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IMMUNOLOGICAL AND BIOCHEMICAL EVIDENCE THAT PR8 IS AN ENVELOPE GLYCOPROTEIN GENE RECOMBINANT BETWEEN FeLV AND ENDOGENOUS XENOTROPIC RD-114 VIRUS

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ABSTRACT

IMMUNOLOGICAL AND BIOCHEMICAL EVIDENCE THAT PR8 IS AN ENVELOPE GLYCOPROTEIN GENE RECOMBINANT BETWEEN FeLV AND ENDOGENOUS XENOTROPIC RD-114 VIRUS

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A feline retrovirus designated PR8, was previously shown to have virus neutralization and interference properties of both FeLV and the endogenous feline xenotropic virus RD-114. Further immunoprecipitation studies show PR8 is indistinguishable from FeLV with the antisera used.

Two dimensional separation of tryptic and chymotryptic peptides suggest PR8 is related to both FeLV and RD-114 in the envelope glycoprotein (gp70). The majority of PR8 gp70 peptides are analogous to FeLV gp70. Several peptides of PR8 are found in RD-114 but not in FeLV gp70, and peptides unique to PR8 are also found. This suggests PR8 gp70 is a recombinant polypeptide.

The major core protein (p27) of PR8 is identical to FeLV p27 and is different from RD-114 p30. These data show the recombinant properties of the virus are not due to phenotypic mixing of FeLV with RD-114. The role of recombinant viruses in leukemogenesis is discussed.

To my family

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INTRODUCTION

The feline leukemia virus (FeLV) is responsible for naturally occurring leukemias and lymphomas in the domestic cat. The virus is contagious, usually transmitted through saliva and urine, and cats exposed to infected cats are at a much higher risk of developing leukemia and other diseases as measured in epidemiological studies. The mechanisms by which FeLV causes leukemia are not understood. Molecular biological studies have provided data concerning virus replication, the viral proteins and arrangement of the genome. Although there exist viruses which cause leukemia in cats, mice, cows and birds, a virus or viral gene specific for human leukemia has not been found.

In the murine system, where leukemias are caused by retroviruses highly related in gross structure and biology to FeLV, a certain class of envelope glycoprotein recombinants may be involved in the pathology of the disease (11). It has been shown that increases in murine xenotropic virus titers occur in the preleukemic mouse, whereas the ecotropic virus titers do not change as the leukemia develops. A recombinant between ecotropic murine leukemia virus (MuLV) and xenotropic virus related sequences has been isolated. This recombinant virus has been shown to have an increase leukemogenic

potential in mice, and the recombination is in the envelope glycoprotein gene env (3).

This thesis reports further investigation of a putative recombinant of FeLV designated PR8. Previous virus neutralization and viral interference tests suggest the recombination is in the envelope glycoprotein gene (10). Herein are further immunological and biochemical tryptic and chymotryptic peptide comparisons of F-161, PR8 and RD-114 viral proteins. PR8 has properties in the feline system analagous to the MCF viruses in the murine system. The role of recombinant viruses in virus-induced leukemia is discussed.

LITERATURE REVIEW

RNA Retroviruses

The feline leukemia virus (FeLV) is a member of the family Retroviridae characterized by RNA viruses which integrate their viral genes into the host cell genome. Various retroviruses are oncogenic, and are capable of inducing leukemias, carcinomas, lymphomas and sarcomas. These oncogenic viruses can be divided into two general classes: viruses with high oncogenic potential (acute) which can induce tumors within weeks to months, and the low oncogenic potential (chronic) viruses which induce leukemias or tumors after a long incubation period, usually six months or more. Chronic viruses are often isolated from animals with leukemias or lymphomas. The viruses replicate in many different types of cells and some of them transform specific target cells at unique stages of differentiation.

The retroviruses have a single stranded RNA genome containing three major genes: gag, pol and env, situated between non-protein coding sequences designated long terminal repeats (LTRs) (63). The gag gene codes for group specific antigens, which are proteins that make up the viral core. The pol gene codes for the viral RNA dependent DNA polymerase, also referred to as reverse transcriptase (RT), and env codes for proteins in the viral envelope. The LTRs are essential for

the insertion of viral gene sequences into the host genome, which is essential for viral replication. The LTRs contain promotor sequences for RNA transcription, and may also be important in cell transformation (63).

Many acute retroviruses have been shown to contain deletions in the pol and env gene regions, and contain cellular genetic sequences instead of the viral genes (85). These v-onc sequences are oncogenic sequences by themselves, capable of transforming cells in transfection experiments (8). The deletions in the acute retroviruses render the virus replication-defective, due to the loss of essential viral information (pol and env). Defective viruses require non-defective leukemia-helper viruses for production of infectious virus. In the feline system FeLV and feline sarcoma virus (FeSV) represent nondefective helper and defective viruses, respectively.

The RT enzyme is an RNA dependent DNA polymerase capable of transcribing the RNA genome into a RNA/DNA duplex. This duplex is converted to a double stranded DNA form which is integrated into the host cellular DNA, and is a prerequisite for viral replication. This viral information can exist in this latent form for many cell generations before activation and production of viral RNA genomes. The exact details of the replication of the retroviruses are not known, however, a current model presented by Gilboa et al (24) may explain certain DNA intermediates that are found in the replication

cycle. For a review on some details of LTRs and integration, please refer to Reddy et al (63).

Retroviruses do not depress synthesis of host proteins or nucleic acids during infection and replication (84). Total viral protein is approximately 1% of total cellular protein synthesis in exponentially growing cells. This is an important consideration when studying the molecular biology of the retroviruses, because host cell proteins are often incorporated into the viral envelope, and may have biological effects which need to be distinguished from virus-specific protein effects. Azocar et al have demonstrated neutralization of FeLV with antisera to normal cell alloantigens (1). This fact helps confuse the subgroup classification when using different group-specific sera in virus neutralization experiments. Viral interference tests may therefore prove more reliable.

Origins of Retrovirus Research

Ludwik Gross first demonstrated the viral etiology of murine leukemia in 1951 (30). Other viruses isolated from mice include the Friend (87), Rauscher (62) and Moloney leukemia viruses (58). The pathogenesis of their associated disease, described by Siegler et al (73) places Gross and Moloney viruses with thymic lymphomas, in contrast to the Friend and Rauscher viruses which involve the spleen. The first murine sarcoma virus to be found was isolated by Jennifer Harvey in 1964 (40). She passed the Moloney strain

of murine leukemia virus (MuLV) in rats, and obtained a virus that produced pleomorphic sarcomas as well as leukemias. This sarcoma virus is the Harvey sarcoma virus. Kirsten and Mayer isolated a murine sarcoma virus from stocks of Kirsten MuLV, which is referred to as the Kirsten sarcoma virus (49).

Hartley and Rowe discovered that the mouse sarcoma virus is a defective virus that only produces infectious virus in the presence of a related leukemia helper virus (38). The sarcoma virus can transform cells in the absence of leukemia virus, but cannot replicate to produce infectious progeny. The helper virus phenomenon is a feature common to many but not all sarcoma viruses. Other examples of helper-defective systems are the Bryan strain of Rous sarcoma virus and avian leukosis virus in chickens (85), FeLV-FeSV in cats, and the Friend virus in mice. The Friend virus is a mixture of two components: spleen-focus forming virus and lymphoid leukosis virus (86). Helper leukemia viruses therefore play an important role in sarcomas as well as leukemias. The sarcoma virus is morphologically identical to leukemia virus particles (85).

Isolation of virus particles from leukemic cats was first performed by Jarrett and colleagues in 1964 (45). Since that time several new virus isolates have been found, including the Rickard strain of FeLV, which transforms thymus (T) cells. Several FeSV strains have been isolated from feline fibrosarcomas, Snyder-Theilen (75), Gardner-Arnstein (21), and McDonough (55). Snyder-Theilen and Gardner-Arnstein seem

closely related, the cell onc gene insertion into the FeSV genome appears to be identical in these two strains, and different from the insert in McDonough FeSV (22).

FeLV-FeSV

The structure of FeLV consists of a central core complex which contains the virion specific RNA. The RNA exists as a dimer (2) and has a sedimentation coefficient of 50-60 Svedberg units. Each strand of the dimer has a length of approximately 8.8 kilobases. The RNA is complexed with RT and is surrounded by gag proteins having molecular weights (m.w.) of 15,000, 12,000, 27,000 and 10,000 daltons (p15-p12-p27-p10) as determined on denaturing polyacrylamide gels. The order listed here is the order they are coded for in the FeLV genome, starting from the 5' end (35). The core complex is surrounded by an outer envelope containing lipids, proteins and glycoproteins, as the core complex buds through the host cell membrane. The major envelope proteins are a glycoprotein with a m.w. of 70,000 daltons, and a 15,000 dalton m.w. protein, referred to as gp70 and p15E, respectively. These envelope proteins attach to cell receptors, are essential for infectivity, and express antigenic determinants which elicit neutralizing antibodies and trigger other immune response mechanisms.

FeLV has a typical type C morphology as observed under an electron microscope. The type C designation is a description of morphological shape, three types have been described, A, B,

and C (85). Type A is probably a precursor which matures and condenses to a type B or C as it buds through the membrane. Type B, represented by the murine mammary tumor virus, has a viral core eccentrically positioned in the envelope, as opposed to type C which is centrally positioned. Type C is the typical form found in chickens, cats and mice with hematopoietic or sarcoma tumors.

Differences between subgroups of type C viruses are related to the envelope gp70. Genetic resistance to viral infection by certain retroviruses may be due to a lack of surface receptors for the viral gp70. Saturation of the receptors by a competing virus may explain the interference phenomenon between viruses of the same type that would otherwise infect.

The classification of FeLV-FeSV into subgroups FeLV-A, FeLV-B and FeLV-C, whether defined by viral interference, host range, or serological techniques provide the same result (please note subgroup A, B, and C is unrelated to morphologic types A, B, and C discussed above). In viral interference experiments FeLV infected feline embryo fibroblasts (FEF) are superinfected with FeSV psuedotypes (virus with a sarcoma genome within an envelope of FeLV) of FeLV subgroup A, B, or C. If interference restricts entry of the FeSV psuedotype, transformation by the sarcoma genome will not occur. For a review see Jarrett (1980), (46). These interference experiments demonstrate three subgroups A, B, and C. All

FeLV isolates to date contain FeLV-A alone, FeLV-A mixed with FeLV-B, FeLV-A mixed with FeLV-C, or a mixture of all three. Neither FeLV-B or FeLV-C occur alone in nature (36).

