MAJOR ARTICLE

Live Attenuated Influenza Vaccine Is Safe and Immunogenic in Immunocompromised Ferrets

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Patients undergoing chemotherapy for cancer are highly susceptible to influenza virus infection. Prevention of influenza virus infection is complicated in the immunocompromised host because of suboptimal responses to the trivalent inactivated influenza vaccine (TIV). A new, live attenuated influenza vaccine (LAIV; FluMist) may offer a more effective alternative to TIV, but the safety of this LAIV in immunocompromised patients must first be established. In the present study, FluMist was administered to ferrets immunocompromised by treatment with dexamethasone and cytarabine. Ferrets exhibited no signs or symptoms attributable to FluMist, and nasal clearance of LAIV strains from immunocompromised ferrets was similar to that from control ferrets. Serum antibody responses against the vaccinating strains were analyzed as a measure of vaccine efficacy. Antibody titers to all 3 vaccine strains in immunocompromised ferrets were similar to those seen in mock-treated control ferrets, as assessed by microneutralization assay. These findings support the potential use of this vaccine in immunocompromised humans.

Influenza virus infections contribute significantly to worldwide morbidity and mortality, causing an estimated 36,000 deaths and 200,000 hospitalizations per year in the United States alone [1–3]. Healthy individuals are less likely to have severe outcomes from influenza virus infections, with most complications being seen in either the very young or the elderly [4–6]. Persons with chronic illnesses or who are immunocompromised are also at significant risk for poor outcomes from influenza virus infections [7–18]. Within these groups, there are increases in hospitalization, significant delays in chemotherapy [8], and progression of the infection to the lower respiratory tract, sometimes resulting in death [7, 11, 14, 19].

Current guidelines of the Advisory Committee on Immunization Practices and the American Association of Pediatrics recommend the administration of the trivalent inactivated influenza vaccine (TIV) on an annual basis to at-risk populations, including children with cancer [3]. However, studies detailing the immune response to TIV in immunocompromised children have reported inconsistent results. In some studies, children receiving chemotherapy responded to influenza vaccination in a manner similar to that of untreated control children [20–22]. In other studies, similar populations showed decreases in either the percentage of patients seroconverting [9, 23, 24] or the mean hemagglutination inhibition (HI) titers [25, 26]. These varied results have engendered the perception that TIV does not work well in these patients [27], leading to reduced use. In fact, only 35% of children 2–17 years old with health conditions that put them at high risk of infection were vaccinated during the 2004–2005 influenza season [28]. In children with cancer in particular, reluctance on the part of oncologists to administer an intramuscular (im) vaccine to patients with low platelet counts and indwelling catheters, in whom needle punctures are discouraged, likely contributes to the low rate of use. Thus, some of the patients at highest risk may be the least likely to receive vaccination with TIV.

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FluMist is a live attenuated influenza vaccine (LAIV) composed of the 3 dominant circulating strains of human influenza virus [3]. This vaccine has been shown to be efficacious and safe for delivery in adults and children [29–40] and has been licensed for use in the United States in healthy individuals between 5 and 49 years old [3, 41]. This vaccine has also been shown to be safe for use in HIV-positive adults and children who are asymptomatic or showing few symptoms [42, 43]. The safety profile of this LAIV in these mildly immunosuppressed individuals provides preliminary evidence in support of a trial of safety and efficacy in patients with cancer. It is feasible that this LAIV could be used in immunocompromised individuals who respond poorly to TIV, and it has the benefit of having a needle-free delivery system, which may make FluMist more desirable for use in patients with low platelet counts.

In the present study, we describe the administration of FluMist in an immunocompromised ferret model. The study was performed to evaluate the safety and immunogenicity of an LAIV in immunocompromised animals before a trial is conducted in humans. Because no such animal model of immunosuppression existed, we gave chemotherapy to young adult ferrets that was analogous to that given to children with acute myeloid leukemia, and we subsequently delivered LAIV to these ferrets during their period of immunosuppression. These data provide a rationale for a trial of the safety and efficacy of this vaccine in human patients.

