Lymphomas and High-Level Expression of Murine Leukemia Viruses in CFW Mice

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Historically, Swiss Webster mice of the CFW subline, both inbred and random-bred stocks, have been considered to have a low spontaneous occurrence of hematopoietic system tumors, and previous reports of infectious expression of murine leukemia viruses (MuLVs) have been rare and unremarkable. In marked contrast, in the present study of CFW mice from one source observed by two laboratories over a 2-year period, nearly 60% developed tumors, 85% of which were lymphomas, the majority of B-cell origin. All tumors tested expressed ecotropic MuLVs, and most expressed mink cell focus-inducing (MCF) MuLVs. Among normal mice of weaning to advanced age, over one-half were positive for ecotropic virus in tail or lymphoid tissues, and MCF virus was frequently present in lymphoid tissue, less often in tail. Patterns of ecotropic proviral integration indicated that natural infection occurred by both genetic and exogenous routes. Lymphomas were induced in NIH Swiss mice infected as neonates with tissue culture-propagated MuLVs isolated from normal and tumor tissue of CFW mice.

The several sublines of so-called Swiss mice in the United States derive from two males and seven females obtained for the Rockefeller Institute in 1926 by Clara Lynch from Andre de Coulon in Lausanne, Switzerland (24). Descendants were distributed over many years to investigators and dealers who, in turn, disseminated these mice widely. Both inbred and outbred stocks have been in extensive use in biomedical research because of their good health, high rate of productivity, low tumor incidence, and sensitivity to certain infectious agents (24). Most inbred strains of mice carry one to six copies of ecotropic murine leukemia virus (MuLV) sequences (5, 8, 20). Among the few strains with no endogenous ecotropic MuLV are the Swiss-related inbred SWR/J (6, 20) and NFS/N (5) and the outbred progenitor of NFS, NIH Swiss (7, 23). Sporadic reports can be found of ecotropic MuLV isolations in certain other Swiss mouse populations, e.g., HA/ICR (3), CF-1 (18), and CFW (1, 18), but to our knowledge, there has been no recent retrovirus evaluation of these widely used stocks.

Because CFW Swiss mice have a low reported incidence of spontaneous lymphomas and leukemias (1, 14, 27), they were chosen for a study of Mycobacterium leprae infection undertaken at the Centers for Disease Control and Prevention (CDC) in collaboration with one of us (D.L.D.). Surprisingly, as early as 5 months of age, mice of both control and M. leprae-infected groups began to develop splenomegaly and lymphadenopathy, and preliminary analysis indicated a high incidence of lymphoma as well as MuLV infection. The present report delineates the histopathology and immunologic and molecular phenotyping of the lymphomas, identifies the MuLV recovered from normal and diseased mice as being both the ecotropic and mink cell focus-inducing (MCF) classes, and describes the natural history of infection as including epigenetic as well as genetic transmission.

MATERIALS AND METHODS

Mice and mouse inoculation. Crt CFW (SW) BR (CFW) mice used at the CDC, Emory University, and the National Institutes of Health were obtained from Charles River Laboratories (CRL; Portage, Mich.), as were strains CD-1 and CF-1. BALB/c mice were obtained from Jackson Laboratory (Bar Harbor, Maine). Mice were inoculated in the right hind footpad with 0.3 ml of M. leprae suspension. Inoculated mice were divided either into control groups and fed regular Purina rodent chow or into drug-treated groups and fed ground chow mixed with antibacterial drugs. Because no obvious difference was noted in the incidence of hematopoietic tumors between the control and drug-treated groups, no further reference will be made to this aspect of the study.

NFS/N and NIH Swiss mice were obtained from the Animal Production Section, National Institutes of Health. Newborn to 2-day-old mice were inoculated with cell-free tissue culture harvests, using approximately 0.04 ml per mouse, divided intraperitoneally and into the area of the thymus.

Tissue sampling and histology. Tail biopsy specimens for DNA extraction and virus testing were obtained from anesthetized mice or at necropsy. At necropsy, affected spleen and/or lymph node was routinely sampled for virus analysis and frozen for DNA extraction. Spleen, thymus, peripheral and/or internal lymph nodes, and nonlymphoid tissues including lung, kidney, and spinal cord with gross evidence of infiltration by tumor were fixed in 10% buffered formalin for sectioning and staining with hematoxylin and eosin. Histopathologic diagnoses were based on previously described criteria (15, 19).