Host range characteristics of FeLV follow the above stated subgroup classification. Subgroup A is restricted to cat cells, subgroup B will grow in cat, human, dog and bovine cells, and subgroup C will grow in all these plus guinea pig cells (46). These results indicate subgroup A may be safer for laboratory use.

Serologically, FeLV contain group, subgroup, type and interspecies specific determinants. The gp70 has the subgroup and the type specific determinants, which for example discriminate the Snyder-Theilen, Gardner-Arnstein and McDonough FeLV-FeSVs (36). Virus neutralization studies using hyperimmune goat sera show there is considerable cross-reactivity between subgroups (34). The major group specific antigen is on the p27 molecule. In addition, interspecies specific determinants common to all mammalian C-type particles are found on the core p27 and p15 molecules.

A significant exception to the distribution of group-specific antigens in the feline system exist on the feline xenotropic virus RD-114. The core antigen of this virus is different from FeLV in both its group antigenic determinants and its molecular weight (54), which is 30,000 daltons (p30), as measured on denaturing polyacrylamide gel electrophoresis. This is the only instance where related viruses

with different size core proteins have been isolated from a single species. RD-114 does not generally produce progeny in feline cells, but will infect and replicate in certain other species. RD-114 will be discussed in more detail later.

Leukemia in Cats

The mechanisms by which FeLV induces a wide variety of neoplastic or non-neoplastic and sometimes degenerative diseases are not well understood. Apparently, different diseases are manifested as a result of the type of target cell infected, further viral-host cell interactions, and/or the degree and timing of immune responses (37). FeLV was first isolated from the serum of a cat with lymphosarcoma. The virus infects lymphocytes in the lymph nodes and then spreads to bone marrow cells. FeLV proliferation is rapid, and persistant viremia ensues. FeLV is then capable of replicating in many rapidly dividing cell types, including lymphocytes, neutrophils, granulocytic leukocytes, megakaryocytes, erythroblasts, salivary epithelial cells, and mucosal cells of the pancreas, intestine, respiratory and pharyngeal systems (37). The number of variations of disease induced by FeLV is large. The mechanisms by which degenerative diseases (thymic atrophy, pancytopenia, thrombocytopenia) occur instead of the analogous proliferative diseases (thymomas, erythroleukemia, megakaryocytic leukemia) is not known. For a review of FeLV induced diseases please refer to Hardy (37). It is possible degenerative disease is caused by

lysis during the process of viral budding, by altering the membrane and allowing lysis by antibody to FeLV or FOCMA (discussed later), or lysed by natural killer lymphocytes.

Self-limiting infections caused by FeLV is accompanied by development of anti-FOCMA and virus neutralizing antibody (66). Persistant infection probably exists due to insufficient amounts of virus neutralizing antibody, anti-FOCMA antibody, or natural killer cell activity. The ability of a cat to resist or succumb to these infections that can lead to disease may simply be a race between development of antibody and virus replication.

Essex and colleagues have shown that 55%-90% of feline tumors have readily detectable retroviruses (13). The other tumors are negative in assays for both virus and viral proteins. It is thought that these virus negative tumors are also caused by FeLV, because virus negative tumors are found in increased percentages in leukemic cat colonies with FeLV. Thus, in the case of the cat, a nonproducer model is also available for study. It is possible the virus negative tumors result from FeLV infection, DNA transcription and cell genome insertion events, but normal FeLV messenger RNA transcription does not occur, or only partial transcripts are expressed, which are sufficient for transformation.

Much of the research with leukemia has been done with mice, due to the ease of maintaining a mouse colony in comparison to a cat colony. The feline system is thought to parallel

the murine system in many ways, especially because retrovirus involvement is apparent in both species. But critical differences between FeLV and MuLV do exist. The fact that FeLV is horizontally transmitted (it is not in the mouse), the outbred nature of the cat, and the natural association of cats to humans, makes understanding the biology of FeLV critically important. Acute lymphoblastic leukemia (ALL) is a common form of lympho-proliferative neoplasia seen in cats. The disease primarily affects young rather than aged cats. Human ALL is also a disease primarily associated with children. The feline retrovirus model may reveal beneficial insights to human leukemogenesis.

Leukemia in Mice

Much of the early work implicating the role of retroviruses in leukemia was done with mice. X-ray exposure of low leukemic inbred strains of mice leads to leukemia in up to 60% of the mice in six to eight months. MuLV were isolated from these irradiated cells, but not from extracts of nonirradiated cells (85). X-ray studies coupled with electron-microscopy show the appearance of viruses before the appearance of appreciable leukemic cells. One step in leukemogenesis therefore may be induction of latent leukemia virus. A subsequent step may be the transformation of blood precursors into leukemic cells.

There are a large number of disease variations induced by MuLV. Newborn mice develop almost exclusively a

generalized myeloid leukemia, with a high percentage of chloroleukemia (38). Passage of mouse virus in rats increased the virulence of the virus, as judged by the reduction of latent period after infection. These viruses, studied by Graffi et al (27), also cause a diversification of the disease characteristics. Virus passed in rats produce, in addition to myeloid leukemias, reticulum cell leukemias, sporadic lymphatic and erythroblastic leukemias. Apparently, the hematologic type of leukemia depends largely on which type of cell was used to pass the virus. The mechanisms for these differences in leukemogenic activity are being studied in mice with Gross passage A virus and the mink cell focus-forming (MCF) virus (described later). AKR mouse derived MCF have increased leukemogenic potential in mice of AKR origin, but not other strains (6). Antigenically different viruses can cause the same pathological response in susceptible hosts, while antigenically related viruses can cause different diseases. For this reason, classification of retroviruses is based on host range and interference, which are properties of the envelope protein (84), and not on the type of disease caused by a given strain.

Although these studies indicate MuLV is the causative agent for a variety of diseases, other studies indicate the relationship between leukemia virus and leukemia is not that simplistic. Ellis et al have shown that expression of ecotropic virus is not prognostic of tumor development in

individual mice (12). It is also a fact that a specific gene encoded by leukemia viruses cannot induce leukemia, in the manner that other v-onc genes have been mapped in other acute retroviral genomes (72).

Three models of MuLV induced leukemogenesis fit current data. These three models are not mutually exclusive, but indicate a study of the biological and biochemical properties of recombinant viruses, and the mechanisms of recombination, is necessary in all animal models.

1. MuLV might initiate leukemogenesis by a transient form of expression. The virus may cause derepression of a gene in a single cell, and that may be sufficient to trigger leukemogenesis.
2. Other MuLV types (e.g., dual-tropic or xenotropic) may play a role either through partial or complete expression of viral information. Recombinational steps other than genome insertion into the host genome may be necessary before a virus becomes truly leukemogenic.
3. MuLV variants or recombinants might only infect and transform cells during a particular developmental state of the cell. Integration at specific stages of cellular gene expression may be necessary, either to promote gene transcription, or to allow integration at specific points in the host genome. Recombinant viruses will be discussed in more detail later.

Endogenous Viruses and RD-114

The fact that type C retroviruses can appear spontaneously led to the hypothesis that viral information can be transmitted from parent to offspring genetically (43). The viral genome is carried "endogenously" as an integrated genome and is not present due to a typical infection event. The information is transmitted from cell to progeny cell in a covert form, and is not reproduced by a typical viral replication cycle. The definition of an endogenous virus is a set of gene sequences comparable to viral sequences that are an integral part of the host's DNA. These "virogenes" are different from those acquired exogenously by infection and integration. Endogenous viruses should also be distinguished from genes which arise by gene duplication and/or recombination mediated by the reverse transcriptase. Many, if not all vertebrate somatic and germ cells contain information homologous to type C retrovirus RNA as determined by nucleic acid hybridization, and are capable of producing these C-type retroviruses (84). Carcinogins, irradiation and the normal aging process favor partial or complete activation of these virogenes and transforming genes (oncogenes), (43).

Often the virus produced by these endogenous viral genomes will not infect homologous cells, that is cells are resistant to infection by their own endogenous viruses (murine AKR is the exception). Viruses with the ability to infect nonhomologous cells are called "xenotropic," in contrast to

"ecotropic" which infect homologous cells. Co-cultivation of cells harboring endogenous viruses with permissive cell lines from a heterologous species is necessary to detect and amplify xenotropic virus production. Clonal cell lines of some species are capable of producing complete virus particles.

The RD-114 virus is a C-type endogenous cat virus which was liberated by co-cultivation with human cells. McAllister (54) inoculated kittens in utero with RD cells, a stable line of human rhabdomyosarcoma cells. Prior to this passage in kittens the RD cells did not contain C-type particles. An endogenous virus from the cat was able to infect and replicate in the human RD cell line. This RD-114 virus does not carry the group specific antigen present in FeLV. The reverse transcriptase is not immunologically related to the enzyme in FeLV, wooly monkey, or gibbon ape type-C viruses. Forty percent of the 60-70s RNA from RD-114 hybridizes to cat cell DNA and not to DNAs from other mammalian cells (17). RD-114 was shown to be a cat endogenous virus, as opposed to a human virus, chiefly through its high degree of nucleic acid homology to Crandell cat cell (CCC) endogenous viruses activated by IUdR (53). The origins of these endogenous viruses have been elucidated by nucleic acid hybridization studies. Genes related to the nucleic acid of RD-114/CCC are found in the cellular DNA of anthropoid primates. These sequences are not found in other members of the cat family Felidea. This indicates that acquisition of this virus by cats from primates is a relatively recent event in feline evolution (81).

Besides the RD-114 endogenous cat virus, another type C virus was acquired during evolution and is now present in the domestic cat germ line ($FeLV_O$). These endogenous $FeLV_O$ appear to have been transmitted from a rat ancestor to ancestors of the cat, another example of trans-species infection. Not all members of Felidae contain this FeLV related DNA, but cats containing RD-114 genes also contain $FeLV_O$ related genes (26). $FeLV_O$ copy number, as well as sites of integration, are conserved among tissues of the same animal, but vary from animal to animal. $FeLV_O$ has not been induced to produce infectious virus, despite repeated attempts (68). Endogenous viruses antigenically related to $FeLV_O$ have not been found in cat embryos. Only the RD-114 class has been detected in nonproducer cat embryo cultures (17). This situation is different from the endogenous viruses found in the murine system, which are highly related in antigenic properties to MuLV. This nonrelatedness may be helpful in attempting to isolate a recombinant virus from the cat analogous to the MCF viruses of mice (discussed later).