MATERIALS AND METHODS

Infectious agents. The 2004–2005 formulation of the LAIV FluMist (MedImmune Vaccines) was used. This vaccine contained $1 \times 10^{6.5}$–$1 \times 10^{7.5}$ $TCID_{50}$ of each of the following strains: A/New Caledonia/20/99 (H1N1), A/Wyoming/3/2003 (A/Fujian/411/02-like; H3N2), and B/Jilin/20/2003 (B/Shanghai/361/2002-like; B). Influenza viruses used in immune assays expressed hemagglutinins from A/New Caledonia/20/99 (H1), A/Fujian/411/02 (H3), and B/Yamanashi/166/98 (B). The A/New Caledonia/20/99 strain was propagated in embryonated chicken eggs, whereas the A/Fujian/411/02 and B/Yamanashi/166/98 strains were grown in MDCK cells (American Type Culture Collection) by use of standard methods. The wild-type *(wt)* influenza virus used was a human H3N2 isolate (A/Sydney/5/97) that was grown in MDCK cells.

Ferrets. Young adult ferrets (Marshall Farms) were selected on the basis of low seroreactivity (HI titer, <1:40) against the circulating H1, H3, and B strains. Three of 4 ferrets in each group were monitored using the PhysioTel Telemetry System (Data Sciences International). An experienced veterinarian implanted the transmitter subcutaneously between the scapulae through a small incision that was closed with sutures. Ferrets were allowed to heal for 3 weeks after implantation. Implanted transmitters were used to continuously monitor the body tem-

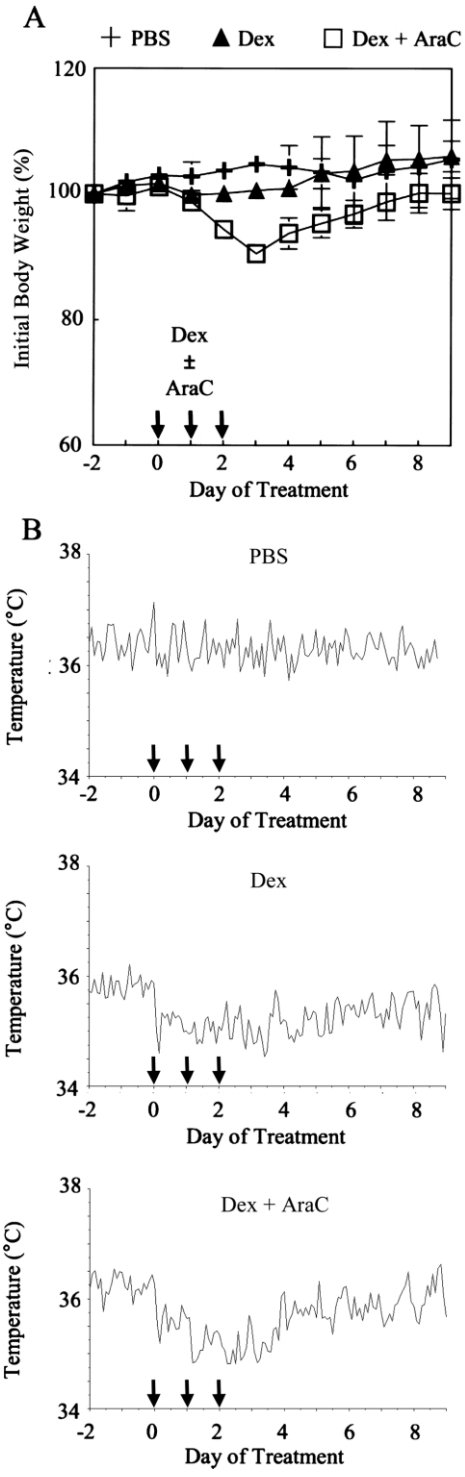

Figure 1. Response of ferrets to chemotherapy. Weight *(A)* and temperature *(B)* of ferrets after treatment with dexamethasone (Dex) either with or without cytarabine (AraC). Four ferrets per group were treated with either Dex *(black triangles)* or Dex and AraC *(white squares)* or were mock-treated with PBS *(plus signs)* and then were monitored for changes in weight. The moving average temperatures for 3 ferrets/group are plotted. Downward arrows indicate the time of administration of chemotherapy.

