Proc. Natl. Acad. Sci. USA
Vol. 73, No. 12, pp. 4633–4635, December 1976
Immunology

Prevention of spontaneous leukemia in AKR mice by type-specific immunosuppression of endogenous ecotropic virogenes

(model systems for *in vivo* oncogenesis/prevention of cancer by passive antibody)

ROBERT J. HUEBNER*, RAYMOND V. GILDEN†, ROBERT TONI†, RICHARD W. HILL*, ROY W. TRIMMER*, DONALD C. FISH†, AND BERNARD SASS‡

* Laboratory of RNA Tumor Viruses, National Cancer Institute, Bethesda, Maryland 20014; † National Cancer Institute-Frederick Cancer Research Center, Frederick, Maryland 21701; and ‡ Microbiological Associates, Bethesda, Maryland 20014

Contributed by Robert J. Huebner, September 17, 1976

ABSTRACT **AKR/J mice, 80–90% of which ordinarily die of spontaneous lymphocytic leukemias by 12 months of age, were significantly protected from developing leukemia in the initial experiment by a single course of treatment with AKR serotype-specific antibodies made in goats and processed as immune gamma globulin (IgG). In experiment 1, IgG was given on the day of birth and on four additional days, and finished on day 14. This schedule resulted in suppression of over 4 logarithms of normal virogene expressions up to 25 days of age and led to partial viral suppression for over 200 days of age. At 365 days of age, 20 of 24 (83.3%) control animals were dead of leukemia whereas six of 30 (20%) treated animals had died of leukemia. In a second experiment, only four inoculations of IgG were given from birth to 20 days, after which they were given three inoculations of radiation-killed vaccine specific for AKR-Gross leukemia virus and one injection of murine sarcoma virus-Gross leukemia virus 10 days later. This combined immunization procedure provided significant virus suppression up to 288 days of age. At 300 days of age, 30 of the 50 (60%) controls had died of leukemia while only 1 of 24 (4.2%) of the immunized mice developed fatal leukemia; the significance of protection for each of the experiments was $P \ll 0.001$. We conclude that these data establish in classical fashion with type-specific immunosuppression the determining role of type-C endogenous virogenes in leukemogenesis and, at the same time, also establish the feasibility of nearly total prevention of leukemia in AKR mice.**

In this communication we report two experiments, both resulting in highly significant prevention of spontaneous leukemia in AKR/J mice. In the first experiment, several injections of virus-specific antisera prepared as immune gamma globulin (IgG) were given shortly after birth and continued to the 14th and 20th days of age, after which the control and immunized mice (starting at 170 days of age) were observed twice daily for development of the leukemia and/or thymic lymphomas which normally account for nearly all deaths in AKR mice by 12 months of age.

PROCEDURES

Preparation of IgG Antisera. The anti-AKR-specific antiviral IgG was prepared by injecting castrated male goats with 1000 times concentrated preparation of banded radiation leukemia virus[§] of Lieberman and Kaplan (1) which had been grown in SC-1 cells (2). The goats, 12–18 months of age, were immunized intramuscularly at 1 to 2 week intervals with 1.0–3.0 mg of purified virus mixed with equal parts of Freund's complete adjuvant. Animals were bled at 10-day intervals beginning 2 weeks after the third inoculation, after which the globulin fraction of pooled sera was precipitated by ammonium sulfate and resuspended in one-half the original volume in phosphate-buffered saline. After dialysis for 48 hr at 4° with several buffer changes, the IgG was filtered through a 0.45 μm filter, dispensed in vials, and stored in liquid nitrogen. Prior to use in mice, it was heat-treated for 30 min at 56°. Two batches of antiradiation leukemia virus IgG appeared to produce toxic deaths in the mice. One of these caused rapid deaths in a proportion of mice inoculated as newborns in experiment 1. The same IgG preparation was used in experiment 2 without producing death in the infant mice. Subsequent experience now indicates that disturbed mothers, those who fail to nurse, or eat their offspring may have been responsible for the deaths during the immunization procedures in experiment 1.

