Proc. Natl. Acad. Sci. USA
Vol. 77, No. 6, pp. 3677–3681, June 1980
Medical Sciences

Passive immunotherapy prevents expression of endogenous Moloney virus and amplification of proviral DNA in BALB/Mo mice

(antiserum treatment/preleukemic virus expression/leukemia/molecular hybridization)

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Communicated by Henry S. Kaplan, March 7, 1980

ABSTRACT **BALB/Mo mice carrying the Moloney murine leukemia virus (M-MuLV) as an endogenous virus become viremic soon after birth and develop leukemia at a later age. M-MuLV-specific gene expression and an increase of virus-specific DNA copies in lymphatic target organs are characteristics of the preleukemic phase. Passive immunotherapy of newborn BALB/Mo mice with anti-gp70 glycoprotein or anti-M-MuLV serum prevented viremia and delayed significantly the subsequent development of leukemia. Molecular hybridization experiments showed that both virus-specific genome transcription and virus-specific DNA amplification could be completely suppressed by antiserum treatment. Thus virus-specific RNA concentrations in target organs of immunized BALB/Mo mice of 6 months or older were as low as in normal BALB/c mice. This is an age at which untreated BALB/Mo mice have already developed malignant lymphoma. Our experiments demonstrate that treatment with antiserum interferes with the early events of virus expression and thus prevents the subsequent steps leading to leukemia.**

The AKR strain of inbred mice has been selected for high incidence of spontaneous leukemia. Gross (1) attributed leukemogenesis to the presence of an endogenous ecotropic leukemia virus. In the AKR mouse this endogenous virus is present at three independent loci (2) and becomes activated around birth (3). Several authors have described strong dependence between the incidence of leukemia and virus titers during early life (4–6).

The observation that the development of leukemia was closely linked to the activation of the Gross murine leukemia virus (G-MuLV) prompted the use of the AKR mouse as a model system to study the effect of immunotherapy on leukemia induction. Huebner and coworkers (7, 8) injected virus vaccines or anti-G-MuLV sera into mice and obtained depression of virus titers and protection against leukemia development. Schäfer and coworkers (9, 10) treated AKR mice with the IgG fraction of a group-specific goat antiserum directed against the Friend murine leukemia virus (F-MuLV) glycoprotein (gp71). The immunized mice were significantly protected against both viremia and the development of leukemia. The results from both of these laboratories clearly demonstrate the importance of an antiserum treatment early during the life-span of AKR mice. However, the molecular events leading to virus activation in young AKR mice are not well characterized, and it is not known which aspects of virus expression are influenced by immunotherapy.

In our laboratory a mouse strain was derived that carries the exogenous Moloney murine leukemia virus (M-MuLV) as an endogenous virus (11, 12). This was achieved by infection of preimplantation mouse embryos and selection for viremic animals. This mouse strain (BALB/Mo) carries one copy of M-MuLV provirus integrated per haploid mouse genome. The age-dependent aspects of the expression of this endogenous virus during the preleukemic and leukemic phase are well characterized. (*i*) Virtually 100% of these mice become viremic during the first 3 weeks after birth and carry infectious virus throughout their life-span. (*ii*) At 3 weeks of age all animals tested express high levels of M-MuLV-specific RNA in the lymphatic target tissues spleen and thymus. (*iii*) Concomitant with the onset of RNA expression, a somatic increase of M-MuLV-specific DNA sequences occurs in the lymphatic target organs spleen and thymus. No somatic amplification of virus-specific sequences or virus genome expression is observed in nontarget tissues such as liver and brain (13). Thus the BALB/Mo mouse represents a well-defined model system to study the molecular characteristics of the different phases in virus-induced leukemogenesis. In this paper, the effects of immunotherapy on virus genome expression leading eventually to disease are analyzed.

