A MAJOR GENETIC LOCUS AFFECTING RESISTANCE TO INFECTION WITH MURINE LEUKEMIA VIRUSES

I. TISSUE CULTURE STUDIES OF NATURALLY OCCURRING VIRUSES

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Genetic control of susceptibility to leukemia viruses has been demonstrated in studies of Gross and Friend murine leukemia viruses (1-7) and the avian leukemia-sarcoma complex (8-10). The recent finding that naturally occurring murine leukemia viruses (MLV) fall into two categories with respect to their growth on mouse embryo (ME) cells of NIH (N) Swiss or BALB/c mice (11) raised the possibility that the susceptibility of these tissue culture cells might be genetically determined. “N-tropic” viruses initiate infection 30-1000 times more efficiently on NIH Swiss than on BALB/c (B) ME cells, while “B-tropic” viruses show the reciprocal pattern. Certain laboratory passaged MLV strains infect both cell types equally well and are designated “NB-tropic” (11).

Further study of host-range susceptibility patterns appeared to be of importance in understanding the natural history of MLV and in establishing optimal conditions for detection of virus from various sources. Conflicting observations regarding leukemogenesis using different naturally occurring MLV, such as those isolated by Gross (1, 12) and Tennant (13, 14), might be understood on the basis of host-range resistance. Experiments were undertaken to characterize the susceptibility of ME cells derived from various inbred strains. All strains tested were found to be similar to either NIH Swiss or BALB/c in showing significantly greater sensitivity to either N- or B-tropic viruses. Studies of cell cultures from F1 and backcross hybrids indicated that a single genetic locus plays a major role in determining in vitro susceptibility to naturally occurring murine leukemia viruses.

Materials and Methods

Mice.—All mice were obtained from the colonies of the National Institutes of Health (N) or The Jackson Laboratory (J), Bar Harbor, Maine, except for ME monolayers of the I and Ha/ICR strains, kindly provided by Dr. Aaron Freeman. The F1 and backcross hybrids

1 Abbreviations used in this paper: B, BALB/c; ME, mouse embryo; MLV, murine leukemia virus; N, NIH; Rad MLV, radiation-induced murine leukemia virus.
were bred in our laboratory. Mouse strains are designated in accordance with standard nomenclature (15). The following abbreviations are used in reference to the F1 hybrids with the female parent listed first (15): BALB/cN, C; DBA/2N, D; C57L/J, L; e.g., (BALB/c X DBA)F1 is expressed as CDF1.

Tissues Cultures.—ME cultures were prepared from embryos in the 14th-16th day of gestation as previously described (16). Embryos were carefully isolated from uterine and placental tissue by cautery and dipped in ether before trypsinization to assure removal of virus from extraembryonic sources. Previous testing had indicated spontaneous MLV growth in secondary cultures of AKR/N and C58/J mice (17), strains known to have a high leukemia incidence (18). Leukemia virus plaques were found in uninoculated secondary ME cultures of these strains, as well as strains RF/J and B10.BR/J. Accordingly, primary ME cultures were utilized for virus titrations on cells derived from strains AKR/N, C58/J, RF/J, and B10.BR/J. Secondary cultures were utilized in virus titrations on ME cells of all other strains, as well as F1 and backcross hybrids.

Preparations of F1 hybrid cells were made in the same manner as the inbred strains; all embryos from a single mother were pooled and regarded as genetically identical. In experiments involving backcross hybrids, each embryo was removed and cultured individually. Cells of certain strains and F1 hybrids were frozen in liquid nitrogen as trypsinized embryo material for use in later virus titrations.

Viruses.—Various naturally occurring MLV strains have been isolated in this laboratory as previously described (17); all have been found to be either N- or B-tropic. These strains and the standard laboratory strains of MLV were grown in appropriate ME cell cultures. Radiation-induced murine leukemia virus (Rad MLV) (Kaplan, 19), kindly provided by Dr. C. Whitmire, and Tennant MLV (13), obtained from the American Type Culture Collection, Rockville, Md. were in the form of tissue extracts from C57BL/6 and BALB/c mice, respectively.

Virus Titrations.—The susceptibility of ME cultures of different strains was determined by measuring the efficiency of plaque induction by various MLV isolates. Cells from each strain were tested with AKR-L1 MLV (N-tropic), BALB/c-S2B MLV (B-tropic), and Moloney MLV (NB-tropic). Additional viruses of the three types were also tested on cultures from a number of the mouse strains. In every test, the viruses were titrated on NIH and BALB/c ME cells to serve as reference titrations.