Flow cytometry. Single-cell suspensions prepared from spleen or lymph node were stained with a panel of previously described antibodies appropriate for two-color analysis, using a FACScan cell sorter (Becton Dickinson) and established techniques (15).

Molecular studies. As previously described (15), high-molecular-weight DNAs were analyzed by Southern blotting for immunoglobulin heavy chain (IgH) rearrangements using EcoRI digestion and hybridization with the J11 J H probe. For T-cell receptor β-chain rearrangements, digestion was with HpaII and the CTB probe was used for hybridization. To detect integrations of ecotropic MuLV, tail and tumor DNAs were digested with EcoRI and hybridized with the ecotropic-specific probe Eco5 (5).

Virus testing. Ecotropic MuLV was detected in SC-1 cells (ATCC CRL 1404) by the Xc cell (ATCC CCL 165) plaque assay (26) utilizing tail extracts or mitomycin C-treated spleen or lymph node cell infectious centers (11). Virus titers are expressed as PFU. Infectious center assays were also used to detect MCF MuLV, employing Mus dunni cells (22) (ATCC CRL 2197) and identification of immunofluorescence with the MCF-reactive monoclonal antibody 514 (ATCC CRL 1914). MCF MuLV was quantitated in M. dunni cells, and titers were expressed as focus-forming units. In a few cases, ecotropic virus expression...
was determined by induction of cultured tail cells with 5-iododeoxyuridine (21). Materials for mouse inoculation were prepared from harvests of SC-1- or M. dunni-passaged isolates.

RESULTS

Hematopoietic neoplasia in CFW mice. During 1995 to 1997, over 1,900 CFW mice, including uninfected control mice and those infected with M. leprae with and without drug treatment, were observed at the CDC. Unexpectedly, many mice developed splenomegaly and lymphadenopathy between 6 and 24 months of age, all test groups being affected similarly. Histopathologic examination indicated a diagnosis of lymphoid neoplasm for most cases. No such disease was seen in a group of BALB/c mice subjected to a similar protocol and housed in the same animal rooms.

Hematopoietic neoplasms also developed in CFW mice bred and aged at the Laboratory of Immunopathology (LIP) from stock received directly from CRL as early as 4.7 months but averaging 1 year. Tumors from both the CDC and CRL-LIP colonies were examined by histopathology, molecular analysis for rearrangements of immunoglobulin and T-cell receptor genes, cell surface antigen profiling, and tissue culture assays to detect expression of ecotropic and MCF MuLV. The incidence of hematopoietic neoplasms was 58% (57 of 99 mice examined). Lymphomas were the preponderant tumor (49 of 57), and both T-cell and B-cell lineages were represented. The lymphoma types seen included T-cell lymphoblastic lymphoma (10 cases) and several classes of B-cell lymphomas, including diffuse large-cell lymphomas, small lymphocytic lymphomas, and follicular lymphomas. The spectrum was similar to that recently reported for strains of NFS mice congenic for ecotropic MuLV loci from AKR, C58, and C3H/Fg mice (NFS.V⁺ mice [19]) except that splenic marginal zone lymphoma (15) was not encountered. Five myelogenous and three erythroid leukemias were also identified.

MuLV in CFW mice. Ecotropic virus was recovered from 100% of tumors tested (44 of 44) and from 14 of 28 normal age-matched mice (χ², 22.1; P = 0.000003). Twenty-eight of 31 mice with a tumor were positive for MCF MuLV, as were 12 of 26 tumor-negative mice (χ², 9.2; P = 0.002). To determine the background of ecotropic MuLV infection in CFW mice bred and maintained at CRL, duplicate tail biopsy specimens taken from 24 randomly selected mice of breeding age, 12 females and 12 males, were received directly from the supplier through the courtesy of William White. One sample was tested for relationships between band transmission and infectious expression. Evidence for both genetic and exogenous routes of infection are active in CRL CFW mice. Genetic virus transmission was clearly associated with at least three germ line integrations, identified as virus-positive mice without germ line viral integration. This band yielded a PstI fragment of about 8.1 kb, suggesting, along with its submolar intensity, some difference in structure compared with other CFW viral genomes. The 8.8-kb genome lacks a HindIII site and, with use of XbaI and the EcoSp probe, generates a 6.4-kb fragment rather than a 7.7-kb fragment as would be generated with Akv and other CFW-derived viral genomes (Table 2).

