Effect of Antilymphocyte Serum on Reovirus Infection of Mice

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Reovirus type 3 infection in mice was investigated, with particular reference to the effect of antilymphocyte serum. With virus inoculation, a characteristic acute disease was manifested in the mice younger than 5 days old but not in mice 7 days old or older. However, a single injection of antilymphocyte serum, which was prepared in rabbits immunized with mouse thymocytes, followed by virus inoculation induced the acute disease in mice 7 days old or older. The latter result suggests that this production of the acute disease may be due to a suppression of the cellular immune mechanism sensitive to antilymphocyte serum.

Reovirus infection has been known to cause an acute, sometimes fatal disease in newborn mice (16, 17) but not in older mice (13, 16). Development or failure of development of acute symptoms of the viral infection may be greatly influenced by an age-dependent defense mechanism of the host. This host defense mechanism may include cellular and humoral immune responses, production of interferon, or other physiological factors (3). Accumulated data have suggested that the cellular immune mechanism may be one of the important defense mechanisms against various viral infections (7). In this respect, it was of interest to investigate the significance of cellular immune mechanisms in reovirus infection of mice, since a suppressive effect of the administration of heterologous antilymphocyte serum (ALS) on thymus-dependent cellular immunity has been well documented in several experimental systems (7, 11).

The present paper describes the findings in studies of the effect of ALS on the pathogenesis of reovirus type 3 infection of mice and discusses the significance of cellular immune mechanism in the viral infection.

MATERIALS AND METHODS

Mice. The dd strain of inbred albino mice obtained from the Mouse Center of Tohoku University was used.

Virus. The Dearing strain (15) of reovirus type 3 kindly supplied by I. Tagaya, National Institute of Health of Japan, was used. The virus was grown in L-cell cultures. The harvested virus was stored at -20°C until used. Infectivity of the virus was assayed by a conventional procedure with L cells in monolayer cultures.

Virus inoculation of the mice. The virus in 10^4 50% tissue culture doses, corresponding to about 100 50%

infectious doses when tested in 2-day-old mice, was inoculated subcutaneously in the dorsal region of the mice. Each litter of suckling mice inoculated with the virus was maintained in one cage with one lactating mother mouse.

Titration of serum antibody. Antibody to reovirus type 3 in sera of the mice was titrated by an indirect immunofluorescence assay. Cover-slip cultures of HeLa cells infected with the virus were first exposed to serially diluted sera of mouse to be tested and then reacted with antimouse globulin goat serum globulin labeled with fluorescein isothiocyanate. The final dilution of the serum showing positive specific staining was expressed as the titer of antibody against the reovirus.

ALS. ALS was prepared by the procedure described by Levey and Medawar (10). Rabbits were injected intravenously on alternate weeks on four occasions with approximately 10^8 lymphocytes collected from thymuses of 4-week-old dd mice. The rabbits were bled 1 week after the last injection. The antisera were pooled and absorbed with the mouse erythrocytes until the hemolytic activity was completely removed. After the absorption, the serum was inactivated at 56°C for 30 min, passed through a membrane filter (450 nm; Millipore Corp., Bedford, Mass.), and then stored at -20°C . A single intraperitoneal injection of 0.02 ml of ALS per g of 2-day-old mouse body weight diminished peripheral lymphocyte counts more than 25% within 4 hr, and this state continued for at least 24 hr. The counts of peripheral granulocytes were not significantly affected. The ALS titered 1:2,560 in agglutination tests with mouse thymocytes containing 10^7 cells/ml.

A 0.02-ml amount of ALS per g of mouse body weight was injected intraperitoneally into each mouse 4 hr before the virus inoculation, unless otherwise stated. For control purpose, normal rabbit sera were tested, and no significant effects on the course of the reovirus infection were observed.

RESULTS

Age dependency of manifestation of the acute disease in reovirus-infected mice. Morbidity and mortality of the reovirus-infected mice were first examined in relation to age. The clinical features of the acute disease observed in the present studies were similar to those described in the previous reports (14, 17). Thus, the incubation period of the disease was between 7 and 10 days, with delay of onset of disease in older mice. The main symptoms were incoordination, disappearance of milk

TABLE 1. *Morbidity and mortality of mice at various ages by the infection with reovirus type 3*

Age of mice (days)	Morbidity	Mortality
2	8/8	8/8
2	9/9	9/9
3	8/8	8/8
3	8/8	7/8
4	6/6	0/6
4	7/7	3/7
5	7/7	5/7
5	9/9	2/9
6	2/8	1/8
6	3/9	1/9
7	0/6	0/6
7	0/8	0/8

TABLE 2. *Effect of antilymphocyte serum (ALS) on morbidity and mortality of 7- and 8-day-old mice infected with reovirus type 3*

Group	Virus inoculation	ALS treatment	Morbidity	Mortality
1	Yes	1× ^a	7/7	1/7
	Yes	1×	6/6	6/6
	Yes	1×	9/9	4/9
	Yes	1×	9/9	9/9
	Yes	1×	9/9	1/9
	Yes	1×	6/6	6/6
	Yes	1×	10/10	3/10
	Yes	2× ^b	10/10	9/10
	Yes	3× ^b	12/12	9/12
2	Yes	3×	10/10	0/10
	Yes	None	0/4	0/4
	Yes	None	0/9	0/9
	Yes	None	0/6	0/6
3	Yes	None	0/8	0/8
	No	1×	0/4	0/4
	No	2×	0/10	0/10
	No	3×	0/5	0/5

^a ALS was injected 4 hr before the virus inoculation.