The natural prevalence of C-type RNA viruses has been elucidated with new virus antigen assay techniques (72). These immunoassay techniques have revealed viral components previously undetectable due to only partial expression of the virogenes. These techniques also show that the expression of endogenous viruses is age related. Since these endogenous viral sequences are integrated in cellular DNA, and are

genetically conserved, the question arises whether viral expression during early stages of differentiation is a normal part of the developmental process (59). The argument contends that only if the gene expression is required will it be conserved. The endogenous viruses do not necessarily have to contribute to a developmental process to prove beneficial. The fact that they are derived by interspecies transfer argues against a developmental role. Todaro has shown in cats that species which acquire primate type C viruses are resistant to infection by endogenous baboon virus (84). Species without the primate derived virus are still susceptible. Therefore the acquired viral genes prove beneficial by providing resistance to related but more virulent viruses.

Other pieces of information concerning endogenous viruses should be noted. The endogenous viruses (except AKR) do not cause any known disease in their respective animals (11). Transformed murine cells release endogenous viruses more readily than untransformed cells. Also, transformed cells releasing high titers of endogenous virus are less able to produce tumors in immunocompetent animals. If viral activation is closely related to the transformed state of the cell, then expression of an endogenous virus under natural conditions may be protective of an immunologic basis against cancer.

Recombinant Viruses

Despite the fact that viral information is a stably inheritable property and genes are conserved in an evolutionary sense, the retroviruses have the ability to recombine with host or other viral sequences. In fact, aberrant expression of MuLV occurs with a high frequency in clonal virus infections (71). Nucleic acid hybridization data has demonstrated genetic recombination between avian leukosis virus and host cellular sequences (41). Certain murine leukemia isolates may represent recombinants between exogenous (ecotropic) and endogenous (xenotropic) murine type-C virus (78). Some of these recombinant viruses have the host range properties of the ecotropic virus plus some of the extended host range of xenotropic viruses (6,7,18). Cloned viruses retaining these "dual-tropic" properties can be explained by recombination in the *env* gene (18). The spleen focus forming virus (SFFV) which is responsible for acute leukemia in Friend virus isolates, is a recombinant in the *env* gene (86).

The envelope glycoprotein (gp70) of the various isolates are recombinant as determined by two-dimensional tryptic peptide mapping (9) and have recombinant genomes as determined by oligo-nucleotide fingerprinting (67).

Hartley suggested a hypothesis that leukemia induction by ecotropic viruses may proceed via recombinant viruses (39). AKR mice increase production of a MuLV antigen in thymocytes at five to six months of age. Increase in the antigen is

not accompanied by increases in viral titers, which are high from birth. This increase in antigen production is not found in the spleen or lymph nodes, but age related changes in the thymus may be responsible for the MuLV antigen amplification (56). During the preleukemic period, there is an increased expression of xenotropic virus. After this event a new class of recombinant viruses emerge. These viruses are capable of inducing characteristic foci on mink cells, and are called mink cell focus-forming (MCF) viruses, and have been isolated from preleukemic and leukemic AKR mice (5). Some MCF viruses have been shown to accelerate the appearance of leukemia in AKR mice (6,88).

The mechanism for these recombinations and their relation to recombinational events accompanying cellular integration if any, are unknown. The acquisition by different ecotropic viruses of endogenous virus-coded glycoprotein genes may be required, but are not necessarily sufficient for oncogenicity (7). Whether the recombinational events occur in a previous cell generation and exist as a preserved genetic locus, or if they are generated independent of cellular replication is still an open question.

The Role of the Thymus in Leukemia

Although retroviruses can replicate in a variety of cell types in an infected animal, leukemia virus target cells are usually limited to the thymus, lymph nodes or spleen in

neonatal animals. The thymus cells may provide a necessary step in some virus-induced leukemias. The thymus appears to alter the virulence of certain viral strains. Although the amount of MuLV in the AKR mouse thymus does not change with age, thymus extracts are more oncogenic than spleen extracts, despite the fact that ecotropic virus titers in the spleen increase with age. Together these facts indicate a dose effect of ecotropic virus is not necessary or important in producing leukemia, and the oncogenic potential of the virus may be altered by the thymus.

The thymus from some strains of mice confer susceptibility to lymphoid leukemia, thymus from other strains do not. This is supported by experiments showing that MuLV induction is determined by loci in the mouse genome (24). The development of lymphoid leukemia in a lymphoid cell population in inbred mice is a stepwise process. Cells pass through phases of dependency, autonomy and responsiveness with respect to thymic influence (57). Although the initial phases of lymphoid leukemia may occur in cells not in the thymus, subsequent migration to and establishment in the thymus may create the observed neoplastic cell population.

Experiments involving removal of the thymus suggest the following considerations: Thymus removal lowers the frequency of X-ray induced leukemia. Grafting a new thymus as late as eight days after irradiation restores a high leukemogenic frequency (4). In fact a thymus graft will restore

susceptability to leukemia by the spontaneous, viral, chemical or radiation induced types. Thus a common feature of mammalian leukemia is the interaction of the thymus with biological, physical or chemical agents.

The sequence of events involving virus infection and integration into the cell genome is not well understood with respect to the regulated expression of genes throughout differentiation. Much of this ignorance is due to the present lack of knowledge in the area of cellular biology of the thymus and lymphoid tissues. If a specific gene in the thymus is repressed or constitutively expressed due to viral integration, it still has not been found. Studies have shown that integration of viral information may occur at multiple sites of the host cell genome (44). However, tumors appear to be of monoclonal origin, both in the avian (H. J. Kung, personal communication) and in the murine system (63). Although viruses can integrate at multiple sites, insertion at specific sites may be necessary for leukemogenesis.

Immunosurveillance and FOCMA

Many cancer cells express antigens foreign to the host. These tumor specific surface antigens are capable in some cases of eliciting an immune response which prevents growth of tumor cells, delaying or preventing tumor development. Many animals, including humans, develop antibody titers against the tumor antigens. Generally, this antibody response is not able to significantly eliminate or arrest a growing

tumor. Also, since antigen-antibody complexes are found in people with progressive cancer, the humoral arm of the immune response receives little attention by tumor immunologists. Investigators suspect the cell-mediated arm of the immune system is more important in tumor immunity. The immunosurveillance hypothesis suggested by Burnet (3) predicted a correlation between cell-mediated immunity and tumor resistance. The manner in which the humoral antibodies are involved is not well understood. Antibody response to virus envelope antigens can eliminate virus through neutralization, but probably has no effect on surveillance of growing tumors.

The naturally occurring feline leukemia seems to be an important exception to the above theorized response to tumors. The feline tumor cells display a tumor specific cell membrane antigen that is not a viral structural protein. Nontransformed FeLV infected fibroblasts and normal lymphoid cells fail to express this antigen. This tumor antigen is called the feline oncornavirus-associated cell membrane antigen (FOCMA) (15) and is chiefly defined by immunoflourescence. For a current review refer to Gardner et al (23). In the cat the immunosurveillance seems to be a function of the humoral arm of the immune system, serum antibody to FOCMA is protective for FeLV related diseases. Protective immunity to FeLV and FeSV is correlated with the titer of antibody against FOCMA. Neonatal cats which acquire maternal FOCMA antibodies are resistant to lethal doses of FeSV. FOCMA antibody

therefore represents a naturally occurring form of immunosurveillance (16).

Essex and colleagues found a correlation between absence of antibodies to FOCMA and tumor progression (14). When uninfected laboratory cats come in contact with FeLV infected cats, many rapidly developed antibody to FOCMA. Most cats developed viremia at least temporarily. A smaller yet significant proportion developed leukemia. These do not have high anti-FOCMA titers (35). FOCMA antiserum is specific for FeLV or FeSV induced tumors from animals, including cats, dogs, monkeys, pigs, goats, sheep and cattle (15). Absorption with purified viral structural proteins does not diminish anti-FOCMA serum activity. There is no cross reaction with antibodies directed against core or envelope protein. Also, FOCMA is distinct from major murine, avian and simian retrovirus induced antigens.

Whether FOCMA antigen is viral encoded or a host antigen derepressed by events accompanying viral transformation is a topic of current debate. None of the findings rule out the possibility that FOCMA is encoded by a cellular rather than a viral gene. The fact that FeSV induces FOCMA expression in other animals is a strong argument that FOCMA is viral coded (74). FOCMA expression in FeSV infected nonproducer cells of mink and rat favor the virus-coded model (77).

Some investigators contend that FOCMA is a polypeptide region fused with the FeSV gag region polypeptides, and is

referred to as gag-X. Gag-X can absorb FOCMA activity in some cases (76). Preliminary molecular weight characterization by these authors assign FOCMA with a protein of 70,000 daltons.

A summary of conclusions derived from epidemiological surveys of FeLV in cats include:

1. The immunosurveillance theory is supported by the immunologic response that works under natural conditions to protect against certain forms of cancer.
2. Transformed producer and nonproducer cells have distinctive antigens that may be exploited for early diagnosis and/or immunotherapy.
3. Immunosurveillance exists in outbred species whose cancers are caused by horizontally transmitted virus.

Neoantigens and Tumor Immunology

Recombination between two nonhomologous protein coding sequences may result in a genetic sequence coding for a hybrid protein, or a shortened polypeptide due to premature translation termination and/or post-translational cleavage. The hybrid protein may contain some of the antigenic determinants of each parental protein, and possibly additional antigenic determinants provided by new amino acids at the splice junction or by a change in protein tertiary structure. These newly created antigenic determinants or neoantigens may aid the host in discriminating self from nonself. These neoantigens may also be involved in a viruses ability to escape an established immunity, at least temporarily (47).

The high degree of polymorphism associated with the envelope gp70 in the murine system is thought to arise due to recombination between members of a multi-gene family of gp70s (10). Members of this family include a serum gp70, a xenotropic gp70 expressed in the spleen, other xenotropic gp70s (G-ERLD), and the genes encoding the type specific determinants of ecotropic gp70 (GIX, G_{RADA1}) (11). Recombinations among these genes may produce neoantigens like the G-ASKL2 associated with the recombinant MCF gp70. The multi-gene family of gp70s may extend across species barriers by way of the RNA retroviruses. This polymorphism is not found in the gag proteins or the gag gene region, therefore the gag gene does not appear to belong to a multi-gene family. The env proteins are translated from a 22s mRNA, which is different in size from the gag gene mRNA (60). It is not known if the recombinants are initially created at the mRNA level, perhaps by mechanisms involving mRNA splicing (32,51,52). The presence of conserved and variable regions within the env gene family of avian RNA retroviruses have been demonstrated by heteroduplex mapping (42). The 3' end is more highly conserved than the 5' end which carries the type specific gp70 determinants.

