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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

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The extent of infectious xenotropic virus expression in homogenized splenic tissue from the high-virus-expressing NZB/BlNJ mice and the non-virus-expressing 129/J mice and their crosses has been examined. The data suggest that a single autosomal "dominant-like" gene controls the spontaneous production and release of infectious xenotropic virus in NZB mice. Analysis of infectious virus production in second-backcross families [(F_1 × 129) × 129] confirmed this conclusion. Variations in the amount of X-tropic virus released were evident in all genetic crosses. Virus titers (expressed as focus-forming units per milliliter) of supernatant fluid ranged from high levels in the NZB mice to somewhat lower levels in crosses involving the 129 mice. In the absence of a definite pattern in the titers observed in the genetic crosses studied, the term dominant-like is proposed for the single gene regulating the expression of X-tropic virus in NZB mice.

All mouse cells contain genome copies of several viruses (for review, see 9, 17). One of these endogenous viruses, the xenotropic (X-tropic) type C virus, has been characterized by its unusual host range. Although it is present in mouse cells, it is unable to productively infect other mouse cells but can be grown in a wide variety of cells heterologous to the host (14-16). The X-tropic virus was first isolated in New Zealand Black (NZB) mice (20), an inbred strain that develops an autoimmune syndrome similar to human lupus erythematosus (3, 11, 20, 23). These mice succumb to immune complex glomerulonephritis or immunoblastic lymphomas (reticulum cell sarcomas) (11, 23). Detection of the X-tropic class of endogenous mouse viruses in this strain was facilitated by the fact that every cell from embryo to adulthood spontaneously produces substantial quantities of the virus (19). Spontaneous virus production can vary up to 100-fold in tissues of male and female NZB mice. Moreover, the titer of NZB type C virus (NZB-murine leukemia virus), produced by cells derived from one-cell clones of NZB embryos cultivated in tissue cultures, ranged from 10^2 to 10^4 infectious particles per ml of supernatant fluid (19). These levels of endogenous virus production are constant for these cell lines after five passages (1). Such quantities of X-tropic virus released are less than those for endogenous ecotropic viruses, which reach titers as high as 10^6 to 10^7 infectious particles per ml of culture supernatants from cultured mouse cells (18). We have also observed that X-tropic viruses in an ecotropic coat can infect mouse cells, but the titer of progeny X-tropic virus formed is never more than 10^3 infectious particles per ml of culture medium (18).

Molecular studies have indicated that most, if not all, members of the *Mus musculus* species contain proviral copies (up to six to nine) of the X-tropic virus in the host chromosome (4; S. K. Chattopadhyay, personal communication). However, other mouse strains differ in their production of X-tropic virus. C57Bl and BALB/c mouse cells spontaneously release moderate amounts, whereas 129/J mice produce very little, if any, detectable infectious X-tropic virus (16). For these reasons, this regulation of spontaneous release of infectious virus appears to be determined by genetic factors within the particular mouse strain.

The purpose of these studies was to examine by genetic analysis the gene(s) regulating the spontaneous production and release of an infectious mouse X-tropic virus whose host range includes human, rat, mink, and dog cells. Results are consistent with the conclusion that a single autosomal dominant-like gene regulates infectious X-tropic virus production in crosses be-

tween the NZB/BlNJ and 129/J strains of mice.

MATERIALS AND METHODS

Mice. The NZB/BlNJ and 129/J mice were obtained from Jackson Laboratories, Bar Harbor, Maine. They were housed in the animal care facilities at Microbiological Associates, Bethesda, Md. They are identified in this report as NZB and 129. Genetic crosses between NZB and 129 were made to study the pattern of segregation of infectious X-tropic virus expression in these mice.

Spleen extracts. Using the technique previously described (12), the mice were hemisplenectomized at 2 months of age, and the fragment of spleen was frozen at −70°C. This segment of spleen was subsequently weighed and prepared as a 10% extract in Eagle minimal essential medium containing antibiotics (100 U of penicillin and 100 μg of streptomycin per ml). The fragment was mixed with the medium to yield a final concentration of approximately 10 mg/ml. Tissues were homogenized with a Potter-Elvehjem homogenizer, and the extracts were clarified by centrifugation at 3,000 × *g*. The supernatant was frozen at −70°C for use in virus assays.