MATERIALS AND METHODS

Mice. The origin of BALB/Mo mice has been described (11, 12). BALB/c mice were obtained from Deutsche Versuchstieranstalt, Hannover. Mice heterozygous at the M-MuLV locus (*Mov-1*) were derived by mating BALB/Mo males with BALB/c females.

Antisera. Goat antiserum to gp70 of Rauscher murine leukemia virus (R-MuLV) was obtained from the Office of Program Resources and Logistics, Viral Oncology, National Cancer Institute.) Rabbit anti-M-MuLV serum was prepared by injecting 1 mg of sucrose gradient-purified clone 1 M-MuLV in complete Freund's adjuvant subcutaneously, followed by four booster injections of 1 mg of purified virus. All antisera used were inactivated at 56°C for 30 min. The IgG fraction purified from goat anti-F-MuLV gp71 serum (14) was a gift of W. Schäfer.

For the preparation of $F(ab)_2$ fragments, IgG was purified from rabbit anti-M-MuLV serum by chromatography over DEAE-cellulose (15). After pepsin digestion the material was applied to a Sephadex G-150 column to separate $F(ab)_2$ fragments from undigested immunoglobulins or Fc fragments (16). To test the extent of cleavage, a radioimmunoprecipitation assay with labeled viral protein was performed: less than 2% of the antibody activity from the $F(ab)_2$ preparation could be precipitated by a *Staphylococcus aureus* immunoadsorbant that would react only with immunoglobulins carrying the Fc part (17).

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Abbreviations: MuLV, murine leukemia virus; G-, F-, M-, and R-, Gross, Friend, Moloney, and Rauscher strains of MuLV.

Antiserum Treatment of Mice. All injections were made intraperitoneally. For the treatment with goat anti-MuLV gp70, the pregnant mice were injected with 200 μl of antiserum at day 15 of gestation. The newborn mice were treated with 30 μl at the day of birth and with 60 μl at weekly intervals (immunization protocol A). The schedule for the treatment with rabbit anti-M-MuLV serum was the following: Pregnant mice were injected with 300 μl of antiserum as above. At the day of birth the newborn mice were injected with 50 μl and then at weekly intervals with 100 μl. From 8 weeks on the treatment was continued with 150 μl antiserum every other week until the mice were sacrificed (protocol B). For the treatment with purified IgG instead of unfractionated antiserum, mice were injected with IgG fractions of goat anti-F-MuLV gp71 serum as described by Schwarz *et al.* (10): The pregnant females received a total of 2.6 ml in nine injections and the baby mice received a total of 2.65 ml of IgG fractions up to an age of 6 weeks (protocol C). For the treatment with $F(ab)_2$ fragments from rabbit anti-M-MuLV serum, the same time protocol and half the volumes as for protocol B were used (protocol D).

Radioimmunoassay for p30 Polypeptides. The concentration of MuLV-specific polypeptide p30 in mouse serum or extracts from organs was determined by competitive radioimmunoassays using radioiodinated M-MuLV p30 and rabbit anti-M-MuLV p30 serum according to Strand *et al.* (18), with the modification that the second antiserum was replaced by *S. aureus* immunoadsorbant (17).

Infectious Center Assay. To obtain single-cell suspensions, spleen and thymus were minced in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. Bone marrow was obtained by flushing the femur with the modified Eagle's medium. The cells were washed twice in medium and centrifuged twice through bovine serum. After resuspension the cells were counted in trypan blue. Dilutions of lymphoid cell suspensions were plated on NIH 3T3 cells grown to 50% confluence in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and Polybrene at 2 μg/ml in Linbro plates. Three days later the cultures were tested by the XC plaque assay (19).

Molecular Hybridization of Nucleic Acids. The extraction of DNA and RNA from mouse organs and the nucleic acid hybridization was performed exactly as described (13).

