PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 173, 217–221 (1983)

Genetics of Xenotropic Virus Expression in Mice. II. Expression of Major Virus Structural Proteins in Crosses Involving NZB/BINJ, SWR/J, and 129/J Strains of Mice[1] (41634)

DONALD L. PUTMAN,* KUNJURAMAN T. NAYAR,* BARBARA O'NEILL,* E. PREMKUMAR-REDDY,† JAY A. LEVY,‡ AND RICHARD E. KOURI*

Division of Toxicology and Oncology, Microbiological Associates, and †Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, Maryland 20816; and ‡Cancer Research Institute, University of California School of Medicine, San Francisco, California 94143

Abstract. The expression of viral structural polypeptides and the production of infectious xenotropic virus were found to segregate together in NZB, 129/J, and SWR/J mice and in crosses between these strains. The viral p30 protein segregation pattern, as measured by competition radioimmunoassay using extracts of frozen spleens from backcross progeny, indicate that xenotropic murine leukemia virus expression is controlled by two dominant genes.

The mouse xenotropic type C virus ($MuLV_x$) was first isolated from New Zealand Black (NZB) mice (1) and characterized as different from other endogenous type C murine viruses by its unusual host range. While spontaneously expressed in mice and in cultured murine tissue, $MuLV_x$ is unable to productively infect mouse cells but is capable of replicating in cells from a number of heterologous species, including human, rat, rabbit, mink, and dog (2–4). Substantial quantities of virus have been demonstrated in embryonic tissue as well as in tissue from mice of all ages (5).

While most, if not all, mouse strains are believed to contain $MuLV_x$ proviral sequences (6), spontaneous production of infectious virus differs among strains. NZB mice produce large quantities of virus, C57BL/6 and BALB/c produce moderate amounts, and 129/J and SWR produce very little, if any, detectable infectious $MuLV_x$ (4). Because of the large variations in infectious virus released by different mouse strains, the regulation of spontaneous infectious virus production appears to be controlled by genetic determinants within the particular mouse strain.

Genetic studies involving crosses between NZB and SWR mice indicated that two independently segregating, autosomal dominant genes were involved in the genetic regulation of $MuLV_x$ expression (7, 8). In our laboratory, crosses between NZB and 129/J mice were studied using cocultivation techniques (9). By these procedures, extracts of frozen spleens were inoculated onto heterologous cell lines which were passed for 3 weeks and then assayed for xenotropic virus. The results suggested that spontaneous production and release of infectious $MuLV_x$ segregated as a single, autosomal dominant-like gene (9). Using radioimmunoassay techniques, the present report will correlate the spontaneous expression of $MuLV_x$ structural protein with the production of infectious virus. The data demonstrate that two independently segregating, dominant genes control xenotropic virus expression in crosses between NZB and 129/J mice as well as in crosses between the as NZB and SWR strains.

Materials and Methods. *Mice.* NZB/BINJ (hereafter called NZB), 129/J, and SWR/J mice were obtained from the Jackson Laboratory, Bar Harbor, Maine. The various F_1 and backcross generation mice were derived in our colony. By convention, the female parent is listed first in designating F_1 and backcross mice. Hemisplenectomies were performed on selected mice by standard procedures (9). Spleens were stored frozen at −70°C until used for the infectious virus and radioimmunoassay described below.

Purification of antigens. Viral proteins were isolated and purified by phosphocellulose column chromatography as described by others (10, 11). Briefly, approximately 5 mg of purified Rauscher murine leukemia virus (R-MuLV) or NZB $MuLV_x$ were disrupted by

[1] This study was supported in part by a contract from The Council for Tobacco Research-USA, Inc., awarded to Microbiological Associates, Bethesda, Maryland.

