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Genetic control of retroviral disease in aging wild mice

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Abstract

Different populations of wild mice (*Mus musculus domesticus*) in Los Angeles and Ventura Counties were observed over their lifespan in captivity for expression of infectious murine leukemia virus (MuLV) and murine mammary tumor virus (MMTV) and for the occurrence of cancer and other diseases. In most populations of feral mice these indigenous retroviruses were infrequently expressed and cancer seldom occurred until later in life (> 2 years old). MMTV was found in the milk of about 50% of wild mice, but was associated with only a low incidence (> 1%) of breast cancer after one year of age. By contrast, in several populations, most notably at a squab farm near Lake Casitas (LC), infectious MuLV acquired at birth via milk was highly prevalent, and the infected mice were prone to leukemia and a lower motor neuron paralytic disease after one year of age. These two diseases were both caused by the same infectious (ecotropic)strain of MuLV and were the principal cause of premature death in these aging LC mice. A dominant gene called FV-4^R restricting the infection with ecotropic MuLV was found segregating in LC mice. Mice inheriting this FV-4^R allele were resistant to the ecotropic MuLV associated lymphoma and paralysis. The FV-4^R allele represents a defective endogenous MuLV provirus DNA segment that expresses an ecotropic MuLV envelope-related glycoprotein (gp70) on the cell surface. This FV-4^R encoded gp70 presumably occupies the receptor for ecotropic MuLV and blocks entry of the virus. The FV-4^R gene was probably acquired by the naturally occurring crossbreeding of LC feral mice with another species of feral mice (*Mus castaneus*) from Southeast Asia. The FV-4^R gp70 does not block entry of the amphotropic MuLV that uses a separate cell surface receptor. Therefore LC mice continued to be susceptible to the highly prevalent but weakly lymphogenic and nonparalytogenic amphotropic strain of MuLV. The study points out the potential of feral populations to reveal genes associated with specific disease resistance.

Introduction

Knowledge about the genetic influence on aging in mammals has been derived in part from inbred mouse genotypes that shorten lifespan by causing an early onset of specific disease rather than generally accelerating senescence (Finch, 1990). An understanding of the molecular genetics of these diseases of short-lived inbred mice often leads to the discovery of other genes or alleles that are associated with longer lived genotypes. On the other hand, other genetic experiments on mammalian aging have employed inbred and wild mouse strains that do not have inordinantly short lifespans (Sacher & Hart, 1978). We have studied several aging populations of long-lived wild mice (*Mus musculus domesticus*) in southern California and have observed one particular population that habitates a squab farm near Lake Casitas (LC) in Ventura County, and in which a highly prevalent infection with murine leukemia virus (MuLV) causes premature death from lymphoma and/or lower motor neuron disease. (For summary, see Gardner, 1978; Gardner & Rasheed, 1982) By classical genetic

breeding experiments between LC wild mice and inbred, lymphoma-prone AKR mice, it was shown that susceptibility or resistance to the MuLV related diseases in individual LC mice depended on the segregation of a polymorphic MuLV resistance gene called FV-4^R (Gardner *et al.*, 1980). This paper reviews the natural history and biology of this particular model and points out the lessons learned and possible relevance to humans.

Rationale for studying retroviruses in aging wild mice

Retroviruses encompass a large family of infectious agents unified by a common structure and mode of replication. Although often noncytopathic and nonpathogenic, they are occasionally associated in animals with a variety of malignancies, immunodeficiencies, neurological degeneration and other effects (Coffin, 1992). Like all retroviruses, the MuLV and MMTV are enveloped particles of about 100 nm diameter with an internal spherical or conical core and a dimeric genome of polyadenylated RNA 7-10 kilobases (Kb) in length. The morphology of the MuLV and MMTV particles was called Type C and Type B, respectively. The virus genes encode a capsid protein (*gag*), the reverse transcriptase enzyme (*pol*) and envelope glycoprotein (*env*). After entry into the cell the RNA genome is copied into a double-stranded DNA molecule by the reverse transcriptase and subsequently this DNA is covalently joined to the genomic DNA of the host cell to form the integrated proviruses. The integrated proviruses are stable and serve as a template for viral mRNA and protein synthesis under the regulation of DNA elements within the long terminal repeats (LTR) flanking the proviral DNA at each end. The promoter sequences within the LTR occasionally activate cellular protooncogenes and thereby trigger lymphogenesis or mammary carcinogenesis. Rarely, some of the MuLV may also transduce cellular protooncogenes to form highly oncogenic viruses. Other MuLV strains (e.g. amphotropic MuLV) are now used for gene therapy.