It is not known how many different neoantigens can arise, or why some recombinants appear more frequently than others, or even if these neoantigens are related to tumor specific cell surface antigens. Ruscetti et al have tested the

cross-reactivity of murine neoantigens with other viruses and normal mouse fibroblasts (69). The results identify neoantigens, determinants not present on ecotropic or xenotropic virus. Fischinger et al have detected a recombinant MuLV gp70 antigen on virus negative thymoma cells (20). It is tempting to postulate that chronic immune stimulation with this altered gp70 could result in blastogenesis (56). In the cat, hybridization kinetics show there are 10-15 nontandem RD-114 related genes (81). The number of feline recombinant envelope gp70s could be very large, if recombination between FeLV and RD-114 in fact occurs.

Whether a recombinant glycoprotein is able to escape a preexisting surveillance mechanism, or whether the recombination allows infection of a different set of cells in the cat is not known. The immunosurveillance theory predicts that production of a neoantigen promotes elimination by cell mediated functions. It is also possible that infection of a small population of lymphoid cells releasing recombinant virus may trigger an amplification of the cell type instead of creating events which eliminate it. It is speculated that this subset of T cells are suppressor T cells which become functionally activated as a result of virus infection. This idea is supported by observations of Kumar et al who found a similar subset of T cells are immunosuppressive for other lymphocytes when exposed to Friend virus in vitro (50). Whether the immunosuppressive events following FeLV infection

are dependent on the above hypothesized events is debatable. FeLV infection is followed by immunosuppression and then neoplastic disease (66). Inactivated FeLV has been directly implicated in immunosuppression by abrogation of tumor immunity mechanisms. The suppression appears to be mediated by FeLV p15 (61). It is likely these immunosuppressive events are independent of virus induced transformation.

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MATERIALS AND METHODS

Cells and Viruses

F-161 virus was originally derived in Rickard's laboratory by passage of virus from a spontaneous thymus lymphoma (the original isolate of Rickard FeLV) in feline embryo fibroblasts (12). Cell-free homogenates from the original tumor were also passed in cats resulting in a high incidence of leukemias and lymphomas. One such tumor from a second passage-infected cat was grown in culture, and produces the F-422 substrain of Rickard FeLV (12). F-161 virus in a chronically infected normal cat embryo fibroblast cell strain (passage 16) was obtained from F. DeNoronha (Cornell). The RD-114 virus, grown in the original RD-114 cell line was obtained from R. M. McAllister (U. of S. Calif.), (11). The feline lung fibroblast line FLF-3 was obtained from W. D. Hardy, Jr. (Sloan Kettering), (8). A clonal subline of FLF-3, FLF-3D, was made by plating a highly diluted cell suspension, followed by trypsinization of well isolated colonies with a cloning cylinder. All feline embryo fibroblast cell lines and the RD-114 cell line were grown in Dulbecco's modified Eagle's medium with 10% heat-inactivated fetal calf serum.

Following are the methods used for biological cloning of the viruses and cell lines used in this study. The cloning

was performed by Dr. A. Haberman and has been briefly described (7), except for the final simultaneous cloning of virus-infected cells (Haberman, Mink, & Velicer, manuscript in preparation).

F-161 virus was cloned by endpoint dilution in microwells. The supernatants of virus-producing cells, determined by RT assay (14), were aliquoted and frozen at -70°C. Two 24 well cluster plates (Costar 16mm well dia.) were seeded with FLF-3 cells. The following day, the cells were pretreated with 25 ug/ml DEAE dextran for 30 min. An aliquot of virus, previously titered on feline S+L- cells (4), was thawed and used to inoculate the cells with 0.06 focus inducing units (fiu) per well. The virus was absorbed at 37°C for 1 hour, 1 ml medium was added to each well, and the cells were allowed to grow to confluency. Two successive overnight medium harvests were then taken from each well and frozen at -70°C. One set was assayed on feline S+L- cells, the other from a positive well was used to inoculate a 60 mm plate which had been seeded with FLF-3 cells. Upon reaching confluency, overnight harvests were obtained, filtered, frozen in aliquots at -70°C, and subjected to a second round of cloning. Supernatant from a positive well from the second round of cloning was used to inoculate fresh cells to produce stocks of F-161 cl 1.

Since the Rickard FeLV substrain F-422 has a 100-fold lower specific infectivity than F-161 in feline fibroblasts,

and appears to consist largely of virus with defective genomes (7), a cell line with an enhanced specific infectivity was derived from F-422 FeLV prior to the cloning of that virus by endpoint dilution. This was done by infecting FLF-3D with a low multiplicity (ca. 0.005 to 0.1 fiu/cell) of F-422 virus. The virus was allowed to spread until maximal virus production, as determined by RT assay, was obtained. These infected cells were then cloned, and the virus production of each of the 11 clones obtained was measured by the RT assay. Two of these clones gave about 3-fold higher virus production than the uncloned mass culture. One of these high-producing cell lines, known as PR8, was found to give about a 10-fold higher virus titer than uncloned F-422 virus-infected FLF-3D cells. Virus from PR8 was then subjected to two rounds of endpoint dilution cloning in microwells as described above. The resulting cloned virus was designated PR8 Cl 1, which was subsequently found to have envelope properties of both FeLV and RD-114 viruses.

F-161 Cl 1 and PR8 Cl 1 were then subjected to a simultaneous cloning of virus/infected cells. Subconfluent cultures (10^5 cells/60 mm plate) of FLF-3D were inoculated on the day after seeding with endpoint dilution-cloned F-161 Cl 1 or PR8 Cl 1, at a multiplicity of infection of 0.2 fiu/cell in medium containing 10 ug/ml Polybrene. Plates were incubated for 1 hour at 37°C. The infected cells were then trypsinized, diluted to 0.6 cells/ml in DMEM with

20% heat inactivated fetal calf serum, and distributed into 24-well plates at 0.5 ml/well. The cells from single colony containing wells were transferred to fresh plates and tested for virus production. The F-161 and PR8 Cl 1 infected cell lines with the highest virus production (designated FLF3D, F161-D6 and FLF3d PR8-B1 respectively), were used to produce virus for these studies. The cells and viruses will be referred to henceforth as F-161 and PR8.

Virus Purification

Virus was harvested from dividing cells by collecting medium every 4 hours on a Smith-Kozoman Autoharvester (Bellco) until the monolayer approached confluency. Media were stored at 0°C on ice until harvest were complete. Four hour harvests were made to minimize loss of virus envelope glycoprotein. The media were clarified by centrifugation at 10,000 rpm (10K) for 10 minutes at 0-4°C in a Sorvall RC2-B centrifuge (DuPont) using a GSA rotor. Media were concentrated to approximately 200 mls using a Pellicon cassette concentrator (Millipore). Media were then layered over discontinuous gradients of 5 mls of 40% and 5 mls 20% (w/w) in TNE (0.01 Tris at pH 7.4, 0.1 M NaCL, 1 mM ethylenediaminetetraacetic acid [EDTA]). Virus was banded at the sucrose interface by centrifugation at 26K for 2 hours at 4°C in a SW 27 rotor (Beckman). The sucrose interfaces were collected and diluted three-fold with TNE and the viruses were pelleted for 1 hour at 26K. Virus pellets were resuspended in

200 ul TNE and layered on a 15%-50% sucrose gradient in a SW27.1 tube and banded to density at 26K overnight at 4°C. The gradients were fractionated on a density gradient analyzer (ISCO). Protein peaks corresponding to a density of 1.14 to 1.16 g/cm^3 were pooled and pelleted for 1 hour at 26K in a SW27.1 tube at 4°C. Pellets were resuspended in water and frozen at -20°C, and used for radioiodination, protein digestion, and two-dimensional peptide mapping experiments.

[^{35}S-]Methionine Labeling of Viruses

Virus used in the immunoprecipitation analysis was labeled in vivo with ^{35}S-methionine (NEN). 490 cm^2 plastic roller bottles (Corning), containing near confluent monolayers were washed with Hank's buffered saline solution. Radiolabeled methionine was incorporated in vivo by adding 500 uCi of ^{35}S-methionine in 5 mls of DMEM medium containing 1/20th normal methionine. Cells and viruses were labeled for 12 hours, and two additional harvests were made with complete medium lacking the radiolabel. Media were clarified at 10K for 10 minutes and stored at 0°C on ice until ultra-centrifugation. Virus was ultra-centrifuge purified on a discontinuous sucrose gradient as described above, except in a SW27.1 tube containing 1 ml of each 20% and 40% sucrose in TNE. Virus was resuspended in 0.2 mls water. One µl of each virus was spotted on a 2.3 cm diameter x 3 mm filter

discs, precipitated with ice cold 20% trichloracetic acid (TCA) for 20 minutes, followed by washings in ice cold 5% TCA. Discs were dried in acetone and added to vials containing 5 ml of phosphor scintillation fluid (2,5, Diphenyloxazole (PPO) 22.7 gm; 1,4,bis-2-(4-methyl-5-phenyloxazolyl)-benzene (POPOP) 1.9 gm, toluene 8 pints) and counted in a liquid scintillation spectrometer (Packard Inst. Co.).

Immunoprecipitation Analysis

Goat antisera against purified FeLV gp70 was obtained from Hilleman (Merck Inst. for Therapeutic Research) and contained some anti-p27 reactivity (13). Goat antisera against Tween-ether disrupted RD-114 was obtained from the Division of Cancer Cause and Prevention, National Cancer Institute. Normal rabbit serum (NRS) was obtained in our own laboratory.

Immunoprecipitations were performed according to the methods of Witte et al (15), with the collection of antigen-antibody complexes by Staphylococcus aureus Cowan I strain prepared for use as an immunoadsorbent according to the method of Kessler (9). This procedure exploits the high specificity and adsorption capacity of the S. aureus bacterium for the Fc region of many IgG subclasses. Prior to use, the prepared S. aureus was pelleted at 3K (International PR6) for 10 minutes and resuspended to its original volume with detergent lysis buffer (0.01M NaH_2PO_4 [pH 7.5], 0.1 M

NaCl containing 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS) containing 1 mg/ml bovine serum albumin (BSA). This S. aureus immunoadsorbant was then used at 10 times the volume of antiserum used for immunoprecipitation.

