Characterization of a Unique Defective Type C Virus Associated with a Moloney Leukemia Virus-Induced Splenic T-Cell Lymphoma Cell Line

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Moloney leukemia virus (MoLV) induces lymphomas in BALB/c mice which either involve an immature thymic T-cell subpopulation or a splenic mature T-cell subpopulation. To investigate further the possible virological and immunological differences in these lymphomas, several lymphoma cell lines were derived. Although the majority of these cell lines expressed only the parental MoLV, one lymphoma cell line (5F4) was found which expressed only a defective virus. 5F4 virions lacked detectable reverse transcriptase activity and by immunoprecipitation lacked a serologically detectable reverse transcriptase. The lack of reverse transcriptase did not appear to be due to a deletion in the viral genome. Intracellularly 5F4 cells synthesized normal *gag* gene precursors but had little, if any, detectable Pr180^{gag-pol} or an altered precursor. These results suggest that the defect of the 5F4 virus is associated with the inability to translate the appropriate precursor for reverse transcriptase. The possible origin of the defective 5F4 virus was also examined by competition radioimmunoassays. These results demonstrate that the type-specific proteins, gp71 and p12, are serologically identical to those of the endogenous ecotropic virus and distinct from the MoLV proteins. Competition assays of 5F4 cell extracts further demonstrated the lack of any detectable MoLV type-specific proteins, although the tumor was presumably induced by MoLV. The significance of these observations to leukemogenesis is discussed.

The involvement of type C viruses in certain types of T-cell leukemias in mice is well documented (32). However, the mechanisms by which these viruses either promote leukemogenesis or are involved in transformation are not known. Because of the long latencies, the apparent monoclonality of T-cell lymphomas, and the lack of *in vitro* transforming activity of murine leukemia viruses (MuLV), it appears that leukemogenesis involves an indirect mechanism. Several possibilities have been presented, including formation of oncogenic recombinant viruses (6, 10, 15), induction of transformation by an effect due to viral integration (6), and promotion of somatic events giving rise to transformation due to chronic immune stimulation brought about by viral replication (20, 34). One way to distinguish among these possibilities is to study the characteristics of individual lymphoma cell lines with regard to their virological and immunological properties.

Moloney leukemia virus (MoLV)-induced lymphomas in BALB/c mice are of particular interest since their pathology is somewhat different from that of AKR spontaneous lympho-

mas. In particular, we have demonstrated that these lymphomas can involve different subpopulations of T cells (25). One form of leukemia involves an immature thymic T cell similar to the AKR type of lymphomas. The second type of leukemia involves a splenic, more immunologically mature T cell. To determine whether these different types of lymphomas might be associated with different types of viruses or virus expression, we have established cell lines from each type. Although the majority of the lymphomas have very few, if any, unique characteristics, one of the lymphoma cell lines (5F4) was found to have unique characteristics. As demonstrated here, this cell line produces only noninfectious virions with characteristics unlike phenotypically similar mutant viruses described to date. Somewhat more surprising, this nonconditional mutant virus did not appear to arise from the MoLV used to induce the lymphoma, but rather from an AKR-MuLV type of endogenous ecotropic virus. The 5F4 lymphoma cell line itself expresses no detectable MoLV type-specific proteins.

MATERIALS AND METHODS

Cells and virus stocks. RL12 cells, an established cell line from a radiation-induced thymoma in C57BL/

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6 mice, was kindly provided by M. Lieberman (Stanford University, Stanford, Calif.). RL12-MoLV cells were chronically infected with MoLV.

AKR-MuLV was isolated from an established line of AKR mouse embryo cells (originally obtained from W. P. Rowe, National Institutes of Health, Bethesda, Md.). MoLV used for the purification of viral proteins was isolated from chronically infected, continuous-passage Swiss mouse embryo cells.