0037-9727/83 $1.50

sonication in 10% Triton X-100 and 0.05 *M* Tris–HCl, pH 9.0. Following incubation for 15 min at room temperature, samples were clarified by centrifugation at 100,000*g* for 1 hr. The supernatant was dialyzed overnight in the cold against 0.01 *M* *N*-bis(2-hydroxy ethyl)-2-aminoethane sulfonic acid, 1.0 m*M* EDTA, and 0.1% Triton, pH 6.5 (BET buffer), and then applied to a 1.6 × 5.0-cm Whatman P11 phosphocellulose column equilibrated at 4°C with BET buffer. The column was washed with 50 ml BET buffer and the bound proteins were eluted with 100 ml linear gradient of 0.0–1.0 *M* KCl in BET buffer. Two-milliliter fractions were collected and compared to marker proteins (BSA, 68,000 mol wt; ovalbumin, 43,000 mol wt; chymotrypsinogen, 24,000 mol wt; and RNase, 14,000 mol wt) by SDS–gel electrophoresis as described by Laemmli (12). The fractions containing each of the viral proteins (p30 and p12 from R-MuLV and gp69/71 from NZB $MuLV_x$) were pooled and aliquots were radioactively labeled with ^{125}I at specific activities of 5–20 $\mu Ci/\mu g$ by the Chloramine-T method of Greenwood *et al.* (13).

Radioimmunoassay for viral proteins. Competition immunoassays for MuLV structural polypeptides were performed as previously described (14) and included a group-specific immunoassay for p30 using goat antiserum against disrupted AKR-MuLV and ^{125}I-labeled R-MuLV p30, a group-specific assay for p12 using goat anti-NIH-Swiss $MuLV_x$ and ^{125}I-labeled R-MuLV p12 protein, and a type-specific immunoassay for the envelope glycoprotein, gp69/71, using goat anti-NZB $MuLV_x$ and ^{125}I-labeled NZB-$MuLV_x$ gp69/71. The antisera were provided by the Resources and Logistics Program of the Virus Cancer Program of the National Cancer Institute, Bethesda, Maryland. The 0.2-ml reaction mixture contained 0.01 *M* Tris–HCl (pH 6.8), 10 m*M* EDTA, 0.01 *M* NaCl, 1% BSA, and 0.4% Triton X-100. Homogenized spleens (unlabeled viral antigens) were adjusted to 250 μg protein/ml 0.1 *M* Tris with 30% Triton X and tested at serial twofold dilutions for their ability to compete with ^{125}I-labeled p30, p12, and gp69/71 for binding limited amounts of antiserum. Antiserum and competing antigens were incubated for 3 hr at 37°C; ^{125}I-labeled antigen (20,000 cpm) was then added, and the incubation continued for 1 hr at 37°C. Following the addition of 0.025 ml of 10% Staph A (Bethesda Research Laboratories, Inc., Bethesda, Md.), the samples were incubated 15 min at room temperature, centrifuged at 2500*g* for 15 min, and the radioactivity was determined in the precipitate. Quantitation of p30 was carried out by comparison of the unknown sample to a R-MuLV p30 standard giving 50% inhibition.

Infectious virus assays. Extracts of frozen spleens were inoculated onto mink lung cells (ATCC CCL64) which were passaged weekly for 3 weeks. Cultures were then examined by fluorescent-antibody techniques for MuLV p30 as previously described (9, 15). The inoculated mink lung cells were also cocultivated with non-virus-producing MSV-infected rat cells (NRK-Harvey cell line), and the culture medium was assayed in NRK cells for the infectious $MuLV_x$ pseudotype of MSV by standard techniques (9). The number of foci formed are proportional to the amount of NZB-$MuLV_x$ present in the monolayer cells (5). Supernatant fluids from mink lung cells at the third passage were also analyzed for infectious $MuLV_x$ by the induction of foci in mink S+L- cells (15).

Results. The presence of the major structural proteins, p30, p12, and gp69/71 in spleens from the parental strains, NZB and 129/J, and their F_1 hybrid was determined by competition radioimmunoassay and is presented in Fig. 1. The major protein, p30, was detected using anti-AKR antiserum and ^{125}I-R-MuLV p30; p12 with anti-NIH Swiss $MuLV_x$ and ^{125}I-NZB p12; and gp69/71 with anti-NZB $MuLV_x$ antiserum and ^{125}I-NZB gp69/71. In each case, the data reflect extracts from a pool of ten spleens. With spleens from NZB and (NZB/129)F_1 mice, the competition with p30 was complete and practically identical for the two strains (Fig. 1A). The amount of spleen protein required to inhibit immune binding by 50% was 16 μg for NZB and 31 μg for (NZB/129)F_1. Spleens from virus-negative 129/J mice demonstrated a very low level of competition with the p30 antigen; 50% inhibition of binding was found only at 250 μg protein.

