SUPPRESSION OF ENDOGENOUS MURINE LEUKEMIA VIRUS BY MATERNAL RESISTANCE FACTOR*

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In crosses of mice of the high lymphoma strain, AKR, with mice of the low lymphoma strain, RF, the lymphoma-resistant phenotype of the parental RF strain is transmitted by both mendelian and nonmendelian mechanisms. The nonmendelian mechanism, identified by comparing lymphoma incidences in reciprocal F_1 crosses of the two strains, involves a maternal resistance factor (MRF)[1] transmitted by RF females to their progeny of both sexes (1). Evidence suggests that the main vehicle for transmission of the factor is the mother's milk (2).

A crucial factor in lymphomagenesis in AKR mice is the life-long expression, beginning soon after birth, of high levels of endogenous ecotropic murine leukemia virus (E-MuLV) in infectious form in lymphoid tissues (3). Recent studies have demonstrated that mice of reciprocal F_1 crosses of AKR and RF differ even more markedly from each other in their levels of E-MuLV expression than in their lymphoma incidences; virus expression in (AKR × RF)F_1 mice was only modestly decreased by comparison with parental strain AKR mice, whereas it was profoundly depressed in (RF × AKR)F_1 mice (4). (By convention, the mother's strain is placed first in designating crosses.) Thus the RF strain may be categorized as MRF$^+$ and the AKR strain as MRF$^-$ with reference to the transmission of maternal resistance both to lymphoma and to endogenous E-MuLV expression, leading to the obvious hypothesis that MRF-mediated suppression of virus expression is the basis of maternally transmitted resistance to lymphoma.

In other genetic studies, we examined crosses of the RF and DBA/2 strains, both of which show only low incidences of spontaneous lymphoma and little or no E-MuLV expression (5). F_1 mice born of MRF$^+$ RF mothers showed the low E-MuLV phenotype of the parental RF strain (i.e., virus-negative until 60–90 d of age, low-level expression thereafter in most mice), whereas F_1 mice born of DBA/2 mothers expressed very high levels of infectious E-MuLV, comparable to those seen in AKR mice, already at weaning age. Thus, the DBA/2 strain is also MRF$^-$ by these criteria.

In the present communication, we have expanded the repertory of mouse strains for these experiments to include SJL (MRF⁺) and ST/b (MRF⁻). We found that inoculation of E-MuLV preparations into 1–4-d-old mice resulted in persistent expression of infectious virus in the MRF⁻ strains, DBA/2 and ST/b, but not in the MRF⁺ strains, RF and SJL. In contrast, inoculation of the virus into 30-d-old mice of MRF⁻ strains did not lead to detectable virus expression but, rather, conferred upon females the MRF⁺ phenotype. Finally, we found that foster-nursing AKR newborns on RF mothers (MRF⁺) resulted not only in a markedly delayed appearance of lymphoma but also in greatly decreased levels of E-MuLV expression in the offspring.

Materials and Methods

Animals. Inbred mice of strains AKR/J, RF/J, SJL/J, and DBA/2J were obtained from The Jackson Laboratory, Bar Harbor, ME. Experiments comparing mice from this source with mice of the same strains bred in our colonies produced indistinguishable results. ST/bN mice were bred in our colony by brother/sister mating starting from breeding pairs obtained through the courtesy of Dr. Carl Hansen, National Institutes of Health. All F_1 mice were bred in our colonies. Only female mice were studied in the experiments reported here.

XC Plaque Assay. Spleens and thymuses of individual mice were homogenized separately in Ten Broeck tissue grinders in 2 ml of minimal essential medium (MEM) with 20% fetal calf serum (FCS) added. Lymphoid cells were separated from supernates by centrifugation at 100 g for 5 min at 4°C. The cells in the pellet were counted in the presence of trypan blue to assess viability (usually >90% viability) and then resuspended at 10^7/ml in fresh medium for use in virus assays. The supernate was used as the cell-free extract after two additional centrifugation steps at 4°C, one at 4,500 g for 15 min and a subsequent one at 7,000 g for 4 min.

