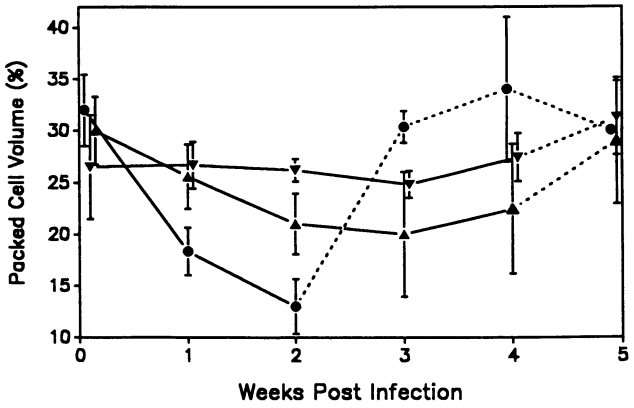

FIG. 2. Packed cell volumes for groups of cats given 120 (●), 60 (▲), and 30 (▼) mg of AZT per kg/day by continuous subcutaneous infusion. Solid lines indicate the period of AZT infusion. AZT was discontinued after 2 weeks in cats that received 120 mg/kg/day. AZT infusion continued for 4 weeks in cats that received 60 or 30 mg/kg/day.

(three cats), and after 2 weeks AZT infusion was discontinued (Fig. 2). Cats given 60 mg/kg/day (seven cats) showed mild regenerative anemia but were able to tolerate the complete 28-day treatment schedule (Fig. 2). Anemia was not detected in cats (six per group) given dosages of 30 mg/kg/day (Fig. 2) or dosages of 15 or 1 mg/kg/day (data not shown). Blood profile analysis values for all groups of cats were within normal values throughout the observation period (data not shown).

Regardless of the AZT dosage, all AZT-treated, FeLV-inoculated cats either seroconverted or developed chronic FeLV antigenemia indicating primary virus infection (Table 1). Cats given 120 mg of AZT per kg/day could not be evaluated for the antiviral efficacy of AZT because of severe toxicity. Six of seven cats given 60 mg of AZT per kg/day, six of six cats given 30 mg/kg/day, and five of six cats given 15 mg/kg/day were significantly protected from chronic antigenemia (P = 0.0047, 0.0006, and 0.0047, respectively) (Table 1). The 1-mg/kg/day AZT dosage was not significantly protective (P = 0.4615), allowing chronic viremia to develop in five of six cats. In challenge control cats and cats receiving 1 mg of AZT per kg/day, chronic antigenemia was first detected during the second or third week after virus inoculation (p.i.). For the two cats in the 15- and 60-mg/kg/day AZT groups that developed chronic antigenemia, virus conversion was delayed to 5 and 7 weeks p.i., respectively. The mean plasma AZT concentrations achieved for groups of cats receiving 15, 30, and 60 mg/kg/day were 0.67, 1.42, and 2.37 μg/ml, respectively, for the 4-week treatment period (Fig. 3). AZT concentrations for the 1-mg/kg/day group were below the detection level and could not be measured. Seven of seven untreated challenge control animals developed chronic antigenemia.

TABLE 1. Effect of AZT treatment on FeLV viremia when administered at various doses or time postinfection

Study	AZT start time (h)[a]	AZT dosage (mg/kg/day)[b]	No. of animals	No. of animals that seroconverted	No. of animals with antigenemia conversion[c]	% Efficacy[d]	Statistical significance (P value)[e]
In vivo titration	−48	120	3	3	NA[f]	NA	NA
	−48	60	7	7	1	86	0.0047
	−48	30	6	6	0	100	0.0006
	−48	15	6	6	1	83	0.0047
	−48	1	6	6	5	17	0.4615
Postexposure chemotherapy	8	60	6	6	1	83	0.0047
	24	60	6	6	2	67	0.0210
	48	60	6	6	1	83	0.0047
	96	60	6	6	0	100	0.0006
	192	60	6	6	5	17	0.4615
Control			7	7	7	0	NA

[a] Time that AZT treatment began relative to virus inoculation.
[b] AZT was administered by continuous subcutaneous infusion at the indicated doses for 28 days, except for the 120-mg/kg/day group, to which it was administered for 14 days.
[c] Antigenemia determined by antigen-capture ELISA.
[d] Efficacy was measured by prevention of chronic antigenemia.
[e] P value was determined by a two-tailed chi-square analysis with the Fisher exact test.
[f] NA, not applicable.