Table 1. White blood cell counts in ferrets after treatment with dexamethasone (Dex) either with or without cytarabine (AraC) and FluMist inoculation.

	Dex			Dex and AraC		
Cell population	Preinoculation	Day 0	Day 14	Preinoculation	Day 0	Day 14
All white blood cells	8950 ± 2420	6695 ± 708	8525 ± 2286	10,805 ± 3525	3570 ± 957[a]	6735 ± 2389
Lymphocytes	5075 ± 1964	1711 ± 444[a]	4378 ± 1366	5103 ± 1129	2191 ± 1111[a]	3495 ± 1250
Neutrophils	3272 ± 527	4122 ± 363	3480 ± 838	4407 ± 1960	1069 ± 556[a]	2686 ± 867

NOTE. Data are cells/microliter of whole blood ± SD.

[a] $P < .05$, compared with preinoculation levels (analysis of variance).

perature of the ferrets per the manufacturer's instructions. All procedures and experiments conducted on ferrets were performed within the biosafety level 2 facility at St. Jude Children's Research Hospital in accordance with guidelines established by the animal care and use committee.

Chemotherapeutic treatment and immunization of ferrets. Ferrets were injected with 5 mg/kg dexamethasone (Dex) (American Pharmaceutical Partners), either alone or at the same time as 100 mg/kg cytarabine (AraC) (Bedford Laboratories), which were individually delivered intraperitoneally for 3 consecutive days. As an inoculation control, PBS was administered to control ferrets in volumes similar to those used for the delivery of either Dex or AraC. Beginning on the final day of the 3-day injection regimen, daily im treatments with 100 mg/kg ceftriaxone (Roche Laboratories) were initiated. Prophylactic antibiotic therapy was continued until white blood cell (WBC) counts increased to at least 5000 cells/μL. Seven days after the final dose of chemotherapy, a single 500-μL dose (250 μL/nostril) of FluMist was delivered by intranasal instillation to ferrets lightly anesthetized with 3% isoflurane (Baxter Pharmaceuticals).

Monitoring ferrets. Throughout the course of the study, PhysioTel-implanted ferrets were monitored daily for body temperature. After inoculation, all ferrets were monitored daily for signs and symptoms associated with toxicity or illness and were given a score using 2 individual criteria—activity and sneezing—in a manner similar to that reported by Reuman et al. [44]. An activity score of 0 was given to ferrets that were alert and playful without stimulation, a 1 was given to ferrets that were alert and playful with stimulation, a 2 was given to ferrets that were alert but not playful with stimulation, and a 3 was given to ferrets that were neither alert nor playful with stimulation. Sneezing was ranked, with a score of 0 given to ferrets who sneezed 0 times, a score of 1 was given to ferrets who sneezed 1–10 times, a score of 2 was given to ferrets who sneezed >10 times, and a score of 3 was given to ferrets who had respiratory distress. Activity and sneezing scores were recorded during a 10-min period and were combined and reported as the clinical score for ferrets.

Blood (1 mL) was collected via the internal mammary vein from isoflurane-anesthetized ferrets before chemotherapeutic treatment (day −11), on the day of inoculation (day 0), and on day 14 after inoculation. A fraction of the blood (~750 μL) was collected into K_2EDTA Vacutainer tubes (Becton Dickinson) and analyzed for complete blood counts and populations. A fraction of the blood that was collected on days 0 and 14 (~250 μL) and a 1-mL sample of blood that was collected on day 35 after inoculation were treated and used for serum antibody analysis, as described below.

Titration of virus in nasal wash fluid. On days 1, 2, 4, 7, and 9 after inoculation, ferrets were anesthetized with 60 mg of im ketamine (Hospira), and nasal wash samples were collected as effluvium into 50-mL tubes (Corning) after the instillation of 1 mL of PBS (500 μL/nostril). For the determination of viral titers, virus was propagated in MDCK cells. Confluent MDCK monolayers were rinsed with PBS and inoculated in quadruplicate with 10-fold dilutions of nasal wash fluid. After 1 h, the inoculum was removed, and 1 mL of infection media supplemented with 1 μg/mL L-(tosylamido-2-phenyl) ethyl chloromethyl ketone–treated typsin (TPCK-trypsin; Worthington Biochemical) was added to the cells. Cells were incubated for 4 days at 33°C in 5% CO_2, and viral titers are reported as the $TCID_{50}$ per milliliter.