The anti-AKR neutralizing titers of the IgG pools were determined in the XC test using the SC-1 cell as the susceptible cell system (2, 5). IgG containing 1:800–1:1600 anti-AKR virus neutralizing titers were given subcutaneously in two experiments according to schedules shown in Table 1.

RESULTS

XC tests on 2% extracts of tail segments of the immunized mice in both experiments as shown in Table 2 revealed almost complete suppression of infectious virus at 25 days of age. The suppression at 34 days of age was also impressive and persisted well beyond 200 days; it was still quite significant at 288 days of age in experiment 2. The almost complete suppression of virus in tail tissues up to 34 days of age was not unexpected since we had observed this in earlier studies using the same or similar IgG preparations made in goats (6).

The protection against leukemia shown in Table 3 was also impressive. Only six of 30 or 20% of the immunized mice developed fatal leukemia, while 20 of 24 or 83.3% of the nonimmunized controls also died of leukemia.

In the second experiment terminated at 300 days, one of 24 (4.2%) of the test group and 30 of 50 (60%) untreated controls died of leukemia. The degree of protection, which was highly significant in both experiments ($P \ll 0.001$), suggested that the serotype-specific immunizations which demonstrably suppressed the normal expression of both the *Akv-1* and *V-2* virogenes (7), affected some crucial step in the establishment of leukemia. This could result from reduction of the number of virogene copies available for subsequent replication and potential reintegration (8). Alternatively, the IgG may have an early effect in partially eliminating the hematopoietic cell clones most likely to be at risk for leukemia as indicated by early

Abbreviations: GLV, Gross leukemia virus; MSV, murine sarcoma virus.

[§] Radiation leukemia virus was selected as the desirable virogene prototype because in addition to having the same serotype specificity as does AKR virus, it replicated to higher titer *in vitro* and, when banded, the virion retained the integrity of its envelope to a much higher degree than did AKR or Gross leukemia virus preparations (3, 4).

Table 1. Immunization dosage schedule

Exp. 1* (IgG only)	Exp. 2† (IgG + vaccines)
Day 0‡, 0.05 ml	Day 0‡, 0.05 ml
Day 3, 0.05 ml	Day 3, 0.05 ml
Day 7, 0.10 ml	Day 10, 0.10 ml
Day 10, 0.10 ml	Day 20, 0.20 ml
Day 14, 0.20 ml	*GLV vaccines*
	Day 25, 0.20 ml SC§ 0.20 ml IP§
	Day 39, 0.40 ml IP¶
	Day 53, 0.40 ml IP¶
	MSV(GLV) vaccine
	Day 63, 0.10 ml SC¶

* In experiment 1, 32 mice were immunized with IgG and 24 controls were not given any immunization. Additional controls (not shown) consisted of AKR mice given comparable IgG made against Rauscher leukemia virus and murine xenotropic virus, neither of which had any detectable suppressive effects on AKR virus at 30 days of age.

† In experiment 2, 24 mice were immunized with IgG and 50 mice were held as controls. At 25 days of age, the mice given IgG were also given additional immunizations consisting of three injections of GLV vaccine at 14-day intervals, followed by MSV(GLV) live virus challenge 10 days later. The vaccine materials, immunization procedures, and assays of viral titers in tail extracts were described in detail in a previous report (3). SC, subcutaneous; IP, intraperitoneal.

‡ Less than 24 hr old.

§ Administered with complete Freund's adjuvant.

¶ Administered without complete Freund's adjuvant.

expression of virogenes (e.g., gp70, p30, etc.). Comparable IgG antibodies prepared against Rauscher leukemia virus and the AT124 (xenotropic) strain of mouse virus, both given in a third experiment using the immunization schedule in Table 1, had no detectable suppressive effects at any time on the titers demonstrated in the tails of test mice.