To determine whether any of the identified endogenous ecotropic MuLV genomes of CFW mice corresponded to identified Env loci of inbred mice (20), Southern blot analysis was performed after digestion with PvuII, XbaI, and HindIII, enzymes that generate diagnostic virus-cell junction fragments. The sizes of hybridization bands obtained using DNA samples that had displayed a single ecotropic virus-specific fragment after EcoRI digestion are given in Table 2. None of the profiles corresponds to those reported elsewhere for 42 inbred mouse strains (20).

The presence of virus-positive mice without germ line viral integrations suggested that infection could be occurring by exogenous routes. This was confirmed in tests of the progeny of matings of phenotypically characterized mice, selected from those in Table 1, in which one parent was both virus and integration negative. Characterization of those integrations that could be tested for relationships between band transmission and infectious expression shown in Table 3 indicates that both genetic and exogenous routes of infection are active in CRL CFW mice. Genetic virus transmission was clearly associated with at least three germ line integrations, identified as 26-kb, 24-kb, and 14-kb EcoRI bands, the 14-kb integration expressing virus with lower efficiency. In the case of a 17-kb EcoRI integration, no spontaneous induction of virus was detected in association with transfer of the gene. Evidence for nongenetic transmission lay in the high frequency of virus-positive progeny derived from matings of negative males with virus-positive females that carried either a 25-kb or a submolar

| TABLE 1. Ecotropic MuLV integrations and infectious ecotropic virus in 24 randomly selected CFW mice
<table>
<thead>
<tr>
<th>Band no.</th>
<th>No. of ecotropic integrations</th>
<th>Band sizes (kb)</th>
<th>Recovery of ecotropic MuLV (no. positive/no. tested)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26.0</td>
<td>6.4</td>
<td>13.3/6.2</td>
</tr>
<tr>
<td>2</td>
<td>24.0</td>
<td>4.1</td>
<td>9.2/6.8</td>
</tr>
<tr>
<td>3</td>
<td>17.0</td>
<td>8.7</td>
<td>11.2/8.0</td>
</tr>
<tr>
<td>4</td>
<td>14.0</td>
<td>2.8</td>
<td>14.0/7.4</td>
</tr>
<tr>
<td>5</td>
<td>8.8</td>
<td>2.6</td>
<td>6.4/8.8</td>
</tr>
</tbody>
</table>

* Tail samples were obtained from adult mice in the CRL colony.
* Measured by Southern blotting of EcoRI-digested tail DNA samples hybridized with EcoSp probe; the 8.8-kb band was of submolar concentration.
* NA, not applicable.

| TABLE 2. Endogenous ecotropic MuLV DNA sequences of CFW mice
<table>
<thead>
<tr>
<th>Band no.</th>
<th>EcoRI</th>
<th>PvuII</th>
<th>XbaI</th>
<th>HindIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26.0</td>
<td>6.4</td>
<td>13.3</td>
<td>6.2</td>
</tr>
<tr>
<td>2</td>
<td>24.0</td>
<td>4.1</td>
<td>9.2</td>
<td>6.8</td>
</tr>
<tr>
<td>3</td>
<td>17.0</td>
<td>8.7</td>
<td>11.2</td>
<td>8.0</td>
</tr>
<tr>
<td>4</td>
<td>14.0</td>
<td>2.8</td>
<td>14.0</td>
<td>7.4</td>
</tr>
<tr>
<td>5</td>
<td>8.8</td>
<td>2.6</td>
<td>6.4</td>
<td>8.8</td>
</tr>
</tbody>
</table>