In the late 1960s, an intense search for human retroviruses was launched in the U.S.A. under the auspices of the National Cancer Institute's War on Cancer. The discovery of the human retroviruses, first the human T-cell lymphotropic virus (HTLV), and then the human immunodeficiency virus (HIV), was still over a decade away. The principal models at that time (1960s) for understanding the relationship of retroviruses to cancer were certain inbred mouse strains (e.g. GR, AKR) that were highly susceptible to breast cancer or leukemia. Most remarkably, for both the Type B breast cancer-causing retrovirus (MMTV) and the Type C leukemia viruses (MuLV), the major route of transmission in these inbred mice was from one generation to the next by genetic inheritance. Horizontal transmission of exogenous Type B or Type C virus in these particular mouse strains played a lesser role in virus spread. The discovery of endogenous (inherited) infectious retroviral genes in inbred mice, and also at about the same time, in domestic chickens, led to the formulation of the Virus Oncogene hypothesis (Huebner & Todaro, 1969) that guided much of cancer virus research in the 1970s. As part of this effort, it was important to determine if similar infectious endogenous retroviruses occurred in the outbred feral progenitors of the inbred lab mice or whether these inherited cancer virus genes were an artifact of laboratory inbreeding and purposeful selection for high cancer incidence strains. With this goal in mind, a number of different populations of feral mice in southern California were studied for their natural history of Type C and Type B retroviruses and associated cancers.

Natural history of type C (MuLV) retroviruses in aging wild mice

Wild mice from about fifteen different and widely separated trapping areas in southern California were allowed to age in the laboratory. Over a decade (1968-1978), about 10,000 mice were housed singly in mason jars and allowed to live out their 'natural' lifespan while under observation for spontaneous tumors or other diseases (Gardner *et al.*, 1973, 1971a, 1971b, 1976; Rongey *et al.*, 1973, 1975; Gardner, Lund & Cardiff, 1980). The mice were autopsied when sick or moribund. Tissues were examined microscopically and samples were collected for Type C and Type B retrovirus assays. The mice were also studied in their natural habitation, bred in the laboratory, and subjected to various experimental procedures to induce latent retro-

virus expression. The details of how these mice were housed, fed, screened for indigenous pathogens and examined for Type B and Type C retrovirus activity are covered elsewhere (Gardner *et al.*, 1973, 1971a, 1971b, 1976; Rongey *et al.*, 1973, 1975; Gardner, Lund & Cardiff, 1980). The rationale for studying aging wild mice proved very valid and led to the realization of a new biology of retroviruses, different in some major respects from that observed in the inbred mice and actually more predictive of the natural history of the Type C human T cell leukemia virus (HTLV) discovered a decade later in humans (Gardner, 1987). By contrast, a mammary tumor virus has never been found in humans (Cardiff & Gardner, 1985).

Spontaneous cancer, lymphoma, motor neuron disease and type C oncovirus (MuLV) infection in aging wild mice

In most (12 of 15) of the aging populations of wild mice, only a few tumors occurred late in life. The total tumor prevalence was 9.5% mostly after two years of age (Gardner *et al.*, 1973). Most of the tumors were lymphomas localized to spleen or lymph node. The only other tumor types observed were pulmonary adenomas, hepatomas, fibrosarcomas and myeloid leukemia. Typical of these wild mouse populations in Los Angeles County were those located at a squab farm in Bouquet Canyon, a birdseed plant (Hartz Mountain) in downtown Los Angeles, an egg ranch at Munneke and a squab farm in Soledad Canyon. The cumulative total mortality and cumulative specific tumor mortality as calculated by life table methods for these three representative aging populations of wild mice in comparison with the lymphoma-paralysis prone Casitas population of wild mice, described in the next paragraph, is shown in Figure 1 and described in detail in Gardner *et al.*, (1976). Other common pathology found in the aging mice included inflammation of the liver portal tracts (cholangitis) due to biliary obstruction from the dwarf tapeworm (*Hymenolepis nana*) and glomerulosclerosis of the kidneys. Neurologic disease did not occur in these particular mice. No amyloidosis was found. No microscopic abnormalities were found in about 25% of the moribund or dead aging mice. In most of the lymphomas, MuLV core (p30) antigen was detected by complement fixation and Type C particles were observed by electron microscopy (EM) (Gardner *et al.*, 1973, 1971a). Virus was not demonstrable in the other tumor types or in spleens of normal aging mice. MuLV p30 antigen and Type C particles were detected in only a few sarcomas induced in older mice by 3-methyl-cholanthrene (Gardner *et al.*, 1971a). The mice were also very resistant to x-irradiation induced tumors. Indigenous polyoma virus infection did not cause any increase in spontaneous tumors in the wild mice (Gardner *et al.*, 1972b). Treatment with antilymphocytic sera caused an activation of latent cytomegalovirus and death but no appreciable activation of latent MuLV (Gardner *et al.*, 1974). These findings indicated that the feral mice were generally very resistant to spontaneous tumor development and that Type C MuLV was strongly repressed in most feral populations and became detectable only infrequently late in life during spontaneous lymphogenesis or chemical sarcomagenesis. The MuLV that was isolated from lymphomas of these resistant mice was shown to be infectious for mouse embryo and other species' cells in tissue culture but only weakly lymphogenic. The wide *in vitro* tropism of this MuLV was designated 'amphotropic' (Rasheed, Gardner & Chan, 1976), in contrast to the infectious ('ecotropic') MuLV of laboratory mice which only grew in rodent cells. Under natural conditions, the amphotropic MuLV was apparently transmitted as a congenital milk-borne infection and the infected mice were specifically immune-tolerant to this virus because of the newborn age of exposure (see below). Following inoculation of susceptible newborn lab mice and wild mice, the amphotropic MuLV induced less than 30% incidence of lymphoma after a latent period of one year or more (Gardner *et al.*, 1978). In retrospect, if we had been forced to work only with these low-tumor incidence, low MuLV expressor populations of wild mice, progress would indeed have been very slow in understanding the biology of Type C retroviruses in wild mice. We could conclude that wild mice, in general, if spared death from predators, fighting, starvation or infectious disease, were long lived (> 2 years) and cancer resistant. However, a few wild mice that had apparently acquired a congenital infection with amphotropic MuLV were prone to develop lymphoma in later life.

