Identification of a Spleen Focus-Forming Virus in Erythroleukemic Mice Infected with a Wild-Mouse Ecotropic Murine Leukemia Virus

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An NFS/N mouse inoculated at birth with an ecotropic murine leukemia virus (MuLV) obtained from wild mice (Cas-Br-M MuLV) developed a lymphoma after 18 weeks. An extract prepared from the lymphomatous spleen was inoculated into newborn NFS/N mice, and these mice developed erythroleukemia within 9 weeks. Splenectomies from the erythroleukemic mice contained ecotropic and mink cell focus-inducing (MCF) MuLVs; however, when these viruses were biologically cloned and reinoculated into newborn NFS/N mice, no erythroleukemia was induced. In contrast, cell-free extracts prepared from the erythroleukemic spleens induced erythroleukemia within 5 weeks. Analysis of cell-free extracts prepared from the erythroleukemic spleens showed that they contained a viral species that induced splenomegaly and spleen focus formation in adult mice, with susceptibility controlled by alleles at the Fv-2 locus. The spleen focus-forming virus coded for a 50,000-dalton protein precipitated by antibodies specific to MCF virus gp70. RNA blot hybridization studies showed the genomic viral RNA to be 7.5 kilobases and to hybridize strongly to a xenotropic or MCF envelope-specific probe but not to hybridize with an ecotropic virus envelope-specific probe. The virus described here appears to be the fourth independent isolate of a MuLV with spleen focus-forming activity.

Wild mice trapped in the Lake Casitas region of Southern California have a high incidence of neurogenic hind limb paralysis and lymphomas, and these diseases are associated with the expression of infectious murine leukemia viruses (MuLVs) (7, 8). Virus isolates from some of these wild mice consisted of a mixture of ecotropic and amphotropic MuLVs (6). The ecotropic MuLVs induce both paralysis and hemopoietic neoplasms after inoculation of susceptible strains of mice, whereas inoculation of amphotropic MuLVs results in the development of lymphomas alone (9). Cas-Br-M is a biologically cloned ecotropic MuLV isolated from wild mice (11) and induces both neurogenic hind limb paralysis and lymphomas when inoculated within 10 days of birth (13). Neurological disease develops after a latent period of 5 to 10 weeks, whereas lymphomas are first evident at 18 to 24 weeks (13). Cas-Br-M-induced lymphomas are predominantly nonthymic and have been found to include T-, B-, and "null" cell lymphomas (H. Morse and P. Hoffman, unpublished data). Both ecotropic and mink lung cell focus-inducing (MCF) MuLVs are produced by cells from these tumors and from spleen cells obtained from mice before the onset of neurological disease or lymphoma (13). However, the precise role of these MuLVs in the etiology of either disease remains unclear.

In the course of studies examining the pathogenesis of the neurological and neoplastic diseases induced by Cas-Br-M MuLV, newborn NFS/N mice were inoculated with a cell-free extract from the lymphomatous spleen of an NFS/N mouse inoculated 18 weeks earlier with Cas-Br-M virus. Unexpectedly, the recipient mice developed erythroleukemia within 9 weeks. This disease was characterized by splenomegaly, lack of lymph node or thymic involvement, and a mild anemia. The association of this erythroleukemia with a defective spleen focus-forming virus (SFFV) derived from Cas-Br-M MuLV and with properties similar to the SFFVs of the Friend virus (FV) complexes is described.

MATERIALS AND METHODS

Mice. Adult and newborn NFS/N mice were obtained from the Small Animal Section, National Insti-
tutes of Health, Bethesda, Md. Newborn litters were received when less than 24 h old. Breeding pairs of DDD and DDD-Fv' mice were a kind gift from Takeshi Odaka and were further bred and maintained in our animal facilities. DDD-Fv' mice have been shown to carry the Fv'-2 allele (20). NFS/N mice congenic for an ecotropic MuLV induction locus of C58 (NFS. C58v-1) were developed by W. P. Rowe and are maintained in our colony.

