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Analysis of Two Strains of Friend Murine Leukemia Viruses Differing in Ability To Induce Early Splenomegaly: Lack of Relationship with Generation of Recombinant Mink Cell Focus-Forming Viruses

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Friend murine leukemia helper viruses (F-MuLV) 57 and B3 were indistinguishable by genomic structural analyses with RNase T_1-resistant oligonucleotide fingerprinting and by antigenic reactivity with a panel of 31 monoclonal antibodies directed against murine leukemia viruses. Nevertheless, F-MuLV 57 and B3 had strikingly different virulences. Approximately 2 months after inoculation, IRW and NFS/N mice inoculated as newborns with F-MuLV 57 had gross splenomegaly caused by erythroid proliferation. In contrast, an equivalent dose of F-MuLV B3 induced spleen or lymph node enlargement 4 to 13 months after inoculation. Although most cases of spleen enlargement in F-MuLV B3-inoculated mice were due to erythroid proliferation, lymphoid or myeloid proliferation was also frequently observed. The replication of both F-MuLV 57 and B3 was equally efficient, and both viruses generated recombinant dual-tropic mink cell focus-forming (MCF) viruses with the same kinetics and efficiency. Moreover, MCF viruses induced by F-MuLV 57 and B3 had the same antigenic patterns. Therefore, the ability of F-MuLV to induce early splenomegaly did not correlate with the generation of recombinant MCF viruses.

Replication-competent ecotropic Friend murine leukemia virus (F-MuLV) induces a high incidence of fatal erythroid and myeloid and lymphoid proliferative diseases in mice of susceptible strains (1, 6, 9, 18, 20, 24, 26, 27, 31, 32). F-MuLV and a number of other ecotropic viruses are able to recombine in some strains of mice with endogenous retroviral sequences to generate recombinant dual-tropic mink cell focus-forming (MCF) viruses (4, 11–14). It is still unclear which virus(es), F-MuLV or Friend MCF, is responsible for induction of the disease observed shortly after inoculation of F-MuLV. In the present study we compared F-MuLV 57, a highly virulent strain isolated by molecular cloning (19, 32), and F-MuLV B3 (17), a biological clone obtained from the same original stock as F-MuLV 57 but exhibiting a low virulence when inoculated into newborn mice of susceptible strains (6, 17).

F-MuLV 57 and B3 were originally obtained from E. M. Scolnick (Merck Sharp & Dohme, West Point, Pa.) and propagated in chronically infected Fischer rat embryo cells. The molecularly cloned Moloney murine leukemia virus (M-MuLV) strain 1387 (17) was propagated in *Mus dunni* cells (16). Virus stocks were titered by using a previously described focal immunofluorescence assay (FIA) on *M. dunni* cells with the appropriate monoclonal antibody (7, 30). The origins of the cell lines used in this study have been previously described (30).

Structural similarities between F-MuLV 57 and B3. To determine if obvious genomic differences between F-MuLV B3 and 57 existed, we compared the RNase T_1-resistant oligonucleotide fingerprints of the virion RNAs, as previously described (11). The large oligonucleotides of these two strains were indistinguishable (Fig. 1). Approximately 50 randomly distributed large oligonucleotides, averaging about 20 bases in length, were compared by fingerprinting. Therefore, the two isolates differed by less than 0.1% in their sequences, probably because of a limited number of point mutations rather than extensive divergence or large substitutions. This analysis was also reflected in the fact that cells infected with either virus had identical antigenic reactivities with a panel of 31 monoclonal antibodies reactive with cell surface viral antigens (5, 7, 22, 23; data not shown). It is noteworthy that similar analyses distinguish all other strains of F-MuLV we have examined (5; L. Evans, unpublished observations), underscoring the similarity between F-MuLV 57 and B3.

Incidence and latency of disease induced by F-MuLV 57 and B3. To compare the relative virulence of F-MuLV 57 and B3, we inoculated newborn IRW (6) or NFS/N mice intraperitoneally with 2.5×10^3 focus forming units (FFU) of either virus. Mice were followed for gross organ enlargement by weekly palpation under ether anesthesia. F-MuLV 57 induced gross splenomegaly as early as 3 to 4 weeks postinoculation, and more than 85% of the mice had gross splenomegaly by 3 months. This incidence of disease in F-MuLV B3-inoculated mice was not observed until 11 months, and organ enlargements were not detected before 4 months (Fig. 2). Mice represented in Fig. 2 were not followed after 11 months; however, other groups of IRW mice inoculated as newborns with F-MuLV B3 showed that 100% of the animals were diseased by 13 months of age. Identical results were observed in NFS/N mice (data not shown).