Fortunately, we discovered three populations of wild mice situated in widely separate locations - a duck farm at La Puente and a grain mill in Norwalk, both in Los Angeles County, and a squab farm near Lake Casitas (LC) in Ventura County - characterized by a high prevalence and level of amphotropic MuLV infection and the more frequent occurrence of lymphomas at a younger age (Gardner *et al.*, 1973, 1976). The LC population was most thoroughly studied. The lymphomas arose in the spleen and were of pre-B or null cell origin (Bryant *et al.*, 1981). These MuLV-infected mice were also prone to a fatal lower motor neuron disease with hind leg paralysis (for summary, see Gardner, 1985). The neurologic disease was characterized by high levels of MuLV in the CNS, and development of a spon-

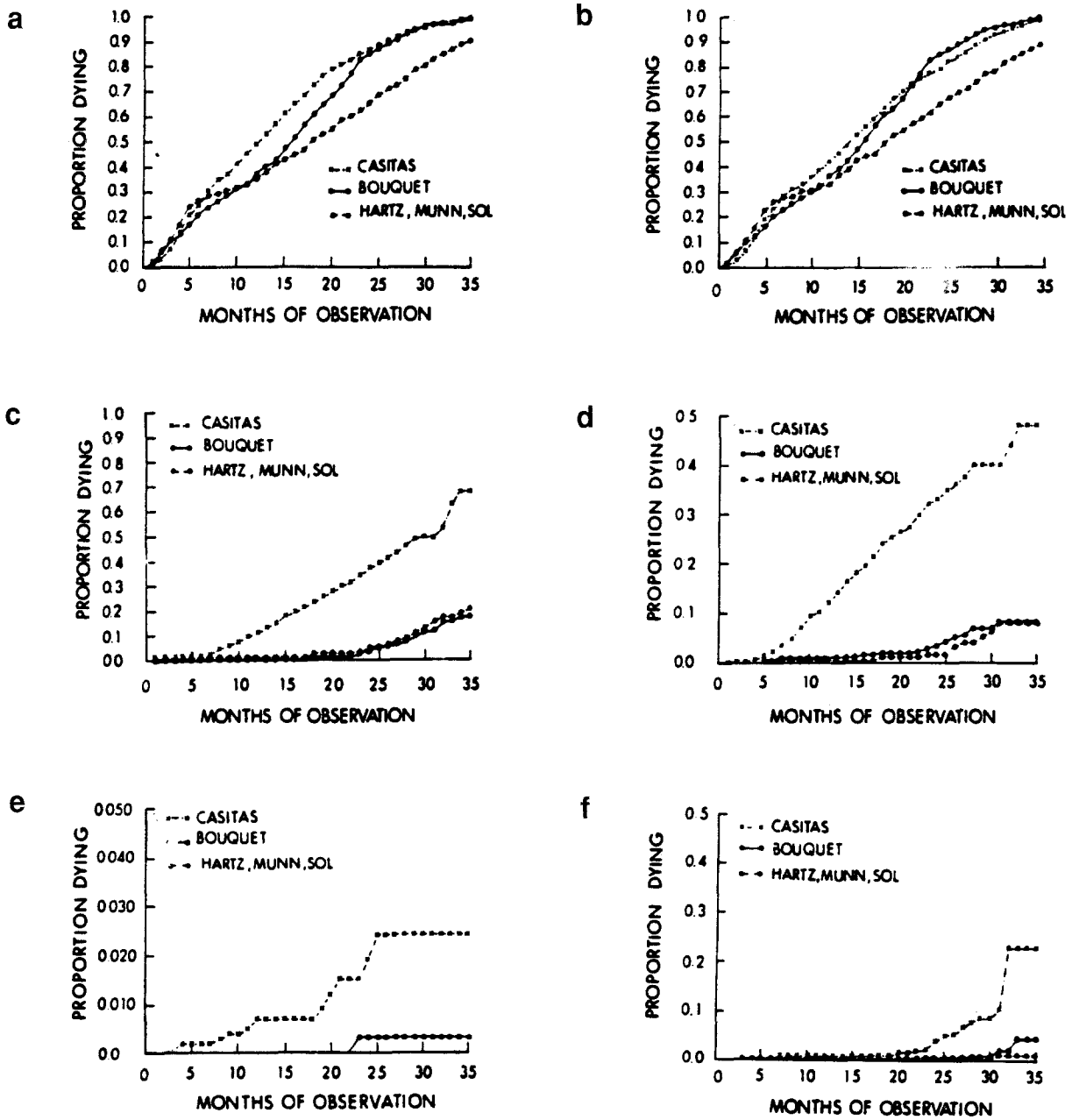

Fig. 1. a. cumulative total mortality from all causes; b. cumulative total mortality excluding deaths from tumors and paralysis; c. cumulative incidence rate for all tumors; d. cumulative incidence rate for lymphoma; e. cumulative incidence rate for carcinoma, excluding hepatoma; f. cumulative incidence rate for hepatoma; g. cumulative incidence rate for lung adenoma; h. cumulative incidence rate for sarcoma; i. cumulative incidence rate for paralysis.

