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# Transmission of Murine Leukemia Virus (Scripps) from Parent to Progeny Mice as Determined by p30 Antigenemia[1]

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## SUMMARY

All mice of C57BL/St, C3H/St, BALB/cSt, NZB/Scr, and NZW/Lac strains developed high levels of p30 antigenemia after inoculation at birth with murine leukemia virus (Scripps). Transmission of virus from neonatally infected parents to their progeny for three successive generations, as evidenced by development of p30 antigenemia, varied among the five strains. Through the three generations, 100% transmission occurred in C3H/St and BALB/cSt mice, 50 to 61% transmission occurred in C57BL/St and NZW/Lac mice, and 11% transmission to the first generation, with no subsequent transmission, occurred in the NZB/Scr mice.

Transmission appeared to occur readily via the milk in all strains. Intrauterine events also played a role with evidence of some viral transfer prior to birth in the C3H/St strain or, conversely, the development of resistance to infection prior to birth in C57BL/St mice. The occurrence of litters from infected parents containing both normal offspring and offspring with elevated p30 appeared to be the result of variable resistance in the intact offspring, perhaps as a result of intrauterine events, and not related to cellular resistance observable in tissue culture or to dominant genetic factors.

## INTRODUCTION

Transmission of MuLV[4] (Gross), MuLV (Graffi), and 3 Friend-Moloney-Rauscher MuLV's (Friend, Moloney, and Buffett) from parent to progeny as evidenced by development of leukemia through successive generations of mouse strains having low natural incidences of leukemia has been well documented (1–5, 7, 10–14, 16, 17). The most successful of these transmission studies used a virulent strain of MuLV inoculated into newborn mice of susceptible strains. Transmission of MuLV occurred primarily via the milk of infected females as established by foster-nursing experiments. However, the lack of rapid and sensitive bioassays for *in vivo* and *in vitro* detection of MuLV prevented previous investigators from obtaining definitive evidence of transmission prior to development of leukemia in the offspring. The purpose of this study was to examine the entire process of transmission and subsequent infection by following the levels of expression of p30, the major MuLV virion protein. Elevated p30 levels in all strains were invariably associated with recovery of infectious virus from spleen cultures.[5] MuLV (Scripps), a Moloney-like virus isolate from NZB lymphoblasts (20), was passed in C3H/St, C57BL/St, BALB/cSt, NZW/Lac, and NZB/Scr strains.

## MATERIALS AND METHODS

**Animals.** Pregnant C3H/St, BALB/cSt, and C57BL/St mice were obtained from L. C. Strong Laboratories, San Diego, Calif. Pregnant NZB/Scr and NZW/Lac mice were bred in the Scripps Clinic and Research Foundation vivarium in La Jolla, Calif. Pregnant C57BL/6J and C57BL/10J mice were received from The Jackson Laboratory, Bar Harbor, Maine.

**Virus.** The isolation and characterization of MuLV (Scripps) have been reported (8, 15). MuLV (Scripps) virus pools were prepared by passing supernatant fluids from SCRF 60A thymoblast continuous suspension cultures through a 0.45-$\mu$m filter and storing them in 1-ml aliquots at −70°. The infectivity of these virus pools was adjusted to $10^2$ to $10^3$ $TCID_{50}$/0.1 ml by virus assay determinations on BALB/c and NIH/Swiss secondary fibroblast cultures using the XC cell test of Klement (9).

**p30 Assays.** Sera and milk samples were analyzed for p30 by a radioimmunoassay that utilizes the double antibody method and is a modification of the procedures described by Oroszlan *et al.* (18) and Parks and Scolnick (19).

**Serum p30.** All mice were bled bimonthly from the retro-orbital plexus. Serum was separated from platelets and blood cells by low-speed centrifugation and stored at −20° until tested by radioimmunoassay for p30 levels.

**Milk p30.** Lactating control and MuLV (Scripps)-infected mice were separated from their offspring approximately 2 weeks postparturition, given 0.2 unit of Oxytocin (Parke-Davis & Co., Detroit Mich.) i.p., and milked after 15 to 20 min using a manually operated suction apparatus (6). Females were then returned to their offspring without apparent interference with nursing. The weight of the collected milk was determined and corrected for moisture lost by the suction apparatus (D. E. Groff, unpublished observations). In order to eliminate milk lipids and concentrate virus, 0.1 to 0.2 ml

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[4] The abbreviations used are: MuLV, murine leukemia virus; G0, the virus-inoculated generation of mice; G1–G3, the offspring in successive generations from G0 mice.

