

IMMUNOPATHOGENESIS OF ACUTE CENTRAL NERVOUS
SYSTEM DISEASE PRODUCED BY LYMPHOCYTIC
CHORIOMENINGITIS VIRUS

II. ADOPTIVE IMMUNIZATION OF VIRUS CARRIERS*

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(Received for publication 24 November 1971)

Lymphocytic choriomeningitis (LCM)¹ virus, when inoculated into the brains of adult mice, produces an acute inflammation of choroid plexus, ependyma, and leptomeninges and a marked convulsive diathesis, with death occurring 6–8 days after infection (1, 2). The immunological nature of this disease has been established by experiments utilizing a variety of immunosuppressive procedures (3–6). Immunosuppressed mice are protected, often permanently, against acute central nervous system (CNS) disease, and fail to show pathological evidence of choriomeningitis. Furthermore, immunosuppression does not alter the replication of virus in the brain (4), and in immunosuppression-induced LCM carriers, virus titers may remain at a high level for a long period (4, 5).

To further document that acute LCM is mediated by an immunological mechanism, attempts have been made to produce disease by adoptive immunization of virus carriers. Volkert and his associates (7, 8) have conducted detailed studies in which they transferred isogenic lymphoid cells from immune donors into neonatally infected carriers. They showed that such transfers could suppress viremia concomitant with the appearance of high titers of neutralizing (N) and complement-fixing (CF) antibodies. However, these successful transfers failed to produce acute CNS disease although there was a marked decrease in virus titers in the brain (9). Similar results have been reported by others (3, 10, 11).

The successful production of acute LCM by adoptive immunization of drug-

* Supported in part by grants AI 09401, NS 05627, and NS 09779 from the US Public Health Service.

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§ Supported in part by research career development award NS 46242 from the US Public Health Service.

|| Supported in part by research career development award NS 21945 from the US Public Health Service.

¹ *Abbreviations used in this paper:* ALS, anti-lymphoid serum; BP, basic proteins; CF, complement fixation; CNS, central nervous system; CY, cyclophosphamide; EAE, experimental allergic encephalitis; LCM, lymphocytic choriomeningitis; MEM, minimal essential medium; N neutralizing.

induced virus carriers is reported in this paper. In the preceding report (12) a comparison was made of the distribution of viral antigen in the brains of "neonatal" virus carriers inoculated within 24 hr of birth, and "drug-induced" carriers infected as adults and given a single dose of cyclophosphamide (CY). Parallel transfer of immune spleen cells into these two types of carriers failed to cause apparent illness in neonatal carriers but regularly caused lethal choriomeningitis in drug-induced carriers. The differing results with carriers produced in different ways suggests an explanation for prior failures, while the drug-induced carrier provides a potent experimental model for further analysis of the immunological mechanisms of classical LCM disease.

Materials and Methods

The virus and methods for virus titration, immunofluorescent staining, histological examination, and complement-fixation (CF) tests have been described previously (12). BALB/c mice (Flow Laboratories, Dublin, Va.) were used for all adoptive immunization experiments.

Virus Carriers.—Neonatal carriers were mice, inoculated intracerebrally within 24 hr of birth with 1000 LD₅₀ of the E-350 strain of LCM virus and held until age 8 wk or older. Drug-induced carriers were 10–12-wk-old mice, inoculated intracerebrally with 1000 LD₅₀ and given a single intraperitoneal injection of CY 150 mg/kg, 3 days later.

Donors for Adoptive Immunization.—Adult BALB/c mice were given an intraperitoneal injection of about 1000 LD₅₀ in 0.5 ml. At intervals of 1–2 wk four additional intraperitoneal doses of virus were administered, the last one 7–10 days before cell transfer.