Viruses. Cas-Br-M MuLV was obtained from an extract of brain from a paralyzed mouse of the eighth serial intracerebral passage of paralytic disease in NIH Swiss mice, initiated with brain extract from a spontaneously paralyzed Lake Casitas (12). Pooling of the cloned virus used for mouse inoculations were found to be free of detectable xenotropic, amphotropic, and MCF MuLV. Cas-Br-M virus titers were determined on SC-1 cells by the XC plaque assay (24). Ecotropic and MCF viruses were also biologically cloned from erythroblastemic spleen extracts by limiting dilution titrations in SC-1 and Mink lung cells, respectively. The titer of the ecotropic virus pool was determined by the XC plaque assay and expressed as PFU per milliliter. The MCF virus was assayed by focus induction in Mink lung cells, and the titer was expressed as focus-forming units per milliliter (12).

Nonproducer NIH 3T3 cells containing SFFV derived from the Mirand strain of polyctyathia-inducing Friend virus complex (Fv-P) were a kind gift from E. M. Scolnick (25). The Friend SFFV was rescued from this nonproducer with an NB-tropic Friend MuLV cloned in NIH Swiss embryo cells by limiting dilution titrations from a stock of FV complex originally supplied by Charlotte Friend.

Spleen extract preparation and inoculations. Cell-free extracts from lymphomatous and erythroblastemic spleens were prepared by homogenization in an appropriate volume of minimal essential medium to make a 10% (wt/vol) suspension. The suspension was clarified twice by centrifugation at 3,000 rpm for 15 min and stored in 1-ml aliquots at -70°C.

Spleen extracts were inoculated intraperitoneally (i.p.) into newborn NFS/N mice in 0.05-ml volumes. Adult mice were inoculated intravenously (i.v.) with 0.25 ml of undiluted or 1:50 diluted extract.

Antisera. Goat anti-Rauscher MuLV gp70 antisem was obtained from the Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, Md. Goat anti-Moloney MCF gp70 antisem, absorbed with ecotropic Friend MuLV to make it MCF specific (26), and a monoclonal antibody to Friend SFFV gp52 (32) have been previously described.

Pulse-labeling, immune precipitation, and polyacrylamide gel electrophoresis. Spleen cells from an erythroblastic mouse inoculated with extract 2906 were pulse-labeled for 60 min with [35S]methionine as previously described (26). Extracts of cells were then immune precipitated with various antisera, and the complexes were precipitated with 10% suspension of Formalin-fixed Staphylococcus aureus (14). S. aureus coated with rabbit anti-rat immunoglobulin G (Cappel Laboratories, Cochranville, Pa.) was used with the monoclonal antibody. Precipitates were electrophoresed on 7% polyacrylamide gels, and the gels were fluorographed and exposed to X-ray film as previously described (1). A pulse-labeled extract prepared from an erythroblastemic cell line established from the spleen of a mouse infected with Friend MuLV-SFFV complex (cell line DS19, reference 27) was used as a control.