Establishment of the 5F4 cell line. Neonatal BALB/c mice were inoculated intraperitoneally with the ICX.H strain of Moloney MuLV (kindly supplied by P. Fischinger, National Cancer Institute, Bethesda, Md.). After 3 months, one of the mice developed a splenic lymphoma which was adapted for growth in tissue culture. Briefly, the enlarged spleen was minced with scissors and meshed through a wire screen into a single cell suspension. The cells were washed several times with complete RPMI 1640 and maintained in the same medium supplemented with 10% fetal calf serum. The culture was left undisturbed for 3 weeks, at which time colonies of nonadherent lymphoid cells formed. The cell line was initially composed of an adherent cell population and a population of lymphoid cells. With continued passage, the culture became a homogeneous lymphoid cell line.

Virus infectivity assays. $S^+ L^-$ focus assays on FG-10 or D56 cells were performed as described (1). Infectability of RL12 cells was assayed by immunofluorescence, using rabbit sera against p30 MoLV antigen. Assays for xenotropic virus on $S^+ L^-$ 81 cells and for masked recombinant virus on cat embryo cells were kindly performed by P. Fischinger as described (12).

Virus purifications. Supernatants of 5F4 cells grown in suspension were harvested every 24 h, and the virus was purified by pelleting through a 25% sucrose cushion and subsequent banding in 15 to 50% linear sucrose gradients as described (17). MoLV and AKR-MuLV were purified from cells grown in roller flasks by the identical procedure.

Viral antigens and antisera. MoLV and AKR-MuLV structural proteins were purified and rabbit or goat antisera were prepared as previously described (9, 11).

Analysis of viral precursor proteins. Exponentially growing cell cultures were labeled in suspension at a density of 5×10^6 cells/ml with 100 μ Ci of [35 S]methionine per ml (specific activity, 1,155 Ci/mmol; Amersham Corp.) in medium containing 1/50 the normal methionine concentration. After a period of incubation (see figure legends), cells were washed twice with ice-cold phosphate-buffered saline and lysed in extraction buffer (1% Triton X-100, 0.5% deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 0.01 M NaH_2PO_4 - Na_2HPO_4 , 0.1 M NaCl, pH 7.5) at 2×10^6 cells/ml. Cell extracts were prepared and immunoprecipitations were carried out as described by Witte and Baltimore (35), using Formalin-fixed *Staphylococcus aureus* (Calbiochem). Immunoprecipitates were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (19) on 10% slab gels. Fluorographs were prepared as described by Bonner and Laskey (3).

Radioimmunoassays. Competition radioimmunoassays were carried out as described previously (11,

12) using ^{125}I -labeled purified viral antigens. Antisera were titrated to precipitate approximately 50% of the labeled antigen. Competing antigens were purified virions or extracts of virus-producing cells. Cells were extracted in an equal volume of 0.5% Triton X-100, 0.5 M KCl, and 0.05 M sodium phosphate buffer, pH 7.0, and clarified by centrifugation for 20 min at $15,000 \times g$.

Reverse transcriptase assays. Exogenous reverse transcriptase assays were carried out with polyribadenylate [poly(rA)] template and oligodeoxythymidylate [oligo(dT)]₁₂₋₁₈ primer as previously described (28). The endogenous reverse transcriptase activity of sucrose gradient-purified virions was performed as described by Rothenberg and Baltimore (29).

Preparation and analysis of virion RNA. Virus-producing cells were labeled with 50 μ Ci of ^{32}P per ml in phosphate-free medium, and virus was harvested after 3 h of incubation at 37°C. Virus was purified as described above. Viral RNA was extracted by the phenol-chloroform procedure, and sucrose gradient analysis was performed as described (18). The peak fractions of 34S RNA were pooled, ethanol precipitated, and treated with glyoxal according to McMaster and Carmichael (23). Glyoxal-denatured RNA was subjected to electrophoresis in a 1% agarose slab gel in 10 mM phosphate buffer, pH 7.0.