RESULTS

Effect of Antiserum Treatment on Development of Viremia. BALB/Mo mice express virus early in life and virtually 100% have become viremic at 4 weeks of age (Table 1*F*). Injections of normal rabbit serum had no effect on the development of viremia (Table 1*E*). The effect of four different protocols of antiserum treatment on development of viremia was studied: treatment with goat anti-R-MuLV gp69/71 serum (Table 1*A*), treatment with rabbit anti-M-MuLV serum (Table 1*B*), treatment with an IgG fraction prepared from goat anti-F-MuLV gp71 serum (Table 1*C*), and treatment with purified $F(ab)_2$ fragments prepared from rabbit anti-M-MuLV serum (Table 1*D*). The results summarized in Table 1 show that development of viremia in BALB/Mo mice could be suppressed by all four immunotherapeutic protocols. Injection of anti-R-MuLV gp70 serum protected approximately 60% of the animals at 4 weeks but only 10–20% at 6–8 weeks of age. Treatment with rabbit anti-M-MuLV serum was more successful and provided longer-lasting protection against viremia. Up to 10 weeks of age none of 56 mice tested was viremic and at 20 weeks only one of 35 had become viremic. Of 22 mice that were 50 weeks old, only 2 developed viremia. Injections of the IgG fraction from F-MuLV gp71 antiserum, using the protocol of Schwarz *et al.* (10), protected all of 14 treated animals against viremia when tested at 6 and 10 weeks of age.

Table 1. Effect of antiserum treatment on viremia in BALB/Mo mice

Treatment	No. of viremic mice/no. of mice tested at age (weeks)						
	4	6	8	10	20	30	50
(*A*) Goat anti-R-MuLV gp70 serum	12/31	11/16	5/6				
(*B*) Rabbit anti-M-MuLV serum		0/58		0/56	1/35	1/26	2/22
(*C*) IgG fraction from goat anti-F-MuLV gp70 serum		0/14		0/14			
(*D*) $F(ab)_2$ fragments from rabbit anti-M-MuLV serum		5/14					
(*E*) Normal rabbit serum		7/7					
(*F*) BALB/Mo untreated	50/50						

BALB/Mo mice were treated with antisera and at different ages the animals were tested by radioimmunoassay for the presence of MuLV p30 in the serum.

Most cytotoxic activity of antisera against cells is mediated by the Fc part of immunoglobulins. To test whether elimination of virus-positive cells by antibody-mediated cytotoxicity would play a major role in protection against viremia, we treated BALB/Mo mice with $F(ab)_2$ fragments prepared from the rabbit anti-M-MuLV serum (half the effective concentration of antibody as used in Table 1*B*). In this group 9 of 14 mice treated were not viremic at 6 weeks of age (Table 1*D*).

Development of viremia ensues when treatment is discontinued after 8 or 20 weeks (Table 2). Five animals were treated with anti-M-MuLV serum up to 8 weeks of age and two animals were treated until they were 20 weeks old. Subsequently these animals were kept without any further antiserum injection. Within 7–10 weeks after discontinuation of the antiserum treatment all seven mice had become viremic.

Expression of Viral p30 in Lymphatic Organs of BALB/Mo Mice Treated with Antiserum. The concentrations of MuLV p30 found in the serum and in lymphatic organs of BALB/c, BALB/Mo, and BALB/Mo mice treated with anti-R-MuLV serum are summarized in Table 3. Approximately 2–3 ng of p30 per mg of protein in spleen and thymus was found in BALB/c mice and 50–300 ng of p30 per mg of protein in organs of 4-week-old BALB/Mo mice. BALB/Mo mice that were protected from viremia by the antiserum treatment had low levels of p30 in spleen and thymus (2.0–2.6 ng/mg of protein) similar to levels in normal BALB/c mice. Animals that became viremic despite antiserum treatment showed high levels of p30 expression comparable to the level of untreated BALB/Mo mice. Animal number 5-5, which had become viremic within the last 2 weeks before sacrifice at 8 weeks of age, expressed as much p30 as untreated BALB/Mo mice.