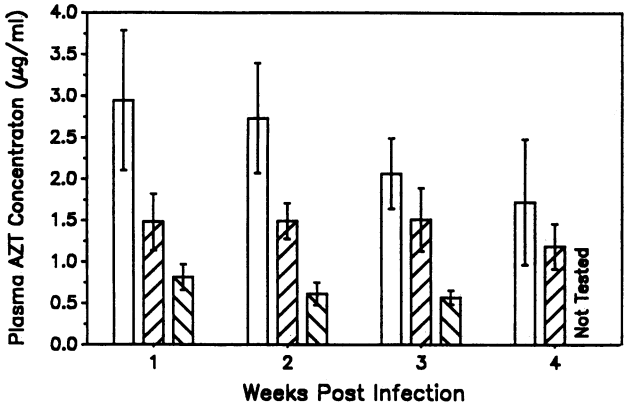

FIG. 3. Mean plasma AZT concentrations for cats infused with 60 (open bars), 30 (bars with right diagonal lines), and 15 (bars with left diagonal lines) mg of AZT per kg/day. Plasma samples from weeks 1, 2, 3, and 4 were assayed for AZT by HPLC. The 4-week 15-mg/kg/day samples were not analyzed.

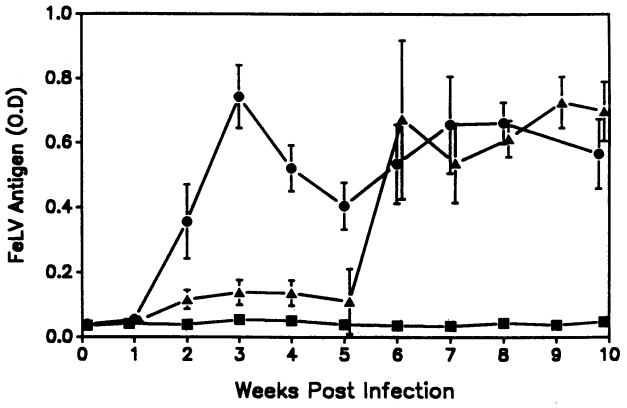

FIG. 4. Mean FeLV antigenemia for groups of cats with AZT treatments beginning at 96 (■) or 192 (▲) h p.i. or untreated challenge controls (●). AZT was administered by continuous subcutaneous infusion at a dosage of 60 mg/kg/day for a period of 4 weeks. Antigenemia was determined weekly by an antigen-capture ELISA. O.D, optical density.

Postexposure chemoprophylaxis. Postexposure chemoprophylaxis was evaluated in cats in which AZT treatment was begun at various times after virus inoculation. AZT was administered by continuous subcutaneous infusion at a dosage of 60 mg/kg/day starting 8, 24, 48, 96, or 192 h p.i. (six cats per group) and continuing for 28 days. AZT initiated at 8, 24, 48, and 96 h p.i. protected five of six ($P = 0.0047$), four of six ($P = 0.021$), five of six ($P = 0.0047$), and six of six ($P = 0.0006$) cats, respectively (Table 1), preventing chronic antigenemia in a total of 20 of 24 animals (83% efficacy) in which treatment was begun at or before 96 h p.i. When AZT was started at 192 h p.i., only one of six animals did not develop chronic antigenemia ($P = 0.4615$) (17% efficacy).

FeLV antigen levels in the plasma of cats in which treatment was begun 96 h p.i. were at low or negative levels during the AZT infusion period and throughout the observation period. However, the five of six cats that developed chronic antigenemia after treatment with AZT beginning at 192 h p.i. had low to moderate levels of antigenemia during the AZT infusion period. In these animals, antigenemia rapidly increased to the levels in challenge control animals once AZT was discontinued (Fig. 4). FeLV antigenemia was detected in challenge control animals by the second week p.i. and rapidly increased to high levels by week 3 p.i. The results indicate that at between 4 and 8 days p.i., the primary FeLV infection had spread to the point where there was a sustained but moderate expression of virus even in the presence of AZT. When AZT was discontinued, the moderate level of virus expression increased sharply, presumably because of the rapid spread of FeLV to previously uninfected cells.