Determination of protein in nasal wash fluid. Nasal wash samples collected from ferrets on days 1, 2, 4, 7, and 9, as described above, were analyzed using the Bradford Protein Assay (Bio-Rad) with bovine serum albumin as a standard (Pierce). Protein values are reported as micrograms of protein per milliliter of nasal wash fluid.

Serum antibody analysis (microneutralization). Serum samples that were obtained from blood collected on days 0, 14, and 35 after inoculation were treated with receptor-destroying enzyme (Accurate Chemical & Scientific), heat-inactivated, and treated with turkey red blood cells. Influenza-specific reactivity was determined using a microneutralization assay, as described elsewhere [45]. Briefly, serum diluted in infection media was incubated with influenza viruses (2000 $TCID_{50}$/mL) expressing hemagglutinins from A/New Caledonia/20/99, A/Fujian/411/02, and B/Yamanashi/166/98 for 2 h. PBS-rinsed MDCK cells (3×10^5 cells/mL) were then exposed to serum-virus mixtures for 2 h. The inoculum was removed, and cells were incubated overnight in infection media supplemented with 2 μg/mL TPCK-trypsin. Cells were rinsed with PBS and fixed with 80%

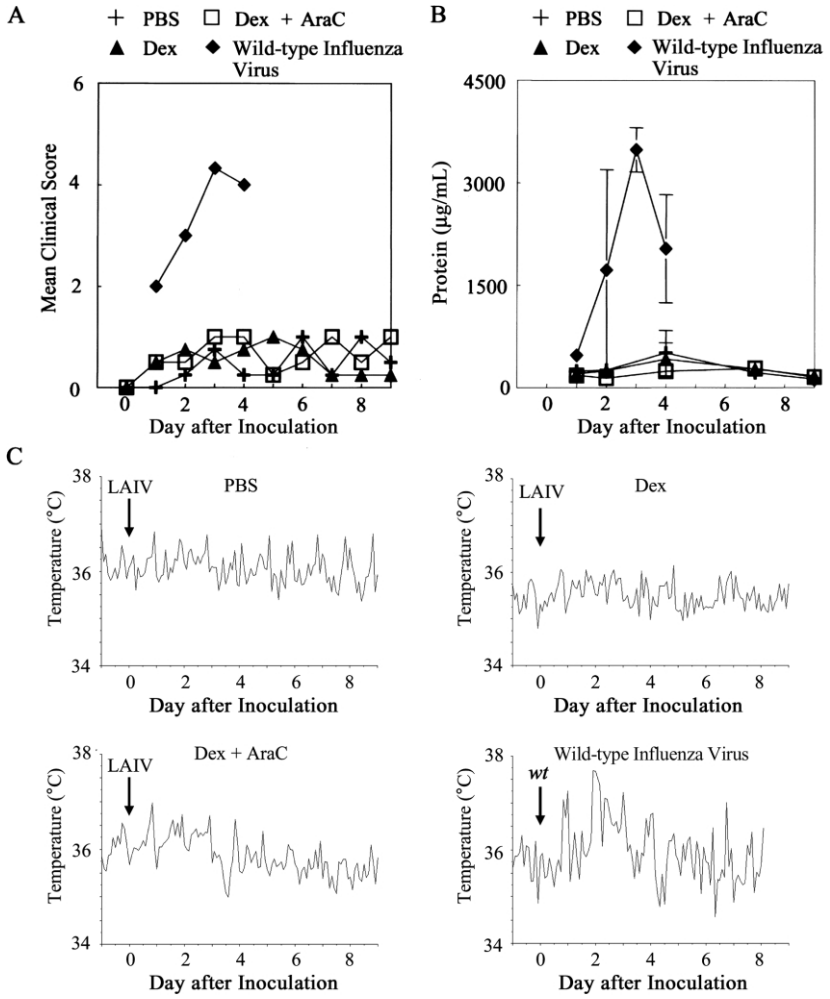

Figure 2. Response of ferrets to inoculation with FluMist. Shown are mean clinical score *(A),* protein in nasal wash fluid *(B),* and body temperature *(C)* after FluMist (live attenuated influenza vaccine; LAIV) inoculation. Four ferrets per group were treated with either dexamethasone (Dex; *black triangles*) or Dex and cytarabine (AraC; *white squares*) or were mock-treated with PBS *(plus signs)* and then were inoculated with FluMist. Additionally, a group of 4 ferrets was inoculated with wild-type *(wt)* influenza virus *(black diamonds).* All 4 ferrets/group were monitored for changes in clinical score and protein expression in nasopharyngeal secretions. The moving average temperatures for 3 ferrets/group are plotted. Downward arrows indicate the time of FluMist or *wt* influenza virus inoculation. Monitoring was performed and samples were collected only on days 1–4 in ferrets inoculated with *wt* influenza virus.