Table 2. Effect of discontinuation of the treatment with rabbit anti-M-MuLV on viremia in BALB/Mo mice

Treatment	No. of viremic mice/no. tested at age (weeks)					
	8	14	15	18	22	26
Treated for 8 wk, then kept without antiserum	0/5	0/5	3/5	5/5	5/5	5/5
Treated for 20 wk	0/2		0/2	0/2	0/2	2/2

BALB/Mo mice were treated with rabbit anti-M-MuLV serum for 8 or 20 weeks. Thereafter the animals were kept without further antiserum injections and tested at intervals for viremia as described in Table 1. Five of these mice developed leukemia between 27 and 45 weeks of age.

Effect of Antiserum Treatment on M-MuLV-Specific DNA Sequences and on Expression of M-MuLV-Specific RNA. We have quantitated virus-specific sequences in organs of 30 individual BALB/Mo mice treated with antiserum. The results obtained with 12 mice are summarized in Tables 3 and 4.

M-MuLV-specific DNA sequences present in different organs of BALB/Mo mice were quantitated by hybridization of cellular DNA to an excess of M-MuLV-specific cDNA as described (13). Nontarget organs of homozygous BALB/Mo mice carry 1 copy per haploid genome, whereas homozygous mice have the expected 0.5 copy equivalent present throughout their life-span. Lymphatic target tissues, however, show an increase of M-MuLV-specific DNA copies to 1.5–2 copies per haploid equivalent during the first 4 weeks of life (Table 3; ref. 13). Tables 3 and 4 summarize hybridization data obtained for individual mice treated with antiserum. Approximately 0.5 copy of M-MuLV-specific DNA was found in target organs of all successfully immunized heterozygous BALB/Mo mice. This value is comparable to that found in nontarget organs, indicating that immunotherapy prevents somatic amplification of M-MuLV-specific DNA sequences.

Table 3. M-MuLV-specific DNA and RNA sequences and p30 protein expression in animals treated with goat anti-R-MuLV gp70 serum (immunization protocol A)

Animal no. (Age) p30 in serum*	Organ tested	p30 in organs, ng/mg protein	M-MuLV-specific sequences: DNA, copies/haploid mouse genome	M-MuLV-specific sequences: RNA, conc. rel. to newborn liver
(*A*) BALB/Mo (homozygous for the *Mov-1* locus)				
1-3	Spleen	2.0	0.9	1.5
(4 wk)	Thymus	2.4	0.9	8.5
<0.01	Liver	0.1	0.9	0.3
Controls	Spleen	50–300	1.5–2	75–300
(4 wk)	Thymus	50–300	1.5–2	50–300
1–10	Liver	0.1–0.5	1	0.6–5
(*B*) F1 (♀ BALB/c × ♂ BALB/Mo) (heterozygous for *Mov-1*)				
5-2	Spleen	2.6	0.45	0.8
(6 wk)	Thymus	5.7	0.45	53
<0.01	Liver	0.3	0.42	0.4
5-3	Spleen	2.0	0.44	1.3
(6 wk)	Thymus	2.5	0.47	NT
<0.01	Liver	0.2	0.48	0.3
5-4	Spleen	NT	0.53	50
(6 wk)	Thymus	NT	0.59	41
0.9	Liver	NT	0.55	0.2
5-5†	Spleen	160	0.9	84
(8 wk)	Thymus	240	0.9	250
8.7	Liver	0.6	0.47	1
Controls	Spleen	50–300	1–2	100–350
(4 wk)	Thymus	50–300	1–2	100–350
1–10	Liver	0.1–0.6	0.5–0.6	1.5–4.0
		(*C*) BALB/c		
Controls	Spleen	1–3	0	0
(4 wk)	Thymus	1–3	0	0
<0.01	Liver	0.1–0.2	0	0

BALB/Mo mice or their F1 hybrids were treated with goat anti-R-MuLV serum. MuLV-specific p30 was determined by radioimmunoassay. M-MuLV-specific DNA sequences were quantitated by using a selected cDNA probe and M-MuLV RNA sequences were quantitated by using a representative cDNA probe (see Fig. 1). DNA copies are given per haploid mouse genome equivalent and RNA concentrations relative to the M-MuLV-specific RNA concentration found in livers of newborn mice, which was set as 1 (see ref. 13). NT, not tested.