Cells for Adoptive Immunization.—Immune or normal donors were killed, spleens removed, and a cell suspension prepared by gently rubbing each spleen against a stainless steel mesh into a sterile Petri dish containing Eagle's minimal essential medium (MEM) in Hanks' balanced salt solution with penicillin (100 units/ml) and streptomycin (0.1 mg/ml). After removing rapidly settling clumps from the suspension, the cells were washed once in MEM and suspended in a volume of 1 ml/spleen, equivalent to about 2×10^8 nucleated cells/ml. About 70% of these cells were viable, by the trypan blue exclusion test. For adoptive immunization of virus carriers a standard dose of one spleen equivalent was injected intraperitoneally.

Immune Mouse Serum.—Serum was prepared by pooling blood from mice immunized as donors for adoptive immunization, obtained when animals were killed for spleen cells. It was inactivated at 56°C for 30 min, and administered undiluted, 0.25 ml/mouse, by tail vein injection.

RESULTS

Adoptive Immunization with Spleen Cells

Drug-Induced Adult Carriers.—When adult BALB/c mice were given a single dose of CY, 150 mg/kg, 3 days after an intracerebral inoculation of 1000 LD₅₀ of LCM virus, approximately 90% became virus carriers, while 10% died of choriomeningitis about 2 wk after infection (12). Carrier mice were given 2×10^8 spleen cells from immune donors, at 5–25 days after infection (Table I). When cells were transferred from 5–9 days after infection, mice consistently died with symptoms typical of acute choriomeningitis. Illness usually began 2 days before death with onset of hunched posture, ruffled fur, and generalized tremulousness. Subsequently, many animals developed convulsions, and dead

animals were often found in the characteristic terminal posture with front legs flexed and hind legs extended. The median interval to death was 6 days after cell transfer. When immune cells were transferred 17–25 days after infection, all mice developed symptoms of acute LCM, but mortality varied from 25 to 100% in different experiments, averaging about 70%. Also, these animals showed a slightly greater survival time (median 8 days after adoptive immunization).

Carriers given cells from normal donors showed much lower mortality, but this varied according to the duration of the carrier state at the time of cell transfer. Transfer 5 days after infection of carriers was followed by mortality

TABLE I
Mortality after Adoptive Immunization of LCM-Carrier Mice with Isogenic Spleen Cells

Type of carrier	Spleen cell donor	Days from infection to spleen cell transfer	Mortality for 30 days after spleen cell transfer	Days from cell transfer to death	
				Median	Range
Drug-induced adult	Immune	5	42/42	6	5–11
		9	16/16	6	5–8
		17–25	47/64	8	4–13
	Normal	5	8/28	9	8–12
		24	1/29	10	
	None*	(5)*	11/124	9	3–19
	(24)*	0/113			
Neonatally infected	Immune	75–138	0/51		
	Normal	75	0/11		

* Drug-induced carriers, held as observed controls; their 30-day mortality was calculated from the 5th and 24th days after infection, respectively.

in about one-quarter of recipients (8/28), somewhat above that seen in observed controls (11/124). When carriers were given normal cells 24 days after infection, mortality was trivial (1/29), and was similar to that seen in observed controls (0/113).

Neonatal Carriers.—Neonatal carriers were inoculated with LCM virus within 24 hr of birth and given spleen cells at 75–138 days of age. There was no mortality after transfer of cells from immune or normal donors (Table I). The transferred cells were aliquots of the same suspensions which produced disease in drug-induced carriers.

Neuropathological Observations after Adoptive Immunization.—

Drug-induced carriers: Drug-induced carriers were adoptively immunized, at 5 and 24 days after infection, and mice were perfused daily. In this experiment all animals died of acute CNS disease, 5 and 7 days, respectively, after

cell transfer. In both groups pathological changes were first noted 48 hr before death, but the CNS lesions showed important differences (Table II) and are described separately.