The plaque assay system (20) is based on the finding that cocultivation of MLV-infected cells with XC cells, a cell line of a Rous sarcoma virus-induced rat tumor (21), results in the formation of syncytia (22). Cell cultures were planted at a concentration of 3.5 X 10⁵/50 mm Petri dish (Falcon Plastics, Los Angeles, Calif.). All cultures were grown and maintained on Eagle's minimal essential medium with 10% unheated fetal calf serum, 2 mEq glutamine, 250 units penicillin/ml, and 250 μg streptomycin/ml. On the day after planting, monolayers were treated with DEAE-dextran (25 μg/ml in medium, 37°C, 1 hr), washed, and inoculated with 0.1 ml of virus. Culture fluids were changed every 2 days, and the cultures were exposed to ultraviolet light (25 sec at 60 ergs/mm² per sec) 6 days after inoculation; this exposure results in selective killing of the ME cells but not the virus (20). Immediately after ultraviolet irradiation, 10⁶ XC cells were added to the dish. The dishes were fixed in methanol 4 days later (after one or two further culture fluid changes) and stained with hematoxylin. Plaques were recognized by the absence of growth of the XC cells in areas of syncytium formation, and we distinguished from nonspecific imperfections in the cell sheet by the presence of two or more syncytia.

It was recognized that variability of cell count and use of previously frozen cells could potentially affect virus titers and invalidate comparative data on ME cells of various strains. Preliminary experiments indicated that titrations on cultures planted with cell numbers within the range of 2.6-5.3 X 10⁵ cells/dish (75-150% of the standard number, and clearly recogniz-
able as different in degree of confluency at time of inoculation), and use of identical lots of fresh and frozen cells did not affect the plaquing efficiency of all MLV types tested. Furthermore, the presence of polymyxin (100 μg/ml) and/or mycostatin (50 μg/ml), which were added at these concentrations in isolated instances when bacteria or yeast were observed, did not affect plaquing efficiency.

RESULTS

Host-Range Patterns of Virus Growth on ME Cells of Various Inbred Strains.—
Initial experiments on ME cells of 16 mouse strains indicated that all resembled

| Cells          | Virus (Log_{10}) |
|----------------|------------------|
|                | AKR-L1 (N-tropic) | BALB/c-S2B (B-tropic) | Moloney (NB-tropic) |
| NIH/N          | 5.2              | 1.5                  | 6.3                |
| AKR/N*         | 5.9              | 2.6                  | 6.6                |
| C3H/HeN        | 5.6              | 1.2                  | 6.3                |
| C58/J*         | 5.3              | 1.1                  | 6.3                |
| C57BR/cdJ      | 5.2              | 1.4                  | 6.3                |
| C57L/J         | 5.2              | 2.0                  | 6.2                |
| DBA/2N         | 5.0              | 2.9                  | 6.3                |
| 129/RtJ        | 4.0              | 1.2                  | 6.2                |
| NZB/N          | 3.3              | 1.4                  | 5.7                |
| BALB/cN        | 2.8              | 4.8                  | 6.2                |
| AL/N           | 2.9              | 4.7                  | 6.1                |
| A/J            | 2.8              | 4.6                  | 6.1                |
| A/HeJ          | 2.6              | 4.5                  | 5.7                |
| B10.QR/J*      | 2.5              | 4.5                  | NT                 |
| C57BL/6N       | 2.0              | 4.3                  | 5.8                |
| I              | 2.0              | 4.3                  | NT                 |

NT = not tested.
* Primary ME cells used as secondary ME cells showed spontaneous plaques.

either NIH Swiss or BALB/c in showing at least 60-fold greater sensitivity to either a representative N-tropic or B-tropic virus (Table I). With the majority of mouse strains, the plaquing efficiency of the reference N-tropic isolate (AKR-L1) or B-tropic isolate (BALB/c-S2B) was quite similar to NIH or BALB/c. Strains 129/J and NZB/N were exceptional in showing significantly lower sensitivity to the N-tropic virus, but nonetheless the N-tropic virus showed an 80-fold greater plaquing efficiency than the B-tropic virus. Titers of the NB-tropic Moloney virus were within a 10-fold range on all strains tested.

Further studies with other N- and B-tropic virus isolates (Table II) indicated
that the susceptibility patterns observed with the initial viruses tested were not unique to those viruses, but more likely a general property of N- and B-tropic viruses. The Kaplan Rad MLV showed B-tropism, with the lower titer characteristic of many tissue extracts before tissue culture passage.

