Interstrain Resistance to Polyoma Virus Oncogenesis and Runting in Inbred Mice Treated with Antilymphocyte Serum (ALS) (34166)

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The inoculation of polyoma virus into newborn mice results in virus multiplication, antipolyoma antibody production and two types of pathological changes (1, 2): a degenerative type which may lead to what is called runting disease (3, 4) and a proliferative type with subsequent appearance of a variety of tumors (1, 3). Although inbred strains injected with a sufficient amount of a potent preparation of polyoma virus may respond with runting disease, tumor development, or both (5), there are quantitative variations which determine the state of resistance and susceptibility of the mouse strains (5–7). These variations are genetically determined (9). One or more genes are thought to control resistance, although exact gene function is not known (9, 10).

The recent use of heterologous antilymphocyte serum (ALS) as an immunosuppressive agent has stimulated interest in the immune response involved in transplantation rejection and tumor development (11–14). Several studies have reported that ALS may potentiate virus pathogenicity and oncogenesis in certain experimental animals (15–19). The present study was designed to examine the immune mechanism after treatment with ALS in two strains of mice genetically different in their resistance to polyoma virus. The C57BL/6J strain of mouse was selected as representative of polyoma tumor resistant and the DBA/2 as representative of polyoma tumor susceptible mice (9). Our primary interest was to determine the factors which mediate the genetically controlled resistance in these two strains of mice.

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Material and Methods. Mouse strains. Inbred C57/BL and DBA/2 mice originally obtained from Jackson Laboratory, Bar Harbor, Maine, were raised and bred in our laboratory. Animals were kept polyoma virus free as indicated by hemagglutination inhibition test (20). Mice exposed to virus were maintained on another floor by different attendants.

Polyoma virus. The LID-1 strain of polyoma virus was obtained from American Type Culture Collection and propagated in mouse embryo tissue culture (2). The amount of polyoma virus in kidneys of infected mice was estimated by determining the infectivity of serial dilutions of extract from emulsified kidney tissues. Virus $TCID_{50}$ was determined by the Reed-Meunch method using tube cultures of mouse embryo cells.

Virus hemagglutination (HA) and hemagglutination inhibition (HI) tests were carried out according to the method of Rowe *et al.* (20). The microtiter technique was used to determine HI antibody titer (21). In this study newborn DBA/2 and C57/BL mice were injected at different times with 5 HA units of virus in a 0.05 ml inoculum. Virus was injected into the subcutaneous space over the dorsal thorax.

Antilymphocyte serum (ALS). The ALS was prepared by hyperimmunizing 6–8 lb female New Zealand white rabbits. These rabbits were given six weekly intramuscular (hamstring) injections of 2×10^7 mouse spleen cells suspended in 2 ml of Hanks' balanced salt solution and complete Freund's adjuvant which was mixed in a 2:3 ratio. Rabbits were bled by cardiac puncture 1 week after the sixth injection. Serum was separated from clotted blood and titrated for

TABLE I. Incidence of Killing, Runting, and Tumor in DBA/2 and C57/BL Mice Treated at Different Intervals with Polyoma Virus and ALS.

Animal group no.	Agents inoculated[a]	Animals inoculated		Survivors 1 month after inoc.		Survivors with runting		Survivors 2 months after inoc.		No. of animals dev. tumors within 6 months after birth	
		DBA	C57	DBA	C57	DBA	C57	DBA	C57	DBA	C57
1	Virus, 1–3 d ALS, 1–3 d	41	25	7	19	7	19	3	14	3[b]	14
2	Virus, 1–3 d ALS, 4–7 d	40	20	13	17	13	0	11	17	11	0
3	Virus, 1–3 d ALS, 14 d	12	11	10	10	0	0	10	10	10	0
4	Virus, 7 d ALS, 1 d	29	9	6	7	6	0	3	7	3[b]	0
5	Virus, 1 d ALS, 3–4 W	14	10	12	10	0	0	12	10	0	0
6	Virus, 3–4 W ALS, 3–4 W	20	14	20	14	0	0	20	14	0	0
7	Virus, 2–3 W ALS, 1 d	10	12	8	11	0	0	8	11	0	0
8	Virus, 1 d ALS, none	15	15	12	14	0	0	12	14	2	0
9	Virus, none ALS, 1 d	8	7	6	7	0	0	6	7	0	0
10	Virus, none ALS, none	8	8	7	8	0	0	7	8	0	0

[a] d = day after birth; W = week after birth.
[b] Latent period 8–9 weeks after infection.

leukoagglutinating activity against spleen cells (22). ALS with leukoagglutinating titers of 1:128 or greater was pooled and stored in 5-ml vials and kept at −20°. ALS was inactivated at 56° for 30 min before use. Newborn mice were injected intraperitoneally with 0.1 ml of ALS twice a week for 4 weeks.