Carriers given immune spleen cells 5 days after infection developed a severe

TABLE II
*Neuropathological Lesions in Mice Inoculated Intracerebrally with LCM Virus: Comparison of Natural Disease with That Produced by Adoptive Immunization**

	LCM only	LCM + CY + immune cells on		LCM + CY + normal cells on	Neonatal LCM + immune cells on	
		PID 5	PID 25	PID 5	PID 75	PID 132
Time from cell transfer to sacrifice	—	5 days	7 days	5 days	8 days	17 days‡
Clinical status at sacrifice	Mori-bund	Mori-bund	Mori-bund	Normal	Normal	Normal
Inflammatory Changes§						
Choroid plexus	3	4	2	—	—	—
Leptomeninges	3	4	2	—	—	—
Vessels	—	1	1	—	—	—
Destructive Changes§						
Cerebellum, granule cells	—	—	1	—	—	—
Cerebellum, molecular layer	—	t	1	—	—	—
Cerebellum, white matter	—	—	1	—	—	—
Olfactory bulb	—	t	—	—	—	—

* LCM only: adult mice inoculated intracerebrally with LCM virus and dying 6-8 days later. LCM + CY + immune cells (or + normal cells): adult mice inoculated with LCM virus, given CY 3 days later, and subsequently adoptively immunized with spleen cells from immune or normal donors. Neonatal LCM: mice inoculated intracerebrally with virus within 24 hr of birth and adoptively immunized as adults. PID: days from infection to adoptive immunization.

‡ See Table IV for viremia and antibody levels in this group of mice.

§ Inflammatory changes: —, no lesions seen; 1, trace; 2, mild; 3, moderate; 4, severe. Destructive changes: —, no lesions seen; t, less than 1% of area affected; 1, 1-25%; 2, 25-50%; 3, 50-75%; 4, 75-100%. Median grade is recorded for each group of mice, based on 6-10 animals per group.

choroiditis (Fig. 1 *a*) and leptomenigitis (Fig. 2 *a*). In comparison with mice dying after receiving virus only, the inflammatory response was much more marked, although its evolution and composition was similar. The infiltrate first appeared in the region of the tenia choroidea and gradually spread to involve the whole choroid plexus. Cells with large, open nuclei (presumably monocytes) predominated, with a substantial minority (less than 25%) of polymorphonuclear leukocytes, and small numbers of lymphocytes. Elsewhere

