Increased Susceptibility to Feline Leukemia Virus Infection in Cats Exposed to Methylnitrosourea¹

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ABSTRACT

Exposure of adult specific-pathogen-free cats to methylnitrosourea resulted in increased susceptibility to infection by feline leukemia virus. A greater proportion of cats exposed to methylnitrosourea and feline leukemia virus (69%) became persistently viremic than those exposed to feline leukemia virus alone (17%). Segmented neutrophils were reduced by 90 to 99% within 3 days following exposure to methylnitrosourea, (15 to 20 mg/kg) whereas the effects on lymphocytes and erythrocytes, although less obvious, were also detected.

INTRODUCTION

Susceptibility of cats to feline sarcoma virus and FeLV² disease is apparently dependent upon multiple host and viral factors. With all variables constant, young cats were found to be more susceptible to FeLV (9, 14) or feline sarcoma virus (16) than were older cats. In addition to virus strain, dose, route of exposure, genetic background of the cat, and age (4, 5, 9, 14), extrinsic factors such as exposure to toxic chemical substances could also influence susceptibility of cats to FeLV infection. In this regard, a positive correlation between FeLV infection and the subsequent development of leukemia or a FeLV-related disease has been well established (4). In earlier studies, the age-related susceptibility pattern was found to vary from 100% susceptibility in newborn kittens to virtually total resistance at 4 months of age (9). This decreased susceptibility of older cats may imply either immune competence or decreased sensitivity of adult tissues to viral infection.

In apparent contrast to these experimentally determined data, naturally occurring lymphosarcoma has a peak incidence of 5.5 years (8). A second peak in age incidence is observed at 2 years, with generally 42% of cases occurring in cats less than 3 years old (8). The basis for a greater incidence of naturally occurring lymphosarcoma in older cats may be concerned with virus or host factors not yet elucidated or may be due to exposure of cats to extrinsic toxic substances that render them susceptible to the ubiquitous FeLV. The objective of this study, therefore, was to determine whether MNU, a known systemic resorptive carcinogen (3, 11), alters susceptibility of young adult cats to FeLV. The long-term objective of these investigations is to develop an experimental model of chemical-viral cocarcinogenesis based on a naturally occurring neoplastic disease.

MATERIALS AND METHODS

Animals. All cats used in this study were from a specific-pathogen-free colony (13), were moved to isolation rooms just prior to use, and were housed in separate cages. The ancestors of these cats were hysterectomy derived from several outbred pregnant queens (13).

Carcinogen Preparation. Solutions of MNU were prepared shortly before administration as described earlier (1).

FeLV. Twenty % thymic tumor homogenates induced by R-FeLV (12) were prepared and clarified at 2,300 x g for 20 min. Supernatants were then recentrifuged at 18,000 x g for 1 min and were made cell free by passing through 450-nm filters and stored in aliquots at -70°. Tumor homogenates contained 5 x 10⁴ focus-forming units/ml on subclone 81-C (6) feline cells as determined with the use of a focus assay described earlier (15).

Animal Inoculation. Young 4- to 7-month-old adult cats were inoculated with either MNU or FeLV, administered separately or in combination. Post-weanling cats of this age were chosen since they were highly resistant to induction of FeLV infection and development of LSA disease (9). Cats were given injections of either 5-, 10-, or 20-mg/kg doses of MNU i.v. (2 cats/dose) while cats receiving R-FeLV alone (12 cats) were given 1 to 2 ml of thymic tumor homogenate i.p. Two groups of 6 cats each received FeLV + MNU (15 mg/kg) or FeLV + MNU (20 mg/kg).

In Vivo Toxicity. Toxicity to MNU was evaluated by frequent testing for changes in gross physical appearance and by frequent hemogram examinations. Hematocrit, hemoglobin, WBC, RBC, and platelet counts were examined on the day of exposure, at 4 days, at weekly intervals thereafter for the first 6 weeks, and biweekly thereafter. Necropsies were performed on all cats dying from MNU-induced toxic effects, as well as on FeLV-infected cats developing LSA disease.

Detection of FeLV Viremia in Peripheral Blood. The presence of FeLV group-specific (gs) antigen in circulating leukocytes was determined as described by Hardy (7), while the presence of infectious virus was determined as described earlier (15), with the use of a sarcoma-positive, leukemia-negative murine sarcoma virus-transformed cell line (6).

RESULTS

Effect of MNU and FeLV on Peripheral Blood. Total peripheral blood leukocytes were depressed within 3 days...
following administration of MNU (20 mg/kg) to young adult cats (5 months old), while doses of 5 and 10 mg/kg, respectively, induced negligible changes throughout the duration of the experiment (Chart 1A). Segmented neutrophils were depressed to within 8% of initial levels within the first 2 weeks following exposure to MNU (20 mg/kg) and by the third week had returned to normal levels (Chart 2). Lower doses of MNU resulted in a transient, less severe neutropenia than that induced with doses of 20 mg/kg. A 30 to 50% depression in lymphocyte populations was observed with the highest MNU dose (20 mg/kg) only and persisted for 5 weeks postinoculation (Chart 3). Similarly, hemoglobin and hematocrit levels were depressed (25%) between the second and third week following exposure to MNU (20 mg/kg) and returned to normal levels by 5 to 7 weeks after exposure.