Histology. Tissues were taken from mice which were runted or had gross tumors and placed immediately into either 10% formalin solution or Carnoy's fixative. Specimens were imbedded in paraffin and 3 μ sections were prepared and stained with hematoxylin–eosin or methyl green pyronine.

Results. The early effect of polyoma virus and ALS on newborn DBA/2 and C57/BL mice. When DBA/2 mice were injected within the first week of life with polyoma virus and ALS a high incidence of killing was observed. Fewer than one-third of the animals survived 1 month and nearly all were dead by the end of the second month (Table I, Groups 1, 2, 4). No significant killing effect was seen if either ALS, virus, or both agents, were given 2 weeks or more after birth (Table I, Groups 3, 5, 6, 7). The peak killing effect occurred between the second and third week of life when virus and ALS were inoculated within the first day of life or when virus was given the first day and ALS a week later. If virus was given a week after birth to mice which received ALS on the first day, the peak killing effect occurred between the third and fourth week of life (Fig. 1). All the mice which died between the second and fourth week of life showed failure to grow,

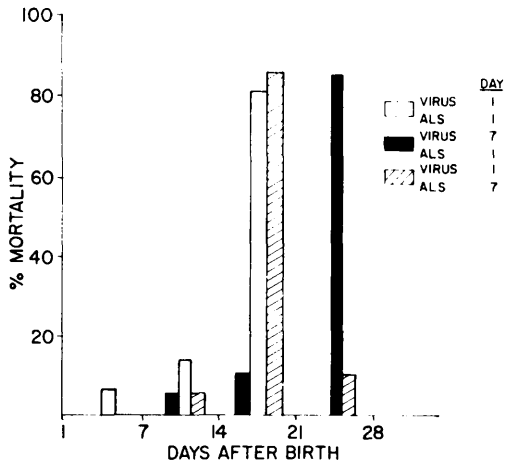

FIG. 1. Occurrence of early mortality in DBA/2 after virus and ALS; neonatally inoculated DBA/2 mice died 2–3 weeks after polyoma virus was given: ALS and virus day 1(Table I, Group 1); ALS day 1, virus day 7(Table I, Group 4); virus day 1, ALS day 7(Table I, Group 2).

lethargy, hair loss, and some of them also had diarrhea previous to their deaths. This syndrome appeared to be early runting.

Treatment of C57/BL newborn mice with ALS, virus, or both, produced no significant killing effect (Table I, Groups 1–10).

The late effect of polyoma virus and ALS on newborn DBA/2 and C57/BL mice. All the DBA/2 mice in Groups 1, 2, and 4 of Table I, which survived 1 month showed runting and all of those mice which survived 2 months or longer developed tumors within 3 months. The DBA/2 mice which were injected with virus on the first day and ALS 2 weeks after birth developed tumor without runting (Table I, Group 3). Two of 12 DBA/2 mice which were neonatally inoculated with polyoma virus alone developed tumor during the sixth month (Table I, Group 8). The DBA/2 mice neonatally inoculated with ALS alone did not develop tumors. The DBA/2 mice treated with either virus, ALS, or both, 3 weeks or later after birth, did not develop tumors (Table I, Groups 5–10).

All C57/BL mice treated with virus and ALS within the first 3 days after birth developed mild runting and only a few died (Table I, Group 1). All mice which survived 2 months or longer developed tumors within 4 months (Table I, Group 1).

The effect of ALS on the latent period of polyoma tumor production in both strains of mice. When newborn DBA/2 mice were inoculated with 5 HA units of polyoma virus, only 2 of 12 mice developed tumors in the next 24 weeks after birth (Table I, Group 8). When ALS and virus were given to DBA/2 mice in the first week of life, 2 of 11 had tumors 9 weeks later and all developed tumors by week 14. When ALS treatment was given 2 weeks after neonatal virus inoculation, 2 of 10 animals developed tumors 14 weeks after virus injection and all had tumors by week 19 of life (Fig. 2).

When C57/BL mice were injected with ALS and virus within 3 days of life, 4 of 14 mice developed tumors 9 weeks after birth. Nine of the 14 mice had tumors by week 15 of life (Fig. 3). When neonatally infected C57/BL mice were treated with ALS on the fourth day or later after birth, or, when ALS was not given, mice did not develop tumors within the 6 months of observation (Fig. 3).