Results of titrations of 14 virus isolates on ME cells of 23 mouse strains, expressed as per cent of the plaquing efficiency found on NIH Swiss or BALB/c cells as simultaneous reference standards, are shown in Table II; ME cells of all strains were tested with the AKR-1 and BALB/c-S2B isolates, in addition to other N- and B-tropic viruses. The median titer of all the tests is given as the percentage of the reference titer; titers showed less than 3-fold differences from the median in all but two of the titrations. Reductions of less than 0.5 log₁₀ (70%) in any single test or series of tests are generally not considered significant due to variables in test conditions; reductions greater than 1.3 log₁₀ (95%) are highly significant.

All strains showed at least a 50-fold difference in sensitivity to N-tropic or B-tropic viruses; in most strains the differences were greater. Again, plaquing efficiency of NB-tropic viruses was within a 10-fold range on all strains tested. The inbred strains similar to NIH Swiss are classified as "N-type," and those similar to BALB/c "B-type." The NZW, 129, RF/J, and particularly the NZB strain were clearly N-type, but were significantly less sensitive to the N-tropic viruses than other N-type mice; however, with the possible exception of NZB, they were fully susceptible to NB-tropic Moloney virus. DBA/2 cells were also somewhat less sensitive than NIH Swiss to N-tropic viruses; while the difference was only 2-fold, it was seen in each of seven tests.

The classification of mouse strains as N- or B-type did not relate to their H-2 type, as the H-2^a, H-2^b, and H-2^k alleles are represented in both categories. (H-2 antigens are expressed on ME cell cultures). All H-2^a strains were N-type, with

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TABLE II
Titer (Log₁₀) of Various N-Tropic and B-Tropic Viruses on Embryo Cells of Different Inbred Mouse Strains

| Cells          | BALB/c-S2N (N-tropic) | BALB/c-S3N (N-tropic) | C57BL/MCT1 (B-tropic) | BALB/c-S2B (B-tropic) | Rad MLV (B-tropic) |
|---------------|-----------------------|-----------------------|-----------------------|-----------------------|-------------------|
| NIH/N         | 5.1                   | 3.8                   | NT                    | <0.5                  | <0.2              |
| C57L/J        | 5.9                   | 4.2                   | 1.7                   | 2.5                   | <0.2              |
| BALB/cN       | 3.2                   | 1.7                   | 4.2                   | 5.5                   | 1.5               |
| A/J           | 3.1                   | 1.1                   | 4.3                   | 5.2                   | 0.6               |
| C57BL/6N      | 2.5                   | 0.2                   | 3.5                   | 4.9                   | 1.3               |

NT = not tested.

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2 Strober, S., and T. Pincus. Unpublished data.
the notable exception of the congenic B10.BR strain, in which the $H-2^k$ allele was introduced into the C57BL/10ScSn strain (a B-type mouse) from the C57BR strain (an N-type mouse) (23). The finding that the B10.BR ME cultures were

| Cells          | H-2 type | No. of tests | N-tropic* | B-tropic† | NB-tropic* |
|---------------|----------|--------------|-----------|-----------|------------|
| N-type        |          |              |           |           |            |
| NIH/N         | —        | 10           | (100)$§$ | <0.1      | (100)$§$   |
| AKR/N         | k        | 2            | 200       | <0.1      | 250        |
| C58/J         | k        | 1            | 120       | <0.1      | 120        |
| C57BR/nedJ    | k        | 2            | 100       | <0.1      | 100        |
| C57L/J        | b        | 5            | 100       | 0.2       | 100        |
| Ha/ICR        | —        | 1            | 80        | 0.5       | 140        |
| ST/jbJ        | k        | 2            | 60        | 0.2       | 80         |
| CBA/J         | k        | 2            | 50        | <0.1      | 100        |
| CE/J          | k        | 2            | 50        | <0.1      | 100        |
| C3H/HeN       | k        | 3            | 50        | <0.1      | 60         |
| DBA/2N        | d        | 7            | 40        | 0.3       | 100        |
| NZW/N         | ?        | 2            | 15        | 0.3       | 100        |
| 129/J         | b        | 4            | 15        | <0.1      | 120        |
| RF/Jj         | k        | 2            | 10        | <0.1      | 100        |
| NZB/N         | d        | 7            | 2         | <0.01     | 30         |
| B-type        |          |              |           |           |            |
| BALB/cN       | d        | 10           | 0.8       | (100)$§$ | 80         |
| A/HeJ         | a        | 1            | 0.3       | 80        | 40         |
| AL/J          | a        | 2            | 0.3       | 60        | 40         |
| B10.BR/J     | k        | 2            | 0.3       | 60        | 50         |
| I             | l        | 1            | 0.1       | 50        | NT         |
| C57BL/10J     | b        | 3            | 0.2       | 30        | 40         |
| A/J           | a        | 6            | 0.4       | 25        | 80         |
| C57BL/6N      | b        | 7            | 0.3       | 25        | 50         |

* Plaquing efficiency relative to NIH ME (per cent).
† Plaquing efficiency relative to BALB/c ME (per cent).
$§$ (By definition).
‖ Primary ME cells used as secondary ME cells showed spontaneous plaques.
NT = not tested.

