Pathogenesis of Cerebellar Hypoplasia Produced by Lymphocytic Choriomeningitis Virus Infection of Neonatal Rats: Protective Effect of Immunosuppression with Anti-Lymphoid Serum

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Intracerebral inoculation of 4-day-old rats with lymphocytic choriomeningitis virus (E-350 strain) produced a nonfatal, acute, severe, and permanent cerebellar necrosis with minimal histological evidence of inflammation. Virus persisted in the brain at high titers for 30 to 40 days and was finally cleared about 120 days after infection. Rabbit anti-rat lymphoid serum prevented cerebellar necrosis, and brain virus titers were higher than in control animals receiving normal rabbit serum. Thus, the immune response which clearly is responsible for the cerebellar lesion also plays a role in the gradual suppression of infection.

Intracerebral inoculation of lymphocytic choriomeningitis (LCM) virus into 4-day-old suckling rats results in a profound destructive lesion of the cerebellum (1, 9). This pathology is associated with a nonlethal transient choriomeningitis. In the mouse, the natural host, intracerebral inoculation of LCM virus results either in an asymptomatic central nervous system infection when given to newborns or in an acute fatal convulsive diathesis with no morphological evidence of central nervous system parenchymal involvement when administered to adults (6). Thus, the pathological picture produced in rats differs sharply from the classical responses of the mouse to LCM virus infection.

Considering the well established immunological nature of LCM virus-induced disease in mice (4-6), the present study was conducted to determine whether the disease in rats also is immune-mediated or whether it is due to a cytotoxic effect of LCM virus on the granule cell population.

MATERIALS AND METHODS

The animals, virus, and methods for virus titration, complement-fixation tests, immunofluorescent staining, and histological procedures have been described previously (9).

Anti-lymphoid serum (ALS). The method of Levey and Medawar (7) was used. Thymus cells, obtained from 6- to 14-week-old rats, were suspended in minimal Eagle medium containing L-glutamine, penicillin, and streptomycin. New Zealand white

rabbits, each about 2.5 kg in weight, were injected intravenously with 4 ml of this suspension, about 4×10^9 cells. A similar booster dose of cells was administered 2 weeks later, and the rabbits were bled the following week. In addition, commercially prepared rabbit anti-rat thymocyte sera (Microbiological Associates, Inc., Bethesda, Md.) was obtained. This serum was certified as extending skin grafts of ACI to Lewis rats from a mean survival time of 11.2 ± 0.8 to 20.3 ± 1.4 days.

Serum was heat inactivated at 56 C for 30 min and absorbed on rat erythrocytes in a ratio of 4 parts serum to 1 part red blood cells. The hemagglutinating titer for rat red blood cells was no more than 1:16. Normal rabbit serum (NRS) was similarly prepared. All sera were stored at -20 C until used.

ALS or NRS (0.1 ml) was inoculated intraperitoneally on alternate days from 1 day before to 20 days after virus.

Experimental design. At 4 days of age, litters were equated at eight pups, and within each litter animals received either LCM virus, 1,000 mean lethal doses, or an equal volume of diluent, 0.75% bovine plasma albumin in pH 7.3 phosphate-buffered saline, injected intracerebrally into the right hemisphere. Half the animals in each litter were given ALS and the other half NRS. Other groups of animals received either LCM virus alone, or LCM virus and intraperitoneal phosphate-buffered saline.

RESULTS

Neuropathological observations. ALS treatment of rats infected intracerebrally at 4 days of age with LCM virus prevented the development of the severe necrotic lesion of the cerebellum which occurred in animals not given

this immunosuppressive regimen (Fig. 1). No clinical signs of disease were apparent in the ALS treatment groups, whereas animals in all the other groups became ataxic 9 to 14 days after infection. A minimal choriomeningitis, first apparent 5 days after inoculation, was seen in animals given ALS and was resolved within the following 10 days. This was in contrast to the severe cellular infiltrate of mononuclear cells seen in the choroid plexuses and leptomeninges of non-immunosuppressed animals during the same period. Both ALS preparations

proved to be equally effective in abrogating the cerebellar pathology.

In order to determine whether pathological alterations would develop with the cerebellum after cessation of immunosuppression, rats were followed histologically for 6 months after termination of ALS. No lesions were found in the ALS-treated animals.

Immunofluorescent observations. The distribution of LCM viral antigen in ALS-treated animals, as assessed by immunofluorescent staining, was similar to that found in the

FIG. 1. Parasagittal views of brains of rats injected intracerebrally with the E-350 strain of LCM virus at 4 days of age and sacrificed 21 days after infection. (A) Animal treated with NRS. Hematoxylin and eosin, $\times 50$. (B) Animal treated with ALS. Hematoxylin and eosin, $\times 35$.

