

IDENTIFICATION OF A VARIANT OF GROSS LEUKEMIA
VIRUS THAT INDUCES DISEASE IN MICE INOCULATED AS
ADULTS*

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Virus-induced leukemogenesis in mice was first described by Gross (1, 2) in studies in which the successful transmission of leukemia (thymic lymphoma) by cell-free extracts from AKR thymomas depended on the inoculation of newborn C3H/Bi mice. Serial passage of this virus resulted in the potent Gross passage A virus (2). Although a very high percentage of newborn mice inoculated with Gross virus (GV) develop thymic lymphomas, adult mice are resistant to the virus and develop no disease. No reason for this susceptibility of neonates has been determined, however, it appears likely that it is related to either (a) an effect on the immature immune system of the neonate or (b) the presence of certain target precursor or stem cells that are not present in the adult.

Resistance to murine leukemia virus (MuLV)-induced disease is mediated by several genes (3). Lilly found that when mice expressing various *H-2* haplotypes were inoculated with high doses of virus, all mice regardless of haplotype developed lymphomas. However, when low doses were used, homozygous *H-2^h* mice were susceptible to disease, whereas homozygous *H-2^b* mice were resistant (4, 5). Backcross analysis and the use of mice with recombinant haplotypes determined that a gene, *Rgv-1*, linked to the *H-2K* region controlled this relative resistance to GV-induced disease.

We have identified an apparently new variant of Gross passage A virus that causes thymic lymphomas in mice inoculated intraperitoneally (i.p.) as adults. In addition, pathogenesis induced by high doses of this virus is very closely regulated by a gene(s) linked to the *H-2* complex.

Materials and Methods

Mice. C57BL/10Li (B10/Li), BALB/c-H-2^k (BALB.K/Li), and BALB/c-H-2^b (BALB.B/Li) mice were bred from pairs obtained from Dr. Frank Lilly, Albert Einstein College of Medicine, Bronx, NY. C57BL/10-H-2^k (B10.K) mice were bred from pairs obtained from Dr. Donald Shreffler, University of Washington, St. Louis, MO.

Virus. The Gross virus variant used in these studies is derived from BALB/c-adapted GV originally obtained from Dr. Frank Lilly. Briefly, this virus is maintained by serial passage in BALB mice inoculated as neonates. Virus stocks are prepared from 10% homogenates in Hanks Balanced Salt Solution (HBSS) of the greatly enlarged thymus,

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spleen, and lymph nodes of susceptible mice inoculated with a high dose of virus. These homogenates kept continuously at 0–4°C, were clarified by centrifugation for 15 min at 2,400 g. The virus supernatants were stored in 2-ml ampules at –70°C until used.

Cytotoxicity. The expression of Thy-1, Gross cell surface antigen (GCSA) and Ia on cultured tumor cell lines derived from thymic lymphomas was examined by trypan blue exclusion in an antibody and complement-mediated cytotoxicity assay. Briefly, 10^5 cells were incubated with antibody in 0.2 ml medium (RPMI 1640 with 10% fetal calf serum) at room temperature for 30 min. The cells were centrifuged, washed in fresh medium, and incubated with guinea pig complement (1:4) for 40 min at 37°C. After complement treatment, the cells were examined for viability by trypan blue exclusion. Testing for Thy-1 and Ia.2 expression was performed by direct cytotoxicity. Testing for GCSA expression was performed by absorption of anti-GCSA antiserum with various cell lines (see Table III) and subsequent testing of the absorbed antiserum for residual anti-GCSA activity by direct cytotoxicity on the E δ Gross (GCSA⁺) tumor cells. C57BL/6 anti-K36 antiserum, which detects GCSA, was kindly supplied by Dr. Elizabeth Stockert, Sloan-Kettering Institute for Cancer Research, New York, NY. Monoclonal antibodies detecting Thy-1.2 and Ia.2 were kindly supplied by Dr. Jonathan Sprent, University of Pennsylvania School of Medicine, Philadelphia, PA.

Results

Adult 8-wk old BALB.K mice were inoculated i.p. with BALB/c-adapted Gross virus that previously had only induced thymic lymphomas in mice inoculated as neonates. 5 mo later, these mice were found to have developed greatly enlarged thymuses and spleens. A cell-free virus stock was prepared from these thymuses by passing homogenates through a 0.20- μ m filter. The virus was serially passaged through BALB.K mice twice. This procedure resulted in an apparent increase in virus potency since the latent period for thymoma development was reduced to 6–8 wk. We have tentatively assigned the designation WB91-GV to this virus.

H-2-Linked Resistance to WB91-GV. 10–12-wk old mice congenic with respect to their *H-2* haplotypes were compared for susceptibility to WB91-GV-induced leukemogenesis (Table I). B10/Li (*H-2^b*) mice appeared to be absolutely resistant to WB91-GV since no mice (0/12) showed any signs of disease 6 mo after inoculation. In contrast 86% (13/15) of congenic B10.K (*H-2^k*) mice developed thymomas. In a similar manner, 91% (11/12) of BALB.K mice inoculated with WB91-GV developed thymomas, whereas only 12% (3/26) of congenic BALB.B (*H-2^b*) mice developed disease after 6 mo. Sublethally irradiated (500 R) BALB.B mice all developed thymic lymphomas after virus inoculation.