The effect of ALS on polyoma virus growth and antipolyoma antibody production in DBA/2 and C57/BL mice. To study the

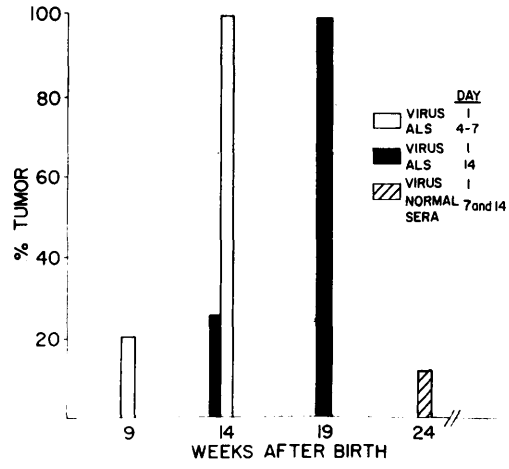

FIG. 2. Occurrence of tumor in DBA/2 after virus and ALS; tumor development was associated with the timing of ALS treatment: virus day 1, ALS day 4–7 (Table I, Group 2); virus day 1, ALS day 14 (Table I, Group 3); virus day 1, normal rabbit sera control series for 7 and 14 (2 of 20 mice in 6 months).

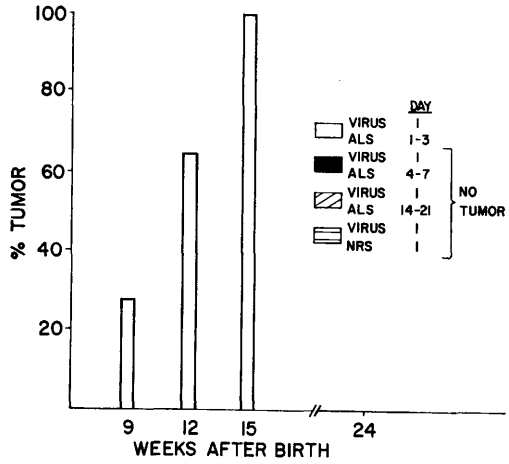

FIG. 3. Occurrence of tumor in C57/BL after virus and ALS; only C57/BL mice given ALS and virus within 3 days of birth developed tumors (Table I, Group 1).

effect of ALS on growth of polyoma virus and antipolyoma antibody production, newborn DBA/2 and C57/BL mice were infected with virus and treated with ALS within the first day of life. Two mice from each strain were sacrificed at 3-day intervals after virus inoculation. The titers of polyoma virus in 0.1 g of kidney tissue and the HI antibody titers of the sera of DBA/2 mice treated with ALS were not significantly different from those of untreated DBA/2 mice. The kidney virus titer and the HI antibody titer of C57/BL treated with ALS were not significantly different from those of the untreated C57/BL mice (Table II).

Gross and microscopic findings in organs of ALS and virus-treated DBA/2 and C57/BL mice. Necropsy examination of organs in ALS-treated mice showed only splenomegaly in both strains of mice. Microscopic section of the lungs, brain, liver, and heart showed no abnormalities. In some mice receiving virus and ALS, or virus alone, nuclear degeneration in tubule cells was noted. The spleens of mice treated with ALS and virus showed reticulocytosis and lymphoid depletion. Lymphoid degeneration was also noted in animals treated with virus.

Discussion. Several workers have reported the development of the runting syndrome in mice infected with polyoma virus during the neonatal period (3, 4). The severity of the syndrome has been correlated with virus dose (3) and its incidence has been considered as one of the criteria to indicate polyoma tumor susceptibility (8). Using only 5 HA units of polyoma virus we found that neonatally inoculated DBA/2 and C57/BL mice grew normally without runting or developing tumor. In other studies, we found that the injection of newborn mice with 0.1 ml of ALS twice a week for 4 weeks did not effect their growth or survival. However, the results were quite different when ALS and virus were given together in the neonatal period.

Combined treatment of DBA/2 mice with virus and ALS results in an accelerated runting-like syndrome which terminates in early death. The mice which died showed loss of

TABLE II. Antihemagglutinin (HI) Titer and Polyoma Virus Titer in ALS Treated and Untreated Newborn Mice.

Animal strain	Titers	Weeks after virus infection 2	3	5	Condition
DBA/2	HI[a]	2560	2560	640	
	Virus[b]	5	4		ALS treated
	HI	2560	1280	640	
	Virus	5	5		ALS untreated
C57/BL	HI	1280	2560	320	
	Virus	5	5		ALS treated
	HI	1280	1280	640	
	Virus	5	3		ALS untreated

[a] Reciprocal dilution of antibody titer.