DISCUSSION

There are a number of early and recent reports describing reductions of spontaneous leukemias in AKR and other high leukemia expressor mouse strains. These effects were achieved with the use of a variety of procedures designed to produce artificial or nonspecific resistance. They include (*i*) removal of the thymus target organ (9, 10), (*ii*) foster nursing of normally high leukemia F_1 mice on milk of a low leukemia strain (11), (*iii*) infections with specified protozoa (12) and (*iv*) treatment with interferon (13). Treatments of AKR mice with a nonspecific adjuvant such as Bacillus Calmette-Guerin resulted in delay in leukemia incidence and variable reduced amounts of leukemia by 365 days of age (14, 15). Except for the interferon reports by Gresser, there have been few successful efforts to reduce spontaneous leukemia or other cancers with procedures designed specifically to suppress endogenous virus expressions.

Experiments involving backcrosses of the F_1's of high and low incidence mice to AKR mice revealed highly significant correlations between genetically determined high and low expressions of type-C virus early in life (30–40 days) with high and low incidences of leukemia late in life (16–18). In studies of combined immunological and genetic influences on leukemia, Aoki *et al.* (19) found that genetic segregation of Gross virus specific antigen provided accurate predictions of leukemia risk in F_1 offspring.

It seems clear that AKR antiviral-specific IgG provided the

Table 2. Log ranges of virus titers in tail segments of immunized and control AKR mice

Age tested (days)	No. tested	Immunized	No. tested	Control
		Experiment 1		
18–25	32	<1.0 (mean)	22	$10^{4.1}$ to $10^{5.1}$
32–34	24	<1.0 to $10^{2.6}$	19	$10^{4.2}$ to $10^{4.6}$
238–248	5	$10^{3.5}$ to $10^{4.0}$	8	$10^{4.1}$ to $10^{5.5}$
		Experiment 2		
25*	15	$<10^{1.0}$ to $10^{2.6}$	8	$10^{5.1}$ (mean)
101–104	24	$10^{2.7}$ to $10^{4.1}$	23	$10^{3.6}$ to $10^{5.6}$
288†	24	$10^{1.3}$ to $10^{2.7}$	30	$10^{1.3}$ to $10^{4.1}$

* Viral titers shown in experiment 2 at 25 days of age were established prior to immunization with inactivated GLV vaccine and MSV(GLV) 10 days later. (See Table 1).

† Mean logarithm difference at 288 days between the immunized and control group was $10^{1.2}$.

determinants for suppression of both endogenous virogene expressions and leukemia. Heterotypic but otherwise comparable Rauscher and xenotropic virus IgG antibodies failed to significantly neutralize AKR virus in neutralization tests and did not suppress virogene expressions in AKR mice. In experiment 2, the killed Gross leukemia virus (GLV) vaccine and live murine sarcoma virus-Gross leukemia virus [MSV(GLV)] may have added protection additional to that furnished by the IgG since the initial IgG suppression of virus at 25 days was poorer than that observed in experiment 1. In any case, these experiments clearly establish that immunoprevention of cancer coded for inherited virogenes (and possible associated oncogenes) is rather readily accomplished with the use of classical antiviral immune mechanisms. These findings, together with our earlier reports of comparable IgG suppression of ecotropic virus in F_1's of several AKR crossbreeding experiments (20), suggest that it may be possible to suppress known or postulated aberrant gene expression in diseases other than cancer.