The disease found in the few normal BALB.B mice that developed disease differed significantly from that found in BALB.K mice. In BALB.K mice, both the thymus and peripheral lymphoid organs showed leukemic involvement, whereas unirradiated BALB.B mice showed the typical thymic enlargement but no involvement of peripheral lymphoid organs. Irradiated BALB.B mice, however, showed the same profile of organ involvement as BALB.K mice.

Growth of Syngeneic Transplanted Tumor Cells. As shown in Table II, syngeneic tumor cells (single cell suspensions), derived from the enlarged thymuses of donor mice, as well as cultured syngeneic tumor cells derived from WB91-GV infected mice, grew well in normal BALB.K mice as well as in sublethally irradiated and virus-infected recipients. However, in BALB.B mice, which express the *H-2^b* haplotype, syngeneic tumor cells grew in sublethally irradiated

TABLE I
H-2-Mediated Resistance to WB91-GV

Strain	H-2 Type	Treatment*	Thymoma development [‡]
B10.K	k	None	13/15
BALB.K	k	None	11/12
BALB.K	k	450R	10/10
B10	b	None	0/12
BALB.B	b	None	3/26
BALB.B	b	450R	5/5

* All animals were inoculated i.p. with 0.2 ml of a 10% homogenate of leukemic thymus and spleen containing $\sim 2 \times 10^4$ PFU as measured in XC assay. Animals were irradiated 24 h before virus inoculation.

[‡] Animals were observed for 6 mo. Those that did not die from thymomagenesis were autopsied and examined for signs of pathogenesis.

TABLE II
H-2-linked Resistance to Growth of Transplanted Syngeneic Tumor Cells Induced with WB91-GV

Strain	H-2 Type	Treatment*	Tumor growth [‡]	
			1° Tumor cells	Cultured tumor cells
BALB.K	k	None	5/5	5/5
BALB.K	k	500R [‡]	5/5	5/5
BALB.K	k	WB91 [§]	5/5	5/5
BALB.B	b	None	0/5	0/5
BALB.B	b	500R	4/5	5/5
BALB.B	b	WB91	0/5	0/5
B10.K	k	None	0/5	0/5
B10.K	k	500R	0/5	0/5
B10.K	k	WB91	2/5	4/5

* Recipients were inoculated subcutaneously with 5×10^6 syngeneic tumor cells from the enlarged thymuses of adult mice inoculated with WB91-GV or 10^5 syngeneic cells from cultured tumor cell lines derived from the enlarged thymuses of adult mice inoculated with WB91-GV (see Table III).

[‡] Recipients were irradiated 24 h before tumor inoculation.

[§] Recipients were inoculated with WB91-GV 2 wk before tumor inoculation.

[‡] Recorded as number of mice in which tumor growth progressed/total inoculated.

but not in WB91-GV-infected recipients, indicating that irradiation may immunosuppress mice, allowing the growth of tumor cells. Surprisingly, both normal and irradiated B10.K recipients rejected syngeneic tumor cells. The failure of tumor cells to grow in irradiated B10.K mice suggests that some radioresistant immune component exists in mice with a B10 but not a BALB genetic background. Only prior inoculation of B10.K mice with WB91-GV allowed tumor growth to progress.

Antigenic Determinants on the Tumor Cell Surface. Cultured tumor cell lines have been derived from WB91-GV-induced thymic lymphomas of BALB.K and B10.K mice. Cells from these lines grow in suspension and, as shown in Table III, express GCSA (Gross cell surface antigen), characteristic of GV-infected

TABLE III
*Expression of Cell Surface Antigens on Virus-induced Tumor Cell Lines**

Cell line	Strain of origin	Inducing virus [‡]	Cytotoxic indices with: [§]		
			Anti-GCSA [†] +C	Anti-Thy-1.2 [†] +C	Anti-Ia.2 [†] +C
B10.K(WB91)-1	B10.K	WB91	3	95	0
BALB.K(WB91)-1	BALB.K	WB91	4	94	3.5
HFL/b (Clone L)	BALB.B	FV	98	0	0
BALB.B gv-1	BALB.B	GV	2	93	1
Normal					
BALB.K lymph node	BALB.K	none	98	56	32

* Assayed by two-step antibody and complement mediated cytotoxicity. See Materials and Methods.

[‡] WB91, Gross virus adult variant; FV, Friend virus (N→NB tropic; polycythemic); GV, Original Gross virus stock.

[§] Cytotoxic indices were calculated as $100 \times \frac{\% \text{ killed (Ab + C)} - \% \text{ killed (C alone)}}{100 - \% \text{ killed (C alone)}}$. Background lysis with complement alone ranged from 0–12%. Controls incubated with antibody alone were >95% viable.