RESULTS

Derivation and characteristics of the 5F4 lymphoma cell line. We have previously demonstrated that MoLV-induced lymphomas may be primarily localized in the thymus and involve immature T cells, or they may be localized in the spleen and involve a more mature T cell (25). To further study the immunological and virological properties of these distinct phenotypes of lymphomas, we have established cell lines from each type. The 5F4 lymphoma cell line was established from a splenic lymphoma induced in BALB/c mice by inoculation of MoLV in newborn mice. The 5F4 cell line is Thy 1.2 positive, demonstrating its T-cell origin. The cells do not contain detectable terminal deoxynucleotidyl transferase (TdT) activity but have readily detectable 20- α -hydroxysteroid dehydrogenase (20 α SDH) activity. The lack of expression of TdT activity and the presence of 20 α SDH activity has previously been demonstrated to be characteristic of the more immunologically mature subpopulation of T cells (25, 33). By immunofluorescence using a monoclonal antibody against p30, >90% of the cells displayed strong immunofluorescence both in the cytoplasm and on the cell membrane. 5F4 cells readily induced lymphomas in syngenic recipients as well as in nude mice demonstrating their malignant properties.

Electron micrographs of 5F4 cells illustrated the typical lymphoblastic morphology expected

for T-cell lymphomas. It was also evident from the electron micrographs that 5F4 cells produced large amounts of particles with the typical morphology of type C viruses. To further confirm the presence of type C particles, virus was concentrated from supernatants by ultracentrifugation and the viral pellet was banded on isopycnic sucrose gradients. A distinct viral peak was evident which banded at a density of 1.16 g/cm³, characteristic of type C particles.

Lack of infectivity of 5F4 particles. To initially characterize the virus associated with the 5F4 cell line, viral infectivity was tested on a variety of indicator cells. The presence of ecotropic type virus was examined by using either FG-10 S⁺ L⁻ cells or D56 S⁺ L⁻ cells. To date, we have been unable to detect any infectivity in these assays. To examine the possibility that the 5F4 virus was a thymotropic ecotropic virus comparable to radiation leukemia virus (9), we examined its infectivity on the RL12 radiation-induced, virus-negative T-cell line by immunofluorescence. Under conditions in which radiation leukemia virus and MoLV gave infectivity for RL12 cells, supernatants from the 5F4 cell line showed no evidence of infectivity. We next examined the 5F4 cells for the presence of xenotropic viruses, using either a cat S⁺ L⁻ cell line or a mink lung cell line assay. In neither assay could infectivity be detected. These observations strongly suggested that the 5F4 cell line produces a virus which is completely defective for infectivity.

Lack of detectable reverse transcriptase activity in 5F4 virions. To determine the basis of the defectiveness of the 5F4 virus, we examined gradient-purified virions for the presence of reverse transcriptase in reactions, using exogenous and endogenous templates. The results obtained with a poly(rA) template, oligo(dT)₁₂₋₁₈ primer, and purified 5F4 virions or AKR-MuLV virions are shown in Fig. 1A. With AKR-MuLV virions, activity was readily detectable by using 2.5, 5, or 10 μ g of viral protein. In contrast, by using either 2.5, 5, or 10 μ g of purified 5F4 virions, no activity was detectable. To be sure that both virion preparations contained comparable levels of virus, they were compared by p30 competition assays; both contained comparable amounts of p30. The results obtained when AKR-MuLV virions and 5F4 virions were compared for endogenously primed reverse transcriptase activity are shown in Fig. 1B. As above, AKR-MuLV virions had readily detectable reverse transcriptase activity with either 50 or 150 μ g of viral protein, whereas comparable amounts of 5F4 virions showed only minimal reverse transcriptase incorporation which was independent

FIG. 1. Reverse transcriptase activity of 5F4 virions. Sucrose gradient-purified 5F4 virions (filled symbols) and control AKR-MuLV (open symbols) were assayed for reverse transcriptase activity with exogenous (A) and endogenous (B) templates as described in Materials and Methods. Symbols: (A) squares, 2.5 μ g; triangles, 5 μ g; circles, 10 μ g of viral protein. (B) Triangles, 50 μ g, circles, 150 μ g of protein.

of the virus concentration used in the reaction. These results strongly suggest that the defectiveness of the 5F4 virus is, in part, due to the lack of reverse transcriptase activity in the virions.