Price and his associates (21) recently reported the prevention of carcinogen-induced malignant transformation of rat cells *in vitro* with the use of specific antiviral IgG prior to treatment with carcinogenic chemicals. This and other successful experiments in which Price and his associates employed both type-specific and heterotypic viral neutralizing antisera for suppression of both virus and transformation provided definitive evidence for the existence of virus specific determinants for transformation, because only type-specific antibodies against virus prevented transformation. These reports provided addi-

Table 3. Prevention of leukemia in AKR mice immunosuppressed by serotype-specific antiviral IgG antibodies. Mice at risk at 170^+ days of age in test and control groups

	Age (days)	Immunized (IgG) leukemia/totals (%)	Controls leukemia/totals (%)	Significance
Exp. 1	300	2/30 (6.6%)	11/24 (45.8%)	$P = 0.001$
	365	6/30 (20%)	20/24 (83.3%)	$P << 0.001$
Exp. 2*	250	0/24 (0%)	9/50 (18%)	$P = 0.02$
	300	1/24 (4.2%)	30/50 (60%)	$P << 0.001$

* The immunized mice in experiment 2 were also given killed GLV vaccine 5 days after completion of IgG treatment followed 10 days later by MSV(GLV) (5).

tional rationale to try immunoprevention of cancer *in vivo* such as is reported in this manuscript.

It would be difficult to explain the complete suppression of virogene expressions in AKR mice (up to 4–5 logarithms of infectious virus units for several weeks after the IgG treatment), unless one presumes that the IgG reacted with critical virogene specified proteins on thymic and bone marrow cell membranes and, in so doing, brought about suppression of normally large amounts of expressed virus. On the other hand, early treatment with IgG antibodies may have eliminated many of the cells which are normally responsible for the critically high levels of virus required to specify high incidences of leukemia by 300–365 days. The level of virus expression, the degree of immunological reactivity towards known viral proteins, and the ultimate cause of death of the surviving 70 treated mice remain to be determined.

The prevention of leukemia in AKR mice suggested additional experiments designed to prevent sarcomas and other cancers which are regularly produced in mice by chemical carcinogens. Thus, we already have evidence that several injections of ecotropic virus-specific IgG antisera given to weanling C3H/f mice before treatment with 200 μg of 3-methylcholanthrene provided significant protection ($P < 0.001$) against subcutaneous sarcomas observed at much higher incidences in controls (unpublished observation).

CONCLUSION

In a sense, the IgG protective antibodies might be viewed as probes providing highly specific information concerning the etiology of cancer in mice. It may, therefore, be possible to utilize these or similar antibody "probes" to identify and isolate products involved in experimental animal and human cancer. If it were possible also to produce high-titered IgG antiserum to tumor-specific glycoprotein antigens that would successfully modify or prevent spontaneous leukemias and/or chemically induced tumors in mice, the possibility that similar specific antisera to tumor cell antigens could be produced for use in humans would perhaps appear to be more feasible.