Approximately 20,000 cpm of virus was used for each immunoprecipitation sample. Prior to incubation with immune serum, the antigen containing material was precleared in order to remove radiolabeled molecules that nonspecifically adsorb to either IgG or the S. aureus immunoadsorbant. Two µl NRS was incubated with virus and 80 µl detergent lysis buffer for 2 hours at 0°C on ice. Twenty µl of the S. aureus was added and incubated for 1 hour on ice, then centrifuged for 2 minutes in an Eppendorf centrifuge. The precleared supernatant was then incubated with 2 µl of the immune antisera, and incubated overnight on ice. Twenty µl of S. aureus was added and incubated for 1 hour on ice, and centrifuged for 2 minutes. The supernatant was carefully removed by vacuum and discarded using a hand drawn, fine-tipped Pasteur pipet, so as not to disturb the bacterial pellet containing the antigen-antibody complex. The pellet was washed three times with detergent lysis buffer at 0-4°C and the wash supernatant carefully removed and discarded as before. The pellets were resuspended in 40 µl sample buffer (SB): 50 mM Tris (pH 6.8), 1% SDS, 1% B 2-mercaptoethanol, 10% glycerol, 5 mg/ml bromphenol blue. Samples were placed in boiling water for 3-5 minutes to liberate the bound antigen.

Nonimmunoprecipitated total virus samples containing 1,000 cpm virus were also suspended in SB and boiled before loading onto the slab gel.

Polyacrylamide Slab Gel Electrophoresis

A 10% polyacrylamide slab gel measuring 1.5 mm x 14 cm x 10 cm (20 ml) was used with a 5% stacking gel as described by Laemmli (10), in a vertical slab gel apparatus (Hoeffer, mode SE-520). Gels were prepared from a stock solution of a 30:0.8 ratio of acrylamide to bisacrylamide mixture. The final concentration of the stacking gel buffer was 0.0626 M Tris-HCl (pH 6.8), and 0.1% SDS. The final concentration of the separating gel buffer was 0.184 M Tris-HCl (pH 8.8), and 0.1% SDS. The electrode buffer was 0.05 M Tris (pH 8.8), 0.38 M glycine, and 0.1% SDS.

Electrophoresis was carried out at 150 volts (constant voltage) until the bromphenol blue dye reached the bottom of the gel. The gel was fixed and stained for 20 minutes in 0.25% Coomassie Blue, 45% methanol, 10% acetic acid, and destained overnight in 10% methanol, 10% acetic acid. Gels used in the radiolabeled immunoprecipitation analysis required flourographic enhancement as described by Bonner and Laskey (1). The PPO was 27.7% in DMSO. The gel was dried using a slab gel dryer (Hoeffer Model SE-540), and exposed to Kodak XAR-5 film at -70°C.

Radioiodination and Protein Digestion

Radioiodination of viral proteins was performed as described by Gibson (5). Protein determinations were performed using the Coomassie Blue technique with crystalline BSA as a standard (2). Twenty-five µg of virus in 25 µl H_2O was added to 25 µl of iodination buffer (0.2% SDS, 8 M urea, 0.1 M Tris [pH 7.5]). Five µl of ^{125}Iodine (Amersham) was added followed by 5 µl Chloramine T (5 mg/ml, Eastman Kodak). The reaction proceeded at room temperature for 30 minutes. Fifteen µl of 0.1 M dithiotreitol (20 mM final concentration) was then added to stop the reaction, and vials were placed on ice for 20 minutes. Five µl of this labeled virus preparation was added to 30 µl SB and 5 µl 2x SB. The samples were divided into two lanes on a 10% slab gel and run as described. ^{125}Iodinated BSA and DNAse were used as molecular weight markers in adjacent slab gel lanes. Non-labeled virus and molecular weight marker proteins were also run on other lanes of the same gel and detected by staining. All manipulations of ^{125}Iodine, including running of the gel, were performed under an exhaust hood.

The iodinated portion of the gel was fixed in 10% methanol, 10% acetic acid for 3 hours on a rocking apparatus. Spots of ^{125}Iodinated ink were made on the gel, and were used to align the gel with the autoradiograph. The wet gel was wrapped in two layers of cellophane, and exposed to Kodak XAR-5 film at -69°C for 3-24 hours.

Trypsinization and chymotrypsinization of iodinated viral protein in gel slices was performed essentially as described by Elder (3). Gel pieces were sliced out, washed extensively with 25% isopropyl alcohol, then 10% methanol, dried under a heat lamp and placed into Eppendorf 1.5 ml capped tubes. The gel from which pieces were removed was re-exposed to film to insure proper protein bands were cut. Dried gel pieces were rehydrated by addition of 0.5 ml Trypsin TPCK or Chymotrypsin DPCC (Sigma) in 0.05 M NH_4HCO_3 buffer (pH 8.0). The gel pieces were incubated at 37°C by flotation in a water bath for 4 hours. Enzyme solutions were removed to 1.5 ml Eppendorf tubes. Gel fragments were re-incubated overnight in 0.5 ml H_2O. Solutions were pooled and lyophilized, washed, lyophilized, and resuspended in 20 µl thin-layer electrophoresis (TLE) buffer, (15% acetic acid, 5% formic acid [v/v]). Samples were stored at -20°C. Counts of solutions and gel pieces in a gamma spectrometer (Packard Inst., Co.) assured quantitative removal of radio-labeled peptides from gel pieces. The specific activity of (^{125}I)protein was 1.09 x 10^6 cpm/µg protein.

Two-Dimensional Peptide Mapping

TLE was done in a high voltage TLE double chamber apparatus (Desaga/Brinkmann), cooled to 0°C by utilizing an external refrigerated circulator (Brinkmann Lauda K-2/R). The cooling block and buffer chambers were modified to accept two 20 x 20 cm TLE plates, so that parallel samples could be run.

Five to 10 µl of a radioiodinated tryptic or chymotryptic peptide sample was spotted on a 0.1 mm x 20 cm x 20 cm plastic-backed cellulose plate (E. Merck). Spots were made by touching a 5 µl pipet to the plate and dispensing 1-2 µl at a time, so that the spot did not exceed 7.5 mm in diameter. The center of the spot was 3 cm from both edges at the lower left corner. The spot was allowed to dry before moistening the plate with TLE buffer from a John spray bottle. A 13 x 100 mm test tube was inverted over the spot while spraying, the spot was then moistened only by capillary action, which prevented spreading of the sample.

Electrophoresis was monitored by a tracking dye mixture containing 2% Orange G (w/v) and 1% acid fuchsin (w/v) in TLE buffer. Tracking dye was applied as a small spot in the upper right-hand corner. Electrophoresis was carried out for 40 minutes at 1000 volts (constant voltage). Plates were air dried.

Ascending thin layer chromatography (TLC) in TLC buffer, (32.5:25:5:20 butanol-pyridine-acetic acid-water, by volume) continued for 3 hours, until the solvent front traveled 15 cm. After TLC, the plates were air dried and exposed to Kodak XAR-5 film for 8-10 days at -70°C. DuPont Cronex intensifying screens were used to shorten the exposure time approximately five-fold.

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RESULTS

The relationship of PR8 virus to F-161 and RD-114 viruses, as determined by immunoprecipitation, is seen in Figure 1. Lanes A represent the total (^{35}S)methionine-labeled proteins in each viral preparation, separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) according to their molecular weight. Goat antiserum to FeLV gp70 was capable of immunoprecipitating F-161 gp70 and PR8 gp70, but not RD-114 gp70 (Figure 1, Lanes B). Goat antiserum to Tween ether disrupted RD-114 virus immunoprecipitated RD-114 gp70 but not F-161 or PR8 gp70s (Figure 1, Lanes C). PR8 virus is therefore indistinguishable from the Rickard F-161 FeLV based on immunoprecipitation and PAGE analysis. F-161 and PR8 are both distinguishable from the endogenous xenotropic RD-114 virus.

Originally it was hoped that RD-114 antiserum would precipitate PR8 gp70 to verify the immunologic relationship as described in previous virus neutralization studies (10). The inability to immunoprecipitate PR8 with RD-114 antiserum is not necessarily in conflict with the finding of PR8 neutralization with RD-114 antiserum. Only a small proportion of RD-114 antiserum antibody is expected to be reactive against PR8's xenotropic determinants. Therefore a PR8 gp70 protein band immunoprecipitated by RD-114

antiserum should appear fainter than the RD-114 gp70 band immunoprecipitated by RD-114 antiserum. Virus neutralization is more sensitive than immunoprecipitation, due to the ability to measure single infection events in a biological assay as opposed to biochemical methods. Although the RD-114 antiserum may neutralize PR8 infectivity, it may not complex with enough PR8 gp70 to visualize the protein on the gel. The viruses labeled with [^{35}S]methionine have a specific activity of 1,860 cpm/μg protein, and approximately 10 ng of a protein is visible in 4 days.

Cross reactivity between the major core proteins when using the RD-114 antiserum is seen in Figure 1. RD-114 antiserum is capable of immunoprecipitating the core proteins of all three viruses (Figure 1, Lanes C), whereas FeLV gp70 antiserum is capable of immunoprecipitating F-161 and PR8 core p27s, but not RD-114 p30 (Figure 1, Lanes B). These results indicate the RD-114 antiserum has a broader specificity than FeLV gp70 antiserum. The FeLV gp70 antiserum was made against partially purified gp70 (17), which may account for its higher specificity. Lutz et al have also shown almost no cross-reactivity of FeLV and RD-114 core proteins with a FeLV antiserum, using an enzyme-linked immunosorbant assay (13).

Other pertinent conclusions derived from the SDS slab gel data results from a comparison of PR8 major core protein to those of F-161 and RD-114. The finding of the

RD-114 major core protein as a p30 instead of p27 supports the findings of Strand and August (19). If PR8 was a phenotypic mixture of FeLV and RD-114, one might expect PR8 to contain both p27 and p30 core proteins. A p30 polypeptide has never been detected in PR8, in either total virus samples or samples immunoprecipitated with the RD-114 antiserum, despite prolonged exposures. A p30 could be detected in PR8 if it contributed as little as 5% of a p27 + p30 protein mixture in this analysis.