* p30 in serum μg/ml.

† This animal was not viremic when tested at 6 weeks of age.

RNA was extracted from the same organs as used for DNA·DNA hybridization. Fig. 1 shows kinetics for hybridization of labeled M-MuLV cDNA to an excess of cellular RNA from different organs. RNA extracted from the spleen of untreated BALB/Mo mice hybridized with a $C_rt_{1/2}$ of 50, whereas RNA from lymphatic organs of successfully immunized mice hybridized with a $C_rt_{1/2}$ of approximately 15,000–30,000. Even at the highest C_rt values tested not more than 40% of the cDNA was protected. RNA extracted from spleens of BALB/c mice hybridized to a maximum of 55% with M-MuLV cDNA. It seems likely, therefore, that the low amount of hybridization seen with RNA from target organs of antiserum-treated BALB/Mo mice is due to expression of endogenous BALB/c virus rather than M-MuLV.

The results are summarized in Tables 3 and 4 and are expressed as M-MuLV-specific RNA concentration relative to the concentration found in livers of newborn BALB/Mo mice. Successfully treated mice have low levels of viral RNA, whereas mice that have developed viremia despite antiserum treatment show full virus RNA expression and increased DNA copy numbers.

These observations indicate that treatment of BALB/Mo mice with antiserum not only prevents the development of viremia but also suppresses both virus-specific genome transcription and the somatic amplification of Mu-MuLV-specific DNA copies observed in untreated BALB/Mo mice.

Effect of *in Vivo* Antiserum Treatment on the Number of Lymphoid Cells Producing Infectious Centers. An infectious

Table 4. M-MuLV-specific DNA and RNA sequences in heterozygous BALB/Mo mice injected with rabbit anti-M-MuLV serum (immunization protocol B)

Animal no. (Age)	Organ tested	M-MuLV-specific sequences in: DNA, copies/haploid mouse genome	M-MuLV-specific sequences in: RNA, conc. rel. to newborn liver
16/B*	Spleen	0.5	0.8
(8 wk)	Thymus	0.51	3.8
	Liver	0.5	0.5
16/C*	Spleen	0.55	3.0
(8 wk)	Thymus	0.52	5.0
	Liver	0.5	0.3
16/D*	Spleen	0.55	0.8
(8 wk)	Thymus	0.48	NT
	Liver	0.6	0.2
16/E†	Spleen	1.28	375
(8 wk)	Thymus	1.32	430
	Liver	0.5	3.8
16/F*	Spleen	0.41	1.7
(8 wk)	Thymus	0.43	5.0
	Liver	0.5	0.2
28/3*	Spleen	0.49	0.5
(25 wk)	Liver	0.48	0.2
28/4*	Spleen	0.47	0.75
(25 wk)	Liver	0.49	0.2
28/3+4	Thymus	0.55	1.0
	Marrow	0.45	1.0

F1 hybrids of BALB/Mo × BALB/c mice were treated with rabbit anti-M-MuLV serum. The presence of MuLV-specific p30 in serum of treated mice was determined as described in *Materials and Methods*. M-MuLV-specific DNA (per haploid genome) and RNA (relative RNA concentration) were determined as described for Table 3. NT, not tested.

* These animals had an excess of anti-p30 in the serum.

† This animal was not viremic when tested at 6 weeks of age. p30 in serum was 6 μg/ml.