1. Lieberman, M. & Kaplan, H. S. (1959) "Leukemogenic activity of filtrates from radiation-induced lymphoid tumors of mice," *Science* **130**, 387–388.
2. Hartley, J. W. & Rowe, W. P. (1975) "Clonal cell lines from a feral mouse embryo which lack host-range restrictions for murine leukemia viruses," *Virology* **65**, 128–134.
3. Huebner, R. J., Todaro, G. J., Sarma, P., Hartley, J. W., Freeman, A. E., Peters, R. L., Whitmire, C. E., Meier, H., & Gilden, R. V. (1970) in *Defectiveness, Rescue and Stimulation of Oncogenic Viruses. Second International Symposium on Tumor Viruses* (Editions du Centre National de la Recherche Scientifique, Paris), pp. 33–57.
4. Huebner, R. J. & Todaro, G. J. (1969) "Oncogenes of RNA tumor viruses as determinants of cancer," *Proc. Natl. Acad. Sci. USA* **64**, 1087–1094.
5. Huebner, R. J., Gilden, R. V., Lane, W. T., Toni, R., Trimmer, R. W. & Hill, P. R. (1976) "Suppression of murine type-C RNA virogenes by type-specific oncornavirus vaccines: Prospects for prevention of cancer," *Proc. Natl. Acad. Sci. USA* **73**, 620–624.
6. Huebner, R. J., Gilden, R. V., Toni, R., Hill, P. R. & Trimmer, R. W. (1976) in *Proceedings of Third International Symposium on Detection and Prevention of Cancer* (Marcel Dekker, Inc., New York), in press.
7. Chattopadhyay, S. K., Rowe, W. P., Teich, N. M. & Lowy, D. R. (1975) "Definitive evidence that the murine C-type virus inducing locus *Akv-1* is viral genetic material," *Proc. Natl. Acad. Sci. USA* **72**, 906–910.
8. Berns, A. & Jaenisch, R. (1976) "Increase of AKR-specific sequences in tumor tissues of leukemic AKR mice," *Proc. Natl. Acad. Sci. USA* **73**, 2448–2452.
9. Furth, J. (1946) "Prolongation of life with prevention of leukemia by thymectomy in mice," *J. Gerontol.* **7**, 46–54.
10. Law, L. W. & Miller, J. H. (1950) "Observations on the effect of thymectomy on spontaneous leukemias in mice of the high-leukemia strains, RIL and C58," *J. Natl. Cancer Inst.* **11**, 253–262.
11. MacDowell, E. C. (1955) "Mouse leukemia. XVI. Spontaneous cases in strain C58 resisted by milk of old STOLI foster nurses," *Cancer Res.* **15**, 23–25.
12. Lunde, M. N. & Gelderman, A. H. (1971) "Resistance of AKR mice to lymphoid leukemia associated with a chronic protozoan infection, *Besnoitia jellisoni*," *J. Natl. Cancer Inst.* **47**, 485–488
13. Gresser, I., Coppey, J. & Chantal, B. (1968) "Action inhibitrice de l'interféron brut sur la leucémie lymphoide souris AKR," *C. R. Hebd. Seances Acad. Sci.* **267**, 127–129.
14. Lemonde, P. (1973) "Protective effects of BCG and other bacteria against neoplasia in mice and hamsters," *Natl. Cancer Inst. Monogr.* **39**, 21–29.
15. Hibbs, J. B., Jr., Lambert, L. W., Jr., & Remington, J. S. (1972) "Adjuvant induced resistance to tumor development in mice," *Proc. Soc. Exp. Biol. Med.* **139**, 1053–1056.
16. Lilly, F., Duran-Reynals, M. & Rowe, W. (1975) "Correlation of early murine leukemia virus titer and H-2 type with spontaneous leukemia in mice of the BALB/c × AKR cross: A genetic analysis," *J. Exp. Med.* **141**, 882–889.
17. Meier, H., Taylor, B. A., Cherry, M. & Huebner, R. J. (1973) "Host gene control of type C RNA tumor virus expression and tumorigenesis in inbred mice," *Proc. Natl. Acad. Sci. USA* **70**, 1450–1455.
18. Heiniger, H. J., Meier, H., Kaliss, N., Cherry, M., Chen, H. W. & Stoner, R. D. (1974) "Hereditary immunodeficiency and leukemogenesis in HRS/J mice," *Cancer Res.* **34**, 201–211.
19. Aoki, T., Boyse, E. A. & Old, L. J. (1968) "Wild-type Gross leukemia virus. II. Influence of immunogenetic factors on natural transmission and on the consequences of infection," *J. Natl. Cancer Inst.* **41**, 97–101.
20. Huebner, R. J., Gilden, R. V., Lane, W. T., Trimmer, R. W. & Hill, P. R. (1976) in *Proceedings of Second Conference on Modulation of Host Resistance in the Prevention or Treatment of Induced Neoplasias*, ed. Chirigos, M. (Raven Press, New York), in press.
21. Price, P. J., Bellew, T. M., King, M. P., Freeman, A. E., Gilden, R. V. & Huebner, R. J. (1976) "Prevention of viral-chemical cocarcinogenesis *in vitro* by type-specific anti-viral antibody," *Proc. Natl. Acad. Sci. USA* **73**, 152–155.