[b] Negative log of polyoma virus end point/0.1 g of kidney.

weight, hair loss, weakness, lethargy, and some diarrhea before death. Microscopically, lymphoid depletion was a persistent finding. The early killing effect was thought to be virus dependent since death usually occurred 2–3 weeks after virus injection (Fig. 1) and variation in the sequence of ALS or virus injection within the first 2 weeks of life did not alter this period. The killing effect was unrelated to antipolyoma antibody level since both strains of mice showed a similar antibody response to virus, and ALS treatment did not significantly suppress antibody production in either one (Table II). Moreover, the killing effect did not seem to be caused by an accelerated growth of virus in the animal tissue, since the virus content in tissues from ALS treated and untreated animals of both strains was the same (Table II). It seems possible that acceleration of runting results from the effect of ALS on a certain type of immune cells and this suggests that immunity to polyoma virus induced runting is of the cell-associated type. This agrees with other reports that immunity to virus infection is mediated by cells associated with delayed type hypersensitivity (19, 23).

The more resistant C57/BL mice did not die when given both ALS and virus in the neonatal period. However, these mice showed mild runting during the second and third weeks after virus injection. This implies that C57/BL mice are more resistant to runting than DBA/2 mice, which is in agreement with our speculation that runting and tumor production are manifestations of alteration of the same mechanism. All DBA/2 mice which survived runting longer than 2 months developed malignant parotid gland tumors. However, tumor development was not always preceded by runting (Table I, Group 3), which suggests that runting represents the most severe stage of immunosuppression that can be tolerated by these animals. Those which survived and developed tumors did so either as persistent runts or after apparent recovery from runting. Two weeks appeared to be the critical period for the development of runting, since inoculation with ALS and virus after this time induced tumor but none of the DBA/2 mice underwent runting. When both agents were given 3 weeks after birth, neither runting or tumor developed within 6 months which implies that DBA/2 mouse resistance to polyoma virus is determined by a developmental level of the lymphoid system. The critical period for inoculation of the C57/BL mice was the first 3 days of life. After that time neither runting nor tumor was observed in the 6-month period (Table I). This implies that the developmental level of resistance of C57/BL lymphoid system to polyoma virus occurs earlier when compared with that of DBA/2 mice. Again, the question of whether the developmental resistance is a manifestation of the number or type of lymphoid cells remains unanswered.

The onset of tumor development appeared to be directly related to the time of ALS inoculation (Figs. 2, 3). This indicates that the potential of polyoma oncogenesis is dependent on the status of the immune mechanism of the animals. The development of tumors in C57/BL mice after treatment with ALS during the first 3 days of life indicates that enough immune suppression occurred to permit the development of mild runting and tumor formation. However, the failure of ALS treatment to initiate tumor production when ALS was given after 3 days (Fig. 3), indicates that a state of immune competence has developed in these animals. This state could be either the development of a threshold number of immune cells beyond which the ALS dosage was not effective, or it could be the maturation of certain immune cells which are not sensitive to ALS treatment. Development of this competent state in DBA/2 mice treated in the same way was delayed. ALS treatment within the first 3 days of life produced the most severe form of lymphoid atrophy and early death from accelerated runting. ALS treatment 4–7 days after birth decreased the mortality from runting and was associated with the earliest development of tumor in the survivors (Fig. 2). When ALS treatment was delayed until 2 weeks after birth, no runting occurred and tumors developed after a longer latent period (Fig. 2). When ALS was given after 3 weeks, no effect on viral oncogenesis was noted.

It seems, perhaps, that a state of immunological competence reflects the development of a threshold number of immune cells. This implies that the resistance in DBA/2 mice to polyoma virus which can be suppressed by ALS treatment sequentially, develops and reaches a competent state during the first 2 weeks of life. If this is the case, then the superiority of the resistance of C57/BL mice to that of the DBA/2 mice results from the rapid development of enough lymphoid cells to produce an immune competent state to polyoma virus. Therefore, this supports Habel's hypothesis which relates interstrain resistance to polyoma virus oncogenesis to differences in the immunological capacity of different mouse strains (24). The genetically controlled resistance to polyoma virus appears to be an expression of a developmental level of a certain number of immune competent cells.

Summary. Newborn DBA/2 mice and C57/BL mice were inoculated at various times in the postnatal period with either polyoma virus, antilymphocyte sera (spleen cell), or both. The DBA/2 mice runted and died in 2–3 weeks when virus and ALS were given within the first week of life. The C57/BL mice treated the same way did not die and some runted temporarily. All DBA/2 mice which received ALS and virus within the first 2 weeks developed malignant parotid gland tumors within 19 weeks after birth. The onset of tumor development was related to the time of ALS treatment. Only C57/BL mice which received ALS and virus within 3 days of birth developed tumors. Early killing, runting, and tumor production in DBA/2 mice and tumor development in C57/BL mice appear to be associated with the developmental level of the animals.

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