

Role of Viruses in the Etiology of Naturally Occurring Feline Leukemia

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A. Introduction

The search to determine if retroviruses cause human leukemia has now been underway for at least 15 years. However, the number of investigators who have conducted direct studies with human materials has been small. By contrast, major emphasis has been given to the study of retrovirus-induced tumors of inbred mice and chickens as models for understanding leukemia of man. At the same time only a limited amount of attention has been given to the study of the agents that are known to cause leukemia in cats and cattle. Having evolved under natural circumstances in outbred species, the feline and bovine retroviruses would appear to be important agents for study. Presumably information derived about potential mechanisms of leukemogenesis in these species would be applicable to many of the questions one might pose about the etiology of human leukemia. In this context we describe recent findings on the biology and natural history of the feline retroviruses and the diseases they cause.

B. Epidemiology

Several forms of leukemia and lymphoma are caused by the feline leukemia viruses (FeLV). These include (a) thymic lymphoma, which is a T-cell neoplasm that originates in the mediastinal cavity; (b) alimentary lymphoma, which is a B cell disease that originates in the gut wall; (c) multicentric lymphoma, which presumably arises in the lymph nodes and is usually a T-cell tumor; (d) acute lymphoblastic leukemia, which originates in the bone marrow and/or blood; (e) various forms of myeloid leuke-

mias; and (f) certain miscellaneous localized lymphomas such as those that occur in the skin or the central nervous system. FeLV also causes aplastic anemia (Mackey et al. 1975), a disease which may be caused by similar imbalances in bone marrow cell populations. Additionally, because FeLV is immunosuppressive, a wide range of infectious diseases that are normally controlled by the immune response may occur more frequently in FeLV-infected animals (Essex et al. 1975a). Another group of feline retroviruses that are closely related to FeLV, the feline sarcoma viruses (FeSV), cause fibrosarcomas. The viruses designated as FeLVs are replication competent and they usually cause no visible pathology in cultured cells. The FeSVs are replication defective and they transform cultured fibroblasts.

The various morphologic forms of feline leukemia and lymphoma occur at different rates in different geographic areas (Essex 1975), an observation that suggests an association between a given form of disease and specific strains of virus. Although FeLVs have been categorized into subgroups by interference (Sarma and Log 1973) or serum antibody neutralization (Russell and Jarrett 1978) and into "strains" by T 1 oligonucleotide fingerprinting (Rosenberg et al. 1980), no association between given morphologic forms of naturally occurring disease and specific strains of subgroups has yet been recognized. One laboratory-passaged strain of FeLV has been shown to induce the thymic form of lymphoma in a consistent manner (Hoover et al. 1976).

Large clusters of feline leukemia and lymphoma were occasionally observed in pet cat populations (Cotter et al. 1973). Since such clusters usually occurred among outbred po-

pulations, it seemed likely that the virus causing the disease was transmitted in a contagious manner. This was confirmed as serologic evidence developed to show that healthy household associates of leukemic animals were very likely to be either infected with FeLV (Hardy et al. 1973) or harboring high levels of antibodies of FeLV (Stephenson et al. 1977a) or to FOCMA, the feline oncornavirus-associated cell membrane antigen (Essex et al. 1975b). Subsequently, it was shown that specific pathogen-free tracer cats introduced into cluster environments soon became viremic themselves with FeLV (Essex et al. 1977). Conversely, the removal of infected cats from such environments prevented the occurrence of future FeLV infections and disease development (Hardy et al. 1976).

The induction period for development of leukemia or lymphoma in cats that become infected with FeLV is prolonged and variable. In one series of 18 cats followed under natural conditions this interval varied from 3 months to 52 months and the mean interval was about 18 months (Francis et al. 1979b). Another issue that confuses the association between FeLV and development of neoplasia is the small proportion of exposed animals that actually develop leukemia or lymphoma. Most cats that become infected rid themselves of FeLV due to an active and efficient immune response to the virus envelope glycoproteins (Essex 1980). Of the majority of animals that do not become persistently viremic, at least some experience transient viremia (Grant et al. 1980). Whether or not all animals that become infected experience viremia is unknown. Even among the 2%–5% of the infected animals that become persistently viremic, most do not develop leukemia. About one-fourth or one-third of these chronically viremic cats get leukemia, while the others develop such diseases as infectious peritonitis, septicemia, and glomerulonephritis (Francis et al. 1980). As mentioned above, this is in part due to the immunosuppressive potential of FeLV and in part because these other diseases have shorter induction periods.