Similar effects on peripheral blood were observed in cats receiving infectious FeLV together with MNU. In 2 groups of 6 cats inoculated with either 15- or 20-mg/kg doses of MNU and R-FeLV, a greater than 70% reduction in total circulating leukocytes was observed between 4 and 14 days after injection (Chart 1B). Return to normal levels was gradual and required approximately 7 weeks. No leukopenia was observed in cats receiving FeLV alone (Chart 1B). As with MNU alone, segmented neutrophils were the more acutely affected leukocyte population, reduced to under 90% of preinoculation levels (Chart 2). Lymphopenia, observed within 3 days in both groups receiving MNU and FeLV together, was more severe in cats receiving the higher dose of MNU (Chart 3). Significant alteration in nonsegmented neutrophil populations were not detected.

Effect of MNU on Susceptibility to FeLV Infection. The influence of MNU on the sensitivity of cats to FeLV infection was assessed by determining the incidence of cats developing viremia at various times after FeLV inoculation (Chart 4). Three cats given injections of MNU (20 mg/kg) died within a 7 to 10-day period from undetermined causes. In those cats remaining, a greater proportion receiving MNU + FeLV developed viremia than those receiving an equal dose of R-FeLV alone from the same pool. By 4 weeks postinjection, 6 of 9 (67%) MNU + FeLV-infected cats developed evidence of viremia compared to only 2 of 12 (17%) cats infected with FeLV alone. Three of 9 (33%) cats infected with MNU + FeLV developed viremia as early as 2 weeks following exposure, while none of 12 FeLV-infected cats had evidence of viremia at that time. One of the 2 viremic cats receiving FeLV alone remained viremic for 2 weeks only and reverted to negative status by 7 weeks. All 6 cats with evidence of viremia remained as such to the present time (6 months postinfection), and 2 of these 6 have died recently with gross and histological evidence of LSA disease.
The proportion of viremic cats at various time intervals following inoculation with FeLV alone or with MNU + FeLV. The number of cats per group were 12 (FeLV alone) and 9 (MNU + FeLV), respectively. Viremia was determined by the detection of gp antigen in peripheral blood leukocytes as well as by direct assay of infectious FeLV on sarcoma-positive, leukemia-negative feline cells.

**DISCUSSION**

These preliminary results suggest that exposure of cats to MNU increases their susceptibility to infection by FeLV. With the use of 15- or 20-mg/kg doses of MNU + FeLV, a higher proportion of 5- to 7-month-old cats became persistently viremic following injection of R-FeLV, in contrast to a control group receiving FeLV only. On the basis of earlier evidence concerning age-related susceptibility (9, 14, 16), older cats (4- to 7-months old) in the current study receiving MNU + FeLV were in effect rendered as susceptible as 1-month-old cats. It is likely that increased susceptibility is due to an MNU-induced immunosuppression (2) or to a consequence of some direct effect on target cells, resulting in increased sensitivity to infection by FeLV. Definite evidence of LSA disease has been confirmed in 2 of the persistently viremic cats that recently became debilitated and died while under sedation.

Toxicity of MNU as analyzed by peripheral blood changes was primarily evidenced by a severe leukopenia as well as by a less severe depression in hemoglobin and hematocrit levels within the first 3 weeks following MNU exposure. With MNU alone, the dose range inducing detectable toxic effects was between 10 and 20 mg/kg. leukopenia was primarily the result of a neutropenia, with the segmented neutrophil population effectively reduced to 90 to 99% of initial levels. The deaths within 7 to 10 days of cats given 20 mg/kg doses were probably the result of severe MNU toxicity, as evidenced by severe neutropenia (less than 1000) just prior to death.

Cats given injections with MNU (20 mg/kg) + FeLV were apparently more leukopenic over a longer time interval than either cats given injections of MNU (20 mg/kg) alone or MNU (15 mg/kg) + FeLV. This effect may be the result of a greater toxic impact on sensitive neutrophilic stem cells.

Factors other than age (9, 14, 16), dose (4, 16) strain of virus (9), and possibly genetic background may also play a role in determining susceptibility of cats to infection by FeLV. In a recent study by Essex et al. (5), a high proportion of post-weaning cats became viremic following horizontal exposure to FeLV-infected cats under natural environmental conditions. Therefore, it appears that the natural route of infection and possibly the duration of exposure may be quite efficient in reducing or eliminating the age resistance to FeLV infection. In addition, results presented here also suggest that exposure to extrinsic compounds, such as MNU together with continued exposure to FeLV, may influence to an even greater extent the susceptibility of cats to infection by FeLV.

The observation of an increased incidence of leukemia following administration of a chemical carcinogen together with a leukemia virus would be highly significant for cancer research. Results presented here of an increased susceptibility to FeLV in cats normally found to be resistant may provide a useful model system for investigation of a potential mechanism of viral-chemical cocarcinogenesis. Whether this increased susceptibility is the result of an insult at some critical step in the generation of an adequate immune response, as suggested by the severe leukotoxicity or by some alteration in the normal functioning of sensitive target cells, which is possibly the result of DNA damage, must wait further study.

Of added significance is the observation that low doses of harmless precursors of MNU added to the food and drinking water of rats resulted in tumors of the nervous system and leukemia in 15 to 45% of the animals (10). Precursors of nitroso compounds are found in nature, and exposure of both humans and animals undoubtedly occurs with regularity.

**REFERENCES**

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