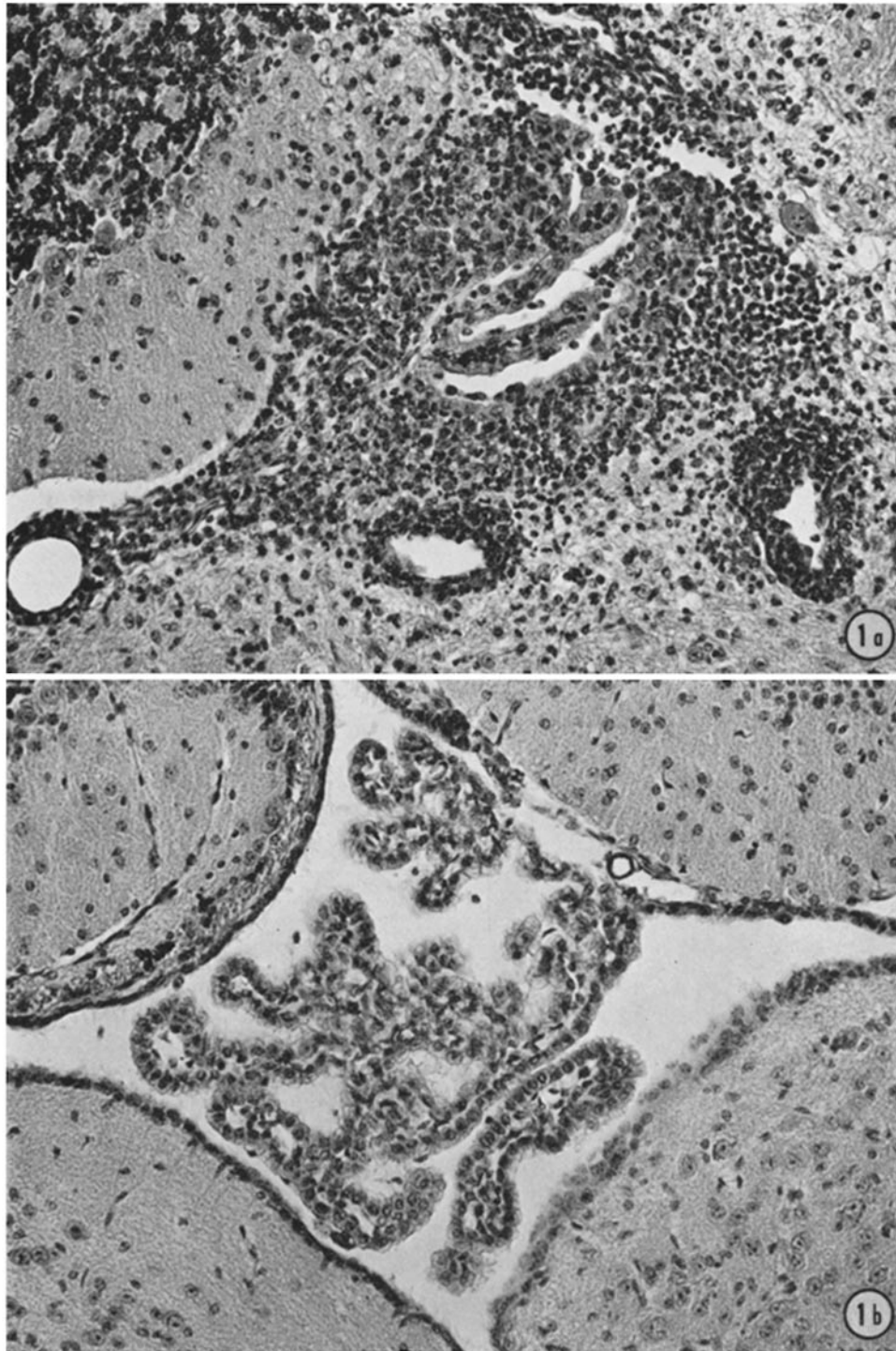


FIG. 1. (a) Severe choroiditis in an adult mouse inoculated with LCM virus intracerebrally (day 0), treated with CY on day 3, adoptively immunized with isogenic spleen cells from an immunized donor on day 5, and perfused when moribund on day 10. Hematoxylin and erythrosin. $\times 225$. (b) Apparently normal choroid plexus from a mouse inoculated with LCM virus intracerebrally within 24 hr of birth, adoptively immunized at 8 wk of age with isogenic spleen cells from an immunized donor, and perfused 5 days later. $\times 225$.