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of milk was then diluted with 4.5 ml of 0.15 M NaCl-0.005 M $Na_2HPO_4$-0.005 M $NaH_2PO_4$ (pH 7.2) at 4° and centrifuged at 1500 rpm for 10 min. The supernatant fluid was then sedimented through 1.0 ml of 15% (w/w) sucrose in 0.01 M Tris-HCl (pH 7.4)-0.1 M NaCl-0.001 M EDTA onto a 0.5-ml cushion of 48% (w/w) sucrose in the same mixture by centrifuging at 45,000 rpm for 45 min at 4° in a Beckman SW 50.1 rotor of the Beckman L4 ultracentrifuge (Beckman Instruments, Fullerton, Calif.) The sucrose interface was collected and stored at −20° until tested by the radioimmunoassay for the total concentration of p30. This amount of p30 was then adjusted to the concentration of p30 (μg/ml) in the original volume of milk. Concentrations of lipids and proteins other than p30 were not determined.

**Experimental Design.** MuLV (Scripps) was transmitted by infected natural and foster mothers within and between inbred strains of mice. Transmission of MuLV (Scripps) to mice of each strain was considered positive when their levels of serum p30 were elevated more than 2 S.D. above control values by 4 months of age since such levels were uniformly associated with recoverable NB-tropic MuLV from the spleens of all strains. Controls were from sex-, age-, and strain-matched unmanipulated mice.

**Transmission within Inbred Strains of Mice.** C3H/St, BALB/cSt, C57BL/St, NZB/Scr, and NZW/Lac litters were inoculated i.p. with $10^2$ to $10^3$ $TCID_{50}$ of MuLV (Scripps) within 24 hr after birth. These 1st-generation mice (G0) were then bred to littermates and their progeny were followed through 3 successive generations (G1-G3) of sibling matings for transmission of MuLV as evidenced by expression of p30 antigenemia. Normal newborn mice were foster-nursed by G0 or G2 females and some newborn G1 or G3 mice were foster-nursed by normal females to distinguish milk transmission of MuLV (Scripps) from *in utero* transmission. Newborn mice to be used in foster-nurse experiments were separated from their G0 and G2 mothers immediately after delivery to prevent milk ingestion. Since newborn mice have transparent skin that allows visualization of stomach contents, entire litters in which any newborn mice had detectable milk or fluid in their stomachs were excluded from the foster-nursing experiments. The fact that this type of separation prevented virus transfer in 100% of C57BL/St mice suggests that it did indeed prevent milk ingestion from the mother.

**Transmission between Inbred Strains of Mice.** Normal C3H/St, C57BL/St, and NZB/Scr mice less than 24-hr old were foster-nursed by lactating homologous G0 females. In addition, normal C3H/St and BALB/cSt neonates were foster-nursed by lactating normal NZB/Scr females. All mice were weaned at 3 weeks of age and segregated according to sex and strain. They were then followed by bimonthly bleedings for evaluation of p30 antigenemia.

## RESULTS

**Normal Serum p30 Values.** The serum p30 values expressed in μg/ml for normal 4-month-old mice of the 5 different strains are shown in Table 1. Values of p30 greater than 2 S.D. from the mean were considered positive (+) for p30 antigenemia in this study; all other values were considered normal (−) for p30 antigenemia.

Table 1
*Normal serum p30 values in 4-month-old mice*

| Strain | No. of mice | Serum p30 (μg/ml) | Normal serum p30 limit (μg/ml) |
|---|---|---|---|
| C3H/St | 31 | 0.026 ± 0.025[a] | 0.076[b] |
| BALB/cSt | 34 | 0.031 ± 0.025 | 0.081 |
| C57BL/St | 11 | 0.063 ± 0.021 | 0.106 |
| NZB/Scr | 45 | 0.036 ± 0.030 | 0.098 |
| NZW/Lac | 51 | 0.049 ± 0.076 | 0.202 |

[a] Mean ± S.D.
[b] Mean + 2 S.D.