Immune assays with the type specific structural protein p12 showed similar results (Fig. 1B). Demonstrable levels of p12 were found

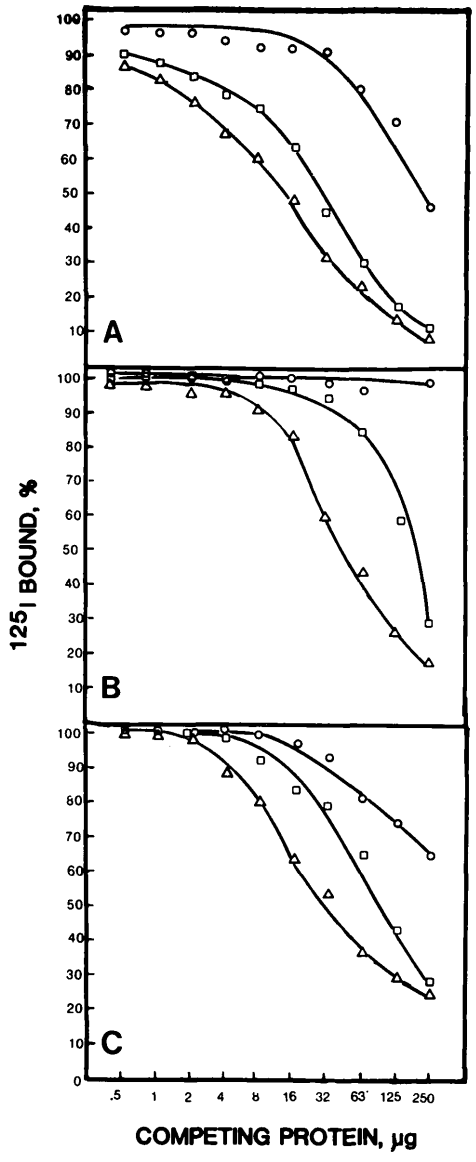

FIG. 1. Competition radioimmunoassay for the major structural proteins p30 (A), p12 (B), and gp69/71 (C) in NZB (△), 129/J (○), and their F_1 hybrids (□).

in spleens from NZB and (NZB/129)F_1, while no detectable level could be found in 129/J spleens (Fig. 1B). The amount of protein required to inhibit 50% of the immune binding was approximately 50 and 125 μg protein for NZB and (NZB/129)F_1 spleens, respectively. Up to 250 μg protein from 129/J spleens failed to inhibit binding of the radiolabeled antigen. Using the envelope glycoprotein, gp69/71 (Fig. 1C), spleens from NZB and (NZB/129)F_1 mice inhibited binding at a level similar to that found with p12. The amount of spleen protein required to inhibit immune binding by 50% was approximately 31 μg for NZB and 95 μg for (NZB/129)F_1 mice. Spleens from 129/J mice inhibited binding by 50% only at the maximum level tested (250 μg protein).

To establish a correlation between the expression of viral structural proteins and the release of infectious virus, the spleens from NZB, 129/J, SWR/J, and crosses between these strains were simultaneously examined for infectious virus and expression of p30 structural protein (Table I). Virus-negative 129/J and SWR/J mice expressed negligible levels of p30 antigen in splenic tissue, 25 and 20 ng/mg protein, respectively. NZB and (NZB/129)F_1 mice were virus-positive with p30 levels of 223 and 270 ng/mg spleen protein, respectively. Virus-positive (NZB/129) × 129 and (NZB/SWR) × SWR backcross mice (BC-1) also demonstrated high levels of p30 (143 and 145 ng/mg protein) whereas the virus-negative 129/J and SWR/J backcross animals demonstrated substantially lower levels, 68 and 84 ng/mg protein, respectively.