Since immunochemical comparison of viral proteins is unsufficient to demonstrate recombinations within a given protein, biochemical separation of peptides from isolated viral proteins was attempted. Tryptic or chymotryptic proteolytic digestion of radiolabeled viral protein, followed by two-dimensional separation of peptides, allow comparison of individual protein structure between separatedly isolated viruses. The ability to separate and identify peptides of PR8 gp70 common to both F-161 and RD-114 gp70s would be strong evidence that PR8 has a recombinant glycoprotein. Also, if PR8 gp70 is a recombinant, some peptides found in F-161 and RD-114 gp70s should be missing. If this is the case, it is further evidence that PR8 is not the product of phenotypic mixing of FeLV with RD-114.

In vitro labeling of protein by radioiodination increases the specific activity of the radiolabel in protein when compared to in vivo labeling with [^{35}S]methionine

(1,860 cpm/μg [^{35}S]methionine vs. 1.1 x 10^6 cpm/μg [^{125}I]labeled protein). It is for this reason iodination is preferred over metabolic labeling when limiting amounts of protein are available.

Since the core proteins of the viruses in this study map in a different genomic region than the env genes, it is likely they are not involved in the putative env gene recombination. Peptide mappings of radioiodinated major core proteins were performed and serve as an internal control for peptide mapping of identical proteins encoded by the F-161 and PR8 viruses. Also, the major core protein (p30) of RD-114 is different in molecular weight, and should produce a peptide map different from F-161 and PR8 p27s.

The tryptic peptide maps of [^{125}I]-labeled F-161 p27, PR8 p27, and RD-114 p30 are seen in Figure 2, and chymotryptic maps in Figure 3. F-161 and PR8 peptide maps show the viruses' core proteins are nearly identical in both tryptic and chymotryptic peptide maps, while RD-114 p30 maps are considerably different. The similarity of F-161 to PR8 in the core protein peptide maps establish the reproducability of peptide mapping of identical proteins from different origins. To the right of each autoradiograph are schematic drawings of the peptide arrays and their composite relationships, which are intended to aid the reader in their analysis of the autoradiographs. Some of the radioiodinated peptides detected on the autoradiographs do not reproduce

well on the photographs, but are indicated in the schematic representations. Slight differences are apparent in the peptide maps when the maps are superimposed, even in the core protein maps which appear identical as judged by the arrangement of peptides in the maps. These differences are slight, and are possibly due to inconsistancies in the TLE plates and/or electric fields during electrophoresis. To simplify comparisons, tracings were made of the peptides from the autoradiographs onto mylar sheets. The separate mylar tracings were superimposed, and spots were compared using slight shiftings of the sheets (not more than 6 mm in any direction). A general comparison of spots in constellation type arrangements is a simplified measure of similarity, as local arrangements are obvious and slight differences in overall dimensions due to the above stated inconsistancies are accommodated. This type of comparison is typically used when analyzing data in two-dimensional peptide mapping experiment (6,9,16).

Table 1 is a summary of data derived from tryptic and chymotryptic peptide maps of the major viral core proteins. The total number of peptides exceeds the number of peptides expected using the following estimation. A protein with a molecular weight of 27,000 daltons (27 K), consists of approximately 225 amino acids. Even if 10% of the amino acids in a 27 K protein were tyrosine, and if all the peptides containing tyrosine were resolvable, with only 1 tyrosine per peptide, only 22 radioiodinated peptides would be

separated on a peptide map. Since chymotrypsin cleaves at the carboxyl end of aromatic amino acids including tyrosine, each radio-labeled peptide should contain only one tyrosine. Trypsin cleaves polypeptides on the carboxyl end of lysine and arginine amino acids, and peptides may contain more than one radio-iodinated tyrosine. The peptide maps of the core proteins generated between 31 and 45 resolvable radio-iodinated peptides (Table 1). It is these results which initially led to the suspicion of contaminating protein. If a contaminating protein does exist, it is of the same molecular weight as the p27s or p30, and the number of spots found should be roughly a multiple of the 22 estimated since the contaminant(s) should donate a similar number of peptides. The number of peptides in the major core protein maps are approximately two-fold higher than this generous estimate, so it is possible more than one contaminant protein exists in the 27 K region. Other possible reasons for the large number of iodinated peptides found might be due to the nature of the iodination reaction. Iodination of proteins is a complex reaction, substitution reactions can occur mainly between active iodine species such as I+ or I_2 and the amino acid side chains of tyrosine, histidine, phenylalanine, tryptophan, and sulfhydryl groups. However, the phenol group of tyrosine is the major target of the reaction at the pH used, and occurs as both mono and diiodinated species (19).

Twelve tryptic and 13 chymotryptic peptides are found which are common between all 3 viruses, and may represent peptides expressing common interspecies determinants, contaminant protein, or nonhomologous peptides determined identical due to the inability to completely resolve nonidentical peptides, and the errors derived from the method of comparison used. Despite the existance of common spots, there is great similarity of F-161 to PR8 p27 when the remaining spots are compared. Of the total number of trypsin derived peptides, 28 of 41 F-161 peptides migrate identically to PR8 peptides, not including those peptides common to all 3 viruses. In fact, only one tryptic peptide in F-161 p27 migrated differently from PR8 p27 (Table 1). The chymotryptic peptides show similar homology, 16 of 31 F-161 p27 peptides migrate similar to PR8 p27, and only 2 peptides are found that migrate unique to F-161 p27. RD-114 has no tryptic or chymotryptic peptides that migrate similar to F-161 or PR8, with the exception of those which migrate similar in all three viruses.

The tryptic peptide maps of radioiodinated F-161, PR8 and RD-114 gp70s are shown in Figure 4, and chymotryptic maps are shown in Figure 5. Table 2 summarizes data derived from analysis of the gp70 peptide maps. The gp70 maps contain the approximate number of peptides that are expected using the estimation procedure described above. Between 32 and 40 peptides are found, which is equivalent to 5.7% to

6.8% of a 70 K protein's amino acids being tyrosine, if all peptides containing tyrosine are separable and each contains only 1 tyrosine. Differences between F-161 and PR8 gp70s are apparent in both tryptic and chymotryptic peptide maps, indicating the proteins are not identical, and a recombinant PR8 glycoprotein is possible. Eighteen tryptic and 16 chymotryptic peptides are common between F-161 and PR8, not including those 6 tryptic and 8 chymotryptic peptides common to all 3 viruses. Of the remaining peptides PR8 has nine tryptic and eight chymotryptic peptides common to RD-114, whereas F-161 has one tryptic and zero chymotryptic peptides common with RD-114 gp70 (not including peptides common to all three). Each peptide map has peptides which are unique to the virus from which it was derived (Table 2). The RD-114 unique peptides are expected due to its biologic and antigenic differences. The unique peptides in F-161 may represent peptide sequences replaced in PR8 by RD-114 sequences. The unique peptides in PR8 however, may represent peptides derived from the sites of recombination. A recombination of nucleotide sequences may encode a new amino acid at the junction, and the new peptide derived may or may not have different electrophoretic and solvent migrating properties. PR8 has seven tryptic and five chymotryptic peptides that are unique, which suggests multiple recombinations have occurred.

Tryptic and chymotryptic peptide analysis of mixtures of F-161 with PR8 gp70, and PR8 with RD-114 gp70, were performed to show proper identity of the peptides of interest (data not shown). The results were not interpretable due to the number of peptides involved, and the inability to resolve the peptides sufficiently.

The full extent of the putative recombination cannot be determined by tryptic or chymotryptic peptide mapping methods. If the RD-114-like region extends into the p15E encoding sequences, or it if involves nonprotein coding sequences, should be determined by nucleic acid analysis, either by nucleotide sequencing, restriction endonuclease mapping, or specific hybridization experiments. However, if the recombination does not extend into the p15E region, it may be determined by peptide mapping if the PR8 map is identical to F-161 and different from RD-114 maps in both tryptic and chymotryptic digestions. Tryptic and chymotryptic peptide maps of 15 K proteins were made and are shown in Figure 6 and 7, respectively. The results are compiled in Table 3. Once again it is apparent contaminant protein may be present due to the large number of peptides observed in contrast to the 12 peptides expected using the previously described estimation method. One likely candidate to contaminate this region is the core p15 polypeptide, which has not been separated from the envelope p15E by these methods. Since there are 24 tryptic and 17 chymotryptic peptides common to all

3 viruses, no conclusion can be drawn. It is possible there is no difference between FeLV and RD-114 p15s, therefore recombination may not be detected even if it did occur. Also, since the similarity and differences may be due to the core p15s, or other possible contaminants, no conclusion can be drawn from peptide mapping without further purification of the viral proteins by isoelectric focusing or immuno-affinity methods.

DISCUSSION

The experiments in this thesis attempt to study the homology of viruses based on comparison of their proteins. Recently in this laboratory, a retrovirus having unique biological and immunological properties has been isolated. The experiments described here further compare this putative recombinant to FeLV and RD-114. The supposition that PR8 virus is a recombinant in the env gene developed from work by Dr. Allan Haberman (10). In virus neutralization and viral interference tests he observed PR8 has properties of both ecotropic FeLV and the feline xenotropic virus RD-114. Antisera to either FeLV or RD-114 were capable of interfering with PR8 infection in cat or mink cells, respectively. Since viral neutralization, viral interference, and host range test the properties of the envelope glycoprotein, Dr. Haberman predicted PR8 gp70 was a recombinant polypeptide.

In one set of experiments, proteins were radiolabeled in vivo with [^{35}S]methionine, and studied by immunoprecipitation techniques followed by SDS-PAGE. Viral proteins were compared after being immunoprecipitated by an antiserum prepared against partially purified FeLV gp70, and an antiserum against Tween-ether disrupted RD-114. It was shown the envelope gp70s of FeLV isolate F-161 is not closely

related to xenotropic RD-114 by demonstrating no common antigenic determinants as defined by these two antisera. PR8 gp70 was indistinguishable from F-161 with both the antisera used, no RD-114 determinants were immunoprecipitated with RD-114 antiserum. The inability of RD-114 antiserum to precipitate PR8 gp70 can be explained by the possible lack of enough antibody directed against the recombinant portions to achieve detectable immunoprecipitation. Virus neutralization is a biological assay capable of detecting single virus infection events, whereas immunoprecipitation is a biochemical technique to purify specific proteins. Neutralization is more sensitive for detection of viral protein necessary for infection.