* Data are ecotropic viral DNA band sizes in kilobases resulting from Southern blot hybridization with EcoSp probe after digestion of DNA with the indicated restriction enzymes.
TABLE 3. Evidence for both genetic and nongenetic transmission of MuLV in CFW mice: ecotropic virus integration and expression

<table>
<thead>
<tr>
<th>Parent</th>
<th>Size of band (kb)</th>
<th>Sexa</th>
<th>Viral expression</th>
<th>Data for offspringb</th>
<th>Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No. of mice</td>
<td>Virus-positive/parental band</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>26</td>
<td>M</td>
<td>+</td>
<td></td>
<td>11</td>
<td>8/8</td>
</tr>
<tr>
<td>25</td>
<td>F</td>
<td>+</td>
<td></td>
<td>10</td>
<td>3/3</td>
</tr>
<tr>
<td>24</td>
<td>M</td>
<td>+</td>
<td></td>
<td>18</td>
<td>8/8</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>-</td>
<td></td>
<td>10</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>-</td>
<td></td>
<td>11</td>
<td>0/6</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>+</td>
<td></td>
<td>18</td>
<td>11/11</td>
</tr>
<tr>
<td>8.8</td>
<td>M</td>
<td>+</td>
<td></td>
<td>21</td>
<td>7/13</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>+</td>
<td></td>
<td>24</td>
<td>0/0</td>
</tr>
<tr>
<td>No band</td>
<td>F</td>
<td>-</td>
<td></td>
<td>15</td>
<td>NAc</td>
</tr>
</tbody>
</table>

a Hybridization band sizes were estimated from Southern blots of EcoRI-digested tail DNA and the EcoSp probe. Ecotropic virus recovery was based on XE plaque assays of tail extracts in the case of parents and tail extract or spleen infectious centers for offspring. Offspring were usually tested at 7 to 12 weeks of age.

b M, male; F, female.

Occurrence of virus cannot be equated with presence of band because of occurrence of maternal exogenous transmission.

c NA, not applicable.

8.8-kb integration. None of these virus-positive offspring displayed germ line ecotropic MuLV integrations. Thus, neither the 25-kb nor the 8.8-kb genome is required for expression of infectious virus, and the offspring must have been infected in utero, during birth, or via ingestion of virus-containing milk.

Characteristics of ecotropic isolates from CFW mice. The ecotropic viruses isolated from tail, spleen, or lymphomas were N tropic in respect to restriction by alleles of Fvl (25) and efficiently induced XC plaques in SC-1 cells. The majority of isolates tested, however, grew very poorly in the clonal line of M. dunni cells used in this laboratory, both XC plaque titers and virus yields being consistently lower than those in SC-1. Of 13 ecotropic virus isolates tested simultaneously in both cell lines, all were restricted, 11 at least 100-fold and some by as much as 5 logs (data not shown). The extent of restriction of these latter isolates was comparable to that seen for Moloney MuLV in these cells (22).

Unintegrated viral DNA prepared by the Hirt procedure from one representative M. dunni-restricted ecotropic isolate was subjected to physical mapping analysis using 20 restriction endonucleases and hybridization with the EcoSp probe as well as with other probes derived from molecularly cloned Akv (5). This isolate, cloned by limiting-dilution titrations in SC-1 cells, was biologically similar to other viruses recovered in the study, being N tropic and restricted in M. dunni cells by at least 5 logs compared with SC-1 cells. As described below, the virus, when inoculated into newborn NIH Swiss mice, induced generation of MCF MuLV and development of lymphomas. The results of endonuclease analysis indicated that this isolate was essentially indistinguishable from prototypical Akv ecotropic MuLV (data not shown and reference 4) and thus clearly distinct from Moloney MuLV.

Recovery of MCF MuLV. Among the 24 mice for which data are listed in Table 1, only two expressed MCF MuLV in tail extract assays; both were ecotropic MuLV-positive, multiple-integration males that were not progeny tested. As shown in Table 4 for normal mice that could be clearly distinguished as having potential for ecotropic virus infection by either endogenous or exogenous routes, MCF virus was recovered from 60% of the ecotropic virus-positive mice (42 of 70). The frequency of isolation increased with age, rising from 5 of 33 (15%) of mice 3 to 8 weeks of age to 34 of 37 (92%) of those 12 weeks of age or older, in contrast to 94 and 83% ecotropic positivity, respectively, at those ages.