Cell lines. The normal rat kidney (NRK) cells (8) were originally obtained from R. Ting, Bethesda, Md. The primary human foreskin (HuF) cells were provided by Mirian Debby and Connie Rees, Cancer Research Institute, San Francisco, Calif. They were used until transfer 10, when sensitivity to the X-tropic viruses diminished (15). The NRK-Harvey cell line is a non-virus-producing NRK cell line transformed by the Harvey strain of murine sarcoma virus (13). A clone of this cell line, B-4, was used because of its high sensitivity for X-tropic virus detection (19). Mink lung (American Type Culture Collection catalog no. CCL64) and mink sarcoma-positive, leukemia-negative (S^+L^-) cells were provided by P. Peebles, Bethesda, Md. D-17 dog osteosarcoma cells were received from P. Arnstein and J. Riggs, Berkeley, Calif.

Medium. Eagle minimal essential medium (GIBCO, Grand Island, N.Y.) supplemented with 10% unheated fetal bovine serum, antibiotics (250 U of penicillin and 250 μg of streptomycin per ml), and 1% glutamine (2 mM; Microbiological Associates) was used for maintenance of most cell lines. Dulbecco and RPMI 1640 media (GIBCO) containing 10% heated (56°C, 30 min) fetal bovine serum were used for maintenance of the mink lung and mink "nonproducer" cells containing the defective genome of the Moloney sarcoma virus S^+L^- cells, respectively. For focus formation assays on NRK and HuF cells, 5% heated calf serum was substituted for the fetal bovine serum in Eagle minimal essential medium.

Cocultivation assays. The spleen extracts were inoculated (0.4 ml) on HuF cells and were passaged once. After 1 week of cultivation, these cells were transferred and cocultivated with the B-4 clone of NRK-Harvey cells by techniques already described (19). To confirm the results obtained from the cocultivation experiments with HuF cells, spleen extracts were also inoculated onto D-17 or mink lung cells. In brief, approximately 3×10^5 of the indicator cells were mixed with 1×10^4 B-4 cells. These cells were maintained in culture for 7 days with fluid changes every 2 to 3 days. On day 7, when the B-4 overlay was confluent, the supernatant was removed, filtered, and frozen at −70°C until it was assayed for focus-forming activity in NRK and HuF cells. The titer of NZB-pseudotype sarcoma virus recovered was expressed as $\log_{10}$ focus-forming units (FFU) per milliliter of culture medium. The number of foci formed are proportional to the amount of NZB-murine leukemia virus present in the monolayer cells (19).

Focus formation assays were conducted as described (19), using NRK and HuF monolayer cells. All cell lines were pretreated with DEAE-dextran (25 μg/ml) for 30 min at 37°C to increase their sensitivity to virus infection (7). Spleens removed from the parental strains NZB and 129 were also placed directly in culture. The fibroblast lines derived from these viable tissues were cocultivated with cells of the B-4 clone.

Fluorescent-antibody assay. The spleen extracts were inoculated on mink lung and D-17 cells. These monolayers were passaged weekly for 3 weeks and then plated in petri dishes containing glass cover slips (12 by 16 mm). After 6 days, these cover slips were removed, washed, fixed, and stained by the procedures of Hartley and Rowe (10). Before staining, they were stored at −70°C. A direct test with fluorescence-labeled anti-murine leukemia virus p30 sera, provided by J. Gruber, National Cancer Institute, was used. The presence of virus was detected by areas of green fluorescence.

S^+L^- assays. Splenic extracts and fluids from human, mink, and D-17 cell cultures receiving these extracts were inoculated onto mink S^+L^- cells. These monolayers were maintained by following the procedure of Peebles (21). After 6 to 10 days, they were examined for the development of foci of cell alteration.

RESULTS

Virus detection. Table 1 demonstrates the spontaneous expression of infectious X-tropic virus in the parental NZB and 129 mice and in progeny mice from specific crosses between these two strains. As determined by the cocultivation assays using the HuF cells inoculated with the spleen extracts, infectious virus could

TABLE 1. *Expression of infectious X-tropic virus in genetic crosses of NZB/BlNJ and 129/J mice*[a]

Strain	No. positive/no. tested
NZB/BlNJ	42/42
129/J	0/51
(NZB × 129)F_1	32/32
(129 × NZB)F_1	4/4
(NZB/129)F_1 × NZB	4/4
NZB × (NZB/129)F_1	14/14
(NZB/129)F_1 × 129	26/50
129 × (NZB/129)F_1	4/5
((NZB/129)F_1 × (NZB/129)F_1)F_2	37/48

[a] In all crosses, the female is listed first. Infectious virus production was measured by cocultivation with HuF and D-17 dog cells, as described in text.

be detected in every one of the NZB mice, whereas the 129 strain was negative. Similar results were observed when the spleen extracts were cocultivated using mink lung or dog cells.