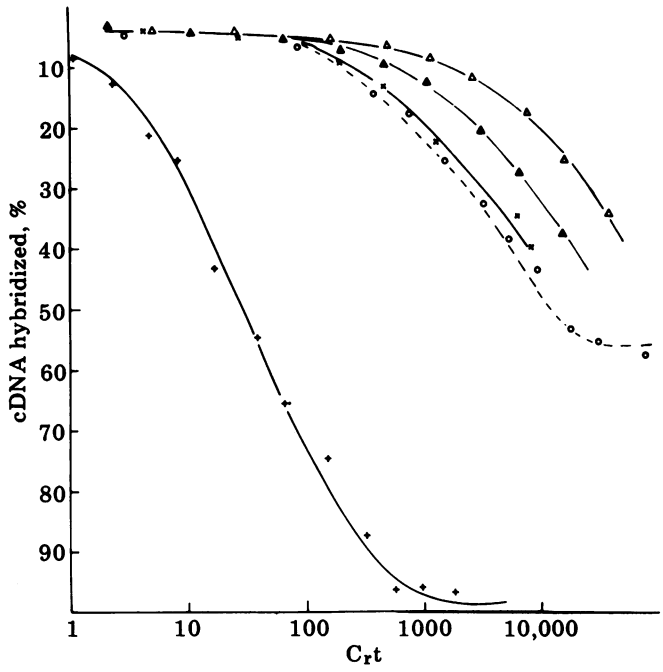

FIG. 1. Hybridization kinetics of M-MuLV cDNA with cellular RNA from various mouse organs. RNA was isolated from spleens of control BALB/Mo (+) and BALB/c (○) mice and from spleen (▲), thymus (×), and liver (△) of a 6-month-old BALB/Mo mouse treated with anti-M-MuLV serum. The RNA was annealed in the presence of representative M-MuLV cDNA, and the results are expressed as percentage hybridized at different C_rt values as described (13). C_rt is the product of RNA concentration (mol of nucleotide/liter) and incubation time (sec).

Effect of *in Vivo* Antiserum Treatment on the Number of Lymphoid Cells Producing Infectious Centers. An infectious center assay was performed to analyze the frequency of lymphoid cells releasing MuLV upon explantation to tissue culture. Serial dilutions of lymphoid cells were plated on NIH 3T3 cells. Five days later the cultures were overlaid with XC cells and XC plaques were read at day 7.

As few as 10 cells from thymus or spleen of 4-week-old BALB/Mo mice yield one infectious center (Table 5*A*). This value did not change when the cells were incubated *in vitro* with antiserum (Table 5*B*) or when the mice were injected with antiserum 2 days prior to sacrifice (Table 5*C*). However, when the same assay was performed with animals successfully treated with antiserum, no infectious centers were observed when 5×10^5 cells were plated (Table 5*D*). These experiments indicate that lymphatic cells from treated animals are unable to produce virus upon *in vitro* cultivation for 5 days in the absence of antiserum.

Effect of Antiserum Treatment on Development of Leukemia. Fig. 2 shows the cumulative mortality of 92 untreated control BALB/Mo mice. At 29 weeks of age 50% of the animals had died from leukemia and at 43 weeks of age 90% were dead. None of 22 animals treated repeatedly with rabbit anti-M-MuLV serum had developed fatal leukemia at 50 weeks of age. Three of 12 animals that were kept longer developed leukemia between 54 and 59 weeks of age.

DISCUSSION

Development of viremia in BALB/Mo mice was efficiently prevented by injecting newborn mice with goat anti-R-MuLV gp70 or rabbit anti-M-MuLV serum. Treatment with R-MuLV gp70 resulted in depression of viremia in more than 50% of 4-week-old animals and in less than 20% of 8-week-old animals. More efficient immunotherapy was achieved by injection of anti-M-MuLV serum: at 10 weeks of age 100% of the treated mice were nonviremic and some mice remained virus-free up to 50 weeks of age (Table 1). This is an age at which nearly all untreated BALB/Mo mice have already developed malignant lymphomas. The reason accounting for the different success rates in immunotherapy cannot be explained easily and could be influenced by interactions between the injected antiserum and the host's own immune system. Such phenomena were observed by Schäfer *et al.* (14): Treatment of F-MuLV-infected mice with immune serum was not successful in inducing complete recovery from leukemia, in contrast to treatment with IgG prepared from the same immune serum. By injecting high concentrations of this purified IgG prepared from goat anti-