Isolation of total cell RNA. Total cellular RNA was prepared by a modification of the method of Strohman et al. (28). Single cell suspensions from the spleen or thymus were washed three times in serum-free medium and resuspended in a small volume of the same medium (ca. half of the packed cell volume). The cells were added to 10 volumes of 8 M guanidine hydrochloride-100 mM sodium acetate and homogenized extensively in a Dounce homogenizer. The RNA was precipitated by the addition of 95% ethanol (one-half of the volume of 8 M guanidine hydrochloride) and left at -20°C for at least 1 h. The precipitate was collected by centrifugation at 5,000 × g for 10 min at -20°C and, after being drained of excess supernatant, was dissolved in 6 M guanidine hydrochloride (one-half of the volume of 8 M guanidine hydrochloride initially used). To the dissolved RNA was added 0.25 ml of 2 M sodium acetate (pH 5.0) per 4 ml of solution, and the RNA was precipitated by 95% ethanol (one-half of the total volume) and left for 1 h or longer at -20°C. This procedure was repeated three or four times to obtain a clean white precipitate. The RNA was then dissolved in 2 to 4 ml of 20 mM EDTA (pH 7.0) and extracted with 2 volumes of chloroform-butanol (4:1). After a brief centrifugation to separate the phases, the chloroform-butanol was reextracted twice with 1 ml of 20 mM EDTA (pH 7.0). The pooled aqueous phases were precipitated by the addition of 2 volumes of 4.5 M sodium acetate (pH 6.0) and left for at least 1 h at -20°C. The precipitate was collected by centrifugation at 12,000 × g for 20 min at 0°C and dissolved in 1 to 2 ml of sterile deionized water. A small portion was reserved for absorption readings to determine the RNA yield, and the remainder was precipitated at -20°C after the addition of 0.1 volume of 2 M sodium acetate (pH 5.0) and 2.5 volumes of 95% ethanol. The RNA was stored in this form until required.

Formaldehyde gels. RNA samples (4 µg) in 10 mM sodium phosphate (pH 7.4)-50% (vol/vol) formamide-2.2 M formaldehyde-0.5 M EDTA were heated at 68°C for 5 min and added to submerged gel slabs (14 by 12.7 by 0.6 cm) consisting of 1% agarose in 10 mM sodium phosphate (pH 7.4)-2.2 M formaldehyde. The samples were electrophoresed at 25 V for 16 to 18 h in a recirculating buffer consisting of 10 mM sodium phosphate (pH 7.4)-2.2 M formaldehyde. The RNA was transferred directly to a nitrocellulose membrane using 20 × SSC buffer (where 2 × SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and procedures outlined by Thomas (29).

Preparation of DNA probes and nucleic acid hybridization. The construction of recombinant plasmids containing subgenomic segments of a cloned infectious AKR ecotropic provirus and an NFS xenotropic provirus has been described previously (2, 3). An ecotropic envelope-specific fragment, pEc-B4 (3), was constructed from the AKR ecotropic provirus (16), and a 500-base pair xenotropic envelope-specific probe, pXenv<sub>S</sub><sub>5,6,7</sub>, was constructed from a cloned NFS-Th-1 xenotropic proviral fragment (2). An MuLV long terminal repeat (LTR) probe was prepared by isolating
FIG. 1. Development and characteristics of Cas-Br-M tumors. All mice were inoculated intracerebrally (i.c.) or i.p. as newborns. Virus doses per mouse were: \(10^4\) PFU of Cas-Br-M MuLV pool 2306, \(10^4\) PFU of ecotropic virus pool 2558, \(10^{3.8}\) focus-forming units of MCF virus pool 2552, or 0.05 ml of 10% spleen extract.

RESULTS

Generation of Cas-Br-M-induced erythroleukemia. These studies were initiated in laboratories (P. M. Hoffman, Charleston, S.C. and H. C. Morse III, Bethesda, Md.) that have never grown or used Friend helper viruses or Friend or Rauscher virus complexes. Mice were housed in animal rooms which had never contained mice infected with any of these agents.

Figure 1 shows the series of inoculations that resulted in the generation of an early erythroleukemia in NFS/N mice. Of five newborn NFS/N mice inoculated intracerebrally with Cas-Br-M virus (P. M. Hoffman, Charleston, S.C.), four developed neurological disease within 7 weeks and one, which did not succumb to neurological disease, developed a lymphoma 18 weeks after inoculation. The diagnosis of lymphoma was established on the basis of splenic and hepatic histology. The white pulp of the spleen was grossly expanded with a fairly monomorphic population of immature lymphocytes (Fig. 2A). The same cell type infiltrated the liver, predominantly in periportal areas. An extract prepared

the 640-base pair KpnI fragment from cloned Harvey sarcoma proviral DNA (10, 18).