giform, non-inflammatory pathology with gliosis and loss of anterior horn motor neurons, mainly in the lumbar spinal cord(Gardner *et al.*, 1973; Andrews & Gardner, 1974). The incidence of lymphomas in these high MuLV expressor mice was ten times greater than that observed in the more common, low MuLV expressor mice (Gardner *et al.*, 1976) (Fig. 1). After about one year of age, 15% of aging MuLV-infected LC mice eventually developed lymphoma, 10% developed paralysis, 2% had both diseases and 5% had epithelial tumors (breast and liver carcinomas, lung adenoma). Virologic studies showed that an ecotropic MuLV, intermixed with the amphotropic MuLV, was uniquely associated with the paralytic disease. Experimental transmission results in laboratory mice proved that the ecotropic MuLV (Officer *et al.*, 1973), including a molecular clone (Jolicoeur *et al.*, 1983), induced both paralysis and lymphoma whereas the amphotropic virus induced only lymphoma (Gardner *et al.*, 1978). In these high MuLV expressor wild mice, both amphotropic and ecotropic viruses were transmitted congenitally via milk leading to a lifelong systemic infection with high titered viremia (10^4 - 10^6 infectious units/ml) and resultant specific immune tolerance (Gardner *et al.*, 1979; Klement *et al.*, 1976). However, general immunity, vigor, and reproductivity were not impaired in the viremic mice. The infectious MuLV was present in many tissues but the major sites of initial virus replication were B cell areas of the spleen (Gardner *et al.*, 1976).

Interestingly, about 15% of LC mice escaped congenital infection with either amphotropic or ecotropic MuLV and remained free of infectious MuLV and related lymphomas or paralysis