To determine if the expression of viral p30 is linked to infectious-virus expression, spleen extracts from individual backcross animals were assayed for infectious virus and quantitated for viral p30 (Table II). The lower 95% confidence limit for p30 in (NZB/129) × 129 virus-positive backcross animals was 95 ng/mg spleen protein (Table I); therefore, 100 ng

TABLE I. CORRELATION OF INFECTIOUS VIRUS EXPRESSION AND EXPRESSION OF VIRUS STRUCTURAL PROTEIN

Strain	MuLVx Status[a]	ng p30/mg spleen protein[b]
129/J	−	25 ± 13
SWR	−	20 ± 9
NZB	+	223 ± 90
(NZB/129)F_1	+	270 ± 130
(NZB/129)129 BC-1	+	143 ± 45
	−	68 ± 24
(NZB/SWR)SWR BC-1	+	145 ± 37
	−	84 ± 34

[a] Infectious virus detection using the cocultivation assay (9).

[b] Mean of three to six mice per group ± standard deviation.

TABLE II. SEGREGATION OF INFECTIOUS VIRUS AND VIRAL p30 IN BACKCROSS PROGENY

Strain	Cocultivation assay[a]	ng p30/mg spleen protein	MuLVx status[b]
(NZB/129)129 (BC-1)	−	40	−
	−	80	−
	−	83	−
	−	55	−
	−	167	+
	−	433	+
	−	290	+
	−	225	+
	−	102	+
	−	308	+
	+	105	+
	+	169	+
	+	118	+
	+	180	+
	+	293	+
	+	290	+
(NZB/SWR)SWR (BC-1)	−	37	−
	−	66	−
	−	69	−
	−	100	+
	−	133	+
	−	100	+
	+	136	+
	+	115	+
	+	107	+
	+	111	+
	+	219	+
	+	146	+
	+	184	+

[a] Infectious virus detection using the cocultivation assay (9).

[b] Virus status according to criteria set forth in text.

of p30/mg protein was set as the minimum limit for classification as positive for infectious virus. In the (NZB/129)F_1 × 129 backcrosses, 38% of the progeny were positive for infectious virus as detected by the cocultivation and mink S+L- cell assays. Virus-positive spleen extracts induced areas of fluorescence in mink lung cells and foci of cell alteration in the S+L- cells. Extracts from virus-negative mice showed no evidence of $MuLV_x$ by these same assays. Quantitation of the viral structural protein, p30, gave a markedly different impression. In the (NZB/129) × 129 BC-1 animals, 75% demonstrated high levels (≥100 ng/mg spleen protein) of p30, suggesting that 75% of the progeny were expressing infectious virus as opposed to the 38% indicated by the cocultivation and mink S+L- assays.

Similar results were found with the (NZB/SWR) × SWR BC-1 mice. A total of 54% of the progeny were positive for infectious $MuLV_x$ by the cocultivation assay, whereas 77% were found to contain high levels of p30 antigen. In all cases examined, every animal that was positive for infectious virus had a p30 level of ≥100 ng/mg spleen protein.

Discussion. Variations in the amount of infectious $MuLV_x$ released from mouse cells have been evident in studies of different mouse strains and of genetic crosses and backcrosses (9). Virus titers ranged from high levels in NZB mice to somewhat lower levels in crosses involving the SWR/J and the 129/J mice (7–9). We have observed that these strain differences in the level of expression of the infectious virus parallel strain differences in expression of the viral structural proteins, p30, p12, and gp69/71.

The correlation between infectious virus production and expression of the major structural protein, p30, in NZB, 129/J, SWR/J, and their F_1 and BC-1 progeny is strong enough to permit use of the competition assay as an alternative approach for virus detection. Not once were high levels (>100 ng/mg tissue protein) of p30 antigen detected in 129/J or SWR/J mice which do not produce infectious $MuLV_x$. Moreover, in backcross generations, presence of p30 antigen segregated as would be expected if virus expression was under the control of two dominant genes.

Data presented from our laboratory indicated that spontaneous production and release of infectious xenotropic virus in crosses between NZB and 129 mice segregated as a single autosomal dominant-like gene (9). The term dominant-like was used because, at that time, the factors affecting the infectious units of virus expressed were not completely understood, therefore we were unable to determine whether the gene was a dominant or codominant (i.e., additive) trait. Datta and Schwartz (7, 8) reported that two independently segregating, autosomal dominant genes (NZV-1, NZV-2) were involved in the genetic regulation of xenotropic virus expression in crosses between NZB and SWR mice. These results have been confirmed (16). The NZV-1 gene controls release of a high-titered virus