The specificity of the radioimmunoassay for normal serum p30 values was demonstrated by showing that labeled MuLV p30 was 80 to 90% precipitable by excess antibody prepared against either MuLV p30 or feline leukemia virus p30; antibody prepared against fetal calf serum reacted less than 10% with labeled MuLV p30. In the quantitative competition radioimmunoassay for unlabeled antigen, normal human placenta and spleen cells; 33% normal rat, rabbit and human serum; and purified gp 70 did not displace labeled MuLV p30. These observations show that the normal p30 values reported in this study were based on p30 detection and not on potential artifacts.

**Transmission within Inbred Strains of Mice.** It was concluded that transmission of MuLV through successive generations of mice had occurred when constant or increasing percentages of progeny mice in each succeeding generation developed serum p30 levels similar to those in the lactating parent. The incidence of p30 antigenemia and average (+) p30 values for transmission of MuLV (Scripps) through successive generations of each mouse strain is expressed as the total incidence of p30 antigenemia and average (+) p30 values in G1-G3 mice nursed on (+) p30 natural mothers (Table 2).

**C3H/St Mice.** All G0 and G1-G3 mice showed high levels of p30 antigenemia by 4 months of age (Table 2). Since 100% of offspring from normal females foster-nursed on G0 and G2 females developed p30 antigenemia (Table 3), MuLV (Scripps) apparently was transmitted through the milk to all G1-G3 mice. When offspring from G0 and G2 females were foster-nursed on normal females, 20 of 43 developed p30 antigenemia. The average (+) p30 value of these 20 mice (1.05 μg/ml) was lower than that of G0 (2.29 μg/ml) and G1-G3 (1.59 μg/ml) mice and the mice receiving MuLV only via the milk (2.53 μg/ml).

**BALB/cSt Mice.** All G0-G3 mice showed very high levels of p30 antigenemia by 4 months of age (Table 2). All offspring from normal females foster-nursed by G2 females developed p30 antigenemia showing that transmission of MuLV (Scripps) in BALB/cSt mice occurred readily through the milk (Table 3). The average p30 value for these mice (4.45 μg/ml) was greater than the average p30 value for G3 mice (3.19 μg/ml).

**C57BL/St Mice.** All G0 C57BL/St mice developed p30 antigenemia (Table 2). In G1-G3 mice nursed by (+) p30 natural mothers, 50% developed p30 antigenemia averaging

Table 2

*Transmission of MuLV (Scripps) by natural mothers (measured in offspring at 4 months of age)*

Incidences of p30 antigenemia and average (+) p30 values in μg/ml for the virus-inoculated generation (G0) and 3 successive generations (G1–G3) of their progeny nursed on (+) p30 natural mothers.

| | G0 | | G1 | | G2 | | G3 | |
|---|---|---|---|---|---|---|---|---|
| Strain | Incidence (+) p30 | Av. p30 (μg/ml) | Incidence (+) p30 | Av. (+) p30 (μg/ml) | Incidence (+) p30 | Av. (+) p30 (μg/ml) | Incidence (+) p30 | Av. (+) p30 (μg/ml) |
| C3H/St | 23/23 | 2.29 ± 0.18[a] | 13/13 | 1.00 ± 0.23 | 24/24 | 1.88 ± 0.25 | 16/16 | 1.67 ± 0.30 |
| BALB/cSt | 12/12 | 4.02 ± 0.28 | 23/23 | 1.83 ± 0.16 | 22/22 | 3.64 ± 0.30 | 8/8 | 3.19 ± 0.51 |
| C57BL/St | 24/24 | 0.92 ± 0.09 | 23/44 | 0.67 ± 0.09 | 18/45 | 1.09 ± 0.23 | 11/16 | 1.19 ± 0.23 |
| NZB/Scr | 15/15 | 2.56 ± 0.22 | 2/18 | 0.24 ± 0.05 | 0/8 | 0.02 | | |
| NZW/Lac | 14/14 | 2.94 ± 0.33 | 10/15 | 1.61 ± 0.40 | 4/7 | 0.59 ± 0.11 | 6/11 | 1.43 ± 0.23 |

[a] Mean ± S.E.