Anti-FeLV antibody responses in cats given pre- and postexposure AZT therapy. All cats which did not convert to chronic antigenemia after receiving either pre- or postexposure AZT therapy developed antibody to FeLV, as determined by indirect immunofluorescence. However, the temporal expression of FeLV antibody relative to virus challenge varied with the AZT initiation time. Cats given preexposure AZT beginning 48 h before virus challenge or AZT therapy beginning 8 h postexposure had delayed antibody responses which were not apparent until after AZT infusion was discontinued at 4 weeks p.i. (Fig. 5). Between weeks 4 and 5 p.i., antibody titers increased rapidly, suggesting that immune sensitization had occurred. In contrast, the antibody responses in the cats given AZT treatments beginning at 24, 48, and 96 h p.i. were apparent beginning at 2 weeks p.i. (Fig. 5).

DISCUSSION

The risk of seroconversion to HIV per episode of percutaneous exposure to HIV-infected blood is estimated to be approximately 0.4% (3, 15, 21). Therefore, the benefits of postexposure AZT prophylaxis following virus exposure may not be justified when considering the known short-term toxicity (29) and the unknown long-term effects of AZT therapy (3). The present study was not intended to either support or refute the validity of postexposure chemoprophylaxis for HIV infection. Instead, the study was meant to extend earlier therapeutic studies in the FeLV-cat model by defining more precisely the limits of postexposure therapy in that model and to better characterize the factors that bestow

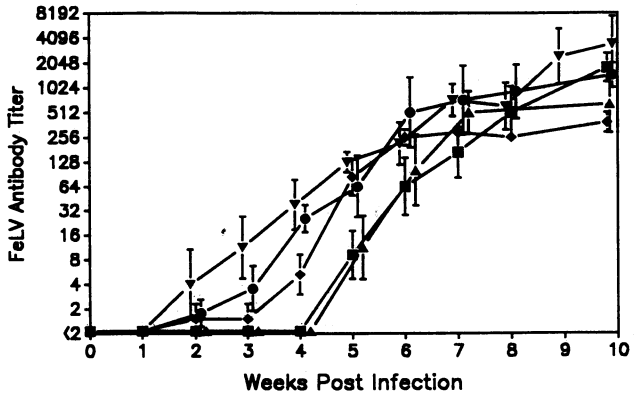

FIG. 5. Comparison of FeLV antibody responses of nonantigenemic cats given pre- and postexposure AZT therapy. AZT was administered by continuous subcutaneous infusion at a dosage of 60 mg/kg/day for 4 weeks. AZT treatment began at −48 (▲), 8 (■), 24 (▼), 48 (◆), or 96 (●) h p.i. Antibody titers were determined in an immunofluorescence assay by using the FeLV-positive lymphoid cell line FL-74 as the target cell.

protection against disease. A previous study in FeLV-infected cats used three dosages in two regimens (10 or 20 mg/kg twice daily and 20 mg/kg thrice daily) and three time points for drug initiation (1 h and 7 and 28 days p.i.) (34). In the present work, a dose titration with five dosages and a postexposure prophylaxis study with five time points after virus exposure were tested; all tests were done by using a uniform regimen. The principal findings were that dosages of ≥15 mg/kg/day, but not dosages of 1 mg/kg/day, effectively prevent antigenemia. The dosage of 15 mg/kg/day produced mean concentrations of AZT in plasma of 0.57 to 0.81 μg/ml (2.13 to 3.03 μM). Thus, the minimum effective dose was fixed at between 1 and 15 mg/kg/day for continuous subcutaneous infusion. These results were comparable to those in a previous report (34), in which a dosage of 10 mg/kg/day was reported to prevent viremia.

In the in vitro studies in which AZT was added to a feline T-lymphocyte cell line 18 h before virus, the IC_{90} for inhibition of FeLV antigen expression was 11.1 nM. In a previous study with FeLV, 90% inhibition of infection required approximately 5 μM AZT when the drug was added 45 min after virus addition (34). The requirement in our assay for less AZT for inhibition of >90% virus infection probably related to the preincubation of cells with AZT. The early addition would allow for cellular uptake of AZT and phosphorylation to its active form prior to the addition of virus.