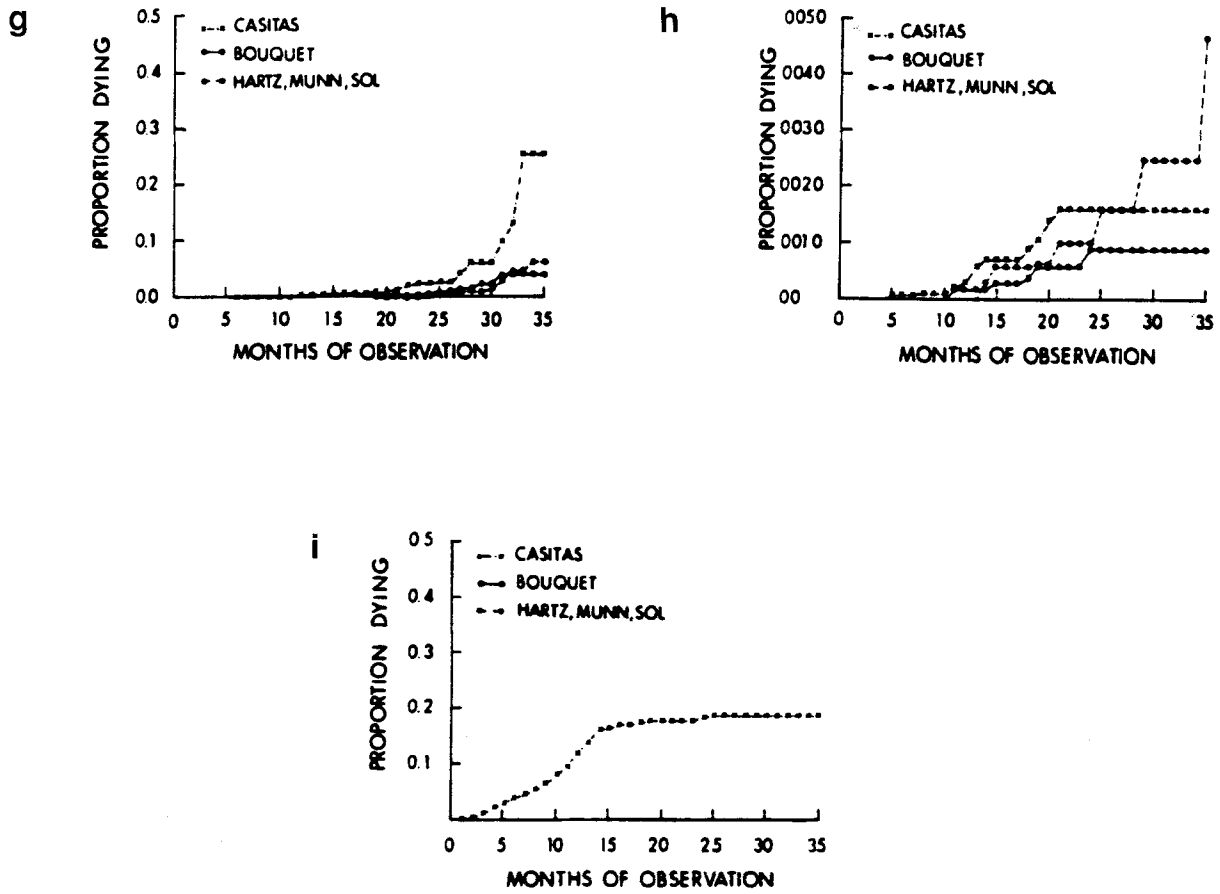

Fig. 1. Continued.

throughout their lifetime (Gardner, 1980). In this respect, they closely resembled the more common cancer resistant, low MuLV populations of wild mice. It was also possible through foster nursing on MuLV-free lab mice to largely eliminate infectious MuLV from the infected LC mice and, thereby, convert them into a long-lived disease resistant population. As with all laboratory mice, all feral mice also contain numerous copies of non-infectious, defective, endogenous MuLV-related proviral DNA in their genome. These endogenous MuLVs are called xenotropic or MCF (mink cell focus forming), and they represent separate envelope classes (Kozak & O'Neill, 1987). These defective proviral genes are thought to be the evolutionary relic of ancient infections with exogenous MuLV. The patterns of these proviral DNA sequences can be used to draw evolutionary lineages between different mouse subpopulations (Kozak & O'Neill, 1987), as will be illustrated later. In certain inbred mouse strains such as AKR, these endogenous, non-infectious MuLV genes recombine with endogenous infectious (ecotropic) MuLV to give rise to highly oncogenic MuLV (Hartley *et al.*, 1977). A similar phenomenon may occur when amphotropic or ecotropic viruses from feral mice are passaged through inbred mice (Rasheed, Pal & Gardner, 1982; Rasheed, Gardner & Lai, 1983). However, this event seldom, if ever, occurs in wild mice (Gardner & Rasheed, 1982). In feral mice, the amphotropic and ecotropic MuLV strains are entirely exogenous (Barbacid, Robbins & Aaronson, 1979; Rassart, Nelbach & Jolicoeur, 1986; O'Neill *et al.*, 1987), genetically stable, do not recombine with endogenous MuLV DNA in the feral mouse genome and remain only weakly oncogenic. Although most of the inherited MuLV proviral genes in laboratory and feral mice have no known biologic function, we shall see shortly a remarkable example in LC mice of a useful proviral function (i.e., the FV-4^R gene).