The difference in virulence of F-MuLV 57 and B3 was also observed by following the decrease in hematocrit values of newborn-inoculated mice. The hematocrit values of mice inoculated with F-MuLV 57 decreased significantly from 40 to 30% as soon as 15 days later, whereas the values in mice

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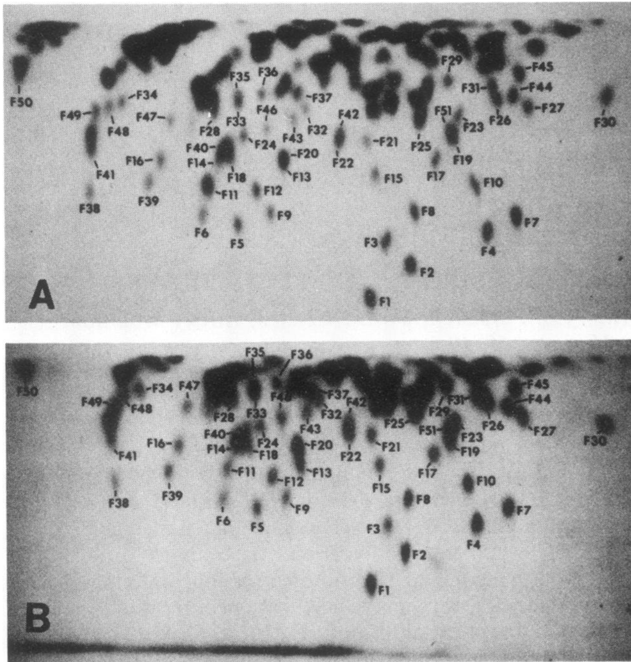

FIG. 1. RNase T_1-resistant oligonucleotides of F-MuLV 57 (A) and B3 (B) produced on *M. dunni* and Fischer rat embryo cells, respectively. The 70S [^{32}P]RNAs of both viruses were digested with RNase T_1 and fingerprinted, and oligonucleotides were numbered as previously reported (11). Electrophoresis was from left to right, and homochromatography was from bottom to top.

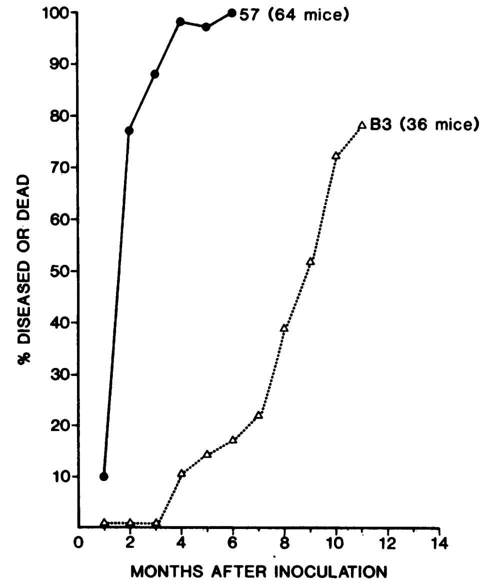

FIG. 2. Kinetics of disease induction (splenomegaly, lymphadenopathy, or death) in IRW mice inoculated as newborns with 2.5 × 10^3 FFU of F-MuLV 57 (●) or 2.5 × 10^3 FFU of F-MuLV B3 (△). Mice were examined weekly for organ enlargements under ether anesthesia.

inoculated with F-MuLV B3 did not decrease to this level until more than 4 months after inoculation.

Replication of F-MuLV 57 and B3 in newborn-inoculated mice. To determine whether the low virulence of F-MuLV B3 was caused by an inability of this virus to replicate efficiently in vivo, spleen cells from newborn-inoculated mice were assayed for production of virus at various times after inoculation. Ecotropic F-MuLV infectious centers (IC) were detected on either SC-1 or *M. dunni* cells, which appeared to be equally susceptible to F-MuLV infection. In all cases, the detection of IC was carried out on live cell monolayers with the FIA with ecotropic-specific monoclonal antibody 48 (7, 30). The number of ecotropic F-MuLV IC observed in the spleens of mice given F-MuLV 57 or B3 was similar from 1 week to 3 months after inoculation (Fig. 3). By this time, most of the F-MuLV 57-inoculated mice had succumbed, and the number of F-MuLV B3 IC remained at the same level until the mice succumbed. No correlation was found between spleen size and the IC titers (data not shown). Therefore, the difference in virulence observed between F-MuLV 57 and B3 was not because of an inability of F-MuLV B3 to replicate in vivo. Plasmas of IRW and NFS/N mice inoculated as newborns with F-MuLV 57 or B3 were assayed for the presence of anti-F-MuLV gp70 antibodies by using a ^{125}I-labeled protein A-binding assay (22) and for presence of infectious F-MuLV by using the FIA on *M. dunni* cells. No detectable anti-F-MuLV antibody response was found in IRW and NFS/N mice 1 to 7 months after inoculation of newborns with either virus, and similar levels of viremia were found in F-MuLV 57- and B3-inoculated mice (10^4 to 10^6 FFU/ml of plasma). Therefore, the different virulences of F-MuLV 57 and B3 were not due to a difference in the elicitation of the immune response resulting in a more efficient clearance of F-MuLV B3 with respect to F-MuLV 57 (25).