Metabolic labeling with [^{35}S]methionine demonstrates that other in vivo labeled proteins associate with the virus when purified by the methods used in these experiments. Specifically, bands of protein are visualized in virus samples precleared with normal rabbit serum (Figure 1, far right-hand lanes). These proteins may represent material which are labeled in vivo and copurify with virus by attachment to the virus, or by cosedimentation of vesicles on sucrose density gradients. The material visualized in the precleared lanes are potential contaminants if they are identical in molecular weight to the proteins isolated in peptide mapping experiments.

A second set of experiments involved comparison of *in vitro* [^{125}I]-labeled viral proteins. Proteins were compared by noting similarities and differences of proteolytic cleavage products from SDS-PAGE separated protein by peptide mapping. Virus samples were radioiodinated in solution, and separated by PAGE prior to excision and enzymatic cleavage into peptides. The peptides were separated by two-dimensional peptide mapping. Radioiodination increases the specific activity of peptides as compared to metabolic labeling with [^{35}S]-methionine (see Materials and Methods), and is required when limiting amounts of protein are available.

Peptide maps were compared by superimposing mylar tracings of the autoradiographs. A general comparison of peptides forming recognizable constellation type arrangements aided the comparison, because slight inconsistancies are apparent despite parallel electrophoresis and chromatography. This method of comparison has disadvantages however. Peptides which are truly nonidentical and not shifted or only shifted slightly are not scored as different in these comparisons. The number of RD-114-like candidate peptides could be reduced due to the inability to resolve peptides sufficiently without small inconsistancies. Also, peptides are found which are considered identical in all three viral proteins, despite the fact that FeLV and RD-114 have only 8% homology as determined by nucleic acid homology. These results can occur for the following three reasons.

First, apparently common peptides are found between non-homologous proteins just due to the nature of the best fit comparison, and chance similarity due to the number of peptides occurring in each map. Secondly, some identical peptides between two different proteins may in fact exist, perhaps coding for the common interspecies antigens. Also, there is the problem of potential contaminating protein, since any virus-associated protein with a molecular weight identical to the viral proteins have not been separated from viral-coded protein. Although Gautsch et al found no serum gp70s associated with any MuLVs (8), Alcozar has been able to neutralize FeLV infectivity with antisera to normal cell alloantigens, indicating FeLV contain cellular protein in their viral envelopes (1). The molecular weight of these cellular proteins was not determined.

Polyacrylamide gel electrophoresis of viral protein may be inadequate to separate viral-coded proteins from other contaminating protein. Protein contaminants from cells or serum can then be radiolabeled in vitro along with the viral proteins, and may obscure the information desired if the contaminant exists in sufficient quantity, and are of a molecular weight similar to the viral proteins analyzed. Further separation of viral proteins by isoelectric focusing and/or immunoaffinity methods may be necessary to insure adequately purified viral proteins. It may be possible to alleviate the contaminant protein problems by immunoaffinity

chromatography enrichment of viral protein, but in this study the number of counts required to produce a peptide map, versus the low number of immunoprecipitable counts, indicate these attempts are impractical with the antisera available. Despite this problem, misleading results could be obtained even if sufficient counts were immunoprecipitated. The FeLV gp70 antiserum used may contain reactivity against cellular protein, so a preclearing with serum directed against normal cells would be required. Monospecific antibodies directed against a viral specific determinant could prevent this problem, but are unavailable at this time.

Tryptic peptide maps of FeLV p27 and RD-114 p30 have been published elsewhere, and are not identical to the maps produced in these experiments (8). The published data show FeLV p27 and RD-114 p30 are similar by having common peptides, and both are similar to baboon and rat retrovirus major core proteins. Although the authors use protein that was immunoselected and SDS-PAGE separated, their method of radioiodination has proven unacceptable in control experiments recently performed (data not shown). Their method of radioiodination in gel slices can produce a tryptic or chymotryptic peptide map even in the absence of viral protein. This result suggests that all of the radioiodination reactants are not sufficiently removed from the gel matrix prior to digestion with trypsin or chymotrypsin. Therefore it is possible the maps produced by these authors contain

iodinated peptides of self-digesting trypsin and chymotrypsin. For this reason radioiodination of viral proteins was performed in solution before separation on the slab gel.

The results of the tryptic and chymotryptic peptide maps of gp70s indicate at least 18 of 38 tryptic and 16 of 33 chymotryptic peptides of F-161 gp70 are homologous to PR8 gp70, and only 1 of 38 tryptic and 0 of 33 chymotryptic peptides of F-161 are homologous to RD-114 (not including peptides common to all 3 gp70s), (see Table 2). In contrast, PR8 gp70 contains peptides homologous to F-161 and RD-114 gp70s. PR8 gp70 has 18 of 40 tryptic and 16 of 37 chymotryptic peptides common to F-161 gp70, and 9 of 40 tryptic and 8 of 37 chymotryptic peptides homologous to RD-114 gp70. The peptide mapping data presented constitute supportive evidence that recombination(s) have occurred in the <u>env</u> gene, by illustrating specific similarities and differences exist between related viruses with different biological properties. The number of PR8 gp70 peptides found homologous to F-161 or RD-114 gp70s is not indicative of the proportion of the two respective viruses' genomes that underwent recombination(s) for form PR8. The peptides were separated by the basis of electrophoretic and solvent properties, and not by a size parameter.

It is important to ascertain that the PR8 virus is a stably inherited genetic entity, and not a product of

phenotypic mixing of envelope proteins of FeLV and endogenous viral or cellular proteins. It is formally possible that the ability to neutralize PR8 with RD-114 antiserum, or PR8's broadened host range, is due to unrecombined RD-114 glycoprotein components in FeLV virion envelope structures. Despite the fact the viruses F-161 and PR8 have been extensively cloned, they were cloned in feline cells; and although RD-114 does not replicate in feline fibroblasts, endogenous expression of RD-114 sequences is possible. Therefore it is possible all FeLV contain RD-114 protein. Several points argue strongly against the possibility that the biological properties of PR8 are due to phenotypic mixing. The F-161 virus was cloned and is propogated in the same feline cell line, and does not demonstrate the recombinant properties. Also, analysis of the F-161 and PR8 proteins indicate no RD-114 major core proteins are present, at the 5% level. The peptide map analysis of gp70s demonstrates there is not a complete set of either F-161 or RD-114 gp70 peptides in PR8 gp70. There is no evidence for the expression of any complete RD-114 sequences in PR8, which suggests the PR8 gp70 is a unique inheritable characteristic.

The number of unique peptides in PR8 gp70 may represent peptides derived from recombinational junction sequences, and may provide insight to the number of recombinations that occurred. The number of recombinations is expected to be even since preliminary restriction mapping data indicate

both PR8 LTRs are FeLV LTRs (A. Haberman, unpublished results). Seven unique tryptic and five unique chymotryptic peptides are found, which are not mutually conflicting results, since the location of proteolytic cleavage sites may or may not separate each recombination sequence. More than two recombinational events may have occurred, but the proof requires genomic sequence analysis, to test nonprotein coding regions.

Protein contamination is suspected in the peptide maps of the p27 and p30 major core proteins and the plSE proteins, due to the large number of peptides resolved. It is possible the large number of peptides are due to labeling of histidine and phenylalanine in addition to tyrosine, allowing additional peptides to be seen. But since correspondingly more peptides are not seen in the gp70 maps, protein contamination is considered more likely. The core proteins of F-161 and PR8 are virtually identical, and considerably different from RD-114 p30. Since the proteins may not be rigorously purified, unequivocal conclusions cannot be obtained from the data. However, the maps of PR8 p27 are near identical to F-161 p27, which suggests the core proteins (and possible contaminants) of PR8 and F-161 are identical. It is interesting to note the number of peptides in the major core protein peptide maps published previously is greater than the numbers derived by the generous estimations used in this study, despite the fact these

proteins were enriched by immunoaffinity chromatography prior to PAGE separation (8). Tryptic peptide maps of p15 murine protein published elsewhere do have the approximate number of peptides derived by this estimation (see results).

Other interesting biological properties of the PR8 virus have been observed by Dr. Haberman (10). PR8 induces syncytial foci in feline S+L- cells, as does the parental F-422 FeLV strain. The F-161 virus produces a normal focus morphology. PR8 therefore retains a biologic property of the thymus tumor isolate, whereas the F-161 virus does not. The unusual syncytial foci may be due to the recombinant envelope glycoprotein. Other viruses that are recombinants in the env gene, such as HIX and MCF MuLV produce syncytial foci on S+L- cells (2,5). Both feline RD-114 and the murine xenotropic viruses do not form syncytium. Whether this phenomenon is in any way related to the pathogenic properties is not known.

By using an antiserum specific for an env gene recombinant MuLV, Fischinger et al (7) detected immunofluorescence on a virus-free lymphoblastic cell line. This cell line (NIXT) does not support the growth of recombinant virus, although pure ecotropic virus growth can occur. These cells exhibited complement-dependent cytotoxicity when treated with the recombinant virus-specific antisera. The antisera precipitates a gp70 which is also found in the media of growing cells. It is possible a recombinant envelope protein is expressed on the cell-surface of the NIXT cells, and

it is responsible for the transformed morphology. The cells used in the Fischinger study are derived from an X-ray induced murine lymphoma which lacks virus production. It is known that X-rays can induce endogenous murine viruses, and it is possible that an altered expression of a membrane protein could result in chronic immune stimulation resulting in blastogenesis (15). If this scenario is true then expression of whole recombinant genomes is not necessary and leukemogenesis can occur in a virus-free state by partial virus expression. This could conveniently explain the virus-free lymphomas observed in natural cat populations (11). The recombinant FeLV PR8 gp70 may be analogous to the aberrant gp70s found on the mouse NIXT cells. Further work is necessary to derive the monoclonal antibodies necessary to determine if virus free lymphoblastic cat cells express recombinant FeLV gp70s.

The antigenic determinants derived by recombinational events between exogenous and endogenous virus or host-cell sequences are not expected to be a simple mixture of the antigens determined by the parental sequences. Cell surface neoantigens which are type-specific and virus-related are unique for different murine dualtropic virus isolates (4), and should also exist in the feline system. Serologic identification of the recombinant peptides encoding the neoantigens in gp70s is possible, and has been done in the murine system (16), using monoclonal antibodies. Molecular

cloning and sequencing of the genomes may prove to be a better method to compare related sequences. If this feline recombinant mimics the recombinants in the murine system, the RD-114 acquired sequences will be found on the -COOH terminal portion of the recombinant gp70 (16).