In in vivo titration studies, AZT was found to be excessively toxic at a dosage of 120 mg/kg/day, causing acute, severe anemia. Anemia also was evident in the 60-mg/kg/day AZT group, but it remained in a tolerable range during the treatment period and was actually less severe in the last sampling before discontinuance of AZT therapy. Our results were compatible with those of a previous study in which mild anemia was observed in FeLV-infected cats given doses of 30 and 60 mg/kg/day by the oral route in three equal doses at 8-h intervals (14). In the present study, no toxicity was detected with 30 mg of AZT per kg/day. AZT was administered by continuous infusion, therefore avoiding the peak concentrations in plasma associated with bolus drug administration. The maximum tolerable dosage in cats given AZT by continuous subcutaneous infusion was 60 mg/kg/day. The improvement in erythrocyte counts at the 4-week treatment sample probably related to the gradual reduction in dosage as body weight increased (the dosage was set at time zero and was not adjusted for weight increases during the 4-week treatment period).

In the postexposure chemoprophylaxis study, AZT therapy (at 60 mg/kg/day) was effective when it was initiated as late as 96 h (4 days) after virus exposure, protecting 20 of 24 animals (83%), but not when it was initiated at 192 h (8 days) p.i., when 5 of 6 animals became chronically antigenemic. The previous report indicated significant protection when AZT was initiated at 7 days p.i. (34). The discordant results between our study and the previous study may be due to (i) the duration of therapy (28 versus 42 days) or (ii) differences in the rate of spread of the challenge virus. The longer treatment period in the previous study may have permitted additional time for immune clearance to proceed; but the substantial difference in protocols in terms of dosage, method of administration (continuous infusion versus intermittent injection), and duration makes direct comparison difficult. Also, our laboratory-adapted isolate of FeLV may have spread more rapidly in vivo than the isolate reported previously, therefore requiring earlier AZT intervention. The continuous infusion protocol allowed us to quantify the concentrations of AZT in plasma during the treatment period. Such measurements were not reported in the previous study.

The moderate level of antigenemia observed in the 192-h postexposure group during the AZT treatment period (Fig. 4) suggested that AZT inhibits further expansion of infection during the 4-week treatment period. When AZT was discontinued at 28 days of treatment, the level of FeLV antigenemia increased rapidly and achieved levels similar to those in the non-AZT-treated cats (Fig. 4). While it was not possible to predict how long virus expansion could have been forestalled with continuation of AZT infusion, it was encouraging to see this pattern during the 4-week treatment period.

The pattern of the antiviral antibody response in the postexposure AZT treatment groups indicated primary virus infection, even though, in some animals, no antigenemia was detected. Interestingly, no antibody was detected until after discontinuance of AZT therapy in cats whose AZT therapy began at −48 and 8 h p.i. However, once AZT was discontinued in these two groups, the antibody titers rapidly increased, and by the time that the next plasma sample was obtained (7 days later), it was at a point comparable to that reached at 3 weeks p.i. in FeLV-challenged animals not given AZT therapy (Fig. 5). The results were compatible with the presence of immune sensitization during AZT therapy, although it was below the level required to give a measurable antibody response. In cats in which AZT therapy was delayed until 24 h p.i. or later, FeLV antibody was detected at week 2 or 3 p.i., a period during which AZT treatment was continuing. These results suggested that when AZT therapy is delayed for 24 h p.i., enough viral antigen accumulates to stimulate antibody production.

The pattern of the immune response noted in our study was compatible with a drug-induced vaccine response previously described by Ruprecht et al. (30) in studies with a Rauscher MuLV-mouse model for AIDS. In the drug-induced vaccine response, drug therapy suppresses but does not eliminate virus expression, allowing enough viral antigen to be processed to prime the immune system. After drug therapy is discontinued, the immune system responds to what presumably would have been a rapid expansion of virus and effectively neutralizes the infection. If drug therapy is delayed too long after exposure, the primary virus infection is able to expand beyond a level which can be controlled by immune factors. In the Rauscher MuLV-mouse system, adoptive cell transfer studies demonstrated that both $CD4^+$ and $CD8^+$ cell types were required for full protection against virus challenge (16). The relative roles of antibody and/or cellular immunity are not defined for HIV, but some studies indicate that antiviral resistance is probably at least partially mediated by neutralizing antibody (36). Passive anti-FeLV antibody has been shown to prevent FeLV infection in vivo (7, 12, 17).