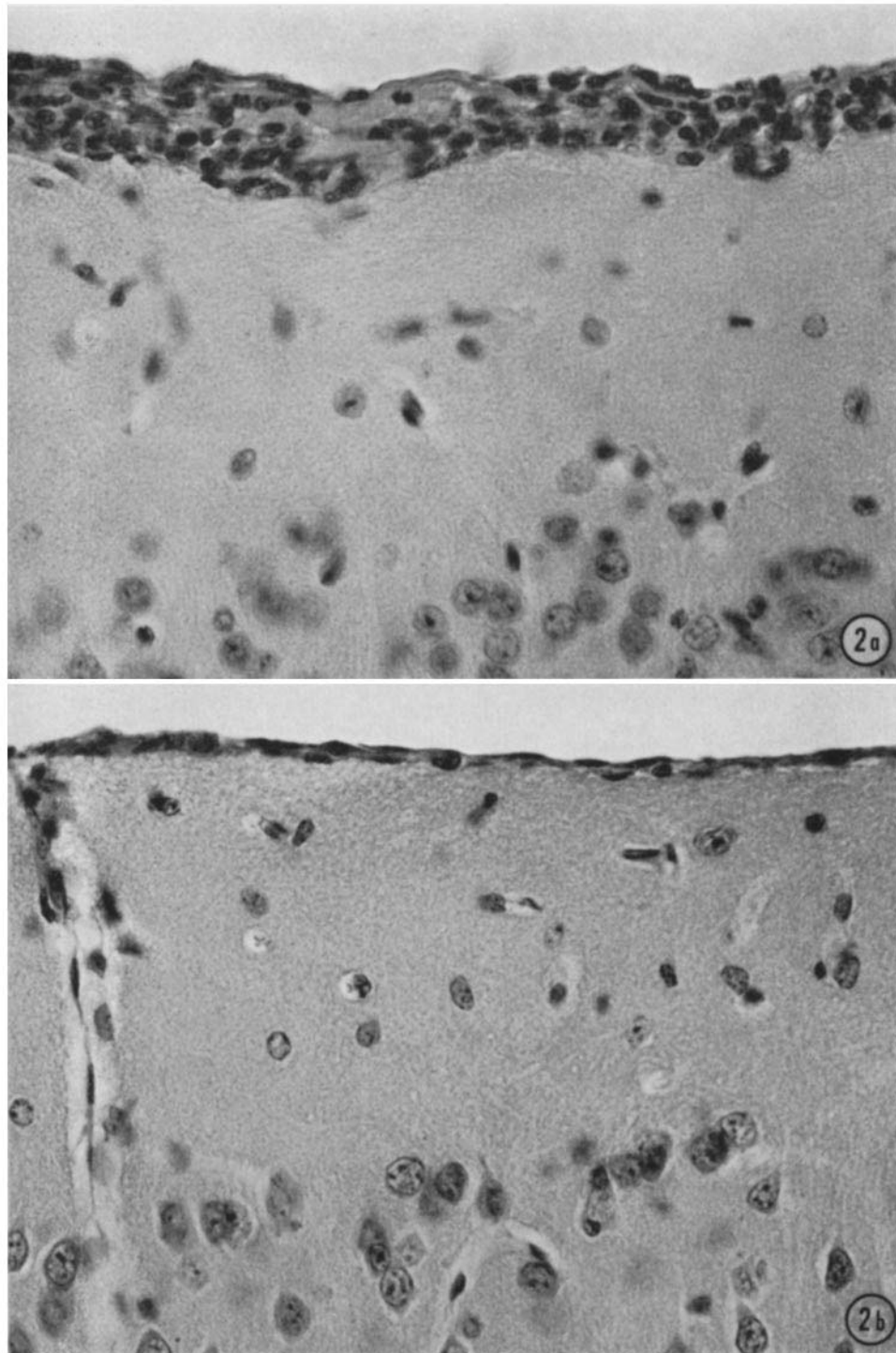


FIG. 2. (a) Severe leptomeningitis in an adult mouse inoculated with LCM virus intracerebrally (day 0), treated with CY on day 3, adoptively immunized with isogenic spleen cells from an immunized donor on day 5, and perfused when moribund on day 10. $\times 500$. (b) Apparently normal leptomeninges from a mouse inoculated intracerebrally with LCM virus within 24 hr of birth, adoptively immunized at 3 wk of age with isogenic spleen cells from an immunized donor, and perfused 8 days later. $\times 500$.