Induction of lymphoma by CFW MuLV. Eight CFW mice were chosen as sources of virus for evaluation of pathogenicity of MuLV isolated from CFW mice by inoculation of neonatal NIH Swiss mice, a strain chosen because it is negative for ecotropic MuLV (7, 19) and has a low incidence of spontaneous lymphoma. These mice comprised four tumor cases (one each of T-cell lymphoblastic lymphoma, diffuse large-cell lymphoma [lymphoblastic lymphoma-like], follicular lymphoma, and myelogenous leukemia) and four with no disease and represented mice from both the CDC and LIP study populations. Except for a biologically cloned ecotropic isolate from the diffuse large-cell lymphoma (lymphoblastic lymphoma-like) case, materials for inoculation represented cell-free pooled harvests of SC-1 cells and M. dunni cells from infectious center tests or passages thereof and thus contained both ecotropic and MCF MuLV classes. The results of inoculation of 10 litters are shown in Table 5. Mice received 103 to 105 PFU of ecotropic virus and approximately 10^3 focus-forming units of MCF MuLV in these cells.

TABLE 4. Frequency of recovery of MCF MuLV from normal CFW mice in relation to age and ecotropic MuLV expression

<table>
<thead>
<tr>
<th>Type of infectious ecotropic virus transmission</th>
<th>Age (wk)</th>
<th>Virus recoveryb (no. of mice positive/no. tested)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ecotropic</td>
</tr>
<tr>
<td>Endogenous</td>
<td>7</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>12–23</td>
<td>18/24</td>
</tr>
<tr>
<td>Exogenous</td>
<td>3</td>
<td>9/10</td>
</tr>
<tr>
<td></td>
<td>7–8</td>
<td>14/15</td>
</tr>
<tr>
<td></td>
<td>12–17</td>
<td>21/23</td>
</tr>
</tbody>
</table>

a Mice at risk for endogenous infection include progeny of virus-negative, sequence-negative females carrying ecotropic integrations coding for infectious ecotropic MuLV (26, 24, and 14 kb) derived from male parent. Mice at risk for exogenous infection include sequence-negative progeny of virus-positive females mated with virus- and sequence-negative males.

b Based on spleen infectious center assay.
NFS mice congenic for ecotropic MuLV proviral loci (15, 19).

The consistent IgM positivity of B-cell lymphomas occurring in the tor of IgH rearrangements in tumor DNA. All expressed surface cytometry, 13 were B-cell lymphomas, confirmed by detection of virus. Induction attempts with cloned ecotropic and MCF viruses will be required to clarify whether both classes, singly or in concert, are lymphomagenic.

MCF MuLV. Lymphomas of both T and B cells, mirroring in type and frequency those seen spontaneously in CFW mice, developed after a latency of at least 6 months in 46 of 65 mice (71%) observed for 1 year or until disease. Harvests from all eight donors induced lymphoma, and there was no significant difference between tumor and nontumor sources. Eleven representative lymphomas were tested for infectious MuLV, and all expressed high levels of both ecotropic and MCF classes, including three mice initially receiving only ecotropic virus. In a separate study (data not shown), at 6 weeks postinfection as neonates with the biologically cloned ecotropic virus, three mice displayed high-level ecotropic MuLV expression in spleen and thymus, and one thymus specimen was positive for MCF MuLV. Thus, as predicted from other studies of exogenous infections (2, 13, 28), MCF MuLV can be generated in vivo shortly after exogenous infection with CFW ecotropic virus. Induction attempts with cloned ecotropic and MCF viruses will be required to clarify whether both classes, singly or in concert, are lymphomagenic.

Phenotypic analysis of lymphomas. Of 19 lymphomas from spontaneous and CFW virus-induced cases analyzed by flow cytometry, 13 were B-cell lymphomas, confirmed by detection of IgK rearrangements in tumor DNA. All expressed surface IgK, but only one-half were surface IgM, in contrast to the consistent IgM positivity of B-cell lymphomas occurring in NFS mice congenic for ecotropic MuLV proviral loci (15, 19). Five of the six T-cell lymphomas were Thy-1 positive, and all were CD4 positive; all displayed rearrangements of the T-cell receptor β-chain loci.