Recombinant plasmid DNAs were labeled with \(^{32}\)P by nick translation (17) and had specific activities of \(6 \times 10^7\) to \(13 \times 10^7\) cpm per \(\mu\)g of DNA. Blot hybridizations were carried out at 42°C for 16 to 20 h using the dextran sulfate procedure of Wahl et al. (31) as modified by Thomas (29). Hybridized membranes were exposed to Kodak AR film at \(-70^\circ\)C with a Du Pont Lightning plus intensifier screen.
FIG. 2. Splenic histology from NFS/N mice with (A) lymphomas induced by Cas-Br-M ecotropic virus and (B) erythroleukemia induced by an extract from the spleen shown in (A). Note the fairly uniform population of immature lymphocytes in (A) compared with the heterogeneous population of immature (large, open nuclei) and mature (small, dense, round nuclei) erythroblasts in (B).
from this spleen was inoculated i.p. into newborn NFS/N mice, and all of these animals developed erythroleukemia within 7 to 9 weeks. In contrast to the lymphoma of the donor, the disease in these first-passage animals had the gross and microscopic character of an erythroleukemia. The splenic histology (of one of these erythroleukemic mice) showed a massively enlarged red pulp containing erythroblasts in various stages of maturation (Fig. 2B) and blood lakes characteristic of disease caused by the anemia-inducing strain of Friend virus complex (FV-A) (19).

A spleen extract from one of these erythroleukemic mice (2906) was inoculated i.p. into a litter of 11 newborn NFS/N mice (H. C. Morse III, Bethesda, Md.). The majority of this study involved analyses of mice from this litter. All of these animals developed enlarged spleens by 5 weeks of age, and three deaths occurred between weeks 5 and 6. At 7 weeks, five mice were sacrificed to determine spleen weights, pathology, and hematology. The mice had enlarged spleens (Table 1) but no sign of lymph node or thymus involvement. The hematocrits ranged from normal to slightly low, and the number of reticulocytes was increased. Blood smears of mice with the most advanced disease showed numerous "smudge" cells as well as recognizable basophilic and polychromatophilic erythroblasts. Spleen and liver impressions stained with Giemsa and histological sections showed marked infiltration of these organs by erythroid precursors.

**Viruses isolated from erythroleukemic and lymphomatous spleens.** Tissue culture assays of a spleen extract (2904) prepared from a mouse of the initial NFS/N litter that developed erythroleukemia recorded both XC-positive ecotropic and MCF dual-tropic MuLVs. After biological cloning by limiting dilution titrations in SC-1 and mink cells, ecotropic virus 2558 and MCF virus 2552 were inoculated into newborn litters of NFS/N and NFS.C58v-1 mice (Fig. 1). These viruses did not induce erythroleukemia in any of the mice inoculated, whereas all mice inoculated with the original spleen extract 2904 developed erythroleukemia within 5 weeks (Fig. 1). The ecotropic virus did, however, produce the spectrum of diseases caused by the parent Cas-Br-M virus, i.e., neurological disease in the majority of mice plus occasional lymphomas (one of eight NFS/N mice at 18 weeks; one of six NFS.C58v-1 mice at 20 weeks). No tumors and no neurological disease were observed in mice inoculated with the MCF virus, even in the presence of the endogenous ecotropic virus in NFS.C58v-1 mice.

The two lymphomas induced by the ecotropic virus 2558 were also analyzed for MuLVs and were found to contain both ecotropic and MCF viruses. Spleen extracts from these lymphomatous mice were inoculated into newborn NFS/N mice to determine whether they could reproduce the original rapid erythroleukemia. Both spleen extracts induced lymphomas within 10 to 12 weeks, but no erythroleukemia was observed in any of the inoculated mice (Fig. 1). This observation indicated that the induction of a rapid erythroleukemia may be a rare event, possibly involving the generation of a defective virus, since the ecotropic or MCF viruses alone, or an extract containing both of these viruses, did not reproduce the original erythroleukemia.