A clonal derivative of SC-1 mouse cells was used for detection of E-MuLV expression. Serial dilutions of both lymphoid cells and cell-free extracts in MEM plus 20% heat-inactivated FCS were used to infect monolayers of SC-1 cells that had been pretreated with Polybrene (16 μg/ml, Abbott Laboratories, North Chicago, IL) for 24 h. SC-1 cells were infected at a density of about 3.5 × 10^5 cells/cm². 1 d later the infectious inoculum was removed and replaced by MEM containing 5% heat-inactivated FCS. 4 d after infection the SC-1 cells were exposed for 25–30 s to ultraviolet irradiation (60 erg/mm² per second) and then overlaid with XC rat cells. 2 d after adding XC cells the plates were fixed with methanol and stained with cresyl violet acetate. Plaques containing syncytial cells were counted under a dissecting microscope. Results were expressed as plaque-forming units (PFU) per 10^7 viable spleen or thymus cells or PFU per 100 mg of tissue extract.

Experimental Infection with E-MuLV. Infectious E-MuLV-containing extracts were prepared from the spleens of 30- to 60-d-old AKR, (DBA/2 × RF)F_1 [D2/RF] or (ST/b × RF)F_1 [ST/RF] mice, all of which express high levels of virus activity from 3 wk of age. Only freshly prepared extracts from three or more donors were used. Spleens were homogenized in five times their weight of MEM plus 5% FCS. The homogenates were centrifuged for 8 min at 500 g; the supernates were removed and centrifuged for 15 min at 1,800 g and then for a further 5 min at 6,000 g. Mice 1–5-d old received 0.1 ml of extract; a 1-in 30 gauge needle was used to make a long subcutaneous track, starting at the chest level, before depositing the inoculum ip. Mice of weanling age or older received a 0.1-ml inoculum directly intraperitoneally.

Induction of the MRF⁺ Phenotype. Beginning at 4–5 wk of age, DBA/2 and ST/b females received three injections of 0.1 ml i.p. of E-MuLV-containing extracts at 1-wk intervals. 1 wk later they were mated with AKR or RF males, and their progeny were tested for E-MuLV expression at 20–300 d of age.

Results

E-MuLV Expression in Mice of Inbred Strains and F_1 Crosses. The XC plaque assay for infectious E-MuLV activity was the method used to examine both cell-free extracts and viable cells, plated as infectious centers, from spleens and thymuses of individual 30-d-old females of selected inbred strains and F_1 crosses (Table I). AKR mice showed the expected high levels of endogenous virus activity in these lymphoid tissues (6), but, except for one DBA/2 mouse, animals of the other four strains tested were completely negative in these experiments. In other experiments not shown, older mice of the RF and SJL strains have sometimes shown low to moderate levels of E-MuLV activity (5, 7), but DBA/2 mice were rarely virus-positive, and ST/b mice have been consistently negative at all ages.

Mice of F_1 crosses of AKR with either DBA/2 or ST/b expressed virus levels that were the same as or only slightly lower than those in parental strain AKR mice (Table I). Reciprocal crosses were indistinguishable in this respect, indicating that DBA/2 and ST/b females are phenotypically MRF⁻. By contrast, pronounced differences existed between the E-MuLV levels of mice of reciprocal F_1 crosses of AKR with both the RF and SJL strains. In these latter intercross generations, mice born of AKR mothers showed AKR-like virus expression; mice born of SJL mothers, on the other hand, showed complete suppression of the virus in the thymus, and those born of RF mothers showed complete suppression in both the spleen and thymus. Thus females of the RF and SJL strains appeared to be, to different degrees, phenotypically MRF⁺.