The lack of reverse transcriptase activity could be due to the presence of an inactive enzyme, an alteration in *pol* gene expression, or a deletion in the *pol* gene region. To determine whether the lack of reverse transcriptase activity was due to a deletion, we compared the size of the 5F4 viral RNA with that of AKR-MuLV and MoLV. For these experiments, the viruses were labeled with ³²P and the 70S RNA was purified on sucrose gradients. The 70S RNA was subsequently heat denatured, and the viral 34S RNA was purified by sucrose gradients. The labeled 34S RNAs of AKR-MuLV, MoLV, and the 5F4 virus were then compared under denaturing conditions on a 1% agarose slab gel. The AKR-MuLV gave two major RNA species of approximately 34S (Fig. 2). The significance of the two bands is not known, although the virus source was not a cloned preparation. The 5F4 virus, however, gave a single RNA band which migrated slightly slower than the AKR-MuLV RNA (Fig. 2, lane B). MoLV also gave a single RNA species which migrated comparably to the

FIG. 2. Comparison of the size of 5F4 virion genomic RNA with genomic RNA of MoLV and AKR virus by agarose gel electrophoresis. ^{32}P -labeled 70S viral RNAs were heat denatured and sedimented in sucrose gradients. The 34S peak fractions were precipitated, treated with glyoxal, and electrophoresed on 1% agarose slab gel as described in Materials and Methods. 23S rRNA (*Escherichia coli*) stained with ethidium bromide was used as a marker. (A) AKR-MuLV RNA; (B) 5F4 viral RNA; (C) MoLV viral RNA

5F4 virion RNA (Fig. 2, lane C). These results suggest that the lack of reverse transcriptase activity in 5F4 virions is not due to a substantial deletion (>5%) of the viral genome.

To determine whether an inactive enzyme might be present, we next examined 5F4 virions for the presence of a serologically detectable reverse transcriptase. For these experiments, 5F4 cells were labeled with ^{35}S methionine and 5F4 virions were purified by sucrose gradients. Disrupted virus was subsequently analyzed by

immunoprecipitation with antisera against reverse transcriptase followed by SDS-PAGE analysis. As a control, ^{35}S methionine-labeled MoLV was also examined (Fig. 3). With MoLV, the antiserum against reverse transcriptase precipitated a single major protein of approximately 85,000 daltons (85K protein), as expected for the p85 reverse transcriptase. In contrast, with comparable amounts of the 5F4 virus, only a very minor band was detectable after electrophoresis of the immune precipitates which migrated slightly slower than the MoLV protein. These results suggest that the 5F4 virions either lack or have very low levels of a serologically detectable *pol* gene product in the virions.

We next examined 5F4 cells and an MoLV-producing cell line for the presence of the Pr180^{*gag-pol*} to determine whether the defect in

FIG. 3. Analysis of 5F4 virions for the presence of p85 reverse transcriptase by immunoprecipitation and SDS-PAGE. ^{35}S methionine-labeled, detergent-disrupted 5F4 and MoLV virions were incubated with antisera against reverse transcriptase or normal rabbit serum, and immunoprecipitates were analyzed on 10% polyacrylamide gel as described in Materials and Methods. (A) MoLV, anti-reverse transcriptase; (B) 5F4 virus, anti-reverse transcriptase; (C) MoLV, normal rabbit serum; (D) 5F4 virus, normal rabbit serum.

the 5F4 cells was associated with altered processing of the precursor polyprotein. The results obtained from SDS-PAGE analysis of various immune precipitates of [35 S]methionine pulsed cells are shown in Fig. 4. Immune precipitates from MoLV-producing cells, using an antiserum against reverse transcriptase (lane D), showed a distinct protein band at approximately 180,000 daltons which was not found in precipitations with normal serum (lane C). This protein appears to correspond to the normal Pr180^{gag-pol} precursor protein (26). Migrating slightly slower than the 180K protein is another major protein band which has been variably seen in our immunoprecipitations and appears to be a cellular contaminant similar to that observed by others (35). When immune precipitates of 5F4 cells were examined by using an antiserum against reverse transcriptase (lane B), a major 180K protein band was not evident and the pattern of proteins precipitated was comparable to that observed with immune precipitates obtained with normal serum (lane A). These results suggested that 5F4 cells produce little or no Pr180^{gag-pol} and that there was no obvious altered *gag-pol* precursor. We next examined both cell lines for the presence of the *gag* gene precursor proteins, using an antiserum against p30. As shown in Fig. 5, SDS-PAGE analysis of immune precipitates using a p30 antiserum of 5F4 cells (lane C) and an MoLV-producing cell line (lane D) showed readily detectable Pr75 and Pr65 protein bands. Also note that the Pr180^{gag-pol} is detectable in the precipitates from the MoLV-producing cell line, but is absent or much reduced in the precipitates from the 5F4 cell line. Therefore, the 5F4 cell line appears to make a normal *gag* precursor, although the *gag-pol* precursor is either absent or much reduced. The above results, taken together, suggest that the defect in the 5F4 cells is associated with an inability to synthesize *gag-pol* precursor protein.