B-type indicates that no gene closely associated with the $H-2$ locus in linkage group IX plays a significant role in determining susceptibility to MLV infection in vitro.

Host-Range Patterns of the Gross and Tennant Murine Leukemia Viruses in Tissue Culture.—Studies of leukemogenesis in vivo indicate that the Gross
Passage A and Tennant MLV have distinctly different host ranges (12, 14). Gross MLV is known to be N-tropic in tissue culture (11), while the pattern of leukemogenesis described for Tennant MLV (14) is suggestive of B-tropism. The plaquing efficiency of these viruses in tissue culture was tested on ME cells of the hosts described in the literature (Table IV), and indicates that the Tennant MLV is B-tropic. There is a broad correlation between the plaquing efficiency of these viruses in tissue culture and their leukemogenic activity.

**Virus Growth on F1 Hybrids of N-type and B-type Mice.**—The findings presented to this point suggested the possibility that two genes, possibly linked or allelic at a single genetic locus, might be responsible for the two overall patterns of virus-cell interaction. Further studies were conducted on F1 hybrid ME cells of N- and B-type mice. These cells were inoculated with N-, B-, and NB-tropic MLV isolates. Table V illustrates results of titrations using the same reference virus pools tested on inbred strains in Table I, and Table VI presents a summary of testing of various MLV types on hybrid embryo cells. In the (N × B) and (B × N)F1 hybrids, sensitivity to N-tropic and B-tropic viruses was reduced approximately 100-fold from that of the sensitive parent, to levels slightly above the titers found in the resistant inbred parent. Thus, resistance appeared to be dominant in regard to both types of viruses. F1 hybrids of two N- or two B-type strains were comparable to the parents; titers were the same as the parents in

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**TABLE IV**

*Plaquing Efficiency of Gross and Tennant Viruses in Mouse Embryo Tissue Culture*

| N-B type | Strain | H-2 type | Plaquing efficiency* | Leukemogenesis† |
|----------|--------|----------|----------------------|-----------------|
|          |        |          | Gross virus§          | Tenant virus¶   |
|          |        |          | Per cent              | Gross virus§    |
|          |        |          |                       | Tenant virus¶   |
|          |        |          |                       | Per cent        |
| N        | C57L   | b        | 80                    | <0.1            |
|          | DBA/2  | d        | 60                    | <0.1            |
|          | C3H    | k        | 100                   | 0.1             |
|          | C57BR  | k        | 100                   | <0.1            |
|          | AKR    | k        | 100                   | <0.1            |
| B        | A      | a        | <0.1                  | 10              |
|          | C57BL/6| b        | <0.1                  | 15              |
|          | BALB/c | d        | 0.6                   | 100             |
| I        |        | l        | NT                    | NT              |

* Experimental results.
† Data from literature.
§ Per cent relative to NIH-ME.
¶ Per cent relative to BALB/c ME.
¶ Per cent relative to NIH-ME.
** See reference 12.
NT = not tested.

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TABLE V

| Cells | Titer (Log_{10}) of Three Murine Leukemia Viruses on Embryo Cells of Various F1 Hybrids of (N-Type X B-Type) Mouse Strains |
|-------|---------------------------------------------------------------------------------------------------------------|
|       | AKR-L1 (N-tropic) | BALB/c-SIB (B-tropic) | Moloney (NB-tropic) |
| N-B type | Mating | | | |
| N | NIH/N | 5.0 | 1.2 | 6.1 |
| B | BALB/c | 2.6 | 4.8 | 6.0 |
| (N X B)F1 | (C57BR/cdJ X C57BL/6N)F1 | 2.7 | 1.8 | 6.0 |
| | (C57BR/cdJ X BALB/cN)F1 | 2.9 | 2.9 | 6.0 |
| | (DBA/2N X C57BL/6N)F1 | 2.9 | 2.5 | 5.8 |
| (B X N)F1 | (BALB/cN X AKR/N)F1 | 3.1 | 3.2 | 5.9 |
| | (BALB/cN X DBA/2N)F1 | 3.1 | 3.2 | 6.0 |
| | (C57BL/6N X AKR/N)F1 | 3.1 | 2.6 | 6.0 |