Table 3

*Transmission of MuLV (Scripps) by G0 or G2 isologous foster mothers to offspring of normal mothers (measured in offspring at 4 months of age)*

| | G1[a] | | G3[b] | |
|---|---|---|---|---|
| Strain | Incidence (+) p30 | Av. (+) p30 (μg/ml) | Incidence (+) p30 | Av. (+) p30 (μg/ml) |
| C3H/St | 9/9 | 3.07 ± 0.59[c] | 8/8 | 1.89 ± 0.26 |
| BALB/cSt | ND[d] | ND | 13/13 | 4.45 ± 0.60 |
| C57BL/St | 21/25 | 1.38 ± 0.13 | 12/17 | 0.85 ± 0.16 |
| NZW/Lac | ND | ND | 4/8 | 2.04 ± 0.40 |

[a] Normal newborns foster-nursed by G0 females.
[b] Normal newborns foster-nursed by (+) p30 G2 females.
[c] Mean serum p30 ± S.E.
[d] Not done.

0.92 μg/ml. All (+) p30 G1–G3 mice came from litters also containing (−) p30 offspring. The (+) p30 and (−) p30 siblings were randomly distributed between sexes. Foster-nursing experiments using uninfected foster mothers showed that transmission of MuLV (Scripps) to G1–G3 mice was dependent upon milk (Table 3). The single (+) p30 offspring of 47 offspring from (+) p30 females foster-nursed by normal females was subsequently shown to have a B-tropic virus rather than an NB-tropic Friend-Moloney-Rauscher-like virus recovered from cultured spleen cells (unpublished observations). When G2 and G3 offspring were nursed by (−) p30 natural mothers, 86 of 87 had normal serum p30 levels.

Because all litters of C57BL/St mice that were nursed on (+) p30 natural mothers or foster-nursed on (+) p30 females had both (−) p30 and (+) p30 offspring, we attempted to determine the basis of this "split litter" phenomenon. To determine whether this occurred at a cellular level reflecting differences among embryos, we titrated pools of both stock MuLV (Scripps) and stock MuLV (Scripps) that had been passaged once through C57BL/St embryo fibroblasts on fibroblast cultures from 8 separate C57BL/St embryos obtained from a single normal pregnant mouse. There was no significant difference either in the infectivity titers of the 2 virus pools titrated on the embryo cultures ($10^{2.8} \pm 10^{0.6}$ and $10^{3.2} \pm 10^{0.4}$ syncytia-forming units/ml, respectively) or in the susceptibility of the cultures from individual embryos to either pool of virus.

We also studied transmission of MuLV (Scripps) in milk to 3 sublines of C57BL mice to determine whether the split litter phenomenon was unique to the C57BL/St strain at the level of the intact animal. Newborn C57BL/6J and C57BL/10J were foster-nursed on G0 C57BL/St females; 4 of 6 of the offspring from each strain developed p30 antigenemia averaging 0.65 and 0.28 μg/ml, respectively. When the offspring of a G2 (−) p30 female and male from the same split litter were foster-nursed by a G0 C57BL/St female, 3 of 5 developed p30 antigenemia averaging 0.93 μg/ml.

**NZB/Scr Mice.** All G0 mice developed high levels of p30 antigenemia (Table 2). However, only 11% of G1 mice nursed on natural G0 mothers developed p30 antigenemia. The average (+) p30 level for these G1 mice (0.24 μg/ml) was lower than that of the other strains. Only 1 (+) p30 NZB/Scr G1 mouse was a female, and she produced 8 G2 offspring, all with normal serum p30 levels. Forty-five G2 and G3 mice nursed by (−) p30 natural mothers also had normal serum p30 levels averaging 0.02 μg/ml. Foster-nursing experiments were not done between control and infected NZB/Scr mice.

**NZW/Lac Mice.** All G0 mice developed high serum p30 levels by 4 months of age (Table 2). Of G1–G3 mice nursed on (+) p30 natural mothers, 61% developed p30 antigenemia averaging 1.43 μg/ml. Although many G1–G3 mice were in litters having (−) p30 and (+) p30 siblings this was not a consistent finding as seen with the C57BL/St litters. Foster-nursing of control NZW/Lac mice by (+) p30 G2 mothers showed that transmission occurs readily via the milk (Table 3). Nine G2 offspring from (−) p30 females had normal p30 levels of 0.09 μg/ml.

**Milk p30.** Simultaneous milk and serum p30 levels were done on lactating control and MuLV (Scripps)-infected mice from the 5 strains (Table 4). Infected mice had increases in both milk and serum p30 levels over those of the controls. However, control NZB/Scr and to a lesser extent NZW/Lac mice had high levels of p30 in the milk. These results strongly suggest that at least some p30 in the milk of control and infected mice is a physical part of MuLV virions.