The results presented here suggest that an important aspect of the control of retrovirus infection is inhibition of the very early expansion of infection by suppressing the viremia associated with most primary retrovirus infections. This initial stage of the virus infection process seeds the susceptible cell population at a time when the immune response has not yet fully peaked. In the case of lentiviruses (i.e., HIV [6], feline immunodeficiency virus [14a], and simian immunodeficiency virus [20]), the immune response that follows is able to clear the viremia but at a point that is too late to prevent the widespread dispersion of virus to the susceptible cell population (6). The immune response to FeLV is different from the general pattern reported for HIV, feline immunodeficiency virus, and simian immunodefi-

ciency virus. Like these lentiviruses, FeLV begins with the induction of viremia which is detected by antigen-capture ELISA at about 2 to 3 weeks postexposure. In some cats, however, FeLV, for reasons not fully understood, expands rapidly, causing acute immunosuppression (5, 26) which prevents the resolution of viremia by immune factors. These cats remain viremic for life (13). Other cats that become immune to FeLV generally have negligible or undetectable levels of viremia (13) and are able to immunologically limit primary infection and develop what appears to be lifelong immunity to FeLV. The lentiviruses are apparently less immunosuppressive in the early stages of infection, such that infected animals or humans develop strong immune responses to the virus. The immune response clears the viremia and suppresses subsequent virus expression (similar to FeLV-infected cats that develop immunity). However, immunity in these systems is subject to the chronic debilitating effects of T-helper cell depletion that eventually leads to overt immunosuppression and AIDS or AIDS-like diseases. With this difference in pathogenesis taken into consideration, the FeLV system provides a sensitive means of evaluating drug efficacy on the basis of the viremia induction end point. The efficacy of immune prophylaxis for the prevention of HIV infection and AIDS may hinge on preventing the initial stage of viremia and, therefore, limiting the early dissemination of virus. The value of the drug-induced vaccine effect in augmenting immunity has not been evaluated.

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REFERENCES

1. **Basham, T., C. D. Rios, T. Holdener, and T. C. Merigan.** 1990. Zidovudine (AZT) reduces virus titer, retards immune dysfunction, and prolongs survival in the LP-BM5 murine induced immunodeficiency model. J. Infect. Dis. **161**:1006–1009.
2. **Burke, D. S., R. R. Redfield, D. C. Bjornson, A. K. Fowler, and C. N. Oster.** 1989. Frequent isolation of HIV-1 from the blood of patients receiving zidovudine (AZT) therapy. N. Engl. J. Med. **323**:467–469.
3. **Centers for Disease Control.** 1990. Public Health Service statement on management of occupational exposure to human immunodeficiency virus, including consideration regarding zidovudine postexposure use. Morbid. Mortal. Weekly Rep. **39**(RR-1):1–14.
4. **Chou, T.-C., and P. Talalay.** 1984. Quantitative analysis of dose-effect relationship: the combined effects of multiple drug or enzyme inhibitors. Adv. Enzyme Regul. **22**:27–55.
5. **Cockerell, G., E. A. Hoover, S. Krakowka, R. G. Olsen, and D. S. Yohn.** 1976. Lymphocyte mitogen reactivity and enumeration of circulating B- and T-cells during feline leukemia virus infection in the cat. J. Natl. Cancer Inst. **57**:1095–1100.
6. **Daar, E. S., T. Moudgil, R. D. Meyer, and D. D. Ho.** 1991. Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. N. Engl. J. Med. **324**:961–964.
7. **de Noronha, F., W. Schafer, M. Essex, and D. P. Bolognesi.** 1978. Influence of antisera to oncornavirus glycoprotein (gp71) on infections of cats with feline leukemia virus. Virology **85**:617–621.
8. **Durand, E., C. Le Jeunne, and F.-C. Hugues.** 1991. Failure of prophylactic zidovudine after suicidal self-inoculation of HIV-infected blood. N. Engl. J. Med. **324**:1062.
9. **Essex, M., G. Klein, S. P. Snyder, and J. B. Harrold.** 1971. Feline sarcoma virus-induced tumors: correlation between humoral antibody and tumor regression. Nature (London) **233**: 195–197.
10. **Fischl, M. A., D. D. Richman, M. H. Greico, M. S. Gottlieb, P. A. Volberding, O. L. Laskin, J. M. Leedom, J. E. Groopman, D. Mildvan, R. T. Schooley, G. G. Jackson, D. T. Durack, D. King, and the AZT Collaborative Working Group.** 1987. The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. N. Engl. J. Med. **317**: 185–191.
11. **Fishinger, P. J., C. S. Blevins, and S. Nomura.** 1974. Simple quantitative assay for xenotropic murine leukemia virus and ecotropic feline leukemia virus. J. Virol. **14**:177–179.
12. **Haley, P. J., E. A. Hoover, S. L. Quackenbush, P. W. Gasper, and D. W. Macy.** 1985. Influence of antibody infusion on pathogenesis of experimental feline leukemia virus infection. J. Natl. Cancer Inst. **74**:821–827.
13. **Hardy, W. D., P. W. Hess, E. G. MacEwen, A. J. McClelland, E. E. Zuckerman, M. Essex, S. M. Cotter, and O. Jarrett.** 1976. Biology of feline leukemia virus in natural environment. Cancer Res. **36**:582–588.
14. **Haskek, W. M., R. M. Weigel, G. Scherba, M. C. DeVera, R. Feinmehl, P. Solter, M. B. Tompkins, and W. F. Tompkins.** 1990. Zidovudine toxicity to cats infected with feline leukemia virus. Fundam. Appl. Toxicol. **14**:764–775.

