

Prevention of Gross Virus-induced Leukemia in Progeny of Immunized Female Rats¹

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SUMMARY

In rats and mice, infection with Gross leukemia virus is age dependent, and leukemogenesis takes place only before and shortly after birth. A shift from complete susceptibility to total resistance occurs at about 2 weeks of age. In order to transmit resistance to induction of leukemia, we gave adult female rats of 3 different strains a single injection of Gross leukemia virus before or during pregnancy. Offspring of these and of nonimmunized control mothers were treated by injection with Gross leukemia virus at 1 to 4 days of age. All 61 rats born from control, nonimmunized mothers died with leukemia 62 to 90 days after injection, while none of the offspring of immunized mothers developed leukemia 7 to 10 months after injection. Sera of protective mothers reacted significantly with the type-specific antigen in complement fixation tests.

INTRODUCTION

Susceptibility to viral induction of leukemia in rodents is with few exceptions related to age (1, 3, 9, 10, 19-21, 27, 28). In rats and mice GV³ is leukemogenic only before and shortly after birth (1, 10, 19-21). Vertical transmission of leukemia in these species has been demonstrated in both the natural and experimental disease; however, the mechanisms of transmission are not entirely clear (8, 12, 18, 23). In the high-leukemic AKR and C58 strains of mice, vertical transmission of GV induces leukemia in over 90% of the progeny. The infection takes place mainly during intrauterine life; therefore, subsequent foster nursing of babies from high-leukemic strains is ineffective in protecting them, and the incidence of leukemia remains high in future generations (23). The highly receptive embryonal tissues contain virus

particles (5) and transmit the disease to newborn mice if used as injectable suspension (11). In contrast to this, leukemia induced by injection of virus is more likely to be transmitted by milk during nursing, and its incidence is unstable and decreasing in future generations (7, 13, 23, 24). Chromosomal transmission (23) suggested mainly by experiments with AKR ova transferred to mice of low-leukemic strains (6) is probably exceptional, and transplacental passage was not supported by experimental evidence (25). Transmission of leukemia viruses through the male was not achieved with Friend virus (25), was insignificant with Moloney virus (23), and occurred variably with the GV (14). However, males of the AKR strain, in which the GV is naturally carried, transmit the disease with a high incidence which indicates that in this case vertical transmission can occur through the spermatozoon as well as through the ovum (14). Since the AKR embryo contains all the antigens of the virus, it can be concluded that it is infected not with a provirus but rather with the complete virion (22).

In the rat a situation more resembling low-leukemia strains of mice is encountered. Rats of strains W/Fu, S-D, and L-E although highly susceptible to GV, display a low incidence of spontaneous leukemia. Induction of leukemia with GV can be achieved almost only within the early postnatal period. A shift from complete susceptibility at ages 0 to 7 days to almost total resistance after 15 days of age was noted in all 3 strains in previous work (19, 20). A typical experiment revealing the age dependence of leukemia induction with GV in rats of 3 different strains is reproduced in Table 1.

For prevention of the infection with GV that takes place at a very early age, immunization of mothers was attempted as the only means of conferring protection to offspring during their period of susceptibility.

MATERIALS AND METHODS

Animals. Female rats 3 to 5 months of age of strains W/Fu, S-D, and L-E were used for virus injections and controls.

Virus. GV used in all experiments was stock, liquid nitrogen stored, cell-free filtrate of rat thymomas induced by rat-adapted GV as previously described (19, 20).

Experimental Procedure. Twelve female rats of all 3 strains (W/Fu, S-D, and L-E) were given a single i.p. injection of 0.5 ml undiluted (1/1) stock GV within 1 month before or during the first 2 weeks of pregnancy. On 3 occasions, the

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³The abbreviations used are: GV, Gross virus; W/Fu, Wistar-Furth; S-D, Sprague-Dawley; L-E, Long-Evans.

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Table 1
Age dependence of Gross virus-leukemia induction in rats

Strain	Age (days)	GV			Latency average (days)
		Dilution	Amount (ml)	L/T ^a	
W/Fu	3	1/10	0.1	4/4	74
S-D	4	1/10	0.1	7/7	71
L-E	5	1/10	0.1	9/9	75
S-D	14	1/1	0.2	1/3	142 ^b
L-E	18	1/1	0.2	0/3	— ^b
W/Fu	64	1/1	0.5	0/3	— ^b
S-D	86	1/1	0.5	0/8	— ^b
L-E	91	1/1	0.5	0/7	— ^b

^aL = number with leukemia; T = total number surviving over 60 days.
^bAlive and well after 7 to 9 months.

injections were given i.v. Three female rats were given injections twice, with a 2-week interval, with a similar amount of virus.

The offspring of these 12 rats and of 8 strain and age-matched noninoculated controls were concomitantly given injections i.p. of 0.1 ml of a 1/10 dilution of GV.

A total of 123 offspring of mothers given injections and 61 of mothers not given injections survived more than 60 days and were considered in this experiment. Of these, 37 were sacrificed after 7 months, autopsied, and carefully examined. The remainder, presently 10 months after infection with GV, will be kept until 14 months and sacrificed thereafter.

Complement-Fixation Tests. Rats were bled from the heart into nonheparinized syringes, and the serum was collected and

frozen after light centrifugation. Sera were assayed in microplate complement-fixation tests according to techniques described by Hartley *et al.* (16, 17) (Table 3).

The antigens used were: mouse sarcoma virus, rat tumor pool no. 4; Moloney leukemia control antigen S 124092; AKR leukemia virus, tissue culture antigen R616; control rat thymus antigen 1397; and control tissue culture antigen 1499.