In leukemia cluster households, where cats are maintained under abnormally crowded conditions, the pattern of disease development becomes shifted (Francis et al. 1980). All of the cats are then exposed to FeLV, half or more become persistently viremic, and up to half may eventually die of leukemia or the immu-

nosuppression-related diseases. This increase in the rate of persistent viremia in such environments is due in large part to the fact that the cats first become exposed to FeLV at a very young age. Presumably higher concentrations of FeLV are also received at the time of exposure. Both factors should substantially increase the chances that persistent viremia and tumor development will occur.

Persistently viremic cats regularly release infectious FeLV in saliva (Francis et al. 1977). The amount of virus they produce ranges from 10^3 to 10^6 infectious units/ml. The virus is reasonably stable for long periods, if it is in a cool and moist environment (Francis et al. 1979c). Healthy, persistently viremic cats may excrete even higher levels than leukemic cats (Francis et al. 1979b). Since the former substantially outnumber the latter in most populations at any given period of time, it is obviously the healthy cats that represent the major link for transmission and maintenance of the agent in the population. As a result it would be impossible to control the disease only by eliminating leukemic or sick animals. By contrast, the elimination of all persistently infected animals from closed populations is effective in preventing disease development (Hardy et al. 1976).

About one-third or one-half of the cases of feline leukemia and lymphoma occur in nonviremic cats (Francis et al. 1979a; Hardy et al. 1980). In some geographic areas certain morphologic forms, especially the alimentary lymphoma, were more likely to occur in nonviremic cats. However, a significant portion of all major forms have been observed in nonviremic animals.

Recently, it was demonstrated that healthy cats that become exposed to FeLV in endemic environments have an increased risk for the development of the "virus-negative" (VN) form of lymphoma. In fact, the increase in relative risk for development of the VN form of lymphoma rises to same proportion (40-fold) as the risk for development of virus-positive feline leukemia following known exposure to FeLV (Hardy et al. 1980). This strongly suggests that FeLV plays an important role in the etiology of VN leukemia. We recently proposed an "immunoselection hypothesis" to postulate one possible mechanism by which such an event might occur (Essex 1980). Since human leukemias occur in individuals that do not harbor replicating

viruses, an understanding of the role that FeLV may play in the etiology of VN feline leukemia may be important.

C. Immune Response

The FeLV particles contain seven distinct proteins designated p15c, p12, p30, p10, p15e, gp70, and reverse transcriptase. All seven have been shown to be immunogenic in cats under natural conditions of exposure (Essex 1980), and several of the proteins have more than one antigenic determinant. The major protein that serves as a target for virus neutralizing antibodies is gp70, although it seems likely that p15e could serve a similar function because of its localization in the backbone of the virion envelope. Although the proteins of the *gag* gene, i.e., p15c, p12, p30, and p10, occur at internal sites in the virus particles, they are expressed at the surface of virus-producing cells (Essex et al. 1978). Many cats that become naturally exposed to FeLV develop high levels of antibodies to such proteins as p30 (Stephenson et al. 1977a). Whether or not this response plays any role in the lysis of virus-producing cells in vivo remains to be determined.

Another antigen or antigen complex associated with FeLV is FOCMA, but only in the sense that it is found on lymphoma cells and/or leukemia cells (Essex et al. 1978; Hardy et al. 1977). In fact, it is not present in either virus-producing, phenotypically normal cells or in FeLV particles. It is present in fibroblasts that are transformed by FeSV (Sliski et al. 1977) and in FeSV particles that have been rescued by a helper other than FeLV (Sherr et al. 1978).

The immune response to FOCMA is correlated with protection against development of leukemia and lymphoma (Essex et al. 1975b) as well as against the development and progression of FeSV-induced fibrosarcomas (Essex et al. 1971) and melanomas (Niederhorn et al. 1980). Antibodies to FOCMA have been identified by membrane immunofluorescence (Essex et al. 1971), by ^{51}Cr release (Grant et al. 1977), and by radioimmunoassay (Snyder et al. 1980). Lymphoma cells can be effectively lysed with antibodies in the presence of complement (Grant et al. 1977). The lysis occurs slowly, requiring up to 20 h, and works most efficiently with cat complement. The pattern of

occurrence of the lytic antibodies coincides almost perfectly with the disease associated pattern first described by membrane immunofluorescence (Essex et al. 1971; Grant et al. 1978), and allows for the possibility that such antibodies may function by complement-mediated lysis in vivo. If such a mechanism is important in vivo, depressions of complement levels might also allow immunogenic tumors to evade an otherwise effective antibody response (Grant et al. 1979). Such antibodies can also be used therapeutically to cause the regression of FeSV-induced fibrosarcomas (Noronha et al. 1980) and prevent early relapse of lymphomas after drug-induced remission (Cotter et al. 1980).

