Inhibition of Erythroid Colony-forming Cells by a M, 15,000 Protein of Feline Leukemia Virus

Maxey L. Wellman, Gary J. Kociba, Mark G. Lewis, Larry E. Mathes, and Richard G. Olsen

Department of Veterinary Pathobiology, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio 43210

ABSTRACT

The effects of concentrated ultraviolet-inactivated feline leukemia virus (FeLV), the purified M, 15,000 envelope protein (p15E) of FeLV, or the purified M, 27,000 structural protein (p27) of FeLV on feline bone marrow mononuclear cells were studied in vitro in methylcellulose cultures. Whole virus and purified viral proteins were from the Kawakami-Theilen isolate of FeLV, which induces erythroid aplasia in cats. Bone marrow mononuclear cells from FeLV-negative young adult cats were preincubated with a medium control, ultraviolet-inactivated whole virus, or the p15E or p27 of FeLV, incubated in methylcellulose cultures for 2 days, and then observed for the formation of colony-forming units-erythroid (CFU-E) and colony-forming units-granulocyte/macrophage. The ultraviolet-inactivated Kawakami-Theilen isolate of FeLV at concentrations of 10 or 20 µg of viral protein/5 x 10⁴ cells decreased CFU-E to 66 to 56% of control values but had no significant effect on proliferation of colony-forming units-granulocyte/macrophage. p15E at concentrations of 0.1 to 0.2 µg/5 x 10⁴ cells decreased CFU-E numbers to 0 to 1% of control values, whereas the same concentration of p27 did not alter CFU-E growth when compared with controls. Neither p15E nor p27 had a significant effect on growth of colony-forming units-granulocyte/macrophage. The erythrosuppressive effects of whole virus and an envelope-derived protein but not a structural core protein suggest that FeLV envelope proteins are important in the selective inhibition of erythropoiesis observed in vivo in FeLV-infected cats.

INTRODUCTION

Erythroid aplasia is a common manifestation of both naturally occurring and experimentally induced FeLV infection (6, 8, 9, 11, 15). The naturally occurring disease is horizontally transmitted and is characterized by a severe, irreversible nonregenerative anemia that often is unaccompanied by granulocytopenia, leukemia, or lymphoma. The bone marrow of these anemic cats is usually hypocellular; however, a hypercellular marrow with ineffective erythropoiesis and myelomegakaryocytic hyperplasia can occur (7). Several isolates of FeLV are associated with selective inhibition of erythroid precursor proliferation in experimentally infected cats. The FeLV-KT (3) and 3 biologically cloned isolates of Subgroup C of FeLV (21) have been shown to induce severe nonregenerative anemia without concurrent granulocytopenia, leukemia, or lymphoma, similar to the naturally occurring disease. The proliferative capacity of hematopoietic progenitor cells can be assayed in vitro (12). Normal feline bone marrow mononuclear cells cultured in vitro in methylcellulose semisolid medium form Day 2 CFU-E and CFU-GM as described for human (12) and mouse (13) bone marrow cells. These clonogenic assays have been used in both humans (1, 20, 23) and cats (3, 21) with aplastic anemia to assess numbers and proliferative capacity of bone marrow mononuclear cells. In vivo FeLV-KT infection in cats causes rapid depletion of CFU-Es but does not decrease the number of CFU-GMs in the bone marrow. The pathogenesis of this selective inhibition of erythropoiesis in FeLV-infected cats is unknown. We report here a similar selective inhibition of erythroid precursor proliferation by incubation in vitro of normal feline bone marrow mononuclear cells with concentrated UV-inactivated FeLV-KT or the purified p15E of FeLV-KT.

MATERIALS AND METHODS

Animals. All cats were obtained at 6 months to 1 year of age from a breeding colony maintained by the Department of Veterinary Pathobiology, The Ohio State University. This colony is uniformly free of infection and immunity to FeLV. The cats were tranquilized with i.m. ketamine prior to bone marrow collection.