which can easily be detected by cocultivation techniques using frozen spleen cell extracts or 10^6 viable spleen cells (7–9). The NZV-2 gene controls production of a very low-titered virus which can only be detected by cocultivation techniques employing at least 10^7 fresh viable spleen cells (7, 8, 16). We have found that low-titered infectious virus associated with NZV-2 cannot be detected with frozen spleen extracts (9, 16); however, data presented in this paper suggest that the NZV-2-associated virus expression can be demonstrated in spleen extracts by radioimmunoassays for the viral structural protein, p30. In the ($F_1 \times 129$) and ($F_1 \times$ SWR) backcrosses, virus expression segregated at a 3:1 ratio of virus-positive to virus-negative progeny based on radioimmunoassay for p30. These virus segregation patterns in backcross progeny clearly indicate that $MuLV_x$ expression is controlled by two genes and confirm the conclusions of Datta and Schwartz (7, 8).

This report documents the correlation between infectious virus production and expression of the viral structural proteins and demonstrates that they cosegregate in crosses between NZB and 129 or SWR mice. This paper also reports that a radioimmunoassay for MuLV p30 can be used to detect NZV-2 gene products in frozen spleen extracts. This approach offers an effective alternative for analyzing viable spleen cells for virus.

We thank Ms. Mini Namboodiri and Ms. Sharon Selwyn for technical assistance and Ms. Jill Bradshaw for her expert assistance in manuscript preparation.

1. Levy JA, Pincus T. Demonstration of biological activity of a murine leukemia virus of New Zealand black mice. Science **170**:326, 1970.
2. Levy JA. Autoimmunity and neoplasia. The possible role of C-type viruses. Am J Clin Pathol **62**:258, 1974.
3. Levy JA. Host range of murine xenotropic virus: replication in avian cells. Nature (London) **252**:140, 1975.
4. Levy JA. Xenotropic C-type viruses and autoimmune disease. J Rheumatol **2**:135, 1975.
5. Levy JA, Kazan P, Varnier O, Kleinman A. Murine xenotropic type C viruses. I. Distribution and further characterization of the virus in NZB mice. J Virol **16**:844, 1975.
6. Chattopadhyay SK, Lowy DR, Teich NM, Levine AS, Rowe WP. Qualitative and quantitative studies of AKR-type murine leukemia virus sequences in mouse DNA. Cold Spring Habor Symp Quant Biol **39**:1085, 1975.
7. Datta SK, Schwartz RS. Genetics of expression of xenotropic virus and autoimmunity in NZB mice. Nature (London) **263**:412, 1976.
8. Datta SK, Schwartz RS. Medelian segregation of loci controlling xenotropic virus production in NZB crosses. Virology **83**:449, 1977.
9. Levy JA, Joyner J, Nayar KT, Kouri RE. Genetics of xenotropic virus expression in mice. I. Evidence for a single locus regulating spontaneous production of infectious virus in crosses involving NZB/BLNJ and 129/J strains of mice. J Virol **30**:754, 1979.
10. Hino S, Stephenson JR, Aaronson SA. Antigenic determinants of the 70,000 molecular weight glycoprotein of Woolly monkey type C RNA virus. J Immunol **115**:922, 1975.
11. Strand M, August T. Structural proteins of ribonucleic acid tumor viruses. Purification of envelope, core, and internal components. J Biol Chem **251**:559, 1976.
12. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680, 1970.
13. Greenwood FC, Hunter WM, Glover JS. The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. Biochem J **89**:114, 1963.
14. Premkumar-Reddy E, Devare SG, Vasudev R, Sarma PS. Simplified radioimmunoassay for viral antigens: Use of *Staphylococcus aureus* as an adsorbent for antigen–antibody complexes. J Nat Cancer Inst **58**:1859, 1977.
15. Avery RJ, Levy JA. Relationship of endogenous murine xenotropic Type C virus production to spontaneous transformation of cultured cells. J Gen Virol **39**:427, 1978.
16. Fishbach M, Volberding P, Talal N, Levy J. Genetic analysis of induction of anti-polyadenylic acid antibodies and xenotropic type-C viruses. Clin Exp Immunol **44**:615, 1981.

Received September 30, 1982. P.S.E.B.M. 1983, Vol. 173.