NRS-treated group. However, in contrast to rats given only virus in which the number of fluorescing cells were markedly reduced beginning 2 weeks after infection, immunosuppressed rats continued to exhibit intensely staining cells in the cerebellum, choroid plexus, hippocampal dentate gyrus, and olfactory bulb. After conclusion of the ALS treatment, viral antigen could be detected in diminishing amounts in these areas. However, even 7 months after infection, fluorescence could still be detected in small numbers of cells throughout the cerebral cortex, diencephalon, midbrain, and cerebellar inner granular cell layer.

Virological studies. The growth curves of virus in the brains of rats receiving ALS and in those receiving NRS were the same over the first postinoculation week (Table 1). Brain virus titers in the NRS-treated animals declined after this time, reaching minimal levels by 50 days after infection. On the other hand, titers remained high in the ALS-treated animals, gradually waning after discontinuance of immunosuppression, with trace levels of infective virus detectable as long as 6 months after inoculation.

Viremia was minimal and transient in the rats given NRS. In contrast, the ALS-treated groups evidenced significant levels of virus in their blood for at least a month after termination of immunosuppression.

Complement-fixing antibody. As in earlier studies, the nonsuppressed LCM virus-infected rats developed observable complement-fixing antibodies by the third week following intracerebral inoculation (Table 1). However, no antibody was detected in the rats given ALS until 3 months after the end of the serum treatment.

DISCUSSION

Intracerebral inoculation of 4-day-old rats

with LCM virus induces a profound cerebellar necrosis. Although the development of this pathology is associated with a severe choriomeningitis, there is a marked absence of more than a minimal inflammation evident by light microscopy within the evolving lesion. Such an observation led, initially, to the assumption that the lesion in the rat was due to a direct cytopathic effect of the virus upon the neuronal cells. The results of this study, however, clearly indicate that the cerebellar destruction is a consequence of immunopathological mechanisms, since immunosuppression prevented development of the lesion in the presence of persistent infection of the neural parenchyma.

ALS treatment markedly slowed virus clearance from the brain, and cessation of treatment was followed by a gradual decline in brain virus titers. Furthermore, suppressed rats had more pronounced viremias. These observations indicate that immune mechanisms play a role in LCM virus clearance. The fact that virus titers gradually decrease concomitantly with the evolution of the circulating antibody response suggests that antibody may contribute to this phenomenon. The simultaneous occurrence of viral clearance from the central nervous system of mice (2) or of rats (unpublished observations) neonatally infected with LCM virus and the appearance in the brain of bound immunoglobulin supports this possibility.

At least two possible reasons exist for the failure of a cerebellar lesion to develop after the termination of the ALS regimen. The establishment of an elevated viremia early in the infection and its persistence resulting from ALS treatment could lead to a transient state of "high zone" immunological unresponsiveness (8) in which virus-specific immune induction is either precluded or masked by the excessive levels of circulating viral antigen(s). As shown

TABLE 1. *Virus and antibody levels in rats after intracerebral inoculation with LCM virus at 4 days of age and effect of immunosuppression with anti-lymphoid serum^a*

Test	Treatment group	Days after inoculation									
		3	5	7	9	15	18	23	52	120	206
Brain virus	NRS	6.2	6.6	6.2	5.6	4.7	2.2	3.6	1.0	0	0
	ALS	6.8	6.4	6.6	6.8	5.6	5.4	5.4	3.8	1.8	tr
Blood virus	NRS	tr	tr	0	0	ND	0	0	0	ND	0
	ALS	0	0	≥1.0	≥1.0	ND	≥1.5	≥1.5	≥1.5	ND	0
Complement-fixing antibody	NRS	<15	<15	<15	<15	ND	<15	60	120	>520	ND
	ALS	<15	<15	<15	<15	ND	<15	<15	<15	168	ND

^a Virus levels expressed as log₁₀ adult mouse mean lethal doses per 0.03 ml of inoculum. ND, not done; tr, trace level. All values are averages of two to five individual specimens.

in Table 1, there was at least a 5- to 6-week interval between the time that ALS administration was discontinued and the time that circulating virus-specific antibody was first detectable. Although this would tend to explain the lack of cerebellar pathology during a period considerably beyond that coinciding with ALS treatment, it cannot account for the failure of pathology to develop after the appearance of an immune response to LCM virus.

The absence of the lesion at this time may be due to alterations in the cell membrane changes induced by LCM virus infection. It has been repeatedly observed that LCM virus-infected cell cultures gradually lose their sensitivity to lymphocyte-mediated cytolysis over a period of time (3); acutely infected populations contain a large proportion of cells with bright punctate immunofluorescent staining granules on their plasma membranes, in contrast to the very low proportion of cells in chronically infected cultures which exhibit such surface staining. The presence of at least two virus-specific antigens produced by LCM virus-infected L cells has been demonstrated (10); one of these appears on the cell surface and the other appears to be restricted to the cytoplasm. These observations suggest that one parameter that may be important in determining the susceptibility of target cells to immune-mediated cytolysis is the presence of the appropriate virus-induced membrane antigens, antigens which tend to be present on the plasma membrane for relatively short periods of time after infection.

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