The (NZB × 129)F_1 and (129 × NZB)F_1 hybrids all showed spontaneous X-tropic virus production by the cocultivation procedure (see Table 1). Only four of the (129 × NZB)F_1 mice were available because the fertility rate with these parental mice was low (see reference 2 for discussion). In the (NZB/129)F_1 × NZB backcross and its reciprocal, all the mice were positive.

In the (NZB/129)F_1 × 129 backcrosses, 52% (26 of 50) of the progeny were positive for X-tropic virus. In the reciprocal backcross, 129 × (NZB/129)F_1, 80% (4 of 5) of the mice were positive for X-tropic virus. The F_2 generation (F_1 × F_1) yielded a population in which 77% were X-tropic virus positive. Other virus assays in mink lung, dog, and mink S^+L^- cells confirmed these cocultivation results. Virus-positive spleen extracts induced areas of fluorescence in mink and dog cells and foci of cell alteration in the S^+L^- cells. Extracts from virus-negative mice as determined by cocultivation assays were negative by these other tests.

Virus expression was not correlated to sex of the animal or the following coat color genes: A^w, c^{ch}, *c*, *p*, *a*. For every cross examined, regardless of the sex of the parents used to generate these crosses, the data are consistent with the conclusion that a single, autosomal gene is responsible for the spontaneous release of this infectious X-tropic virus by the NZB mouse strains.

To confirm that the virus expression is regulated as a single Mendelian trait, expression of infectious virus in the second-backcross generations were studied (Table 2). Three virus-positive (F_1 × 129) mice were crossed to 129, and a total of 36 animals were generated from these three families. In each family approximately 50% of the progeny were virus expressing. The three virus-negative (F_1 × 129) mice mated to a strain 129 mouse generated progeny which were all virus negative (0 of 34).

In none of the aforementioned crosses did the viral status of the mother influence the virus segregation pattern of the resulting progeny. This can be seen from the first and second backcrosses. That is, when the mother was virus positive, a total of 37 of 74 or 50% of the progeny expressed infectious virus (26 of 50 + 11 of 24; see Tables 1 and 2, respectively). When the mother was virus negative, a total of 10 of 17 or 59% of the progeny expressed infectious virus (4 of 5 + 6 of 12; see Tables 1 and 2, respectively).

To confirm that the virus status remained stable, spleens from animals previously tested for their viral status as weanlings were reexamined when these mice were 1 year old. Twelve virus-positive animals and 12 virus-negative animals were tested. In every case, the virus status remained unchanged (data not shown).

To determine whether the single gene controlling X-tropic virus expression functions in a dominant or a codominant fashion, attempts at quantitating the level of virus expression were made. However, the cocultivation assays used are not easy methods for the true quantitation of degree of viral expression (see Table 3).

Variations in the amount of X-tropic virus released were evident in all genetic crosses. Virus titers (in FFU per milliliter of supernatant fluid) ranged from high levels in the NZB mice to somewhat lower levels in crosses involving the 129 mice. Eighty-one percent of the NZB gave a titer of >500 FFU/ml and 19% gave a titer ranging between 200 and 500 FFU/ml. The F_1 hybrids derived from an NZB female parent produced a virus titer as high as the NZB,

TABLE 2. *X-tropic virus expression in second-backcross progeny of NZB and 129/J mice*

Family	Parental strain[a]	No. positive/no. tested
1	129 × (F_1 × 129)$^+$	6/12
2	(F_1 × 129)$^+$ × 129	7/16
3	(F_1 × 129)$^+$ × 129	4/8
4	129 × (F_1 × 129)$^-$	0/22
5	129 × (F_1 × 129)$^-$	0/7
6	(F_1 × 129)$^-$ × 129	0/5

[a] Mouse whose spleen showed presence of infectious virus; −, mouse whose spleen showed no infectious virus.