Table 5. Frequency of infectious virus-releasing cells from the organs of antiserum-treated and control BALB/Mo mice

Treatment	Organ	Frequency of infectious centers producing lymphoid cells
(*A*) Control	Spleen	$\geq 10^{-1}$
	Thymus	$\geq 10^{-1}$
	Marrow	$\geq 10^{-1}$
(*B*) Mouse as *A*; cells incubated with antiserum *in vitro*	Spleen	$\geq 10^{-1}$
(*C*) Viremic mouse; injected with 500 μl of anti-M-MuLV serum 2 days before sacrifice	Spleen	$\geq 10^{-1}$
	Thymus	$\geq 10^{-1}$
(*D*) Mouse continuously treated with anti-M-MuLV serum from before birth	Spleen	$< 2 \times 10^{-6}$
	Thymus	$< 2 \times 10^{-6}$
	Marrow	$< 2 \times 10^{-6}$

(*A*) Lymphoid cells from an untreated viremic BALB/Mo mouse at 6 weeks of age. (*B*) About 30×10^6 spleen cells of *A* were incubated in 2 ml of Dulbecco's modified Eagle's medium supplemented with 25% rabbit anti-M-MuLV serum at 20°C for 1 hr. Then the cells were washed as usual. (*C*) A viremic littermate of the BALB/Mo mouse used for *A* was injected with 500 μl of rabbit anti-M-MuLV serum. Two days later the animal was sacrificed and lymphoid cells were isolated from the spleen and thymus. At sacrifice the serum of the animal contained an excess of anti-p30 activity. (*D*) The lymphoid cells were isolated from an antiserum-treated BALB/Mo mouse (protocol B) that had not developed viremia when sacrificed at 6 weeks of age.

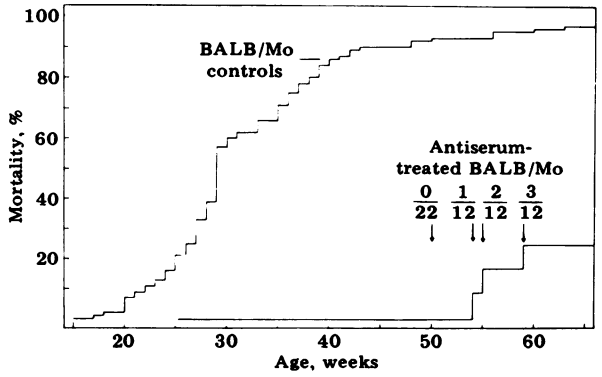

FIG. 2. Effect of antiserum treatment on mortality. The 92 BALB/Mo control mice were kept for up to 65 weeks of age. The 12 experimental animals were treated with rabbit anti-M-MuLV serum (protocol B, Table 2) and kept for 55 or 65 weeks. Occurrence of viremia was monitored by radioimmunoassay and development of leukemia by weekly inspection of both groups of animals. Autopsies were performed on diseased animals to verify the presence of malignant lymphoma.

F-MuLV gp71 serum, Schäfer and coworkers (9, 10) also achieved long-lasting protection of AKR mice from development of viremia and leukemia. A large fraction of the animals remained virus-free even after treatment was discontinued. A similar protective effect of the IgG fraction from goat anti-F-MuLV gp71 serum has been observed in the present experiments which BALB/Mo mice (Table 1C). These results suggest that injection of purified neutralizing antibodies at an early age may enable the animals to develop their own antibody response to the virus, leading to a long-lasting immunity.