Leukemic transformation of lymphocytes has not been found to be a function of a transforming gene in any of the chronic leukemia viruses, as cellular transformation has been related to v-onc sequences in the acute retroviruses. Due to this lack of an onc gene, researchers have looked at the viral promoters as possible promoters for pre-existing cellular onc sequences. It is also possible the recombinant env gene viruses are responsible for cell transformation. Recombinant env gene viruses are implicated in leukemia due to the emergence of recombinant virus in preleukemic mouse thymus (12). The presence of these viruses is intriguing in attempting to identify leukemia-specific immunosurveillance targets. Whether induction of a specific class of neoantigens is sufficient to induce leukemogenesis in cells in certain animals is not known.

There are advantages and understandings that can be derived from use of the feline recombinant virus in tumor virology. The ecotropic FeLVs and endogenous xenotropic RD-114 virus are unrelated in the cat, which is not the case in the murine system. Therefore nucleotide homology may not be required for recombination. Antigenic differences

are also less vague. FeLV is also horizontally transmitted, and the cat is an outbred animal; whereas MuLVs are vertically transmitted (from parent to offspring) in highly inbred mice lines. Therefore alterations in gene expression of leukemic cats are not due to effects of highly inbred genes. FeLV is not the product of radiation induction, and FeLV produces a leukemia which is pathologically similar to human acute lymphoblastic leukemia (11). Recombinant retroviruses in cats are therefore an important finding for future study to the role of recombinant viruses in oncogenesis.

The mechanisms underlying recombinations in the env gene are not known. The unrelatedness of FeLV and RD-114 may indicate nucleotide sequence homology is not necessary for recombination. There is also evidence the recombination of retroviruses occurs at the RNA level (19), because recombinants are not found in avian cells having avian virus-related DNA sequences which do not produce virus specific RNA.

The existence of PR8 supports the possibility that variant forms of glycoprotein exist on many retrovirus induced tumors, and that viruses encoding recombinant glycoprotein may be intermediates in leukemogenesis. In order to verify the former point, recombinant envelope virus glycoprotein should be identified on cat tumors, especially tumors induced by FeLV. To verify the latter point that recombinant viruses are intermediates in leukemogenesis,

infection experiments should demonstrate increased frequency of developing leukemia, or have a decreased latency period.

The experiments reported here do not show that recombination is necessary for FeLV induced leukemia. These experiments suggest that a recombinant virus exists in a feline thymoma isolate. All viruses isolated having a recombinant glycoprotein are not expected to be leukemogenic, because not all MCF murine viruses have increased leukemogenic potential. It is possible that the critical event for leukemogenesis is the recombination with the host or endogenous viral sequences, the emergence of a dual-tropic class of viruses could then be considered as a product of the event, unimportant to leukemogenesis. On the other hand, it is possible the recombinant envelope glycoprotein is necessary to escape the immunosurveillance mechanisms, or to infect certain types of cells. The fact that many feline lymphomas do not express virus, does not indicate whether or not virus-induced recombination has occurred. Further experiments involving FeLV infections in laboratory cats will be necessary to establish the frequency of generating new <u>env</u> gene recombinants. An understanding of the genetic variations which produce observable biologic properties is fundamental to learning how virus interact with cells and how diseases are caused.

Figure 1. Immunoprecipitation analysis of [^{35}S]methionine labeled viral proteins on a 10% polyacrylamide slab gel. Viruses F-161, PR8 and RD-114 were immunoprecipitated with anti-FeLV, anti-RD-114 or normal rabbit sera as described in Materials and Methods. Lanes A, total virus; B, anti-FeLV; C, anti-RD-114; D, normal rabbit sera. Outside lanes contain ^{14}C-labeled BSA (m.w. 69Kd) and chymotrypsinogen A (m.w. 25.7 Kd) as molecular weight markers. Electrophoresis was performed at 150 V (constant voltage) until a bromphenol blue dye ran off the gel. The gel was fixed, stained, destained, treated for fluorography, dried, and exposed to Kodak XAR-5 film with Cronex intensifying screens for 8 days as detailed in Materials and Methods.

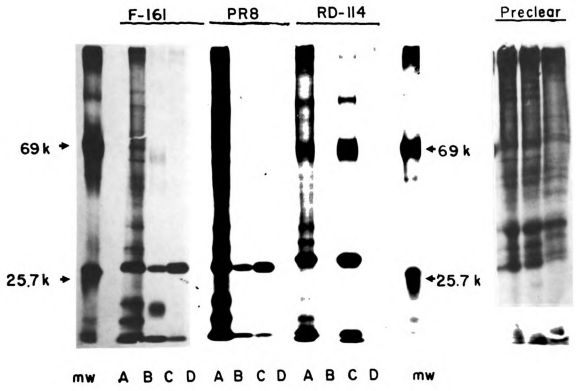
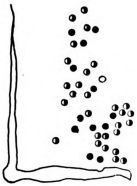

F-161
PR8
RD-114
Preclear
69k
25.7k
69k
25.7k
mw A B C D A B C D A B C D mw

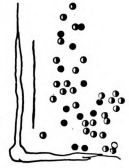
Figure 2. Autoradiographs of tryptic peptide maps of ^{125}I-labeled F-161 and PR8 p27s and RD-114 p30. In this figure and subsequent figures peptides were resolved in the 1st dimension by TLE from left to right, cathode at right, at 1,000 volts for 40 minutes at 0°C. Plates were air dried and followed by ascending TLC in the 2nd dimension (bottom to top) as described in Materials and Methods. Plates were exposed to Kodak XAR-5 film with Cronex intensifying screens at -70°C for 10 days or as needed. Symbols: ○, indicates peptides unique to each map; ◑, peptides common between F-161 and PR8 maps; ◒, peptides common to PR8 and RD-114 maps; ●, peptides common to all three peptide maps; as determined by comparisons as described in the text.

TRYPSIN p27s & p30

F161



PR8



RD-114

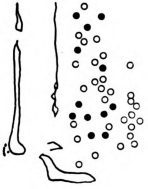
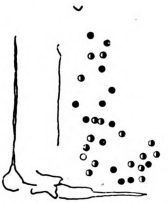


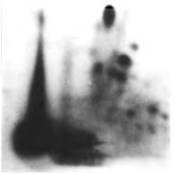
Figure 3. Autoradiographs of chymotryptic peptide maps of ^{125}I-labeled F-161 and PR8 viral p27s and RD-114 viral p30. Symbols: ○, peptides unique to each map; ◑, peptides common between F-161 and PR8; ●, peptides common to all three.

CHYMOTRYPSIN p27s & p30

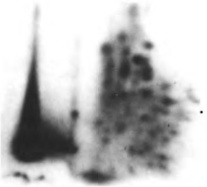
F161



PR8



RD-114



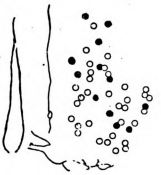
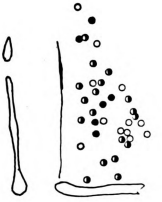


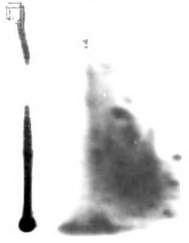
Figure 4. Autoradiographs of tryptic peptide maps of ^{125}I-labeled gp70s. Symbols: ○, peptides unique to each map; ◑, peptides common to F-161 and PR8 maps; ◒, peptides common to PR8 and RD-114 maps; ●, peptides common to all three peptide maps; as determined by comparisons as described in the text.

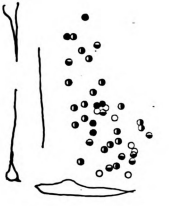
TRYPSIN gp70

F161



PR8





RD-114

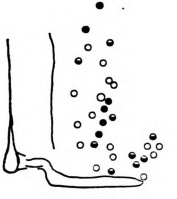
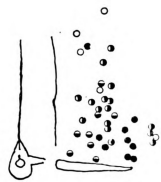


Figure 5. Autoradiographs of chymotryptic peptide maps of ^{125}I-labeled viral gp70s. Symbols: ○, peptides unique to each map; ◑, peptides common to F-161 and PR8; ◒, peptides common to PR8 and RD-114; ●, peptides common to all three maps.

CHYMOTRYPSIN gp 70

F161

PR8



RD-114

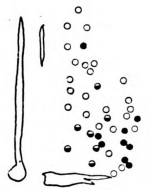
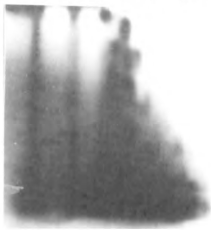
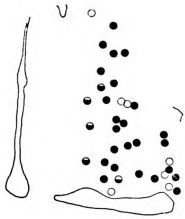


Figure 6. Autoradiographs of tryptic peptide maps of ^{125}I-labeled viral p15s. Symbols: ○, peptides unique to each map; ◑, peptides common to F-161 and PR8; ●, peptides common to all three.

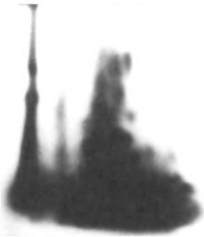
TRYPSIN pI5

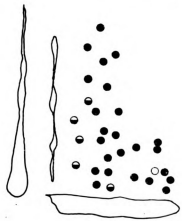
FI6I





PR8





RD-114

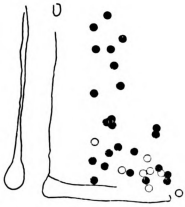
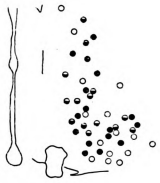


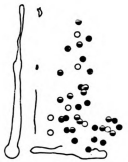
Figure 7. Autoradiographs of chymotryptic peptide maps of ^{125}I-labeled viral p15s. Symbols: ○, peptides unique to each map; ◒, peptides common to F-161 and PR8; ●, peptides common to all three.

CHYMOTRYPSIN pI5

F161



PR8



RD-114

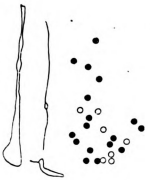


Table 2.--Summary of envelope glycoprotein peptide mapping experiments.

F-161 gp70	Trypsin	Chymotrypsin
Like PR8 only	18	16
Like RD-114 only	1	0
Common to all 3	6	8
Unique	13	9
Total	38	33

PR8 gp70	Trypsin	Chymotrypsin
Like F-161 only	18	16
Like RD-114 only	9	8
Common to all 3	6	8
Unique	7	5
Total	40	37

RD-114 gp70	Trypsin	Chymotrypsin
Like F-161 only	1	0
Like PR8 only	9	8
Common to all 3	6	8
Unique	16	23
Total	32	39

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