^b ALS was injected two or three times once a day from 1 or 2 days before virus inoculation.

TABLE 3. *Effect of antilymphocyte serum (ALS) on morbidity and mortality of 4-week-old mice infected with reovirus type 3*

Group	ALS treatment	Morbidity	Mortality
1	None	0/9	0/9
2	1×	5/5	0/5
	1×	6/6	5/6

tank, emaciation, ataxia, cramp, and diarrhea with occasional steatorrhea. Oily hair and jaundice occurred less frequently. In most cases, the period of the acute illness continued 7 to 10 days after the onset, and death usually occurred during this period. Retardation of the growth was also seen in most mice. This was evident even in the mice which had recovered from the acute symptoms and which survived for more than 1 month.

The results of a typical series of experiments are summarized in Table 1. Manifestation of the acute symptoms was evident in all 2- to 5-day-old mice but only in a portion of 6-day-old mice. None of the acute symptoms appeared in the 7-day-old mice during the 4 weeks of observation. Mortality of the infected mice in each age group was roughly parallel to morbidity. The appearance of characteristic acute symptoms did not always terminate fatally, and it was evident that morbidity was more significant than mortality for evaluating the significance of the reovirus infection.

Effect of treatment with ALS on the virus infection of mice. The effect of ALS on the reovirus infection of mice was investigated. Mice aged 7 and 8 days were injected with ALS at daily intervals on one, two, or three occasions and inoculated with the virus 4 hr after the last treatment (group 1). Two groups served as controls, one with virus only (group 2) and the other with ALS-treatment only (group 3). Table 2 summarizes the findings. None of the mice in group 2 showed signs of the acute disease and this agreed with the preceding findings. Symptoms or signs were not observed on the animals in group 3, except for lymphopenia. By contrast, the characteristic acute disease caused by reovirus infection was observed in all of the mice treated with ALS followed by virus inoculation (group 1). Morbidity in this group was 100%, and the mortality was usually high also. The symptoms which appeared in the mice of this group were comparable to those observed in the younger mice infected with the virus alone. The frequency of administration of ALS did not significantly affect the morbidity, showing that the dose of ALS used in a present single injection

TABLE 4. *Development of antibody against reovirus type 3 in sera of mice 4 weeks after the virus inoculation*

Group	No. of tested mice	Antibody titer ^a
Six-week-old noninfected control mice	5	<10, <10, <10, <10, <10
Two-week-old mice infected with virus	6	2,560, 2,560, 640, 640, 640, 640
Two-week-old mice infected with virus after pre-treatment with ALS	3	>2,560, >2,560, 2,560

^a Reciprocals of serum dilution.

was sufficient for the induction of this viral disease.

The capability of ALS to induce the acute illness in older mice was also investigated. Table 3 shows the results obtained from experiments with the 4-week-old mice. All of the mice treated with ALS and then inoculated with virus manifested symptoms similar to those observed in the case of younger mice.

Humoral antibody response in the virus-infected mice. It was of interest to determine whether the treatment of mice with ALS caused a change in humoral antibody response against infection with reovirus. The antiviral antibody titer of sera from mice in the three groups was examined. Apparently healthy mice (group 1) aged 6 weeks showed no detectable antibody to reovirus type 3 (Table 4). All of the mice inoculated with the virus at the age of 2 weeks (group 2) showed high titers of the antibody 4 weeks after the virus inoculation. The three survivors, which had been inoculated with the virus after pretreatment with ALS at the age of 2 weeks (group 3), were tested for antibody 4 weeks after the virus inoculation. The antibody titers in this group were somewhat higher than those in group 2.

DISCUSSION

The present studies clearly showed the ability of ALS to potentiate the pathological consequences of reovirus type 3 infection in 7-day-old or older mice infected with the virus. Such illness did not occur in untreated animals. It may be emphasized that the single injection of a small dose of ALS was able to induce the acute disease in essentially all of the mice. The effectiveness of ALS in suppressing cellular immune response has been shown in many viral infections, such as vaccinia (9), herpes simplex (12, 18), yellow fever (5), lymphocytic choriomeningitis (4, 8), murine leukemias (2, 6), adenovirus type 12 (1), and polyoma (2), although the mechanism of action of ALS was not fully resolved. The marked amplification of reovirus infection by pretreatment with ALS may be similarly explained as a consequence

of suppression of cellular immunity. If this is true, the following may be considered. First, the higher susceptibility to reovirus infection at younger ages of mice seems to indicate the difference between the maturation of the cell-mediated immune mechanism in young infant mice and that in older mice. In the present studies, the 5-day-old or younger mice might have been immunologically immature for induction of such cell-mediated immunity, whereas the 7-day-old or older mice might possibly have been mature. Second, at least a part of the mechanisms of both the silent infection in older mice and the recovery from acute illness in younger mice after reovirus infection can be explained by the action of cellular immune mechanisms. It may be concluded from the present and previously accumulated data that a cellular immune mechanism plays an important role on the recovery from the acute disease.

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