Characterization of 5F4 virion structural proteins. To determine the origin of the 5F4 virus and to possibly gain further insight into the cause of the defectiveness of the virus, we next examined the serological characteristics of 5F4 virions in various radioimmune competition assays. The results obtained in competition assays for p10 and p30 are shown in Fig. 6. When purified 5F4 virus was compared with either AKR-MuLV or MoLV for p10(A) or p30(B), the competitions were comparable to those with the other viruses. Since the 5F4 cell line was derived from an MoLV-induced lymphoma, the virus was next examined in competition assays for the type-specific MoLV proteins p12 and gp71. As shown in Fig. 7, 5F4 virus competed only par-

FIG. 4. Analysis of viral precursor proteins by immunoprecipitation with anti-reverse transcriptase sera. 5F4 cells and RL12-MoLV cells were labeled for 20 min with [35 S]methionine, and immunoprecipitates were prepared and analyzed on SDS-PAGE as described in Materials and Methods. The autoradiograph shows immunoprecipitation of 5F4 extracts (lanes A and C) and RL12-MoLV extracts (lanes B and D) with normal rabbit serum (lanes A and B) or with antisera against reverse transcriptase (lanes C and D).

tially in the assays for MoLV p12(A) and did not compete in the assay for MoLV gp71(B). Therefore, although the 5F4 cell line was derived from a presumed MoLV-induced lymphoma, the virus particles were clearly not of MoLV origin. To determine the possible origin of the 5F4 virus, we next examined its ability to compete in assays for AKR-MuLV p12 and gp71. The 5F4 virus competed both in the assay for AKR-MuLV p12(C) and in the assay for AKR-MuLV gp71(D) (Fig. 7). In both cases, the 5F4 virus competed with slopes of competition comparable to those for the AKR-MuLV proteins or AKR-MuLV virions. Since AKR-MuLV has viral proteins serologically indistinguishable from the endogenous ecotropic virus of BALB/c mice (BALB: virus-1) (16), the results suggest that the 5F4 defective virus was probably derived from the BALB/c endogenous ecotropic virus.

The competition assays using 5F4 virus clearly

FIG. 5. Analysis of viral precursor proteins by immunoprecipitation with antisera against p30. [35 S]-methionine labeled 5F4 and RL12-MoLV cell extracts were prepared and analyzed as described in Materials and Methods and in Fig. 4. The autoradiograph shows immunoprecipitation of 5F4 extracts (lanes A and C) and RL12-MoLV extracts (lanes B and D) with normal rabbit serum (lanes A and B) and with antisera against MoLV p30 (lanes C and D).

demonstrated that MoLV-specific proteins could not be detected in purified virions. However, it was conceivable that the 5F4 cell line was also infected with defective MoLV. We therefore examined extracts of 5F4 cells for the presence of MoLV proteins. When cell extracts from AKR-MuLV- and MoLV-producing cell lines were examined for MoLV gp71(A) and p12(B), both antigens were detectable in the MoLV producer and gave competition curves equivalent to the standard proteins, whereas the AKR-MuLV producer gave only partial or no competition in these assays (Fig. 8). With extracts of 5F4 cells, MoLV p12 and gp71 were not detected; rather, these extracts gave only partial or no competition equivalent to the AKR-producing cell line, suggesting that MoLV p12 and gp71, if present, are at levels lower than approximately 10 and 25 ng per mg of protein. These results demonstrate, therefore, that the 5F4 lymphoma cell line does not express, either in the cells or in the virus, MoLV-specific proteins.