TABLE VI

| Cells | Summary of Plaquing Efficiency of N-, B-, and NB-Tropic Viruses on Embryo Cells of F1 Hybrids of (N-Type X B-Type) Mouse Strains |
|-------|---------------------------------------------------------------------------------------------------------------|
|       | N-tropic* | B-tropic* | NB-tropic* |
|       | Parents§ | F1 | Parents§ | F1 | Parents§ | F1 |
| N-B type | Mating | | | | | |
| N | NIH/N | 100 | 0.1 | 100 | 100 | 100 | 100 |
| B | BALB/cN | 0.8 | 100 | 100 | 100 | 100 | 100 |
| (N X N)F1 | (C57BR/cdJ X C57L/J)F1 | 100 | 100 | 100 | <0.1 | 0.1 | 100 | 100 | 100 |
| | (C57BR/cdJ X NZB/N)F1 | 100 | 2 | 10 | <0.1 | <0.01 | 0.1 | 100 | 32 | 100 |
| (B X B)F1 | (A/J X BALB/cN)F1 | 0.4 | 0.8 | 0.6 | 25 | 100 | 50 | 80 | 100 | 50 |
| | (BALB/cN X A/J)F1 | 0.8 | 0.4 | 0.6 | 100 | 25 | 50 | 100 | 80 | 40 |
| (N X B)F1 | (C57BR/cdJ X BALB/cN)F1 | 100 | 0.8 | 0.5 | <0.1 | 0.1 | 100 | 100 | 100 |
| | (C57L/J X A/J)F1 | 100 | 0.4 | 0.2 | 0.2 | 25 | 100 | 50 | 80 | 100 |
| | (C57L/J X BALB/cN)F1 | 100 | 0.8 | 0.2 | 0.2 | 100 | 100 | 100 | 100 |
| | (DBA/2N X C57BL/6N)F1 | 40 | 0.3 | 0.3 | 0.3 | 25 | 0.5 | 100 | 50 | 60 |
| (B X N)F1 | (BALB/cN X AKR/N)F1 | 0.8 | 200 | 1.0 | 100 | <0.1 | 1.0 | 100 | 250 | 60 |
| | (BALB/cN X C57BR/cdJ)F1 | 0.8 | 100 | 0.2 | 100 | <0.1 | 0.4 | 100 | 100 | 100 |
| | (BALB/cN X DBA/2N)F1 | 0.8 | 40 | 0.4 | 100 | 0.3 | 1.0 | 100 | 100 | 100 |
| | (C57BL/6N X AKR/N)F1 | 0.3 | 200 | 1.0 | 25 | <0.1 | 0.5 | 50 | 250 | 80 |

* Plaquing efficiency relative to NIH ME. (per cent).
§ Plaquing efficiency relative to BALB/c ME. (per cent).
¶ Data derived from Table III.

As with the inbred strains, titers of NB-tropic viruses were quite similar on F1 hybrids, indicating that the factors controlling sensitivity and resistance to the
N- and B-tropic viruses do not affect growth of NB-tropic viruses. Studies of four additional NB-tropic MLV isolates on (N × B)F₁ hybrid ME cells (Table VII) further reflect this finding.

Virus Titrations on Individual Backcross Embryos.—The possibility of two genes, both dominant for resistance to N- and B-tropic viruses, had been established by the above studies of inbred strains and F₁ hybrids. If a single genetic locus (or linkage group) is involved in determination of sensitivity to N-tropic and B-tropic viruses, mendelian type of segregation should be seen in backcross hybrids, with a 1:1 ratio of homozygotes to heterozygotes.

(B × N)F₁ hybrid mice were backcrossed to parental N- and B-type strains, as well as to nonparental N-type mice; cultures from individual backcross embryos were tested for plaquing efficiency of N- and B-tropic viruses.

| Table VII |
|-----------|
| *Titer (Log₁₀) of Various NB-Tropic Viruses on Embryo Cells of F₁ Hybrids* |

| Cells | Viruses |
|-------|---------|
| N-B Type | Mating | Friend-BALB/c* | BALB/c-Tj | Rauscher | WM1-B |
| N | NIH/N | 5.1 | 4.9 | 6.2 | 6.6 |
| B | BALB/cN | 4.8 | 5.4 | 6.2 | 6.6 |
| (N × B)F₁ | (C57BR/cdJ × BALB/cN)F₁ | 5.2 | 5.5 | 6.2 | 6.7 |
| (B × N)F₁ | (BALB/cN × AKR/N)F₁ | 4.8 | 5.3 | 5.7 | 6.4 |
| (B × N)F₁ | (C57BL/6N × AKR/N)F₁ | 4.8 | 5.2 | 5.9 | 6.6 |

* This Friend MLV variant has been passaged in our laboratory and is distinct from the Friend MLV variants described in the accompanying report (24).