### Transmission between Inbred Strains of Mice

**Controls.** Because of the high milk p30 level of 58.7 μg/ml in normal lactating NZB/Scr mice, we attempted to determine whether or not endogenous MuLV could be transmitted via the milk of these females to C3H/St and BALB/cSt

neonates. The 7 C3H/St and 7 BALB/cSt mice foster-nursed on normal NZB/Scr females did not develop p30 antigenemia.

**MuLV (Scripps)-infected Mice.** N-tropic viruses selectively infect N-type mouse cells, B-tropic viruses selectively infect B-type mouse cells, NB-tropic viruses like MuLV (Scripps) infect N- and B-type mouse cells, and xenotropic murine viruses do not infect any mouse cells (20). In order to establish that an NB-tropic virus was being transmitted via the milk by virus-inoculated females, offspring from N-type mice (C3H/St and NZB/Scr) and B-type mice (C57BL/St) were foster-nursed on G0 C3H/St, C57BL/St, and NZB/Scr females and were followed for development of p30 antigenemia by 4 months of age. As can be seen in Table 5, the incidences of p30 antigenemia for each offspring strain were not significantly different regardless of the foster-mother strain.

## DISCUSSION

Although some control and all infected lactating mice had levels of milk p30 elevated over their sera p30, effective milk transmission of MuLV to neonates as measured by p30 antigenemia only occurred from MuLV (Scripps)-infected mice. Control C3H/St, NZW/Lac, and NZB/Scr females had levels of p30 in the milk 15- to 1500-fold greater than in the sera, raising the possibility that milk-transmitted MuLV could have a role in the natural transmission of endogenous MuLV. Since these lactating mice did not effectively transmit MuLV to isologous offspring, and in the case of NZB/Scr mice, to homologous neonates as well, we concluded that these milk p30 values represented the noninfectious expression of endogenous MuLV. The levels of p30 in the milk of all virus-inoculated G0 and G2 mice, however, were increased by 15 to 45 μg/ml over isologous controls. Since MuLV (Scripps)-inoculated C3H/St, C57BL/St, and NZB/Scr mice transmitted MuLV via the milk to both isologous neonates and homologous N- and B-type neonates, the MuLV transmitted from virus-inoculated mice was an NB-tropic virus, presumably MuLV (Scripps). Thus it appears that lactating mammary glands can selectively express and excrete not only endogenous but also exogenous MuLV p30.

Table 4

*Levels of p30 in milk of lactating control and MuLV (Scripps)-infected mice*

| | Control mice | | Infected mice[a] | |
|---|---|---|---|---|
| Strains | Milk p30 (μg/ml) | Serum p30 (μg/ml) | Milk p30 (μg/ml) | Serum p30 (μg/ml) |
| C3H/St | 0.92[b] | 0.06 | 45.7 | 6.15 |
| BALB/cSt | 0.08 | 0.03 | 38.1 | 5.67 |
| C57BL/St | 0.03 | 0.06 | 18.2 | 2.45 |
| NZB/Scr | 58.7 | 0.04 | 75.3 | 1.23 |
| NZW/Lac | 3.92 | 0.05 | 19.2 | 4.62 |

[a] Lactating G0 and (+) p30 G2 females.
[b] Each value represents the average of 2 or 3 mice.

It is clear that tranmission occurred readily via milk, but some intrauterine events also appeared to play a role. Milk transmission alone could account for transmission through successive generations of the 5 strains of mice: 100% transmission in C3H/St and BALB/cSt mice, 50 to 61% transmission in C57BL/St and NZW/Lac mice, and 11% transmission to the 1st generation with no subsequent transmission in NZB/Scr mice. However, prior intrauterine events have modified the expected results from milk transmission as was evidenced by normal C57BL/St neonates foster-nursed on (+) p30 females showing a significantly ($p < 0.002$) higher percentage of animals with p30 antigenemia (79%) than G1–G3 mice nursed on (+) p30 natural mothers (50%). Since there was no effective *in utero* transmission of MuLV (Scripps) in infected C57BL/St mice, the most feasible explanations of this finding would be either passive transplacental transfer of either maternal anti-MuLV antibodies or transfer of soluble MuLV subviral antigens. The latter explanation would assume either an ensuing immunological response by the fetus and subsequent protection against milk-transmitted MuLV or the binding of MuLV antigens to cellular receptor sites in target organs of the fetus that blocked effective absorption and infection of these cells by MuLV transmitted via the milk. In contrast to G1–G3 C57BL/St mice, at least 47% of G1 and G3 C3H/St mice exhibited effective *in utero* transmission of MuLV (Scripps). *In utero* transmission has been reported for MuLV (Buffett) (1) but apparently rarely with MuLV (Moloney) (11), MuLV (Graffi) (10), and MuLV (Gross) (5). Buffett *et al.* (1) recovered MuLV from 14% of embryos of MuLV-infected Ha/ICR females as indicated by the development of leukemia in assay mice.