DISCUSSION

Initially our interest in studying the properties of the 5F4 virus were prompted by the observation that in BALB/c mice MoLV can induce lymphomas that involve different subpopulations of T cells (25). It was conceivable that the different types of lymphomas might be associated with different viruses, particularly different types of recombinant viruses (5, 27). We therefore established a variety of lymphoma cell lines that could be used to characterize the viruses associated with particular types of lymphomas. The 5F4 cell line was one such line which was

FIG. 6. Radioimmunoassays for viral structural proteins. Radioimmuno competition assays were as described in Materials and Methods. The results are expressed as percentage of total 125 I counts per minute in the antigen-antibody precipitate normalized to 100% for maximal binding at infinite dilution of the competing antigen. Detergent-disrupted viruses and purified homologous proteins were tested as competing antigens. (A) Competition assays for 125 I-p10 MoLV: MoLV (○), 5F4 virus (●), AKR-MuLV (△), p10 MoLV (□). (B) Competition assays for 125 I-p30 MoLV: MoLV (○), 5F4 virus (●), AKR-MuLV (△), p30 MoLV (□).

FIG. 7. Radioimmunoassays for determination of serological specificity of p12 and gp71 proteins of 5F4 virus. Assays were performed as described in Material and Methods and in Fig. 6. (A) Competition assays for ^{125}I -p12 MoLV: unlabeled MoLV p12 ($\square$); 5F4 virus ($\bullet$); AKR-MuLV (Δ); MoLV ($\circ$). (B) Competition assays for ^{125}I -gp71 MoLV: unlabeled MoLV gp71 ($\square$); 5F4 virus ($\bullet$); MoLV ($\circ$). (C) Competition assays for ^{125}I -p12 AKR: unlabeled AKR p12 ($\square$); 5F4 virus ($\bullet$); AKR-MuLV (Δ); MoLV ($\circ$). (D) Competition assays for ^{125}I -gp71 AKR: unlabeled AKR gp71 ($\square$); 5F4 virus ($\bullet$); AKR-MuLV ($\circ$).

FIG. 8. Radioimmunoassays for determination of MoLV-specific gp71 and p12 proteins in the cell extracts. Assays were performed as described in the legend of Fig. 6. (A) Competition assays for ^{125}I -gp71 MoLV: unlabeled MoLV gp71 ($\square$). (B) Competition assays for ^{125}I -p12 MoLV: unlabeled MoLV p12 ($\square$). The competing antigens included cell extracts from RL12-MoLV cells ($\circ$), uninfected RL12 cells (Δ), and 5F4 cells ($\bullet$).

established from a splenic lymphoma induced in a BALB/c mouse by MoLV. This particular lymphoma was interesting since, unlike most T-cell lymphomas which involve a TdT-positive, 20 α SDH-negative immature thymocyte, this T-cell lymphoma involved a more mature TdT-negative, 20 α SDH-positive lymphoma T-cell subpopulation. As demonstrated by the results, when the virus associated with this lymphoma was characterized, a number of unexpected properties were observed. First, the 5F4 lymphoma appears to produce only a noninfectious defective virus; second, although the lymphoma was presumably induced by MoLV, the virus appears to not have been derived from MoLV but rather from an AKR-MuLV ecotropic-type virus.

The virus produced by the 5F4 lymphoma cell line appears to be completely defective for infectivity. Using a variety of assay systems that would have detected ecotropic, xenotropic, or recombinant type viruses, we observed no viral infectivity. The basis of the defectiveness appears to be related to the lack of reverse transcriptase activity. Phenotypically, therefore, the 5F4 virus is comparable to other previously described noninfectious mutant viruses (2, 14); however, the defect affecting the *pol* gene appears to be somewhat different. In particular, Besmer et al. (2) described a defective virus which arose by a deletion of part of the *gag* gene and most or all of the *pol* gene. This deletion resulted in a reduction in the size of the viral RNA, the absence of Pr180^{*gag-pol*}, and a reduction in the size of the *gag* precursor to 45,000 daltons. Gerwin et al. (14) also characterized a replication-defective virus which had only low levels of reverse transcriptase activity. In this case, the cells did not make a Pr180^{*gag-pol*} but did appear to make an altered *gag-pol* precursor of 147,000 and 114,000 daltons. Consistent with this observation they also detected a defective *pol* gene product in virions of 45,000 daltons. The defective 5F4 virus differs from these viruses in that there was no major deletion in the viral RNA evident, there was no alteration of the *gag* precursor, and there was little or no Pr180^{*gag-pol*} or an altered *gag-pol* precursor.