14a. **Hayes, K.** Unpublished data.

15. **Henderson, D. K., and J. L. Gerberding.** 1989. Prophylactic zidovudine after occupational exposure to the human immunodeficiency virus: an interim analysis. J. Infect. Dis. **160**:321–327.
16. **Hom, R. C., R. W. Finberg, S. Mullaney, and R. M. Ruprecht.** 1991. Protective cellular retroviral immunity requires both $CD4^+$ and $CD8^+$ immune T cells. J. Virol. **65**:220–224.
17. **Hoover, E. A., J. P. Schaller, L. E. Mathes, and R. G. Olsen.** 1977. Passive immunity to feline leukemia: evaluation of immunity from dams naturally infected and experimentally vaccinated. Infect. Immun. **16**:54–59.
18. **Lange, J. M. A., C. A. B. Boucher, C. E. M. Hollak, E. H. H. Wiltink, P. Reiss, E. A. van Royen, M. Roos, S. A. Danner, and J. Goudsmit.** 1990. Failure of zidovudine prophylaxis after accidental exposure to HIV-1. N. Engl. J. Med. **322**:1375–1377.
19. **Looke, D. F. M., and D. I. Grove.** 1990. Failed prophylactic zidovudine after needlestick injury. Lancet **335**:1280.
20. **Lundgren, B., D. Bottiger, E. Ljungdahl-Stahle, E. Norrby, L. Stahle, B. Wahren, and B. Oberg.** 1991. Antiviral effects of 3′-fluorothymidine and 3′-azidothymidine in cynomolgus monkeys infected with simian immunodeficiency virus. J. Acquired Immune Defic. Syndr. **4**:489–498.
21. **Marcus, R., and the CDC Cooperative Needlestick Surveillance Group.** 1988. Surveillance of health care workers exposed to blood from patients infected with human immunodeficiency virus. N. Engl. J. Med. **319**:1118–1123.
22. **Martin, L. N., M. Murphey-Corb, K. F. Soike, and B. Davison-Fairburn.** 1990. Effects of 28 day treatment with azidothymidine (AZT) initiated 1 to 72 hours after infection with simian immunodeficiency virus (SIV). 3rd International Conference on Antiviral Research, Brussels, Belgium, 22 to 27 April 1990. Antiviral Res. **14**(Suppl. 1):S114.
23. **McCune, J. M., R. Namikawa, C.-C. Shih, L. Rabin, and H. Kaneshima.** 1990. Suppression of HIV infection in AZT-treated SCID-hu mice. Science **247**:564–566.
24. **Mitsuya, H., K. J. Weinhold, P. A. Furman, M. H. St. Clair, S. N. Lehrman, R. C. Gallo, D. Bolognesi, D. W. Barry, and S. Broder.** 1985. 3′-Azido-3′-deoxythymidine (BW A509U): an antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus in vitro. Proc. Natl. Acad. Sci. USA **82**:7096–7100.
25. **Morrey, J. D., K. M. Okleberry, and R. W. Sidwell.** 1991. Early-initiated zidovudine therapy prevents disease but not low levels of persistent retrovirus in mice. J. Acquired Immune Defic. Syndr. **4**:505–512.