Previous studies of intercrosses of the DBA/2 and RF strains have shown that 20-d-old F_1 mice born of MRF⁻ DBA/2 mothers show high levels of E-MuLV activity (5) that are expressed from proviruses of the RF strain genome (8). By contrast, F_1 mice born of MRF⁺ RF mothers remain virus negative until late in life (5). Two parallels to these findings emerge from the results in Table I. First, reciprocal F_1 crosses of RF and ST/b mice showed the same marked differences in E-MuLV expression seen in the crosses of RF with DBA/2, confirming that ST/b, like DBA/2, mothers were phenotypically MRF⁻. Second, crosses of SJL with either DBA/2 or ST/b mice also resembled those of RF with DBA/2 or ST/b in that E-MuLV was expressed in the progeny if the SJL parent was the father but not if it was the mother (cf. reference 9). In the DBA/2 by RF crosses studied earlier, the E-MuLV expressed was shown to originate from one or more proviruses of the RF parental strain (5). By analogy, the virus activity detected in the present crosses of SJL with DBA/2 or ST/b was presumably expressed from SJL provirus(es). Since both the incidences and the titers of E-MuLV detected in these SJL crosses were lower than those in the analogous RF crosses, it appears that the SJL provirus(es) are less readily expressed than those of the RF strain.

E-MuLV Infection of Neonates and Juveniles. Freshly prepared extracts of the spleens of AKR, D2/RF, or ST/RF young adults were used to infect recipient mice of various ages. Since extracts from the different sources produced indistinguishable results in mice of any one strain, these results were pooled (Table II). Mice of the four strains that had proved to be E-MuLV negative at 30 d of age received 0.1 ml i.p. of spleen extract at 1–5 d of age. Tests of their lymphoid

TABLE I

E-MuLV Expression in Lymphoid Organs of Mice of Various Inbred Strains and of Their Reciprocal F_1 Crosses at 30 D of Age

| Mice | n | Percent E-MuLV-positive mice ($\log_{10}$ virus titer) | | | |
| | | Spleen | | Thymus | |
		Cells*	Extract‡	Cells	Extract
ST/b	10	0	0	0	0
DBA/2	10	10 (2.2)	0	0	0
SJL	10	0	0	0	0
RF	10	0	0	0	0
AKR	10	100 (5.5)	100 (4.6)	100 (3.0)	100 (2.5)
(AKR × ST/b)F_1	10	100 (5.3)	100 (4.5)	100 (4.0)	60 (2.8)
(ST/b × AKR)F_1	10	100 (5.2)	100 (4.8)	100 (3.6)	60 (2.5)
(AKR × DBA/2)F_1	10	100 (4.9)	100 (3.9)	100 (2.7)	100 (2.6)
(DBA/2 × AKR)F_1	10	100 (5.0)	100 (4.6)	100 (3.4)	20 (2.5)
(AKR × SJL)F_1	10	100 (5.6)	100 (4.8)	100 (5.0)	100 (2.6)
(SJL × AKR)F_1	12	50 (4.6)	42 (4.2)	0	0
(AKR × RF)F_1	15	100 (4.7)	100 (3.5)	33 (2.5)	20 (2.2)
(RF × AKR)F_1	10	0	0	0	0
(ST/b × RF)F_1	10	100 (5.5)	100 (4.9)	100 (3.7)	50 (3.0)
(RF × ST/b)F_1	10	0	0	0	0
(DBA/2 × RF)F_1	11	100 (4.6)	100 (3.2)	100 (2.2)	0
(RF × DBA/2)F_1	10	0	0	0	0
(ST/b × SJL)F_1	10	80 (4.4)	50 (2.8)	40 (3.6)	0
(SJL × ST/b)F_1	10	0	0	0	0
(DBA/2 × SJL)F_1	20	80 (3.9)	35 (3.1)	0	0
(SJL × DBA/2)F_1	10	0	0	0	0

* Values for cells are the geometric mean numbers ($\log_{10}$) of infectious centers induced in the XC assay per 10^7 viable cells plated.
‡ Values for tissue extracts are the geometric mean titers ($\log_{10}$) in the XC assay of virus-induced plaques per 0.4 ml of extract.

tissues at 18–38 d of age showed that mice of the MRF$^+$ strains, RF and SJL, remained virus negative except for 3 of 13 SJL mice that showed low virus titers in their spleen cells. However, mice of MRF$^-$ strains DBA/2 and ST/b became infected, showing high virus titers in the spleen and often moderate titers in the thymus; this infection was long-lasting, since neonatally inoculated MRF$^-$ mice were tested at 2–8 mo of age showed a similar pattern of virus expression. ST/b mice were slightly more susceptible to E-MuLV infection than DBA/2 mice. The susceptibility of MRF$^-$ mice to E-MuLV infection disappeared with age, however; DBA/2 and ST/b mice inoculated at ~30 d of age remained virus negative when tested 1 mo later (Table II).