Natural history of Type B viruses in wild mice

The prevalence of Type B virus (mammary tumor virus) and of spontaneous breast tumors was uniformly low in all of the populations of wild mice, regardless of the level of MuLV infection. Less than 1% of wild mice developed breast tumors after one year of age. Type B particles and MMTV antigen were detectable in about 50% of the spontaneous breast tumors and in about 50% of normal lactating breast tissue from these mice (Rongey *et al.*, 1973; Gardner, Lund & Cardiff, 1980). Type B particles were also detected by EM in seminal vesicles and salivary glands of normal wild mice (Rongey *et al.*, 1975). MMTV in normal wild mouse milk was only weakly tumorigenic in foster nursed laboratory mice. In wild mice, the MMTV, like MuLV, was transmitted by milk and not genetically. Interestingly, some LC wild mice were discovered that completely lacked any MMTV, either in the form of infectious viruses or proviral DNA (Cohen & Varmus, 1979). Breast development and lactation were normal in these mice, although a few did develop hyperplastic mammary lesions (Faulkin *et al.*, 1984). Thus, among wild mice, MMTV exists in low prevalence and causes a few breast tumors, but it plays an insignificant role in the overall biology or causes of death (Cardiff & Gardner, 1985).

Non-genetic control of Type C virus and associated disease in wild mice

Because the Type C virus (MuLV) was transmitted epigenetically in wild mice, it was possible to largely eliminate the virus by immunologic or animal husbandry methods. Passive immunization of newborn LC mice with goat antisera against the LC ecotropic MuLV markedly reduced the titer of ecotropic virus in the progeny of viremic mothers and completely prevented the development of paralysis (Gardner *et al.*, 1980). However, since the amphotropic MuLV of these mice was not neutralized by this antisera, the progeny did develop some lymphomas later in life. Insofar as adult LC mice were already highly viremic, it was not possible to actively immunize them with inactivated whole amphotropic or ecotropic MuLV vaccines (Klement *et al.*, 1976). In LC mice the ecotropic and amphotropic MuLV both replicated early in life primarily in the spleen. Therefore, splenectomy at six weeks of age significantly reduced the serum titer of both classes of MuLV. Since development of the neurologic disease depended on obtaining a high level of ecotropic virus in the CNS early in life, this disease was prevented by the splenectomy

(Gardner *et al.*, 1978). The reduction of viremia also reduced the incidence but did not eliminate the later occurrence of lymphomas in the splenectomized LC mice.

Perhaps the most dramatic means of non-genetic control of MuLV in these mice was by foster nursing on nonviremic laboratory mice (Gardner *et al.*, 1979). Because the amphotropic and ecotropic MuLV were transmitted almost, if not entirely, by milk, it was possible to virtually eliminate these viruses by foster nursing. Conversely, it was possible to introduce the LC MuLV into uninfected lab mice by foster nursing on viremic LC females (Gardner *et al.*, 1979). These infected lab mice then developed the lymphoma and paralytic diseases that were typical of naturally infected LC mice.

Selective breeding of nonviremic LC mice, which constituted about 15% of the total population, also was a very effective measure in that these mice remained free of infectious MuLV throughout their lifespan and did not develop the associated diseases (Gardner *et al.*, 1980). These nonviremic mice were susceptible, however, to the ecotropic and amphotropic MuLV when it was introduced by nursing on viremic LC mothers (Gardner *et al.*, 1979). The ability of this noninfected minority of LC mice to remain uninfected while living among the majority of infected LC mice is further evidence against the horizontal transmission of MuLV among unrelated mice and supports the contention that milk-borne virus is the major route of transmission of MuLV in nature. These findings also support the absence of activated endogenous MuLV as infectious agents in the aging uninfected wild mice.

Genetic control of Type C virus in wild mice by the introduction of the FV-1^b MuLV-resistance allele from inbred mice

Before Type C virus (MuLV) was discovered in wild mice, it had been shown that this type of virus was subject to genetic control in inbred laboratory mice. The gene in lab mice primarily responsible for this control was called FV-1 because it restricted the growth of Friend MuLV (Lilly & Pincus, 1973). Two alleles, FV-1^n and FV-1^b, present in different strains of inbred mice, restricted B-tropic or N-tropic MuLV growth. B-tropic and N-tropic MuLV were defined by their relative susceptibility to growth in Balb/c or NIH Swiss cells, respectively. This dominant gene effect was exerted after viral entry during the process of reverse transcription. All of the wild mouse ecotropic and amphotropic viruses were found to be N-tropic and all of the LC wild mice tested were monomorphic for the FV-1^n genotype. Therefore, the FV-1 locus was fully permissive for the infectious MuLV present naturally in these wild mice and differences in prevalence of ecotropic or amphotropic MuLV in the LC wild mice could not be accounted for by segregation of the FV-1 alleles, as seen in lab mice. However, it was possible to show by cross breeding that the FV-1^b allele from the C57Bl inbred mouse strain could block the growth of the N-tropic LC-MuLV, because the F1 progeny of crosses between viremic LC wild mice and uninfected C57Bl mice were completely free of any infectious Type C virus, even after nursing on the infected LC mothers (Gardner *et al.*, 1976). Backcrosses of the F1 hybrids to the LC parental strain showed that this virus resistance segregated with the FV-1^b allele from the C57Bl parental strain.