**Erythroleukemia and spleen focus induction in adult mice.** To determine whether adult mice were susceptible to Cas-Br-M-associated erythroleukemia, NFS/N mice aged 7 weeks were inoculated i.v. with a spleen extract from an erythroleukemic mouse (4794, Table 1). Recipient mice were sacrificed 10 days after inoculation, and their spleens were weighed and examined for the presence of the splenic foci characteristic of other rapid virus-induced erythroleukemias. Mice inoculated with the undiluted extract developed enlarged spleens with numerous foci (Table 2). Mice inoculated with a 1:50 dilution of the extract developed discrete foci in sufficiently low numbers to permit accurate quantitation.

**TABLE 1. Spleen weights, hematocrits, and reticulocyte counts of erythroleukemic NFS/N mice**

<table>
<thead>
<tr>
<th>NFS/N no.</th>
<th>Spleen wt (g)</th>
<th>Hematocrit (%)</th>
<th>Reticulocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4784</td>
<td>0.30</td>
<td>42</td>
<td>13</td>
</tr>
<tr>
<td>4785</td>
<td>1.17</td>
<td>41</td>
<td>10</td>
</tr>
<tr>
<td>4789</td>
<td>1.53</td>
<td>43</td>
<td>11</td>
</tr>
<tr>
<td>4793</td>
<td>1.32</td>
<td>43</td>
<td>8</td>
</tr>
<tr>
<td>4794</td>
<td>1.60</td>
<td>45</td>
<td>9</td>
</tr>
<tr>
<td>Control</td>
<td>0.11</td>
<td>45</td>
<td>2</td>
</tr>
</tbody>
</table>

*Newborn NFS/N mice were inoculated i.p. with 0.05 ml of spleen extract 2906 (Fig. 1). The mice were bled, and spleen weights were determined at 7 weeks.

**TABLE 2. Erythroleukemia and spleen focus induction in adult NFS/N mice**

<table>
<thead>
<tr>
<th>Spleen extract dilution</th>
<th>Spleen wt (g)</th>
<th>No. of foci per spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiluted</td>
<td>0.45, 0.53, 0.20, 0.60</td>
<td>TNTC, b TNTC, 86, TNTC</td>
</tr>
<tr>
<td>1:50</td>
<td>0.11, 0.09, 0.10, 0.11</td>
<td>24, 26, 9, 12</td>
</tr>
</tbody>
</table>

*All mice were inoculated i.v. with 0.25 ml of erythroleukemic spleen extract from NFS/N mouse 4794 (undiluted or diluted 1:50) and sacrificed at day 10.

b TNTC, Too numerous to count.
Spleen extracts from two Cas-Br-M-induced lymphomas (bottom of Fig. 1) were tested in adult mice, and no splenic foci or enlargement was detected (data not shown).

**Fv-2 control of susceptibility to Cas-Br-M erythroleukemia.** To examine whether alleles at the Fv-2 locus would determine susceptibility to Cas-Br-M-associated erythroleukemia, adult DDD (Fv-2+) and congenic DDD.Fv-2+ mice were inoculated i.v. with the erythroleukemic spleen extract 4794, and their spleens were examined 10 days later. All DDD mice had splenic enlargement with focus formation, whereas the DDD.Fv-2+ mice had normal spleens (Table 3).