The serum samples were heated 30 min at 56° before use and screened at 1/10 serum dilutions.

RESULTS

Prevention of leukemia induction by GV in the offspring of immunized mothers was achieved in 100% of cases, as summarized by Table 2.

All 61 control rats born from the 8 mothers not given injections died with thymoma and lymphoid leukemia at 62 to 90 days after injection of GV.

All 123 rats born from the 12 virus-inoculated mothers were negative for leukemia 210 to 300 days after injection of GV. At 7 months, 37 of them were sacrificed and were found negative for leukemia on careful examination. The remaining 86 are living and well at present, 10 months after being given injections as newborns with GV. There was no difference between litters born to mothers of different strains, given injections i.p. or i.v., once or twice, before or during pregnancy.

In the complement-fixation tests (Table 3), 4 of 5 sera from immunized mothers gave a 4+ type-specific reaction with the AKR virus antigen to which sera from the 2 nonimmunized mothers were negative. Reactions against all other antigens in the complement-fixation tests were negative.

Table 2

GV-induced leukemia in offspring of immunized and nonimmunized rats

Strain	Mothers		Age (days)	Offspring		Results L/T ^d
	Total no. ^a	Material injected (ml) ^b		Total no.	Material injected (ml) ^c	
S-D	7	GV 1/1 0.5	1-4	71	GV 1/10 0.1	0/71
S-D	4	None	1-4	38	GV 1/10 0.1	38/38
W/Fu	1	GV 1/1 0.5	1-4	6	GV 1/10 0.1	0/6
W/Fu	1	None	1-4	5	GV 1/10 0.1	5/5
L-E	4	GV 1/1 0.5	1-4	46	GV 1/10 0.1	0/46
L-E	3	None	1-4	18	GV 1/10 0.1	18/18
Total of mothers immunized was 12 with total no. of offspring at 123						0/23
Total of mothers nonimmunized was 8 with total no. of offspring at 61						61/61

^aThree immunized mothers were remated and second litters were similarly used.
^bRoute of injection was i.v. and i.p.; time of injection was before and during pregnancy.
^cRoute of injection was i.p.
^dL = number with leukemia; T = total number injected.

Table 3

Complement-fixation test—microplate^a

Unit of hemolysin, 0.2 X 1 to 8000; unit of complement, 0.08 X 1 to 40.

Rat sera	Antigens ^b				
	A MSV-R 1-10	B ML-TC 1-10	C AKR-TC 1-10	D CRThy 1-10	E CTC 1-10
1. Control	0	0	0	0	0
2. Control	0	0	0	0	0
3. Immunized	0	0	4	0	0
4. Immunized	0	0	4	0	0
5. Immunized	0	0	4	0	0
6. Immunized	0	0	0	0	0
7. Immunized	0	0	4	0	0

^aCourtesy of Dr. Padman S. Sarma, National Cancer Institute.^bA, mouse sarcoma virus, rat tumor pool no. 4; B, Moloney leukemia control antigen S 124092; C, AKR leukemia virus, tissue culture antigen R 616; D, control rat thymus antigen 1397; E, control tissue culture antigen 1499.

DISCUSSION

The present experiments demonstrate that full protection against induction of leukemia by GV can be conferred to newborn rats by immunizing their mothers at any time before birth with as little as 1 i.p. injection of GV.

The detection by complement-fixation tests of specific antibodies against the type-specific antigen in the sera of immunized mothers was in total agreement with the development of a capacity to protect their offspring against GV-induced leukemia.

Although virulent enough to induce leukemia in 100% of controls, GV was entirely inactivated in newborns of immunized mothers, bringing leukemia induction down to zero. Transfer of inactivating antibodies from mother to offspring had apparently occurred during intrauterine life and/or nursing. Transfer of passive immunity has been similarly reported to occur in Swiss mice immunized with Friend virus (26) and with a virus isolated from spontaneous leukemia (4). Protection against virus induction of Friend disease was obtained in 60 to 70% of Swiss and 20 to 30% of DBA mice but lasted only the first 15 days of life (26). In a similar experiment, with the use of a virus isolated from Swiss mouse leukemia to challenge newborns of non-immunized and immunized mothers, leukemia with a latency period of 9 months was induced in 60 to 90% versus 0 to 24%, respectively (4).

Leukemia in the rat compared to that of the mouse exhibits several particular features which may be more relevant to the study of the human disease. High-leukemia strains of rats do not exist and spontaneous leukemia, as in man, is uncommon. Pathological entities corresponding to Friend's and Rauscher's disease to which adult mice are susceptible are unknown in rats. Infection by GV is almost entirely restricted to the embryonal and early postnatal period, after which strong natural resistance develops. It is

not known whether the susceptibility to virus induction of leukemia manifested by very young organisms is caused by an incompetent immunological system or due to an increased ability of immature target cells for neoplastic transformation. The experience of cellular transformation *in vitro* seems to favor the latter hypothesis. Regardless of the mechanism involved, if the initial events related to induction of leukemia, as well as of cancer in general, occur at a very early age, effective protection could come only passively, through transfer of immunity from the mother. Various antibodies present in the maternal circulation are transmitted to offspring and can attain similar concentration in their serum (2). The transfer takes place before, after or both before and after birth with characteristic species variations. In the rat, immunoglobulins pass from the maternal circulation into the uterine cavity; however, most of the transfer takes place after birth through colostrum and milk (15). In man, it appears that prenatal transmission of immune substance plays the major role (2). If eventually a viral agent will be related etiologically to leukemia and cancer in man, maternal immunization may prove to be the means of protecting fetus and newborn during the critical period of susceptibility.

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