Genetic control of Type C virus in wild mice by natural segregation of the FV-4 MuLV-resistance allele

As noted above, the LC wild mice are monophoric for the FV-1^n genotype and their amphotropic and ecotropic MuLV are N-tropic. Therefore, this locus could not account for the control of ecotropic MuLV in these animals. Surprisingly, another dominant gene, called FV-4 was found to control replication of ecotropic MuLV in these mice (Gardner *et al.*, 1980). This gene is distinct from other MuLV restriction genes (e.g., FV-2 and FV-3) described in laboratory mice (Lilly & Pincus, 1973), that have not been described in LC or other wild mice. When FV-4 was first discovered in LC mice it was called Akvr-1 because it restricted replication of the AKR endogenous ecotropic MuLV and prevented lymphoma in AKR $\times$ LC F1 hybrids (Gardner *et al.*, 1980). Later, it became clear that Akvr-1 was allelic with and identical in sequence to the FV-4 dominant resistance gene which was first described as preventing exogenous infection by N- and NB-tropic Friend MuLV in Japanese wild mice (*M. m. molossinus*) and the derivative G inbred line

Table 1. Properties of FV-4 restriction gene.

1.	Segregates as a dominant gene in LC mice. Allele frequency 0.56.
2.	Present also on chromosome 12 in Asian wild mice - *M.m. castaneus* and *M.m. molossinus.*
3.	Not present in laboratory mice (except G strain derived from *M.m. molossinus*). Acquired in LC mice by interbreeding with *M.m. castaneus.*
4.	Represents a defective endogenous MuLV provirus expressing an ecotropic envelope glycoprotein (gp70) on the surface of uninfected cells.
5.	Interferes with infection by all ecotropic MuLV by blocking of the receptors.
6.	Determines susceptibility or resistance to exotropic MuLV-caused paralysis or lymphoma in individual LC mice.

(Suzuki, 1974; Odaka *et al.*, 1981). Genetic studies then confirmed that the ecotropic MuLV resistance gene of G mice, *M. m. molossinus* and LC mice were alleles of a single locus on chromosome 12 (Odaka *et al.*, 1981; O'Brien *et al.*, 1983).

The FV-4 resistance gene was discovered serendipitously while cross-breeding LC mice with AKR inbred mice (Gardner *et al.*, 1980). Three patterns of MuLV viremia were observed in the F1 progeny of the individual AKR × LC crosses; at two months of age, either all of the F1 progeny were viremic, all were nonviremic, or about 50% were viremic. The viremia was entirely due to AKR ecotropic MuLV because only LC males or nonviremic LC females were bred to the AKR mice and we had already determined that infectious MuLV is transmitted only by LC females (Gardner *et al.*, 1979). In F1 backcrosses to AKR mice and in F2 progeny, viremia was present in about 50% and 25%, respectively, thus indicating the segregation in LC mice of a dominant gene capable of strongly blocking the expression of the endogenous ecotropic MuLV inherited from the AKR parent. The dominant MuLV-restrictive allele was called FV-4^R, and the recessive allele FV-4^S. The FV-4^R restriction effect was long lasting (> 18 mo) and associated with prevention of lymphoma in the AKR x LC F1 progeny. The properties of this MuLV restriction gene are summarized in Table 1. FV-4 (Akvr-1) strongly blocks cell to cell spread of all exogenous and endogenous infectious ecotropic MuLVs (N-tropic, B-tropic or NB-tropic) both *in vivo* and *in vitro* (Odaka *et al.*, 1981; Rasheed & Gardner, 1983). All ecotropic MuLVs use the same receptor encoded by the Rec-1 locus on mouse chromosome 5 (Sarma, 1967) and thus would be interfered with by binding of the FV-4 encoded gp70 to this receptor. This ecotropic MuLV receptor has recently been cloned and sequenced (Albritton *et al.*, 1989) and its normal function shown to be that of a basic amino acid transporter (Kim, *et al.*, 1991; Wang *et al.*, 1991). The homologous human gene has also been cloned (Yoshimoto, Yoshimoto & Meruelo, 1991), and both mouse and human genes are activated in rapidly proliferating cells (Yoshimoto, Yoshimoto & Meruelo, 1992). FV-4^R does not, however, block amphotropic MuLV, which uses a different receptor encoded by a gene on chromosome 8 (Gazdar *et al.*, 1977). Restriction is generally stronger *in vivo* than *in vitro* and stronger in hematopoietic cells than in fibroblasts.