Effect of Foster Nursing. Newborn AKR mice were transferred for nursing to

TABLE II

E-MuLV Expression in Mice of Various Strains after Virus Inoculation Neonatally or at 30 D of Age*

Mice	Age of mice (d) at the time of:		n	Percent E-MuLV-positive mice ($\log_{10}$ virus titer)			
	Virus inocu-lation	Virus assay		Spleen		Thymus	
				Cells[‡]	Extract[‡]	Cells	Extract
SJL	1–2	22–30	13	23 (3.4)	0	0	0
RF	1–4	18–30	25	0	0	0	0
ST/b	1–4	24–34	24	100 (5.2)	96 (4.2)	88 (3.7)	42 (3.1)
ST/b	1–4	108–252	24	100 (4.9)	86 (4.5)	82 (3.8)	50 (3.3)
ST/b	30–32	55–60	15	0	0	0	0
DBA/2	1–5	22–38	32	100 (4.7)	94 (3.2)	44 (3.2)	13 (3.8)
DBA/2	1–5	72–248	37	100 (4.3)	59 (3.2)	24 (2.7)	0
DBA/2	30–34	54–60	15	0	0	0	0

* E-MuLV preparations inoculated consisted of freshly prepared cell-free extracts of spleens of young adult AKR, D2/RF, and ST/RF mice.
[‡] See footnotes to Table I.

TABLE III

Effect of Foster-nursing on E-MuLV Expression in Weanling AKR Mice

Newborn mice	Foster mother	Age (d) at virus assay	n	Percent E-MuLV-positive mice ($\log_{10}$ virus titer)			
				Spleen		Thymus	
				Cells	Extract	Cells	Extract
AKR	—	20–32	10	100 (5.5)	100 (4.6)	100 (3.0)	100 (2.5)
AKR	RF	23–34	18	38 (3.7)	22 (2.9)	0	0
AKR	DBA/2	26–30	10	100 (5.8)	100 (5.0)	100 (3.5)	100 (2.0)

foster mothers of either the RF or DBA/2 strains. At 20–34 d of age, E-MuLV activity detected in their spleens and thymuses was measured and compared with that in tissues of AKR progeny of the same age reared by their own mothers (Table III). AKR mice fostered by MRF⁻ DBA/2 mothers showed levels of virus activity indistinguishable from those in unfostered controls. Those fostered by MRF⁺ RF mothers, on the other hand, showed nearly total suppression of the virus and, in a separate group, a markedly lower and later lymphoma incidence (Fig. 1) by comparison with controls nursed by their own mothers.

Induction of the MRF⁺ Phenotype. Female DBA/2 and ST/b mice that had been thrice inoculated with E-MuLV as weanlings were mated at 2 mo of age with either AKR or RF males. Levels of expression of endogenous E-MuLV were determined in the F_1 progeny of these matings and were compared with those in genetically identical populations of F_1 mice born of uninoculated DBA/2 and ST/b mothers. The results (Table IV) show that the virus-inoculated females produced progeny that were significantly protected against endogenous virus expression by comparison with the control progeny of uninoculated females.
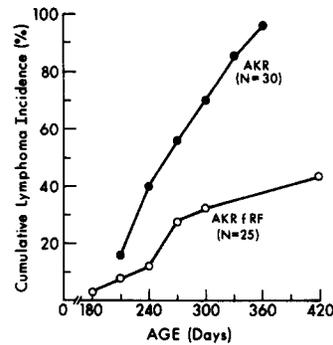
FIGURE 1. Cumulative incidence of lymphoma in AKR mice nursed by their own mothers (●) and in AKR mice foster-nursed by RF mothers (O).