Virus and Viral Protein Preparations. The concentration (18), UV inactivation (10), and viral protein purification (17) have been described previously. The UV-inactivated virus was used as a whole virus preparation. The p15E and p27 proteins were used to study the effects of an envelope-associated and a structural core protein, respectively. These viral protein preparations did not contain detectable endotoxin as assayed by the Limulus amebocyte assay (M. A. Bioproducts, Walkersville, MD)

Bone Marrow Culture. Bone marrow was aspirated from the humerus or femur of FeLV-negative cats and suspended in 50% (v/v) RPMI 1640 (Grand Island Biological Co., Grand Island, NY) and Dulbecco's phosphate-buffered saline (Grand Island Biological Co.) containing preservative-free heparin (Abbott Laboratories, North Chicago, IL). Mononuclear cells were separated by Ficoll-Hypaque density gradient separation (LSM; Litton Bionetics, Kensington, MD) for 30 min at 400 x g and resuspended in RPMI 1640 at 5 x 10⁶ cells/ml. These cells were preincubated 30 min at 37° with RPMI 1640 (control), or various concentrations of UV-inactivated FeLV-KT (10, 20, 100, or 200 µg/ml RPMI 1640), p15E (0.02, 0.2, 1, or 2 µg/ml RPMI 1640), or p27 (0.02, 0.2, or 2 µg/ml RPMI 1640). The cell-virus suspensions were then cultured in methylcellulose for CFU-E and CFU-GM as described by Iscove et al. (12) and modified by Boyce et al. (3) for the cat. The final culture mixture for the UV-inactivated virus contained 30% horse serum (KC Biological, Lenexa, KS), 13% Iscove's modified Dulbecco's medium (IMDM; Grand Island Biological Co.), 10% bone marrow-conditioned medium, 0.2% bovine serum albumin (Sigma Chemical Co., St. Louis, MO), 2 x 10⁻⁴ µglutamine (Grand Island Biological Co.), 0.8% methylcellulose (Dow Chemical Co., Midland, MI), 0.01% Fungizone (E. R. Squibb and Sons, Inc., Princeton, NJ), 1% penicillin-streptomycin mixture (M. A. Bioproducts, Walkersville, MD).
M. L. Wellman et al.

The final culture mixture for the p15E and p27 proteins contained 30% fetal calf serum (Flow Laboratories, McLean, VA), 10% horse serum, 10% bone marrow-conditioned medium, 3% α-medium (Grand Island Biological Co.), 0.2% bovine serum albumin, 2 × 10^{-4} M glutamine, 0.5% methylcellulose, 2700 units penicillin, 2700 μg streptomycin, and the appropriate cell-virus suspensions. Each bone marrow cell-viral protein combination was plated in triplicate in 96-well microtiter plates (Costar Plastics, Cambridge, MA) with 5 × 10^4 cells/well. The final cell concentrations of the virus and viral proteins are shown in Charts 1 and 2. The plates were incubated at 37° in a humidified atmosphere containing 5% CO_2. CFU-E and CFU-GM were counted in the same wells by inversion phase-contrast microscopy as described previously for the cat (3). Triplicate well counts were averaged to obtain mean colony counts. Mean colony counts from cells incubated without viral protein represent 100% growth. Mean colony counts of cells incubated with viral protein were transformed to percent of control colony growth as follows.

Mean colony counts of cells incubated with viral protein
Mean colony counts of cells incubated without viral protein × 100

**RESULTS**

**UV-inactivated FeLV.** Inhibition of CFU-E but not CFU-GM occurred when normal feline bone marrow mononuclear cells were incubated with UV-inactivated FeLV-KT (Chart 1). Although preincubation of mononuclear cells with 1 or 2 μg UV-inactivated FeLV-KT per 5 × 10^4 cells resulted in erythroid colony numbers similar to the control, preincubation with 10 or 20 μg UV-inactivated FeLV-KT suppressed erythroid colonies to 66 and 56% of control colony growth, respectively. Due to variation in colony counts, only the suppression noted with 20 μg of virus was significant (p < 0.01) using Fisher’s test of least significant differences. In contrast, each concentration of UV-inactivated FeLV-KT tested had no significant effect on numbers of CFU-GM.