acetone (Fisher Scientific) for 10 min at room temperature. Air-dried plates were washed with PBS containing 0.05% Tween 20 (PBS-T; Sigma), and mouse monoclonal antibodies specific for the nucleoprotein of either influenza A or influenza B virus (gift of Robert G. Webster, St. Jude Children's Research Hospital, Memphis, Tennessee) were diluted in blocking buffer (1% bovine serum albumin [Invitrogen] in PBS-T) and then were added to the plates. After 1 h, the plates were washed with PBS-T, horseradish peroxidase–conjugated goat anti–mouse IgG antibody (Fc-specific; Sigma) that was diluted in blocking buffer was added, and the plates were incubated for 1 h. The plates were washed with PBS-T, and *o*-phenyldiamine substrate in phosphate-citrate buffer containing sodium perborate (Sigma) was added. On the addition of 1 N H_2SO_4, the OD at 492 nm was measured on a Multiskan Ascent plate reader (Labsystems). Microneutralization titers are reported as the reciprocal of the final serum dilution that neutralized virus to a level less than one-half of that seen in virus control wells.

Statistical analysis. Comparisons of WBC counts and viral titers in nasal wash fluid were performed using 1-way analysis of variance. SigmaStat for Windows (version 3.11; SysStat Software) was used for all statistical analyses. $P < .05$ was considered to be statistically significant for these comparisons.

RESULTS

Pilot study of the effects of AraC on ferrets. In a pilot study set up to determine the toxicity of AraC in this model, 3 young

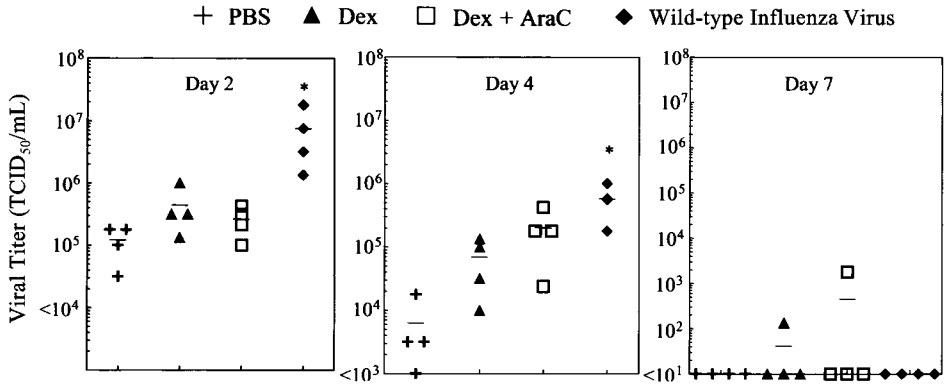

Figure 3. Viral titers in nasal wash fluid from ferrets after inoculation with FluMist. Four ferrets per group were treated with either dexamethasone (Dex; *black triangles*) or Dex and cytarabine (AraC; *white squares*) or were mock-treated with PBS *(plus signs)* and then were inoculated with FluMist. Additionally, a group of 4 ferrets was inoculated with wild-type influenza virus *(black diamonds)*. Viral titers ($TCID_{50}$/mL) for 4 ferrets/group are reported for days 2, 4, and 7 after inoculation. *$P < .05$, compared with PBS-treated ferrets inoculated with FluMist (analysis of variance).