TABLE IV

Induction of the MRF-positive Phenotype by Inoculation with E-MuLV-containing Spleen Extracts

Mice	Mother inoculated with virus from:	Age (d) at virus assay	n	Percent E-MuLV-positive mice ($\log_{10}$ E-MuLV titer)			
				Spleen		Thymus	
				Cells	Extract	Cells	Extract
(ST/b × AKR)F₁	—	28–55	12	100 (5.2)	100 (4.5)	100 (3.7)	50 (2.5)
(ST/b × AKR)F₁	AKR	28–66	17	53 (4.3)	29 (2.7)	0	0
(ST/b × RF)F₁	—	23–86	10	100 (5.5)	100 (4.9)	100 (3.7)	30 (3.0)
(ST/b × RF)F₁	AKR	39–83	15	13 (2.0)	0	0	0
(DBA/2 × AKR)F₁	—	31–36	11	100 (5.0)	100 (4.6)	100 (3.4)	18 (2.5)
(DBA/2 × AKR)F₁	AKR	25–48	17	88 (4.5)	29 (3.2)	12 (2.4)	0
(DBA/2 × RF)F₁	—	23–80	20	100 (4.4)	100 (3.1)	80 (2.4)	10 (2.0)
(DBA/2 × RF)F₁	AKR	31–80	16	13 (3.7)	0	0	0
(DBA/2 × RF)F₁	ST/RF	20–35	27	27 (2.3)	0	0	0
(DBA/2 × RF)F₁	D2/RF	21–30	17	29 (2.9)	0	0	0

Reduced virus expression was apparent in both the spleen and the thymus in all four crosses studied. It appears that the inoculation of E-MuLV into these young adult females of MRF⁻ strains, which did not lead to active virus infection, did lead to induction of the MRF⁺ phenotype.

Virus-containing extracts from the three different sources used (AKR, ST/RF, and D2/RF) were equally effective in inducing the MRF⁺ phenotype in DBA/2 females, as revealed in studies of E-MuLV expression among their progeny by RF males (Table IV). However, the MRF⁺ phenotype induced in DBA/2 and ST/b females by AKR extracts was less effective in suppressing E-MuLV expression in their progeny by AKR than by RF males (Table IV). This latter finding is consistent with results from a small number of tests of E-MuLV expression in 6–8-mo-old mice born of DBA/2 or ST/b females treated with AKR extracts and then mated with AKR or RF males (data not shown); in these experiments, these older progeny of AKR fathers showed a tendency to escape

from MRF-induced virus suppression, whereas this escape was not seen in the older progeny of RF fathers.

Discussion

Both the RF and SJL mouse strains have been shown to be phenotypically MRF$^+$; F$_1$ progeny of either of these strains with the high E-MuLV AKR strain exhibited marked suppression of infectious E-MuLV expression by comparison with the AKR parent, but only if the mother, and not the father, was the RF or SJL parent. The transmission of this MRF within the inbred RF and SJL strains appears to be the main factor responsible for two related characteristics of these mice. First, mice of both strains, like AKR mice, possess one or more inducible E-MuLV proviruses in their genomes (10). However, in contrast to AKR mice, these mice showed complete suppression of virus expression by the MRF until $\sim$3 mo of age in RF and even later in SJL mice, after which some individuals manifested escape from suppression and expressed low to moderate levels of infectious virus in their tissues. Second, in contrast with mice of MRF$^-$ strains, RF and SJL mice inoculated soon after birth with infectious E-MuLV-containing extracts remained virus-negative at 30 d of age. These findings provide support for the hypothesis that the MRF is the product of an immune response and consists of antiviral antibodies able to suppress endogenous E-MuLV expression in infant mice long enough to permit maturation of a native antiviral immune capacity in the developing babies. Such antibodies would be expected to cross-react strongly among the E-MuLV originating from proviruses of the AKR, RF, and SJL strains, and our data bear out this prediction.