Induction of MCF viruses in mice inoculated with F-MuLV 57 or B3. We assayed normal and enlarged spleens of newborn-inoculated mice with F-MuLV 57 or B3 for the presence of MCF IC at different times after inoculation. MCF IC were detected by using the FIA with the broadly reactive MCF virus-specific monoclonal antibody 514 on *M. dunni* cells (5, 6). Because *M. dunni* cells are highly permis-

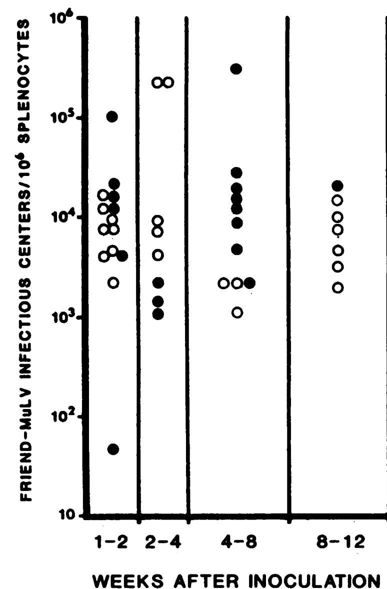

FIG. 3. Replication of F-MuLV 57 (●) and B3 (○) in IRW mice inoculated as newborns with 2.5 × 10^3 FFU per mouse. Dilutions of spleen cells were seeded on SC-1 or *M. dunni* cells and assayed by using the FIA with ecotropic-specific monoclonal antibody 48 (7).

sive to both dual-tropic MCF viruses and ecotropic F-MuLV (16), both pseudotyped and unpseudotyped MCF viruses could be detected (29). We observed that F-MuLV B3-inoculated mice were positive for MCF IC as early as F-MuLV 57-inoculated mice (Fig. 4). The appearance of MCF IC in both cases was independent of splenomegaly (data not shown), and the number of MCF IC present in the spleens of mice inoculated with F-MuLV 57 or B3 was similar at all times studied (Fig. 4).

Different families of Friend MCF and Moloney MCF viruses have been identified by virus RNA fingerprinting and by their reactivity with monoclonal antibodies (11, 12). Moreover, it has been shown that different ecotropic murine leukemia viruses may preferentially induce different types of MCF viruses (11, 12). With a panel of nine monoclonal antibodies reacting with MCF viruses, we found that MCF IC detected after inoculation with F-MuLV 57 or B3 had an identical pattern of reactivity (Table 1). In addition, RNA fingerprinting analysis of one F-MuLV B3-induced MCF virus identified the endogenously derived oligonucleotides characteristic of F-MuLV 57-induced MCF viruses (X12, X37, X39, X40, X41, X42) (11, 12). These data indicated that F-MuLV B3-induced MCF viruses were generated by recombination with the same endogenous sequences found in F-MuLV 57-induced MCF viruses. Therefore, it is unlikely that the differences in virulence observed between F-MuLV 57 and B3 was because of a qualitative or quantitative difference in the induction of recombinant MCF viruses.

Cell types in enlarged spleens from F-MuLV 57- and B3-inoculated mice. Another explanation for the differences in virulence is the possible inability of F-MuLV B3 to induce erythroid proliferative disease. Therefore, we analyzed the different cell types present in enlarged spleens after inoculation with F-MuLV 57 or B3. At different times after infection, splenomegalic mice were killed, and single spleen cell suspensions were processed for staining with Giemsa, Sudan black, and nonspecific esterase reactions as previously described (6). Because spleens from control mice had less than 5% erythroid or myeloid cells, enlarged spleens with more than 10% of erythroid or myeloid cells were considered positive for erythroid or myeloid proliferative diseases. If more than 80% of the cells were not erythroid or myeloid, the proliferative disease was designated as lymphoid. We found that splenomegaly in mice inoculated with F-MuLV 57 was caused by an increased frequency of erythroid cells in 96% of the cases (Table 2). Erythroid cells in these spleens ranged from 30 to 95% of the total cells. Abnormal myeloid cell distribution was sometimes observed, but was generally associated with an abnormal erythroid cell distribution (Table 2). No abnormal lymphoid distributions were observed in this group. In the case of F-MuLV B3-inoculated mice, the involvement of nonerythroid cells in spleen enlargement was more frequent than with F-MuLV 57. Neverthelsss, the majority (61%) of F-MuLV B3-inoculated mice had erythroid proliferative disease. Therefore, this virus strain was able to induce a significant incidence of erythroleukemias.