As expected, ME cells from (B × N)F₁ × N backcross mice showed no segregation for sensitivity to B-tropic viruses. Similarly, there was no segregation for N-type sensitivity in (B × N)F₁ × B backcross mice.

Representative titrations of embryos taken from two mothers are shown in Table VIII. All backcross embryo ME cells fell into two categories in regard to: (a) efficiency of plaquing relative to that seen on ME cells of reference N- and B-type strains; (b) comparative plaquing efficiencies of the N- and B-tropic viruses. Certain ME cell cultures showed plaquing efficiencies within a 10-fold range of the N- or B-type reference titration; the plaquing efficiency of the second virus was invariably at least 20-fold lower (usually more). These backcross embryos (Nos. 1, 6, 9, 10, 13, 14, and 17) resembled the inbred strain and are regarded as homozygous, designated “NN” or “BB.” Other backcross hybrid ME cells showed plaquing efficiencies at least 30-fold lower than those seen in the reference titrations, with efficiencies of both the N- and B-tropic viruses within a 10-fold range. These backcross embryos (Nos. 2–5, 7, 8, 11, 12, 15, and 16) resembled the pattern seen in the F₁ hybrid and are regarded as heterozygous, designated “NB.”
All of 100 individual backcross ME cells tested were found to fit clearly into one of the two categories described above. As shown in Table IX, 52 were heterozygous and 48 were homozygous. These results are compatible with the expected 1:1 ratio if a single major locus is involved in determination of N- and B-type resistance.

The NB-tropic Moloney virus was tested for plaquing efficiency on ME cells of 40 of these backcross embryos. Plaquing efficiency on all these cells was within a 2-fold range of that found on ME cells of inbred strains and F1 hybrids. This finding indicates further that the gene system described for N- and B-tropic MLV is not relevant to the growth in vitro of NB-tropic MLV.

In 8 of the 12 backcrosses there was a tendency for ratios of greater than 2:1 among heterozygotes and homozygotes, despite the totals of all mice tested agreeing with the 1:1 ratio. A chi-square analysis revealed a P value between 0.05 and 0.10 for this suspected maternal influence on the type of progeny. No clear patterns were observed (e.g. hybrid vs. inbred mothers) and the signifi-

TABLE VIII
Titer (Log10) of N-Tropic and B-Tropic Viruses on Mouse Embryo Cells of Individual Backcross Hybrids

| Genotype (Homozygous or Heterozygous) | N-tropic | B-tropic | Embryo number |
|--------------------------------------|----------|----------|---------------|
| N                                   | 5.1      | 1.3      | NN            |
| B                                   | 2.8      | 4.8      | BB            |
| (B × N)F1                           | 3.3      | 3.1      | NB            |
| (B × N)F1 × N                       | 5.4      | 2.8      | NN            |
| CDF1                                | 3.3      | 3.8      | NB            |
| C57L                                | 3.4      | 3.2      | NB            |
| 1                                    | 3.4      | 3.4      | NB            |
| 2                                    | 3.5      | 3.3      | NB            |
| 3                                    | 5.2      | 2.7      | NN            |
| 4                                    | 3.5      | 3.2      | NB            |
| 5                                    | 3.6      | 3.6      | NB            |
| 6                                    | 4.8      | 2.8      | NN            |
| B × (B × N)F1                       | 10       | 2.7      | 4.0 BB        |
| BALB/c × CLF1                       | 11       | 2.9      | 2.0 BB        |
| 12                                   | 2.7      | 1.8      | NB            |
| 13                                   | 2.7      | 4.6      | BB            |
| 14                                   | 2.3      | 4.2      | BB            |
| 15                                   | 2.7      | 1.9      | NB            |
| 16                                   | 2.4      | 1.8      | NB            |
| 17                                   | 2.4      | 4.5      | BB            |
cance of this observation remains unclear. The segregation of the total embryos into two categories is quite clear, however, and provides considerable evidence for the influence of a single major gene in determination of the type of host-range susceptibility to different naturally occurring leukemia viruses.