adult ferrets were treated with 100 mg/kg AraC for 3 consecutive days, evaluated, and then treated with 200 mg/kg for 3 additional days. AraC treatment resulted in anorexia, weight loss, lethargy, and decreases in total WBC, lymphocyte, and neutrophil counts. Toxicity, including copious diarrhea, was severe during treatment with the higher dosage of AraC (200 mg/kg/day) but not during treatment with the lower dosage (100 mg/kg/day). Seven days after the start of treatment, all 3 ferrets had elevated body temperatures in association with *Escherichia coli* bacteremia, which resulted in death. Necropsy of the ferrets revealed severe enterocolitis and hypoplastic bone marrow (data not shown), which confirmed the myelosuppressive and enterotoxic effects of the chemotherapeutic regimen. On the basis of the severity of clinical symptoms during treatment with 200 mg/kg/day of AraC, the 100 mg/kg/day dosage was chosen for experiments using FluMist. To prevent the complication of bacteremia and sepsis in subsequent experiments, broad-spectrum antibiotic prophylaxis was used immediately after chemotherapeutic treatment.

Safety of FluMist in immunocompromised ferrets. Groups of 4 ferrets were given either mock therapy with PBS, 5 mg/kg Dex, or 5 mg/kg Dex and 100 mg/kg AraC daily for 3 days. In accordance with the findings of the pilot study, all 4 ferrets treated with Dex and AraC showed a decrease in weight, whereas the weights of ferrets in other groups increased or remained stable (figure 1*A*). During this period, ferrets did not appear to be anorexic or lethargic. The ferrets regained weight during the week after the last Dex and AraC treatment. Treatment with Dex, either with or without AraC, caused transient hypothermia (figure 1*B*), which stabilized after treatment was discontinued.

On the day of inoculation (day 0), ferrets treated with Dex had decreased total WBC counts and absolute lymphocyte counts (table 1), compared with preinoculation counts. Similarly, ferrets treated with Dex and AraC had decreased total WBC and absolute lymphocyte counts. However, ferrets treated with Dex and AraC also had decreased absolute neutrophil counts, and this was not seen in ferrets treated with Dex alone. By 14 days after inoculation, WBC counts, including lymphocyte and neutrophil counts, had returned to the levels seen before chemotherapy. Because blood was drawn from ferrets only once a week during this experiment, the nadir of the WBC counts and subset counts is not known.

No clinical signs suggestive of influenza virus infection. After inoculation, ferrets treated with either Dex (7.0% ± 2.7%) or Dex and AraC (6.0% ± 4.2%) had small weight gains, compared with mock-treated ferrets (2.6% ± 1.5%), and no decrease in body weight was observed in any ferret. Mean clinical scores of ferrets treated with Dex with or without AraC were similar to those seen in mock-treated ferrets and were lower than those seen in ferrets inoculated with *wt* influenza virus (figure 2*A*). After inoculation, protein expression in the nasal wash fluid of ferrets treated with Dex with or without AraC were similar to the expression seen in mock-treated ferrets, and protein expression in both groups was lower than that in ferrets inoculated with *wt* influenza virus (figure 2*B*). The mean body temperature after inoculation was similar in all treatment groups, and the spike in body temperature seen 48 h after inoculation of ferrets with *wt* influenza virus was not seen in any ferret inoculated with FluMist (figure 2*C*).

Although ferrets did not exhibit any signs of illness after FluMist inoculation, virus was recovered from the upper respiratory tract of all animals, as was demonstrated by titers in nasal wash fluid (figure 3). Mock-treated ferrets had lower mean viral titers on days 2 and 4, compared with those in ferrets treated with either Dex or Dex and AraC, although the titers remained lower than those in ferrets inoculated with *wt* influenza virus. Only the titers in ferrets inoculated with *wt* influ-