The most important result of this study was the observation that all molecular aspects of early virus activation and virus spread—i.e., somatic virus DNA amplification and virus genome expression—could be completely suppressed by antiserum treatment. Successfully immunized BALB/Mo mice of 6 months of age had as low concentrations of virus-specific RNA in their target organs spleen and thymus as normal BALB/c mice. Similarly, no increase of virus-specific DNA copies occurred in these animals. Both virus genome transcription and somatic DNA amplification are early and characteristic events of virus expression and leukemia development in BALB/Mo mice (13). Earlier studies have suggested that reintegration of M-MuLV into the genome of lymphatic target cells may be a necessary step leading to high virus expression. The age-dependent occurrence of unintegrated proviral DNA in thymus and spleens of BALB/Mo mice supports the hypothesis that somatic DNA amplification is due to superinfection of target cells with virus (20).

One possibility to explain the effect of antiserum treatment on virus genome expression is the assumption that infectious virus synthesized early after birth is neutralized. Thus neither reinfection of target cells nor subsequent viral DNA integration can occur, and the chain of events leading to full virus expression is interrupted. If antiserum treatment is initiated after the primary steps of DNA amplification have occurred or if infectious virus is incompletely neutralized, virus expression cannot be suppressed. We have observed some animals with beginning signs of virus expression in the thymus or spleen prior to significant amplification of viral DNA. The results in Tables 3 and 4 also indicate that DNA amplification and full virus expression occur rapidly once animals become viremic. These results suggest that virus has to be continuously neutralized in order to prevent viremia, and, once this immunity breaks down, virus spreads rapidly. The results of Schäfer and coworkers (10) indicated that treatment of AKR mice with antibody is effective only when initiated during the first days of life. Because virus DNA amplification is characteristic for leukemogenesis in AKR mice as well as in BALB/Mo mice (21), it is likely that immunotherapy has similar effects on virus activation in both systems.

Alternatively, passive immunotherapy may work by cytotoxicity directed against cells that express virus. We cannot rule out this possibility; however, two considerations are not compatible with this interpretation: (*i*) Viremia was suppressed by purified $F(ab)_2$ fragments, arguing against antibody-dependent cytotoxicity directed against virus-releasing cells. (*ii*) Some animals became precipitously viremic after prolonged treatment with antiserum. This argues that cells *in vivo* retain the potential to express virus for several months. The failure to observe infectious centers in lymphatic cells from antiserum-treated animals indicated that the M-MuLV genome cannot be activated by incubating the cells *in vitro* in the absence of antibody (Table 5). Transplantation to normal BALB/c mice will have to be done in order to demonstrate whether activation of virus can be induced under *in vivo* conditions.

A third alternative to explain our results should be mentioned. Antibody may cause cell surface alterations that prevent virus genome expression by some as yet undefined regulatory mechanism. Indeed, antibody to gp70 can influence virus expression, as has been reported in a number of systems (22–25).

Our results clearly demonstrate that passive immunization of BALB/Mo mice with M-MuLV antiserum can prevent development of leukemia in the great majority of mice. None of 22 treated animals had developed leukemia at 55 weeks of age, at which 93% of untreated BALB/Mo mice had already succumbed to fatal leukemia (Fig. 2).

The results in this paper provide a molecular basis for understanding the mechanisms that may be responsible for immunotherapy of virus-induced leukemia. It will be interesting to explore whether a combination of passive and active immunotherapy could be used to protect BALB/Mo mice permanently against activation of their endogenous leukemia virus.

We thank E. Hoffman and K. Haase for expert technical assistance and Dr. J. Bilello for critical comments. This work was supported in part by a grant from the Deutsche Forschungsgemeinschaft and by a research contract from the National Cancer Institute (NOICP 71008). The Heinrich-Pette-Institut is supported by Freie und Hansestadt Hamburg and Bundesministerium für Jugend, Familie und Gesundheit, Bonn, West Germany.

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