Expression of the FV-4^R locus in uninfected cells is associated with the presence of an ecotropic MuLV-related envelope glycoprotein of about 70,000 kD (gp70) on the cell surface (Yoshikura & Odaka, 1982; Ikeda & Odaka, 1983; Dandekar *et al.*, 1987). Hirt analysis of FV-4^R resistant cells shows no proviral DNA after ecotropic virus challenge, whereas the same cells do show proviral DNA after challenge with amphotropic virus (Dandekar *et al.*, 1987). The block to infection at the cell surface level presumably occurs by receptor interference. Using an AKR ecotropic env probe, the FV-4^R gene was cloned and shown to be a truncated proviral genome containing a small segment of *pol*, the entire *env* gene (gp70) and a 3' LTR (Dandekar, S. 1987; Kozak, C.A. *et al.*, 1984; Ikeda, H. *et al.*, 1985). Sequence analysis showed that the FV-4^R gp70 was 70% related to the AKR ecotropic gp70, 58% similar to endogenous xenotropic and MCF envelope sequences, but 90% related to the LC ecotropic gp70. The protein encoding sequence of the FV-4^R allele, cloned from LC mice, was identical to that of the FV-4^R gene from *M. m. molossinus* (Dandekar *et al.*, 1987). Southern blot hybridization showed that *M. m. molossinus*, *M. m. castaneus* and LC mice each contained the same FV-4^R provirus (Kozak & O'Neill, 1987). *M. m. mollosinus* contains a single FV-4^R provirus whereas LC mice and *M. m. castaneus* each carry several additional copies of the FV-4^R gene. Moreover, *M. m. castaneus* and LC

mice share two specific FV-4 proviral integrations. Therefore, the FV-4 resistance gene was probably introduced into *M. m. molossinus* and LC mice by natural interbreeding with *M. m. castaneus* during this past century. LC feral mice are thus hybrids between *M. m. domesticus* from Europe and *M. m. castaneus* from Asia (Kozak & O'Neill, 1987). Opportunities for such interbreeding would have occurred via the shipping trade or immigration from Asian countries.

The observed frequency of the FV-4 resistance allele in LC mice randomly trapped at the squab farm was 56% which does not vary from expectation of the Hardy-Weinberg equilibrium (Gardner *et al.*, 1980). The probable frequency of LC mice that contain at least one FV-4 restriction allele is 80% and all of these mice would therefore be resistant to the LC ecotropic MuLV and associated paralysis and lymphoma. They would not, however, be totally resistant to lymphoma, because they may still be congenitally infected with amphotropic MuLV. Compared to the ecotropic MuLV, however, the amphotropic MuLV is less lymphogenic. The 20% of LC mice not inheriting this resistance allele would be considered homozygous for the susceptibility allele (i.e. FV-4^{ss}) and would be vulnerable to ecotropic virus congenital infection and the associated diseases later in life. Inheritance of FV-4^{R} would thus have a protective value on the survival of individual LC mice. Although manifested after the onset of breeding, evolutionary conservation of this locus might be expected because of its beneficial effect on survival and reproductive life span. In summary, this fascinating wild mouse model (summarized recently in Gardner, Kozak & O'Brien, 1991) has taught us that susceptibility or resistance to lymphoma and a slow neurologic disease, both developing after midlife (> 1 year of age) is determined, not only by exposure at birth to maternally transmitted ecotropic-MuLV, but ultimately by inheritance of an ecotropic-MuLV resistance gene (FV-4^{R}) segregating in this outbred population.

Lessons learned and possible relevance to humans

A major lesson learned from this study is that feral mice are indeed a valuable model for studying the biology of retroviruses in relation to etiologically-associated tumors and non-oncogenic diseases. The natural history of MuLV in wild mice, discovered in the 1970s, resembled the natural history of HTLV discovered in humans a decade later (Gardner, 1987). Remarkable similarities between the Type C leukemia viruses of wild mice and humans include the primary transmission via maternal milk early in life, the long latent period before onset of lymphoma, the genetic stability of the virus, and the ability of the same virus to induce both lymphoma and a non-neoplastic, degenerative neurologic disease. As shown with MuLV in this wild mouse model, avoidance of breast feeding appears effective in preventing transmission of HTLV infection from carrier mothers to their children in endemic regions of southern Japan (Hino & Doi, 1989).

In respect to human retrovirology, it is possible that natural resistance to HTLV and HIV infection may someday be found attributable to resistance genes such as FV-4^{R}. The human genome certainly contains a large complement of endogenous defective HTLV-related proviral DNA (Shih, Misra & Rush, 1989). Introduction of such cloned resistance genes into bone marrow progenitor cells might become a future application of gene therapy directed at control of human retroviral diseases. A counterpart to the mammary tumor virus of mice has not been found in humans, and this virus has only a minimal oncogenic potential in feral mice. In respect to aging, these studies point out the potential of feral populations to uncover other loci associated with increased longevity by selection for specific disease resistance. However, it remains unclear if FV-4 and other virus resistance genes have any effect on general senescence.

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