**p15E and p27 Proteins of FeLV-KT.** As shown in Chart 2, a similar but more potent selective suppression of CFU-E was observed when normal bone marrow mononuclear cells were incubated with the p15E protein of FeLV-KT. p15E at concentrations of 0.1 or 0.2 μg/5 × 10^4 cells dramatically decreased CFU-E numbers to 0 to 1% of control colony growth (p < 0.01). In marked contrast, p27 of FeLV-KT at the same concentrations did not alter CFU-E growth when compared to control wells. CFU-Es were moderately decreased when incubated with 0.002 or 0.02 μg of either p15E or p27/5 × 10^4 cells. The effects of p15E and p27 on CFU-GMs were variable and not significant. Note that, at the p15E concentration (0.2 μg/5 × 10^4 cells) which suppressed CFU-E numbers to 0% of control growth, CFU-GM numbers were no different from control growth.

**DISCUSSION**

Bone marrow mononuclear cells from cats experimentally infected with FeLV Subgroup C have decreased proliferative capacity of erythroid progenitors but normal proliferative capacity of granulocyte macrophage progenitors (3, 21). The mechanism of this selective inhibition of erythroid progenitors is unknown. The association of erythroid aplasia with certain viral envelope protein-defined subgroups of FeLV suggests that envelope proteins influence the selective inhibition of erythropoiesis. Our results indicate that both intact, UV-inactivated FeLV-KT and an M, 15,000 envelope protein, p15E, from the Kawakami-Thelen isolate of FeLV inhibit early erythroid colony formation (CFU-E) without inhibiting granulocyte/macrophage colony (CFU-GM) formation. The purified subviral p15E protein mediated this inhibition at concentrations 100-fold more dilute than the intact inactivated virus. This p15E protein has been shown to suppress lymphocyte blastogenesis in vivo in cats (17). In contrast, p27, a nonenvelope structural protein, did not suppress erythropoiesis or lymphocyte blastogenesis. While the purification methods used to isolate p15E may include some nonenvelope M, 15,000 viral components or may alter the structure of the p15E envelope protein, these results do support the hypothesis that viral envelope proteins influence the selective inhibition of erythropoiesis.

Several hypotheses have been postulated to explain the discriminative inhibition of erythroid precursor proliferation observed previously in vivo and in vitro with some isolates of FeLV and
reproduced here using FeLV-KT in vitro. These include direct viral effects on early erythroid precursors and indirect viral effects on specific regulatory cells required for erythropoiesis (21). Although the p15E used in these experiments is not cytotoxic to peripheral blood lymphocytes (17), this protein may be selectively cytotoxic to erythroid progenitors. Selective erythroid progenitor cytotoxicity has been suggested recently as the mechanism of aplastic anemia associated with a parvovirus-like virus in humans (19). Alternatively, p15E could bind to membrane receptors on erythroid progenitors, resulting in a defective response to molecules regulating proliferation and differentiation. Viral protein effects on hematopoietic regulatory cells have been described. An M<sub>15</sub>, 15,000 virus envelope protein from a murine leukemia virus has been shown to inhibit macrophage function in vivo in the mouse (4). In contrast, UV-inactivated FeLV does not appear to inhibit macrophage function in vitro but instead inhibits interleukin I-stimulated proliferation of human T-cells via impaired interleukin II production by, and decreased interleukin II response of, T-cells (5). These anti-T-cell-proliferative effects of inactivated FeLV are mediated by p15E. In contrast to inhibition of a T-cell that may normally stimulate erythropoiesis, selective erythrosuppression may involve viral-related proliferation of T-cells which inhibit erythroid proliferation and differentiation. The role of T-cells in both normal and abnormal bone marrow progenitor cell proliferation has been described in humans (2, 14, 16, 20, 22). However, none of the studies involving abnormal T-cell regulation of hematopoiesis in humans has been associated with viral-mediated suppression. Further studies will be required to elucidate the mechanism by which p15E from FeLV-KT mediates selective erythrosuppression and to determine if envelope proteins from isolates of FeLV that are not associated with erythroid aplasia have similar erythroid suppressive effects.

REFERENCES


FeLV p15E-mediated Erythroid Inhibition
Inhibition of Erythroid Colony-forming Cells by a $M_r$ 15,000 Protein of Feline Leukemia Virus

Maxey L. Wellman, Gary J. Kociba, Mark G. Lewis, et al.