Table 5

*Transmission of MuLV (Scripps) by infected G0 foster mothers to control homologous neonates (p30 determined in offspring at 4 months of age)*

| | | Offspring strain | | | | | |
|---|---|---|---|---|---|---|---|
| | | C3H/St | | C57BL/St | | NZB/Scr | |
| Foster mother strain | Type[a] | Incidence (+) p30 | Av. (+) p30 (μg/ml) | Incidence (+) p30 | Av. (+) p30 (μg/ml) | Incidence (+) p30 | Av. (+) p30 (μg/ml) |
| C3H/St | N | 17/17 | 2.52 ± 0.34[b] | 7/8 | 1.76 ± 0.33 | 3/7 | 0.35 ± 0.16 |
| C57BL/St | B | 9/12 | 2.23 ± 0.44 | 33/42 | 1.19 ± 0.10 | 2/9 | 0.34 ± 0.20 |
| NZB/Scr | N | 3/4 | 1.79 ± 0.49 | 3/4 | 0.77 ± 0.01 | 2/18[c] | 0.24 ± 0.05 |

[a] N-type strain has *Fv-1*$^n$ locus; B-type strain has *Fv-1*$^b$ locus.
[b] Mean serum p30 ± S.E.
[c] G1 NZB/Scr offspring nursed on G0 natural mothers.

Under the special conditions of inoculating young pregnant A/LN and BALB/c mice with MuLV (Moloney) *in utero* transmission does occur in a low percentage of offspring (7). However, *in utero* transmission of MuLV (Friend) in Ha/ICR females under similar conditions was not seen (17). The results of our study suggest that intrauterine events of (+) p30 females from the C57BL/St strain may impart an increased resistance to development of p30 antigenemia after milk transmission of MuLV. On the other hand, in C3H/St mice no protective intrauterine effect can be detected but rather transmission occurs by both intrauterine and mammary routes.

The basis for the difference in tranmission among the 5 strains appears to be dependent upon the resistance of the intact offspring. This conclusion was reached by comparing the effectiveness of milk transmission from virus-inoculated C3H/St, C57BL/St, and NZB/Scr females to isologous and homologous normal neonates. The incidence of p30 antigenemia and average (+) p30 values in each offspring strain was similar regardless of the strain of the foster mother. Therefore, the offspring susceptibility is the most important factor in accounting for the variable responses to milk-transmitted MuLV (Scripps).

The occurrence of C57BL/St litters split into normal and high p30 offspring after nursing on virus-inoculated females appeared to be the result of the variable resistance among offspring. We attempted to determine whether or not the basis for the split litter phenomenon occurred at a cellular level or at the level of the intact animal. Since there was no significant difference in viral infectivity of separate pools of MuLV (Scripps) titrated on cultures of 8 individual C57BL/St embryos, we concluded that this phenomenon occurred only at the level of the intact animal. C57BL/6J, C57BL/10J, and C57BL/St offspring from G2 (−) p30 female mated with a G2 (−) p30 male from the same split litter when foster-nursed by G0 C57BL/St females were also in litters split into (−) and (+) p30 offspring. These observations, and the fact that the C57BL/St strain is an inbred strain, indicate that the split litter phenomenon probably does not have a genetic basis. In addition, the occurrence of split litters of C57BL/St neonates with similar incidences of p30 antigenemia and (+) p30 values foster-nursed on virus-inoculated C3H/St, a susceptible strain of mice, and NZB/Scr, a resistant strain of mice, support the contention that C57BL/St offspring exhibit a variable resistance to milk-transmitted MuLV.