D. Tumor and Transformation Specific Antigens

Attempts to detect specific molecular species of antigens that react with typical high-titered FOCMA-type antisera from healthy »regressor» type cats led to the recognition of two general classes of molecules. The first, which was found initially on FeSV-transformed mink nonproducer fibroblasts, was a polyprotein that contained the 5' portions of the *gag* gene products (p15, p12, and parts of p30) covalently linked to a molecule of 60,000–70,000 daltons which presumably harbored the FOCMA determinants (Stephenson et al. 1977b). Such *gag*-x polyproteins have now been described for several oncogenic retroviruses and the possibility that the "x" portion of this molecule contains determinants that would be analogous to FOCMA has been considered. In the feline system two classes of *gag* x proteins have been found that represent the three partially characterized isolates of FeSV (Porzig et al. 1979). One class of *gag*-x protein contained shared antigenic cross-reactivity in the "x" portion for the Gardner-Arnstein and Snyder-Theilen viruses. The second reacts with cells transformed by the McDonough strain of FeSV and contains little if any cross-reactivity with cells transformed by the Gardner and/or Snyder strains of FeSV. These results appear analogous to those obtained by nucleic acid hybridization concerning the detection of suspected "src" sequences in fibroblasts transformed by the same three strains of FeSV (Frankel et al. 1979).

The second general class of proteins that

contains antigens which react with FOCMA-type antisera are those molecules of 65,000–70,000 daltons that have been detected in the membranes of lymphoma cells and FeSV-transformed nonproducer fibroblasts (Snyder et al. 1978; Worley and Essex 1980). Antiserum made in rabbits to the 65,000-dalton protein purified from FeSV-transformed cells could be used to precipitate an analogous 68,000-dalton protein in the membrane of cultured feline lymphoma cells. Cat antisera containing FOCMA antibodies also precipitate both the 65,000-dalton protein of transformed mink cells and the 68,000-dalton protein of lymphoid cells (Worley and Essex 1980). Earlier, Snyder et al. (1978) found that the ¹²⁵I-lactoperoxidase technique revealed a 70,000-dalton protein in membranes of feline lymphoma cells which reacted with FOCMA antisera (Chen et al. 1980). This 70,000-dalton protein may be the same as the 68,000-dalton protein described above.

Cats which were repeatedly immunized with their own cells that had been transformed in culture with FeSV developed high titers of antibodies to the appropriate gag-x protein. Although such sera usually contain antibodies to the virion structural proteins, they also contain antibodies to the “x” specific portion of the molecule. Thus, hyperimmune cat sera which initially reacted with the 85,000-dalton gag-x protein characteristic of the ST strain of FeSV still immunoprecipitate the same molecule after the removal of all antibodies to viral proteins by passage of the serum sample over an immunoadsorbent column (Chen et al. 1980).

The 85,000-dalton gag x polyprotein is expressed in FeSV-transformed cat cells as well as mink cells and in FeSV-transformed cells that replicate helper FeLV as well as nonproducers. This gag x protein is expressed in primary cultures of explanted FeSV-induced fibrosarcomas, suggesting that the protein may play a role in vivo as well as in transformation in vitro. Cultured explanted cells from FeSV-induced melanomas also contain the same protein, suggesting that the gag x type gene products may be expressed concordantly with malignant phenotype even in tumors originating from different embryonic germ cell layers. The latter observation suggests that at least some “x” type genes may have a pleiotropic effect and is compatible with the concept that the same or related FOCMA-type se-

quence may cause malignant alterations in both stromal cells and various lymphoid and hematopoietic cells (Chen et al. 1980). Several types of spontaneous tumors that were not associated with FeLV or FeSV were also checked for FOCMA and gag x type antigens and all were negative. Similarly, cat and mink cells that were transformed with agents other than FeSV did not contain these proteins.

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