The precise defect in the 5F4 virus resulting in the absence of reverse transcriptase activity or the Pr180^{*gag-pol*} is not known. One possibility is that a deletion or substitution occurred within the *pol* gene region which we could not detect by comparing the size of the viral RNAs. However, such a deletion or substitution might have been expected to alter either the *gag* or *gag-pol* precursor. A second possibility is that a mutation or small deletion occurred which specifically af-

fected the translation of the *pol* gene region. Since the Pr180^{*gag-pol*} is currently thought to be a read-through translation product of *gag* gene translation (26), a mutational event may have introduced additional termination codons such that the frequency of translating the *pol* gene region was much reduced. Alternatively, it is conceivable that a splicing event near the *pol* gene similar to that required for *env* gene mRNA (30) is required to generate an appropriate mRNA from which a Pr180^{*gag-pol*} precursor can be translated. In this case, either a mutation or a small deletion in the splicing site could result in the absence of a Pr180^{*gag-pol*}. Last, the possibility exists that there is a cellular defect which, as above, affects either the processing of the viral RNA or its efficient translation. Nevertheless, the 5F4 cell line should prove useful in examining the factors affecting the synthesis of the Pr180^{*gag-pol*} precursor protein.

The lack of expression of any MoLV type-specific proteins in the 5F4 lymphoma cells, although the tumor was induced by MoLV, was quite surprising. The MoLV stocks used for tumor induction were uncloned, animal-passaged preparations which are known to contain recombinant-type viruses equivalent to the HIX virus (13) as well as the parental MoLV. In the majority of lymphomas induced by this virus preparation or in the extracts used as a source of virus, however, we have only detected the parental MoLV by competition radioimmunoassays. Since the data demonstrate that the 5F4 virus is serologically indistinguishable from AKR-MuLV, it would appear that the virus arose as a defective variant of an endogenous ecotropic-type virus either present as a contaminant of our MoLV stock or from the mouse in which the tumor arose.

The observation that the 5F4 cells only produce a defective AKR-MuLV type of virus is also of interest. Since this type of infectious event in MoLV-infected mice would be expected to be quite rare, the observation suggests that the 5F4 lymphoma arose from a single cell and thus is monoclonal. This conclusion is consistent with recent studies (24, 31) which have demonstrated the monoclonality of lymphomas based on patterns of virus integration in individual lymphomas. In spite of the lack of expression of MoLV type-specific proteins, it is conceivable that MoLV proviruses are integrated in the cell and not expressed. In this regard, experiments using restriction analysis and Southern blotting have demonstrated that no MoLV provirus specific restriction fragments are detectable in 5F4 cells (D. Steffen, personal communication).

The relevance of our observations with the

5F4 lymphoma to leukemogenesis are difficult to assess. Because of the short latency (3 months) and the observation that the tumor was a splenic rather than the typical thymic lymphoma, the pathogenesis is quite unlike that seen normally with AKR-MuLV-type viruses and is more like that seen with MoLV. Therefore, the lack of MoLV expression is quite anomalous. Several possibilities exist, however, that can be examined. Clearly, one possibility is that the defective virus has acquired a substitution involving a transforming function. Because of the lack of infectivity and our inability to date to rescue the virus, we have not yet been able to examine this aspect. Second, it is possible that transformation in leukemogenesis is mediated by viral integration and does not require viral expression. Third, recent evidence has been presented which suggests that type C viruses promote leukemogenesis by chronic immune stimulation (21, 34). In this case, there is no a priori reason that the transformed cell would express the virus associated with induction of the lymphoma. In the case of the 5F4 lymphoma, the initial infection with a defective AKR-MuLV provided a strong interference preventing a productive infection with MoLV. Nevertheless, the properties of this lymphoma cell line may make it particularly useful in subsequent studies of possible mechanisms in viral leukemogenesis.

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