26. **Perryman, L. E., E. A. Hoover, and D. S. Yohn.** 1972. Immunologic reactivity of the cat: immunosuppression in experimental feline leukemia. J. Natl. Cancer Inst. **49:**1357–1365.
27. **Polas, P. J., C. L. Swenson, R. A. Sams, C. M. Cheney, K. A. Hayes, M. J. Tarr, G. J. Kociba, and L. E. Mathes.** 1990. In vitro and in vivo evidence that the antiviral activity of 2′,3′-dideoxycytidine is target cell dependent in a feline retrovirus animal model. Antimicrob. Agents Chemother. **34:**1414–1421.
28. **Portnoi, D., A. M. Stall, D. Schwartz, T. C. Merigan, L. A. Herzenberg, and T. Basham.** 1990. Zidovudine (azido dideoxythymidine) inhibits characteristic early alterations of lymphoid cell populations in retrovirus-induced murine AIDS. J. Immunol. **144:**1705–1710.
29. **Richman, D. D., M. A. Fischl, M. H. Greico, M. S. Gottlieb, P. A. Volberding, O. L. Laskin, J. M. Leedom, J. E. Groopman, D. Mildvan, M. S. Hirsch, G. G. Jackson, D. T. Durack, S. Nusinoff-Lehrman, and the AZT Collaborative Working Group.** 1987. The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. N. Engl. J. Med. **317:**192–197.
30. **Ruprecht, R. M., S. Mullaney, L. D. Bernard, M. A. G. Sosa, R. C. Hom, and R. W. Finberg.** 1990. Vaccination with a live retrovirus: the nature of the protective immune response. Proc. Natl. Acad. Sci. USA **87:**5558–5562.
31. **Ruprecht, R. M., L. G. O'Brien, L. D. Rossoni, and S. Nusinoff-Lehrman.** 1986. Suppression of mouse viraemia and retroviral disease by 3′-azido-3′-deoxythymidine. Nature (London) **323:** 467–469.
32. **St. Clair, M. H., C. A. Richards, T. Spector, K. J. Weinhold, W. H. Miller, A. J. Laglois, and P. A. Furman.** 1987. 3′-Azido-3′-deoxythymidine triphosphate as an inhibitor and substrate of purified human immunodeficiency virus reverse transcriptase. Antimicrob. Agents Chemother. **31:**1972–1977.
33. **Swenson, C. L., P. J. Polas, and L. E. Mathes.** 1989. A technique for continuous intravenous infusion in cats. Lab. Anim. Sci. **39:**615–617.
34. **Tavares, L., C. Roneker, K. Johnston, S. M. Lehrman, and F. de Noronha.** 1987. 3′-Azido-3′-deoxythymidine in feline leukemia virus-infected cats: a model for therapy and prophylaxis of AIDS. Cancer Res. **47:**3190–3194.
35. **Theilen, G. H., T. G. Kawakami, J. D. Rush, and R. J. Munn.** 1969. Replication of cat leukemia virus in cell suspension culture. Nature (London) **222:**589–590.
36. **Tremblay, M., K. Numazaki, X. Li, M. Gornitsky, J. Hiscott, and M. Wainberg.** 1990. Resistance to infection by HIV-1 of peripheral blood mononuclear cells from HIV-1-infected patients is probably mediated by neutralizing antibody. J. Immunol. **145:**2896–2901.
37. **Yarchoan, R., H. Mitsuya, C. E. Myers, and S. Broder.** 1989. Clinical pharmacology of 3′-azido-2′,3′-dideoxythymidine (zidovudine) and related dideoxynucleosides. N. Engl. J. Med. **321:**726–738.