Preliminary studies using a radioimmunoassay for detection of anti-MuLV antibodies reactive with AKR E-MuLV particles indicate that such antibodies were indeed present in high titers in the sera of RF mice but were also present at equal levels in the sera of MRF$^-$ DBA/2 mice. It would follow from this observation that, if the MRF does consist of antiviral antibodies, these antibodies must represent a particular subset of the possible repertory of antiviral antibodies. The basis for the distinction between MRF-associated and MRF-independent antiviral antibodies remains to be clarified.

The MRF$^-$ DBA/2 strain belongs to a different category of mice. It possesses a competent E-MuLV provirus (10) but expresses infectious virus only rarely and at very low levels. If, by analogy with MRF$^+$ strains, the low virus phenotype of DBA/2 mice is due to suppression by antiviral antibodies, these antibodies must be non–cross-reactive with E-MuLV originating from AKR, RF, and SJL proviruses, since (a) DBA/2 mothers provide no detectable anti–E-MuLV protection to their offspring by fathers of these strains, and (b) DBA/2 neonates are highly susceptible to chronic infection by exogenous E-MuLV. It seems more likely that the DBA/2 provirus is suppressed by some cis-acting mechanism. The ST/b strain resembles DBA/2 in all these characteristics, except that mice of this strain do not express detectable E-MuLV activity, consistent with their apparent lack of a complete E-MuLV provirus in their genome (10).

Although DBA/2 and ST/b mice were highly susceptible to infection with exogenous E-MuLV soon after birth, they had become resistant to the virus at 30 d of age. This resistance apparently developed as a function of the acquisition

of immunocompetence with respect to the virus; females hyperimmunized with virus-containing extracts became phenotypically MRF$^+$ and, in crosses with RF males, produced up to seven litters of F$_1$ progeny that remained markedly E-MuLV-suppressed to at least the age of 8 mo. This result bears comparison with recent findings of Schwarz et al. (11), who succeeded in inducing the MRF$^+$ phenotype in some AKR females by repeated inoculation, beginning immediately after birth, of a goat antiserum to the purified Friend MuLV envelope glycoprotein, gp70, which cross-reacts with the homologous E-MuLV gene product. AKR mice are presumably MRF$^-$ because they produce infectious E-MuLV in large quantities soon after birth and thus become tolerant or paralyzed with respect to its antigens; either inoculation of heterologous anti-gp70 antibodies, as in the experiments of Schwarz et al. (11), or maternal transmission of appropriate antiviral antibodies, as in AKR babies foster-nursed on RF mothers (Table IV), can interfere with E-MuLV expression until a native immune response can be established. This decreased virus expression coincides with a significant delay and decrease in lymphoma incidence in both sets of experiments, thereby providing further evidence that expression, and not the mere presence, of endogenous E-MuLV proviruses is crucial in spontaneous AKR lymphomagenesis.

Summary

Females of the RF and SJL inbred mouse strains transmit to their progeny of both sexes a nonmendelian maternal resistance factor (MRF) able to suppress the expression of endogenous ecotropic murine leukemia virus (E-MuLV). This MRF is demonstrable in crosses with AKR mice by comparing E-MuLV expression in the spleens and thymuses of reciprocal F$_1$ generations. DBA/2 and ST/b mice are MRF negative by these criteria. Neonatal inoculation of E-MuLV-containing spleen extracts gives rise to persistent expression of infectious virus in mice of the MRF$^-$ but not the MRF$^+$ strains. However, inoculation of the virus in 30-d-old females of the MRF$^-$ strains no longer leads to a state of persistent infection; instead, these females become MRF$^+$ and transmit protection against E-MuLV expression to their progeny by AKR and RF males. The MRF appears to be transmitted to the progeny mainly through the milk, since foster-nursing AKR neonates on RF (but not DBA/2) mothers greatly reduces E-MuLV expression in the progeny. These RF-fostered AKR mice also show a reduced and delayed lymphoma incidence, a finding consistent with the idea that maternally transmitted resistance to E-MuLV expression is the basis for the classic maternal resistance to lymphomagenesis seen in the progeny of RF mothers.

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