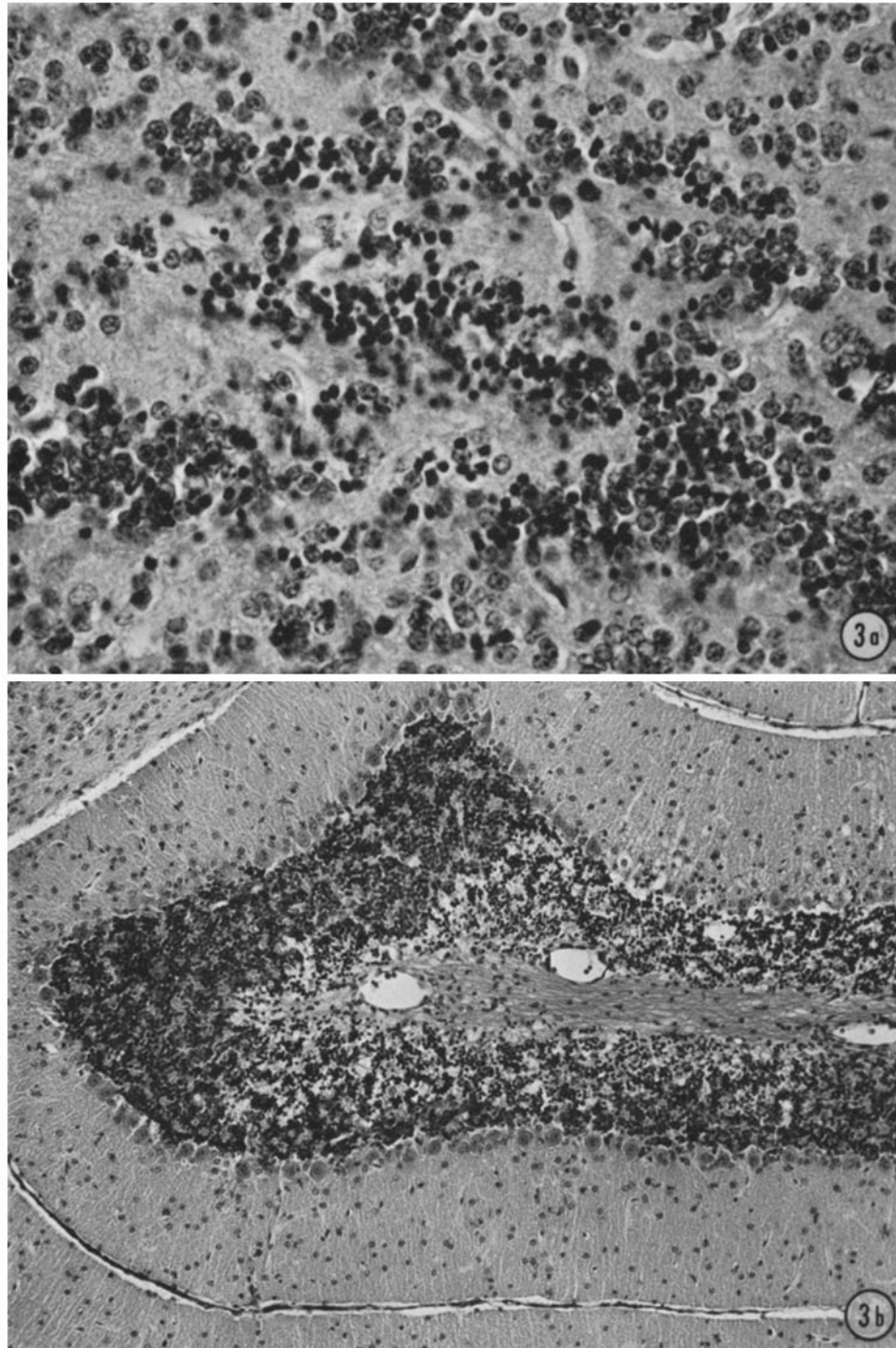


FIG. 3. (a) Olfactory bulb, to show focal necrosis of granule cells, in an adult mouse inoculated with LCM virus intracerebrally (day 0), treated with CY on day 3, adoptively immunized with isogenic spleen cells from an immune donor on day 5, and perfused when moribund on day 10. $\times 450$. (b) Folium of cerebellum to show necrosis of granule cells, in an adult mouse inoculated with LCM virus intracerebrally (day 0), treated with CY on day 3, adoptively immunized with isogenic spleen cells from an immune donor on day 24, and perfused when moribund on day 30. $\times 125$.