**TABLE IX**

*Classification of Backcross Hybrid Progeny of 12 Mothers by Plaque Titrations on Cell Cultures from Individual Embryos*

| N-B type Mating | Number of embryos in litter | Genotype (Homozygous or heterozygous) |
|-----------------|-----------------------------|--------------------------------------|
|                 |                             | NN | NB |
| N × (B × N)F₁   | DBA/2N × CDF₁               | 9  | 3  | 6  |
|                 | C57L/J × CDF₁              | 9  | 7  | 2  |
|                 | C57L/J × CLF₁              | 10 | 8  | 2  |
|                 | C57BR/cdJ × CLF₁           | 6  | 3  | 3  |
| Total           |                             | 34 | 21 | 13 |
| (B × N)F₁ × N   | CDF₁ × DBA/2N              | 8  | 2  | 6  |
|                 | CDF₁ × C57L/J              | 9  | 3  | 6  |
|                 | CLF₁ × C57L/J              | 7  | 2  | 5  |
|                 | CLF₁ × C57BR/cdJ           | 11 | 7  | 4  |
| Total           |                             | 35 | 14 | 21 |
| B × (B × N)F₁   | BALB/cN × CDF₁             | 9  | 1  | 8  |
|                 | BALB/cN × CLF₁             | 8  | 4  | 4  |
| Total           |                             | 17 | 5  | 12 |
| (B × N)F₁ × B   | CDF₁ × BALB/cN             | 9  | 6  | 3  |
|                 | CLF₁ × BALB/cN             | 5  | 2  | 3  |
| Total           |                             | 14 | 8  | 6  |
| Total of all embryos |                     | 100 | 48 | 52 |

**DISCUSSION**

The studies presented indicate that a single genetic locus is the major determinant of in vitro plaquing efficiency of N- and B-tropic viruses on embryo cells of inbred strains. On the basis of over 100 pooled embryo cultures tested, the noninbred NIH strain appears to be homozygous for N-type sensitivity. It is striking that all 23 mouse strains tested showed one of two reciprocal patterns of sensitivity. This suggests that the genes determining N- and B-type sensitivity
are allelic or on the same linkage group; further data supporting this hypothesis are presented in the accompanying report (24).

It is apparent that overall patterns are defined by the N-B locus described, but that significant variations are present within these patterns. B-type strains were all somewhat less sensitive to B-tropic viruses than BALB/c. This could possibly be an artifact due to the passage of most of these viruses on BALB/c ME cells; however, Rad MLV, derived from a C57BL mouse with radiation-induced leukemia (19) and carried only in C57BL mice, was also found to propagate somewhat better on BALB/c than on C57BL/6 cells (Table II). The decreased sensitivity of cells from the B-type C57BL and A strains, as well as those from the N-type DBA/2, NZW, 129, RF, and NZB strains could result from additional genes, non-genetically determined variables, or quantitatively different alleles at the same genetic locus. The presumed N-B locus may be complex, involving partial or multiple alleles which cannot be clearly distinguished with presently available techniques. N- and B-tropic viruses are genetically stable upon passage in cells of a resistant type (11), and it appears that viral genes determine the tropisms observed at the mouse N-B locus. It is not known whether all MLV of the same host range are genotypically identical.

The striking resistance of cells from the NZB strain is especially intriguing in view of the autoimmune phenomena seen in these mice and their F1 hybrids with the NZW strain (25). Although MLV "C-type" particles (26) and antigens (27) are found in these mice, MLV biological activity cannot be detected in NZB tissues by standard methods used to detect other MLV types (28). MLV activity has been demonstrated in NZB tissue using a viral rescue technique, but activity is not seen on embryo cells of mouse strains (including NIH Swiss, BALB/c, and NZB) but rather on rat embryo cells (28). This finding apparently represents a host-range phenomenon not involving the N-B locus. Other examples of such phenomena, as well as other allelic variants at the N-B locus, may exist.

Studies of the F1 hybrids indicate that both genes (or alleles) show autosomal dominance for resistance, and the data regarding the backcross hybrids indicate a pattern of mendelian segregation. This genetic locus has no effect in the determination of plaquing efficiency of certain laboratory-passaged strains, e.g. Moloney MLV, which are designated NB-tropic. The resistance of the F1 hybrids can be applied to distinguish a mixture of an N-tropic and B-tropic MLV from an NB-tropic MLV. Both preparations show similar plaquing efficiencies on N- and B-type cells. However, a 30-fold or greater reduction on F1 cells is seen in the case of a mixture, as opposed to no reduction in the case of the NB-tropic MLV.