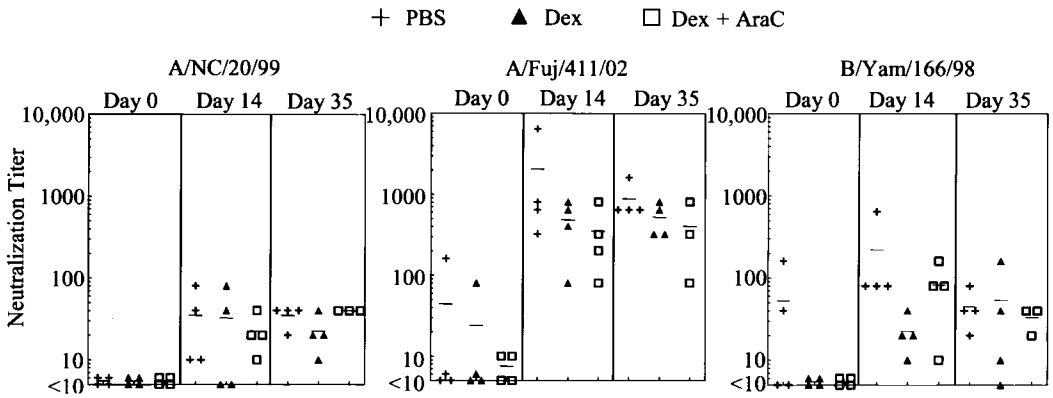

Figure 4. Antibody response of ferrets after inoculation with FluMist. Four ferrets per group were treated with either dexamethasone (Dex; *black triangles*) or Dex and cytarabine (AraC; *white squares*) or were mock-treated with PBS *(plus signs)* and then were inoculated with FluMist. Serum antibody responses to hemagglutinin are reported for days 0, 14, and 35 after FluMist inoculation. Virus neutralization titers are reported as the reciprocal of the final dilution of serum that neutralized virus to a level below one-half of that seen in virus control wells. All groups contained 4 ferrets at each time point, except for the Dex and AraC group, which contained 4 ferrets on days 0 and 14 and only 3 ferrets on day 35. A/Fuj/411/02, A/Fujian/411/02; A/NC/20/99, A/New Caledonia/20/99; B/Yam/166/98, B/Yamanashi/166/98.

enza virus were statistically different, compared with those in the other groups. On day 7 after inoculation, all 4 mock-treated ferrets had undetectable levels of influenza virus, which were similar to the levels in ferrets inoculated with *wt* influenza virus. Influenza virus was still detected in 2 ferrets treated with Dex and in 2 ferrets treated with Dex and AraC at day 7, although all ferrets had undetectable levels by day 9. Viruses obtained from ferrets were either of the H1 or B subtype, and no ferret tested positive for virus of the H3 subtype.

Immunogenicity of FluMist in immunosuppressed ferrets. Ferrets in all groups responded to all 3 vaccine strains, as measured by the induction of neutralizing antibodies (figure 4). After chemotherapy was completed, and on the day of inoculation, some ferrets had low levels of preexisting antibodies to influenza virus hemagglutinin, as was determined by microneutralization assay. The highest preimmunization titer seen was 160. After inoculation, antibodies against the H1 and B hemagglutinin were detectable but had moderate titers, whereas the immune response to the H3 hemagglutinin was more pronounced.

In response to the H1 subtype included in the vaccine, all ferrets went from having undetectable levels of neutralizing antibodies at day 0 to having detectable levels at day 35 after inoculation. This increase was statistically significant by day 14 in the Dex and AraC group and by day 35 in the PBS and Dex groups. In response to the H3 component of the vaccine, at least 1 ferret in each group exhibited detectable H3-specific neutralizing antibodies at day 0, but all ferrets showed at least a 10-fold increase in antibody titers by day 14. The increase in titers was statistically significant by day 14 in all 3 groups and persisted until at least day 35. Antibody responses to the B component of the vaccine were similar to those seen to the H1 component of the vaccine. Two control ferrets had titers of 40 and 160 on day 0. These titers increased to 80 and 640, respectively, at day 14, which indicated that these ferrets had a response to this antigen. Ferrets with undetectable levels of antibodies against the B component of the vaccine exhibited detectable levels by day 14, and all of the ferrets except 1 (in the Dex group) still had detectable antibodies against the B component of the vaccine at 35 days after inoculation. These increases in titers were statistically significant in only the Dex and AraC group at day 14. The measurement of serum antibody titers by HI assay revealed similar trends in influenza-neutralizing activity (data not shown). Analysis by ELISA of serum antibody responses showed high titers for antibodies against all 3 subtypes included in the vaccine at day 35 (data not shown), which is to be expected with this more sensitive assay [45]. Surprisingly, we were unable to detect influenza-specific antibody expression at mucosal surfaces in ferrets that received FluMist, even when attempts were made to measure the expression of total antibodies and antibodies of the IgA isotype by use of ELISA.