TABLE 3. *Extent of infectious virus production by the NZB/BlNJ mice and their genetic crosses with 129/J mice*

Strain	No. (%) of mice whose spleens gave virus titer (FFU/ml) of:		
	<200	200–500	>500
NZB	— (0)	8 (19)	34 (81)
(NZB × 129)F_1	1 (7)	2 (14)	11 (79)
(129 × NZB)F_1	4 (100)	— (0)	— (0)
(NZB/129)F_1 × NZB	1 (25)	1 (25)	2 (50)
NZB × (NZB/129)F_1	1 (9)	8 (73)	2 (18)
(NZB/129)F_1 × 129	9 (50)	5 (28)	4 (22)
129 × (NZB/129)F_1	4 (100)	— (0)	— (0)
[(NZB/129)F_1 × (NZB/129)F_1]F_2	7 (19)	12 (32)	18 (49)

[a] The production of infectious X-tropic virus is estimated by the extent of the pseudotype murine sarcoma virus formation by the cocultivation assay. The titer of virus is given as focus-forming particles of culture supernatant as determined in NRK cells (20).

whereas the F_1 hybrid derived from a 129 female parent seemed to express a much lower virus titer (<200 FFU/ml). The amount of infectious X-tropic virus produced by backcross progeny derived from (NZB/129)F_1 × NZB was consistently higher than the virus titer among (NZB/129)F_1 × 129. The F_2 progeny showed virus titers somewhat similar to those for F_1 progeny. No definite pattern of virus titer emerges from these crosses, thus obfuscating any conclusion as to the dominant or codominant nature of the gene. For this reason, the term dominant-like is proposed to explain the nature of inheritance of this single autosomal gene.

DISCUSSION

Data presented in this paper indicate that spontaneous production and release of infectious X-tropic virus in crosses between the NZB and 129 strains segregate as a single, autosomal dominant-like gene. The term dominant-like is preferred because those factors which affect the titer of virus expression in these genetic crosses are not fully understood at the present time, and whether this gene is a fully dominant or a codominant (i.e., additive) trait cannot be established unequivocally. This problem is illustrated by the data presented in Table 3, where no clear-cut regulation of virus titer is observed.

However, the fact that virus expression is controlled by a single autosomal gene is clearly established by the observations of virus segregation patterns in backcross and F_2 progeny. In (F_1 × NZB) backcrosses, all the progeny were virus positive, whereas in the (F_1 × 129) backcrosses virus expression segregated on a 1:1 ratio of virus-positive–to–virus-negative progeny. In the F_2 population, virus-positive–to–virus-negative progeny were observed on a 3:1 ratio. Confirmation of the single-gene nature of the virus expression and the observation that this single gene did in fact "breed true" is seen in the results from the second-backcross families (see Table 2). Virus-positive animals mated to strain 129 mice always yielded progeny of which half were virus positive, and virus-negative animals mated to strain 129 mice yielded progeny of which all were negative for virus expression.

These results are consistent with those obtained by Stephenson and Aaronson (22) in their study using cell cultures from embryos derived from NZB and NIH Swiss strains of mice and their genetic crosses. Thus, the difference in virus expression that exists between NZB and NIH Swiss and between NZB and 129 both result from the action of a single autosomal dominant-like gene. Whether this gene is exactly the same in both crosses remains to be determined.

In their studies involving crosses between NZB and SWR mice, Datta and Schwartz (5, 6) reported that two independently segregating, autosomal dominant genes were involved in the genetic regulation of X-tropic virus expression. The method used by these authors (5, 6) for detection of X-tropic virus expression consisted of a cocultivation assay using spleen cell suspensions on clonal cells (clone 81 cell line) derived from a murine sarcoma virus-transformed feline cell line.

Since our data showed the presence of only one gene, we verified these results by using the same techniques described by Datta and Schwartz (5, 6). A fragment from the same spleen that had previously been tested on HuF cells was now tested on the cat cell line. The data obtained were consistent with our original observations.

To ascertain whether the parental strain SWR contributed to the difference in the number of genes, genetic crosses involving NZB and SWR were additionally tested (manuscript in preparation). The spleens were assayed with both the HuF and cat cell lines. The results from both assays conformed to the one-gene hypothesis described in this paper. Since neither the assay system nor the strain is shown to be the contributing factor, we are unable to explain the discrepancy in the number of genes regulating X-tropic virus expression, unless the N*zv*-1 and N*zv*-2 genes described by Datta and Schwartz (6) are totally different from the single, autosomal dominant-like gene responsible for the spontaneous production of infectious virus in our crosses. However, since this gene could very well be identical to either the N*zv*-1 or the N*zv*-2 gene, we have not proposed a name for the locus described in this paper.

Since all mouse strains seem to contain the same number (six to nine) of proviral copies of X-tropic viruses (4; Chattopadhyay, personal communication), the data from these genetic studies suggest that this gene may be regulatory rather than structural. Derivation of congenic lines, which are now underway in our laboratory, may prove useful in delineating the nature of these gene functions and provide a system relatively free of modifying influence so that the role of X-tropic viruses in such diseases as autoimmunity and cancer can be addressed.

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