**Precipitation of pulse-labeled erythroleukemia spleen cells by anti-gp70 antibodies.** Samples of pulse-labeled extract prepared from spleen cells from one of the NFS/N mice inoculated with extract 2906 were precipitated with anti-gp70 antisera and monoclonal antibody prepared to Friend SFFV gp52 (26, 32). The precipitates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and an autoradiograph of the gel is shown in Fig. 3. A pulse-labeled extract from the Friend SFFV erythroleukemia cell line DS19 was used as a control (Fig. 3, lanes 6 and 7). The Cas erythroleukemic cells contained a gp70 and a 50,000-dalton protein (lanes 1, 2, and 4), slightly smaller in size than the gp52 of Friend SFFV (lane 6). The gp70 and the 50,000-dalton protein present in the Cas-Br-M erythroleukemic spleen were precipitated by the MCF-specific antisemur (lane 2) and monoclonal antibody to Friend SFFV gp52 (lane 4). This antisemur and monoclonal antibody have previously been shown to precipitate MCF virus gp70 and Friend SFFV gp52 (26, 32).

**Molecular studies of RNAs from erythroleukemic and lymphomatous spleens.** RNA was prepared from the erythroleukemic spleen of an NFS/N mouse inoculated with extract 2906 and from two Cas-Br-M-induced lymphomas (first passage of extracts from lymphomas induced by ecotropic virus 2558, Fig. 1). RNAs from a spontaneous AKR thymoma and an FV-P-induced erythroleukemic spleen from an NFS/N mouse were used as controls.

The results of the hybridization with the LTR probe are shown in Fig. 4A. The LTR probe was used as a generalized probe since it will hybridize LTRs from all MuLV classes. The Cas-Br-M erythroleukemic spleen contained RNA species consistent with the presence of two distinct viruses, a full-length helper virus plus a 7.5-kilobase (kb) defective virus (lane 3) slightly larger than the 7.4-kb SFFV of FV-P (lane 2). In addition to the genomic RNAs, smaller RNA species consistent with envelope messages were seen: a 2.85-kb RNA for the helper virus envelope message and a 2.3-kb RNA for the defective virus envelope message. The defective Cas RNA had an envelope message of identical size to the FV-P SFFV RNA envelope message. The defective virus genomic and envelope RNAs were absent in the Cas-Br-M-induced lymphomas (Fig. 4A, lanes 4 and 5).

When these RNAs were probed with envelope-specific probes, the defective Cas virus hybridized strongly with the xenotropic envelope-specific probe, pXenv (Fig. 4C, lane 3), but not with the ecotropic envelope-specific probe, pEc-B4 (Fig. 4B, lane 3). The same pattern of hybridization was observed with the FV-P RNA (Fig. 4B and C, lane 2). The pEc-B4 probe also

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**TABLE 3.** *Fv-2 control of Cas-Br-M erythroleukemia*<sup>a</sup>

<table>
<thead>
<tr>
<th>Mice</th>
<th>Spleen wt (g)</th>
<th>No. of foci per spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDD (Fv-2+)</td>
<td>0.85, 0.53, 0.18, TNTC&lt;sup&gt;b&lt;/sup&gt;, 0.19</td>
<td>90, 76</td>
</tr>
<tr>
<td>DDD.Fv-2+</td>
<td>0.14, 0.16, 0.16, 0.12, 0.10</td>
<td>No foci</td>
</tr>
</tbody>
</table>

<sup>a</sup> All mice were inoculated i.v. with 0.25 ml of erythroleukemic spleen extract from NFS/N mouse 4794.

<sup>b</sup> TNTC, Too numerous to count.

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FIG. 3. Autoradiograph of labeled Cas 2906 spleen cells immune precipitated with various antisera. Cas 2906 spleen cells (lanes 1 to 5) or DS19 Friend SFFV erythroleukemia cells (lanes 6 and 7) were pulse-labeled with [35S]methionine and then immune precipitated with either goat anti-Rauscher MuLV gp70 serum (lanes 1 and 6), goat anti-Moloney MCF gp70 serum absorbed with Friend MuLV (lane 2), normal goat serum (lanes 3 and 7), monoclonal antibody prepared to Friend SFFV gp52 (lane 4), or control culture supernatant from the SP2 myeloma line (lane 5). Immune precipitates were then electrophoresed on 7% polyacrylamide gels in the presence of sodium dodecyl sulfate.
showed poor hybridization to the Friend and Cas-Br-M helper viruses present in the erythroleukemic spleens. Even after a long exposure (Fig. 4B, lanes 2 and 3), only faint helper envelope messages could be detected. This was probably due in part to diminished homology between pEc-B4 (constructed from an AKR ecotropic virus) and the Friend and Cas-Br-M ecotropic envelope region, in combination with insufficient helper virus RNAs to permit adequate detection.