DISCUSSION

Traditionally, the use of live viral vaccines in immunocompromised individuals during their period of immunosuppression has been discouraged. FluMist is not presently licensed or recommended for persons with chronic diseases [3]. However, if they can be demonstrated to be safe, live viral vaccines may offer some benefits in immunocompromised populations. Thus, the primary goal of the present study was to assess the safety of an LAIV in an animal model of mild-to-moderate immunosuppression.

In the present study, we described the treatment of ferrets with toxic chemotherapeutic agents in a manner similar to that

used for combination chemotherapy in children with acute myeloid leukemia [46–48]. This model included many of the adverse effects, including depressed leukocyte counts, gut toxicity, and increased risk for gram-negative sepsis, that are seen in humans receiving such chemotherapy, although the less toxic of the 2 treatment doses that we tested was used during the testing of the LAIV. To avoid secondary infections during immunosuppression, ferrets were given prophylactic antibiotic treatment. At the point when both lymphocyte and neutrophil counts were decreased because of treatment, ferrets were inoculated with FluMist, and the safety and immunogenicity of this LAIV in this model was assessed. Because influenza vaccine is typically administered to immunocompromised patients after WBC counts return to normal, and not when they are at their nadir, this time point approximates the clinical situation well. FluMist was safe in this model, because no differences in clinical symptoms or virological parameters were seen. Although immunocompromised ferrets did not show symptoms of increased severity of infection after the administration of this vaccine, there was a suggestion that they had prolonged infection with the vaccinating strains. This was evidenced by the higher viral titers at 4 days after infection and the positive cultures at day 7 after inoculation in 4 immunosuppressed ferrets (2 in the Dex group and 2 in the Dex and AraC group). This prolonged replication was not associated with any increased signs of illness, the viral titers were very low, and the infection was cleared by day 9.

The response of immunocompromised humans to TIV is generally poor, with studies reporting lack of seroconversion [9, 23, 24] and low HI titers [25, 26]. Reduced titers of influenza-specific antibodies [18, 25, 26] may be due to a lack of B cells [23]—a condition that also affects the ability of HIV-infected individuals to respond to TIV [49]. LAIV offers the theoretical advantage of being a live vaccine that may be able to elicit strong CD8 cytotoxic T cell responses [29, 31]. Additionally, strong influenza-specific immune stimulation is demonstrated through increases in both serum antibody and nasal IgA antibody expression [31, 50, 51]. Although increases in IgA expression have been suggested to occur in humans [50, 51], we were unable to detect an increase in influenza-specific antibodies in the nasal wash fluid from infected ferrets.

As was described earlier, there appears to be a bias of the immune system toward neutralizing antibodies against the circulating H3 hemagglutinin [37]. However, the level of antibody required to define efficacy against influenza virus has yet to be determined, with HI titers as low as 1:10 correlating with protection [51], and an HI titer of 1:32 is generally considered to be protective [50]. In previous studies of FluMist, the immune response to the H1 hemagglutinin was suboptimal after a single exposure to the vaccine [36, 38]. The data presented in the present study indicated that there was lower immunity to both the H1 and B subtypes of influenza virus that were included in the vaccine, which further supports the hypothesis that delivery of a boosting dose may lead to optimal responses to all 3 subtypes included in the vaccine [30, 36, 38, 43].

Our utilization of a ferret model for immunosuppression represents the first example, to our knowledge, of using chemotherapy to reduce immunity before delivery of an LAIV. This model could also be useful for examining antiviral treatment of immunosuppressed individuals exposed to *wt* influenza virus. Using this model, we have demonstrated the safety and efficacy of LAIV in immunocompromised animals. These data will provide a basis for the testing of this vaccine in humans with cancer, as a way to prevent influenza virus infection in this high-risk population.

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