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## REFERENCES

1. Buffett, R. F., Grace, J. T., Jr., DiBerardino, L. A., and Mirand, E. A. Vertical Transmission of Murine Leukemia Virus. Cancer Res., *29:* 588–595, 1969.
2. Buffett, R. F., Grace, J. T., Jr., DiBerardino, L. A., and Mirand, E. A. Vertical Transmission of Murine Leukemia Virus through Successive Generations. Cancer Res., *29:* 596–602, 1969.
3. Gross, L. Transmissible Mouse Leukemia Agent. *In:* G. E. W. Wolstenholme and M. P. Cameron (eds.), Ciba Foundation Symposium on Leukemia Research, pp. 76–104. Boston: Little Brown and Co., 1954.
4. Gross, L. Vertical Transmission of Passage A Leukemia Virus from Inoculated C3H Mice to Their Untreated Offspring. Proc. Soc. Exptl. Biol. Med., *107:* 90–93, 1961.
5. Gross, L. Transmission of Mouse Leukemia Virus through Milk of Virus-injected C3H Female Mice. Proc. Soc. Exptl. Biol. Med. *109:* 830–836, 1962.
6. Haberman, B. H. Mechanical Milk Collected from Mice for Bittner Virus Isolation. Lab Animal Sci., *24:* 935–937, 1974.
7. Ida, N., Fukuhara, A., and Ohba, Y. Several Aspects of Vertical Transmission of Moloney Virus. Natl. Cancer Inst. Monograph, *22:* 287–311, 1966.
8. Kennel, S. J., Del Villano, B. C., Levy, R. L., and Lerner, R. A. Properties of an Oncornavirus Glycoprotein: Evidence for Its Presence on the Surface of Virions and Infected Cells. Virology, *55:* 464–475, 1973.
9. Klement, V., Rowe, W. P., Hartley, J. W., and Pugh, W. E. Mixed Culture Cytopathogenicity. A New Test for Growth of Murine Leukemia Viruses in Tissue Culture. Proc. Natl. Acad. Sci. U. S., *63:* 753–758, 1969.
10. Krischke, W., and Graffi, A. The Transmission of the Virus of Myeloid Leukemia of Mice by the Milk. Acta Unio Intern. Contra Cancrum, *19:* 360–361, 1963.
11. Law, L. W. Influence of Foster-Nursing on Virus-induced and Spontaneous Leukemia in Mice. Proc. Soc. Exptl. Biol. Med., *111:* 615–623, 1962.
12. Law, L. W. Studies of Experimental Transmission of Leukemogenic Virus Infection in Mice. J. Natl. Cancer Inst., *34:* 543–549, 1965.
13. Law, L. W. Transmission Studies of a Leukemogenic Virus, MLV, in Mice. Natl. Cancer Inst. Monograph, *22:* 267–285, 1966.
14. Law, L. W., and Moloney, J. B. Studies of Congenital Transmission of a Leukemia Virus in Mice. Proc. Soc. Exptl. Biol. Med., *108:* 715–723, 1961.
15. Lerner, R. A., Jensen, F. C., Kennel, S. J., Dixon, F. J., Des Roches, G., and Franke, U. Karyotypic, Virologic and Immunologic Analyses of Two-Continuous Lymphocyte Lines Established from New Zealand Black Mice. Possible Relationship of Chromosomal Mosaicism to Autoimmunity. Proc. Natl. Acad. Sci. U. S., *69:* 2965–2969, 1972.
16. Mirand, E. A., Buffett, R. F., and Grace, J. T., Jr. Mode of Transmission of Friend Virus Disease. Proc. Soc. Exptl. Biol. Med., *121:* 970–976, 1966.
17. Mirand, E. A., Grace, J. T., Jr., and Buffett, R. F. Passive and Active Immunity to Friend Virus Disease. Nature, *209:* 696–698, 1966.
18. Oroszlan, S., White, M. M. H., Gilden, R. V., and Charman, H. P. A Rapid Direct Radioimmunoassay for Type C Group Specific Antigen and Antibody. Virology, *50:* 294–296, 1972.
19. Parks, W. P., and Scolnick, E. M. Radioimmunoassay of Mammalian Type C Viral Proteins: Interspecies Antigenic Reactivities of the Major Internal Polypeptide. Proc. Natl. Acad. Sci. U. S., *69:* 1766–1770, 1972.
20. Sarma, P. S., and Gazdar, A. F. Recent Progress in Studies of Mouse Type-C Viruses. Current Topics Microbiol. Immunol., *68:* 1–28, 1974.