**DISCUSSION**

In this study of tumors induced by the wild-mouse ecotropic virus, Cas-Br-M, we report the identification of a new SFFV associated with the development of rapid erythroleukemia. Replication-defective SFFVs have previously been identified in Friend (5), Rauscher (23), and myeloproliferative sarcoma virus (21) complexes and were probably present in WM1-B virus (22). The present virus, which we propose to call Cas SFFV, appears to be the fourth or fifth independent isolate of a murine SFFV.

The Cas SFFV was first detected, and most probably generated, after inoculation of newborn mice with a spleen extract prepared from a mouse with a lymphoma induced by Cas-Br-M virus. The mice developed rapid erythroleukemia presumably due to the presence of the Cas SFFV genome formally detected in mice of the subsequent passage. The spleens from erythroleukemic mice contained a virus with spleen focus-forming activity, a 50,000-dalton viral glycoprotein, and small viral RNA species consistent with the properties of a defective virus. Ecotropic and MCF viruses isolated and biologically cloned from an erythroleukemic spleen did not induce erythroleukemia. Furthermore, Cas-Br-M-induced lymphomas showed no biochemical evidence of a defective virus, and extracts from lymphomatous spleens had no spleen focus-forming activity and were unable to induce erythroleukemia in newborn or adult mice. More definitive proof that the Cas SFFV is responsible for the erythroleukemia will come from studies with nonproducer cells containing the Cas SFFV (manuscript in preparation).

The Cas SFFV has biological, immunological, and biochemical properties similar to those of the Friend SFFVs. The pathological characteristics of the Cas erythroleukemia are similar to those induced by the FV-A complex. Newborn and adult mice are susceptible, and susceptibility to disease is controlled by alleles at the Fv-2 locus, properties common to both FV-A and FV-P erythroleukemias (15). Subtle differences between the Cas SFFV and FV-associated SFFVs were found on the viral protein and RNA level. Cas SFFV induced a 50,000-dalton glycoprotein slightly smaller than the gp52 of Friend SFFV (26). The Cas gp50 reacted with MCF-specific antibodies and monoclonal antibody to Friend SFFV gp52 in the same manner as has been reported for Friend SFFV gp52 (26, 32).

The Cas SFFV genomic RNA was found to be 7.5 kb, slightly larger than the 7.4-kb SFFV in the Mirand strain of FV-P and considerably larger than the 6.6-kb SFFV in FV-A (4). Hybridizations with envelope-specific DNA probes showed that both the Cas and FV-P SFFVs hybridize strongly to a xenotropic or MCF envelope-specific probe but not to an ecotropic envelope-specific probe. This pattern of hybridization and
the precipitation of Cas SFFV gp50 with MCF-specific antibodies suggests that Cas SFFV, like Friend SFFVs (30), is an envelope gene recombinant with the Cas-Br-M ectotropic virus and endogenous xenotropic- or MCF-related sequences.

We wish to emphasize that these studies were carried out in laboratories and animal rooms that had never been used for work with Friend or Rauscher SFFVs. This fact, plus the results showing the Cas SFFV to cause an erythroleukemia similar to FV-A-induced erythroleukemia but to be more related in size to FV-P SFFV, make it highly improbable that Cas SFFV arose through contamination. Studies are currently in progress to determine whether Cas-Br-M ectropic virus generates a SFFV with high frequency.

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LITERATURE CITED