Hartley, J. W., T. Pincus, and W. P. Rowe. Unpublished data.
In a study of C3H (N-type) and C57BL/6 (B-type) mice, segregation patterns in the F₂ generation indicated that two genes are involved in susceptibility to leukemogenesis by Gross MLV (2). One gene, rgr-1, is linked to the H-2 locus, while little is known regarding the other, rgr-2. The absence of H-2 association excludes the possibility that the locus identified in the present studies is rgr-1. It is possible that rgr-2, which has been recognized on the basis of statistical considerations (2), may be the N-B locus.

No association with the H-2 locus is found in the N- and B-type patterns, but the interaction of H-2otype and MLV infection, summarized elegantly by Snell (29), is seen in the present data: all mice showing MLV plaques in secondary ME cultures without superinfection, i.e. AKR, C58, RF, and B10.BR (spontaneous emergence of MLV in secondary cultures of C3H ME has also been noted [17]), possess the H-2 allele, and all H-2o mice with the exception of the congenic B10.BR are N-type in sensitivity. However, the B-type sensitivity of the B10.BR strain indicates that H-2 is not a major genetic factor in this in vitro tissue culture system. Clearly, leukemogenesis is a far more complex process than tissue culture infection of fibroblasts. The role of the H-2 locus may be of great importance in in vivo leukemogenesis, after initial infection with MLV (2).

On the basis of studies presented in the accompanying report, there is strong evidence that the N-B locus is identical or closely linked to a major gene described for Friend virus susceptibility, Fv-I (30). All mouse strains known to possess the Fv-I allele are N-type while all Fv-I strains are B-type (24).

Susceptibility to infection of chick cells by viruses of the avian leukosis-sarcoma complex has been shown to be genetically determined (8-10). Although these viruses show striking similarities to the murine leukemia and sarcoma viruses in many respects, the patterns of genetically determined sensitivity show several significant differences between the avian and murine systems: (a) resistance is dominant in the murine system while susceptibility has been found to be dominant in the avian system; (b) resistance is generally over a greater range in the avian system than the 100-1000-fold differences seen in the murine system and may involve an absolute block; (c) viruses of different host range are serologically distinguishable in the avian system, while all naturally occurring MLV isolates are immunologically of the Gross type, whether N- or B-tropic (17).

It should be recognized that our ability to identify genes affecting MLV infection rests largely on the availability of inbred mouse strains. The two genes presently known to influence susceptibility to infection and leukemia induction by naturally occurring MLV, rgr-I (2) and the N-B locus (which could be rgr-2 [2]), are both dominant for resistance. If other unrecognized genes concerned with leukemia virus susceptibility are dominant for resistance, the difficulty of detection of leukemia viruses in an outbred population is apparent.
The findings regarding Gross and Tennant leukemia viruses present an application of N- and B-type patterns to understanding leukemogenesis by MLV strains. The need for an appropriate host for leukemia induction studies was recognized in the original studies of Gross (12) and Tennant (14). Gross virus was isolated from a C3H mouse inoculated with an AKR tissue extract (both N-types) (1), while Tennant virus was isolated from the spleen of an elderly BALB/c (B-type) mouse (13). The apparent conflict in host-range sensitivity of the C3H, C57BR, and A strains to these two leukemia viruses is understood through recognition of the tropisms of the two viruses. Earlier studies have indicated that pseudotype sarcoma viruses, contained in coats of N- and B-tropic MLV isolates, lead to sarcoma formation in NIH Swiss and BALB/c mice, respectively (11). The experiments presented thus identify an important genetic determinant affecting control of susceptibility to MLV infection, and provide a model for further study of early biologic events in leukemia induction.

SUMMARY

Previous studies have indicated that all naturally occurring murine leukemia viruses propagate significantly more efficiently on embryo cells of either NIH Swiss or BALB/c mice.

Studies of the plaquing efficiency of representative viruses on embryo cells of various inbred and hybrid mice indicate that the pattern of sensitivity of the cells is genetically determined. All of 23 strains tested were found to resemble either NIH Swiss (N-type) or BALB/c (B-type) with respect to plaquing efficiency of these viruses. Virus growth on embryo cells derived from (N-type × B-type)F1 hybrids indicated dominance of resistance to both types of viruses. Backcross hybrid studies indicated that a single locus is the primary determinant of the host-range patterns observed. This locus has no effect on growth of certain laboratory-passaged leukemia viruses which propagate equally well on embryo cells of all mouse strains, F1, and backcross hybrids. Though other genetic and nongenetic factors influence viral growth or expression in vitro and in vivo, the genetic locus described appears of major significance in the biology of murine leukemia.

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