TABLE 1. Monoclonal antibody reactivity of MCF viruses induced in mice inoculated as newborns with F-MuLV 57, F-MuLV B3, or M-MuLV[a]

Ecotropic MuLV inoculated	MCF-reactive monoclonal antibodies[b]								
	Hy7	350	502	508	513	514	516	518	522
F-MuLV 57	+	−	+	−	−	+	−	−	−
F-MuLV B3	+	−	+	−	−	+	−	−	−
M-MuLV 1387	−	−	±	+	+	+	+	−	+

[a] NFS/N mice were inoculated intraperitoneally within 48 h after birth. Spleen or thymus IC were seeded on *M. dunni* cells. Fluorescent foci were detected 4 to 6 days later by using an FIA (30) with the different monoclonal antibodies.

[b] All monoclonal antibodies react with envelope antigens of MCF from different origins (5–7, 12). +, Presence of fluorescent foci; −, absence of fluorescent foci; ±, dim fluorescent foci were sometimes observed.

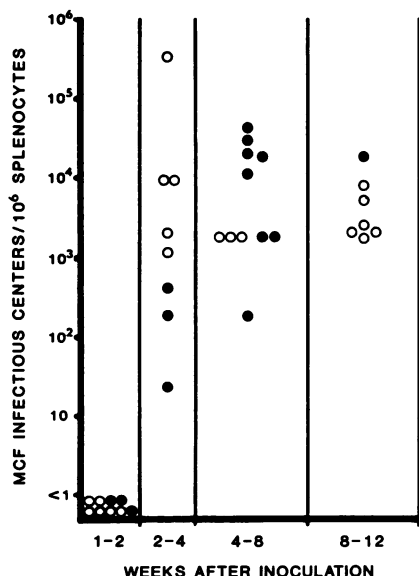

FIG. 4. Detection of MCF viruses in IRW mice at different times after inoculation as newborns with F-MuLV 57 (●) or B3 (○). Spleen IC were seeded on *M. dunni* cells and assayed by using the FIA with MCF virus-specific monoclonal antibody 514 (5).

Different factors have been described which can influence the type of cell induced to proliferate after inoculation with murine leukemia viruses. The virus strain and more specifically the long terminal repeat and the envelope genes seem to play a role in the cell specificity of leukemia (2, 3, 10, 15, 21). However, the mouse strain (6, 24, 26–28, 33) and the age of inoculation (8, 33) also influence the type of disease induced after inoculation with F-MuLV. It is still not clear what mechanisms determine the type of cell proliferation after inoculation with F-MuLV 57 or B3. It seems that a longer latency of disease observed in relatively resistant strains of mice inoculated as newborns or after inoculation of susceptible strains of mice as adults favors a more frequent involvement of nonerythroid proliferation (27, 33; P. Tambourin, personal communication). Also in agreement with this observation is our finding that incidence of nonerythroid leukemias, particularly lymphoid leukemias, was increased in mice inoculated with F-MuLV B3, which had a longer latency of disease.

The present results suggest that the generation of recombinant F-MCF viruses is not sufficient to induce early splenomegaly observed with F-MuLV 57 and other fully virulent strains of F-MuLV, since F-MuLV B3 did induce Friend MCF viruses as efficiently as F-MuLV 57. Since F-MuLV B3 induced only late leukemias, whereas F-MuLV 57 and other fully virulent strains of F-MuLV are able to induce both early splenomegaly and late transplantable leukemias (6, 18, 20, 26, 32, 33), it is likely that the pathogenic effect of F-MuLV 57 which leads to induction of

TABLE 2. Proliferative cell types in enlarged spleens of mice inoculated at birth with F-MuLV 57 or B3[a]

F-MuLV strain (no.)	Proliferative cell type (%)[b]					
	Erythroid	Erythroid/ myeloid	Erythroid/ lymphoid	Myeloid	Lymphoid	Myeloid/ lymphoid
F-MuLV 57 (27)	23 (85)	3 (11)	0 (0)	1 (4)	0 (0)	0 (0)
F-MuLV B3 (36)	8 (22)	10 (28)	4 (11)	2 (5.5)	10 (28)	2 (5.5)

[a] Grossly enlarged spleens from NFS/N or IRW mice inoculated as newborns with 2.5×10^3 FFU of either virus strain were obtained 1 to 8 months after inoculation.

[b] Proliferative cell type was determined as described in the text (6).

early splenomegaly develops through a nonneoplastic mechanism, different from the leukemogenic process. This is supported by the observations that spleen cells from newborn-inoculated animals are more easily transplantable late in disease and that early F-MuLV 57-induced splenomegaly can often be reversed by the transfusion of mature erythrocytes (20, 26, 33).

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