[†] Anti-GCSA antiserum was absorbed with cells from the appropriate cell line and tested for residual anti-GCSA activity on E δ Gross (GCSA⁺) tumor cells.

[†] Assayed with unabsorbed antiserum by direct cytotoxicity.

cells, and Thy-1.2 but do not express Ia.2 as determined by antibody and complement-mediated cytotoxicity.

Discussion

The results of these studies appear to indicate that we have isolated a variant of Gross virus which, unlike the original virus, induces thymic lymphomas when inoculated i.p. into adult mice. That this virus is a variant of GV and not a completely new virus is suggested by the fact that (a) tumor cells induced in animals inoculated as adults express GCSA characteristic of GV (Table III) and (b) the profile of organ involvement is the same as that observed in mice inoculated with GV as neonates.

In our studies with BALB mice, there appears to be a correlation between resistance to virus-induced thymomagenesis and the ability to reject syngeneic tumor cell grafts, i.e., BALB.K mice are susceptible to virus and fail to reject transplanted tumor cells, whereas BALB.B mice are resistant to virus and are resistant to the growth of tumor cell grafts (Tables I and II). In addition, BALB.B mice immunosuppressed by irradiation are both susceptible to virus and fail to reject transplanted tumor cells (Tables I and II). These studies, therefore, strongly suggest that in mice with a BALB genetic background, *H-2*-mediated resistance to WB91-GV is regulated by radiosensitive immune cells, most likely cytotoxic T cells and/or antibody-producing B cells, which can either generate an effective anti-viral immune response as in BALB.B mice or cannot as in BALB.K mice. In B10.K mice, however, there is a dissociation between susceptibility to virus and the ability to reject syngeneic tumor cells, i.e., both normal and irradiated B10.K mice reject syngeneic tumor grafts (Table II) although untreated B10.K mice are susceptible to virus (Table I). It, therefore, appears that in B10.K mice there are radioresistant cells, perhaps NK cells or macro-

phages, that can effectively inhibit tumor cell growth. Transplanted tumor cells do grow in B10.K recipients that are pretreated with WB91-GV (Table I) suggesting that virus expression and/or production in these mice may suppress their radioresistant immune cells. Interestingly, this virus-induced suppression of tumor cell graft rejection is not observed in BALB.B mice pretreated with virus (Table II). A likely explanation for this difference between B10.K and BALB.B mice may be that BALB.B mice, by virtue of their *H-2^b* haplotype, eliminate replicating virus by mounting an effective anti-virus response, whereas B10.K (*H-2^k*) may fail to generate this response. The consequence of this failure to respond could be the continued expression of virus in B10.K hosts, which may then result in the suppression of cells responsible for tumor cell rejection. In BALB.B mice, however, with the virus cleared, tumor cell rejection could proceed effectively. It is possible that these events, which may occur during tumor cell graft rejection, may also affect the process of thymomagenesis by controlling the generation of immune responses to autochthonous tumor cells in virus-infected mice.

It is interesting to note that a few untreated BALB.B mice develop thymic lymphomas (Table I) but there is no spread of malignant cells to the peripheral lymphoid organs as apparently occurs in BALB.K and irradiated BALB.B mice. This finding may reflect the presence of immune cells in the periphery that effectively eliminate tumor cells as they leave the thymus.

The leukemogenic viruses isolated from AKR thymomas have been found to be dualtropic mink cell focus-forming (MCF) viruses (6, 7) although some appear to be ecotropic (8, 9). These viruses contain *gag*, *pol*, and *env* regions but no transforming genes (9–11). In this respect, they are similar to avian leukosis virus (ALV), which induces B cell transformation in chickens, apparently by a promoter-insertion mechanism in which the proviral genome integrates adjacent to *c-myc* resulting in the increased transcription of this gene (12–14). Like ALV, MCF viruses have recently been shown to have a common region of provirus integration (11). It has not yet been demonstrated whether some gene similar to *c-myc*, whose constitutive expression in T cells might result in transformation, is located in this region of MCF proviral integration. However, if this were the case, it is possible that in the long passage history of the BALB/c-adapted GV, this virus has acquired a *v-onc* from the cell genome which now acts as a viral transforming gene similar to others previously described (see reference 15). Such an event might account for the potency of WB91-GV in adult animals.

Alternatively, the leukemogenicity in adult mice of WB91-GV may be associated with certain unique changes that have occurred in the 3' (*env*-U3) region of the virus genome, either as the result of mutation or recombination with some endogenous virus. Differences within this region have previously been demonstrated to affect the leukemogenicity and potency of retroviruses (9, 10).

Summary

Gross murine leukemia virus normally induces leukemia (thymic lymphoma) in mice inoculated as neonates, but not as adults. We have isolated an apparent variant of this virus which induces thymomas when inoculated i.p. into susceptible adult mice. Using *H-2* congenic BALB and C57BL mice, susceptibility to virus-

induced thymomagenesis was found to be linked to the *H-2* complex. In addition, a radioresistant immune mechanism leading to inhibition of tumor growth was observed in mice with a C57BL but not a BALB background.

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