MuLV in other commercially available Swiss mouse strains. It is of potential importance that two additional Swiss mouse strains, CD-1 and CF-1, maintained by CRL express infectious MuLV and display genomic integrations of ecotropic MuLV. In Southern blots of EcoRI-digested tail DNAs, five of six retired breeder CD-1 mice displayed one or two ecotropic virus-specific fragments, of 13.5, 17.5, or 24 kb; ecotropic MuLV was recovered from four mice, one of which also expressed MCF virus. CD-1 mice, widely used in toxicologic studies, have reported incidence of spontaneous hematopoietic neoplasms that ranges in untreated controls from 2 to 24% (16). No studies of MuLV expression have been described, to our knowledge. In CF-1 mice, for which we have found no report of spontaneous lymphoma, multiple EcoSp-reactive fragments, usually nine or more ranging in size from about 8.8 to 30 kb, were detected in EcoRI digests of tail DNA of 14 of 14 retired breeder and 9 of 9 3- to 4-week-old mice. Only one-fourth of the mice expressed ecotropic virus in spleen, but 5-iododeoxyuridine-treated tail cultures were uniformly positive, indicating the presence of competent ecotropic proviral genomes.

<table>
<thead>
<tr>
<th>Source of virus</th>
<th>No. of mice with lymphoma/ no. of mice observed</th>
<th>Latencya (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoma</td>
<td>29/41a</td>
<td>334 ± 73</td>
</tr>
<tr>
<td>Nonlymphoma</td>
<td>17/24a</td>
<td>340 ± 51</td>
</tr>
<tr>
<td>Total</td>
<td>46/65</td>
<td>336 ± 66</td>
</tr>
</tbody>
</table>

a No significant difference by χ² test.

b Average age at necropsy ± standard deviation.

DISCUSSION

These studies establish that at least one colony of CFW Swiss mice has a high but not universal rate of infection with MuLV of both ecotropic and MCF classes and a high frequency of hematopoietic system neoplasms that develop within 4.7 months to over 1 year of age. Ecotropic and usually MCF MuLVs were isolated from all lymphomas tested, compared to 43% of spleens of comparably aged CFW mice without disease, and expression of ecotropic virus early in life was strongly predictive of development of T- or B-cell lymphoma. As previously found in characterizing the spontaneous lymphomas of NFS.V mice (19), the majority of CFW lymphomas were of B-cell lineage. In other high-lymphoma-incidence, highly ecotropic MuLV-expressing mouse strains such as AKR and HRS, virtually only T-cell lymphoma is found, and its occurrence is strongly correlated with the generation of pathogenic MCF MuLV genomes in the thymus. In contrast, the MCF MuLVs that have been isolated from B-cell lymphomas are rarely or only weakly pathogenic. Because most pathogenic MCF MuLVs require the presence of ecotropic virus for efficient in vivo infection (11), in studying the lymphoma-inducing potential of CFW viruses we tested mixtures of ecotropic and MCF MuLVs isolated from B-cell lymphomas or normal tissue of CFW mice. Although all mixtures induced in NIH Swiss mice a variety of lymphomas comparable to those arising spontaneously in CFW mice, the efficiency was relatively low. No conclusion could be drawn about the pathogenic potential of the MCF isolates per se because of the general long latency to disease and thus abundant opportunity for generation of new MuLV viruses by recombination between ecotropic env gene sequences from the input virus and endogenous nonecotropic, polytropic MuLV sequences (28). In addition to effects that might be specified by viral structure, the genetic background of strains such as NFS.V and CFW could be an important factor in disease phenotype. As strains of Swiss origin, NFS and CFW might have genes in common that influence B-cell lymphomagenesis. Both host and viral factors could influence the putative mechanism of viral lymphomagenesis, i.e., by affecting which cellular genes are altered or the type of mutation that is induced by retroviral insertion.

Several phenotypic patterns of virus expression and presence or absence of genomic ecotropic MuLV integrations were found in individual mice in the colony, and progeny testing of typed parents indicated that both exogenous and endogenous routes of infection occur. Females without detectable germ line proviral sequences can express high titers of ecotropic and MCF MuLV and transmit at least ecotropic and probably both viruses efficiently to the progeny produced following mating with virus- and sequence-negative males. On the other hand, certain ecotropic proviral integrations identified in genomic DNA were found to be associated with transmission of ecotropic virus by males mated with virus- and sequence-negative females. Other mice were negative for both proviral sequences and expression of virus. The large-scale, random-breeding husbandry of these mice (W. White, personal communication) would be expected to maintain a population of highly diverse virologic phenotypes.

The source of ecotropic MuLV infection in the population of CFW mice studied here is unknown. According to the CRL product catalog, the origin of their stock was a single pair from an inbred subline of the original Rockefeller Institute Swiss mouse colony, acquired by Carworth Farms. The present CRL colony was Caesarean derived in 1974 from “a representative cross-section of the Carworth CFW colony.” It is not possible to document the time at which infection was introduced.
MuLV was detected in one of 14 CFW mice from Carworth Farms tested in the late 1960s (18). Whether this represented endogenous or exogenous infection cannot be determined. One possible source would be accidental interbreeding with a high-virus mouse strain and subsequent establishment of endogenous, readily expressed infection in offspring, presumably at some point before Caesarean rederivation in 1974. Based on restriction enzyme fragment size comparison, however, none of the ecotropic MuLV integrations analyzed in the present study corresponds to those established for inbred mouse strains. A known \textit{Env} (20) could have been missed because of insufficient sampling or obscured by the complex pattern of highly efficient infection that is indicated by the presence of at least three different expressed ecotropic MuLV genomes and ready spread by exogenous route(s). High frequency of exogenous spread is not characteristically observed for laboratory mice but has been documented for certain California wild mouse populations (17). Akv-like virus has been isolated from wild mice in Japan (9), but DNAs of ecotropic viruses from the California demes are less similar (10).

The clear association of virus expression with lymphoma and leukemia development in these mice strongly indicates that their use will be problematic in studies that require long-term observation, as exemplified by the \textit{M. leprae} experience, and will necessitate monitoring for the influence of MuLV. For example, development of lymphoma in CFW mice of the same source studied here was attributed to chronic exposure to a strong low-frequency electromagnetic field (14). Control, unexposed mice did not develop lymphoma, and based on electron microscopy, MuLV was not detected in the one tumor examined. The protocol for the study, however, established two sublines of mice, derived from different breeders and maintained separately as “control” and “exposed” lines. Although the likelihood of selection of one or more virus-expressing breeders for the “exposed” line of mice and negative breeders for the control group may not be high, the possibility clearly exists. In a sampling of female and male breeding-age mice, we detected expression in 7 of 12 females and 8 of 12 males.

The reduced sensitivity of the \textit{M. dunni} cell line to infection by Moloney MuLV but not a broad range of other ecotropic MuLVs is at the level of viral envelope gene-cell receptor interaction and has been ascribed to a single amino acid substitution in the ecotropic MuLV receptor compared to that of fully sensitive NIH 3T3 cells (12). Although differences in viral envelope sequences can be identified in the Moloney MuLV genome compared to \textit{M. dunni}-permissive ecotropic isolates, none has been clearly associated with the difference in phenotype. Site-directed mutagenesis studies showed that a single amino acid substitution at \textit{env} position 82 could render Moloney MuLV able to infect \textit{M. dunni} cells with increased efficiency, but this change was accompanied by reduced ability to infect SC-1 and NIH 3T3 cells (T. Torrey, personal communication). No differences at 20 restriction enzyme sites throughout the virus genome were found between Akv ecotropic MuLV, which is unrestricted in \textit{M. dunni} cells, and a highly restricted lymphomagenic CFW ecotropic isolate; sequence comparisons within the \textit{env} gene have not been made.

It should be noted that the several strains of outbred and inbred Swiss Webster mice designated as CFW in use in the United States and in Europe should not be considered to be identical. We have examined only one population for the high-lymphoma–high-MuLV-expression phenotype.

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