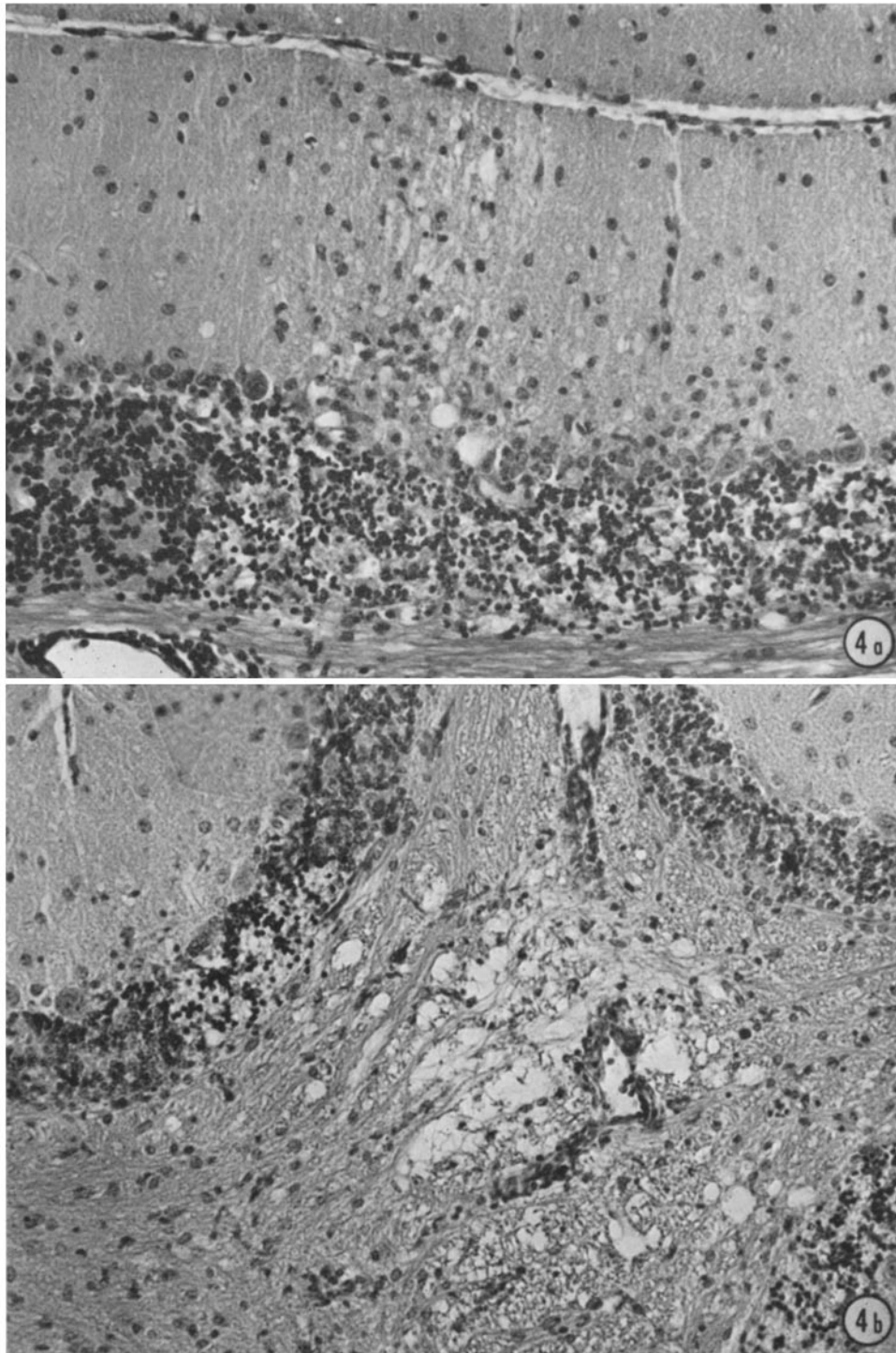


FIG 4. (a) Cerebellum, to show focal necrosis involving inner molecular layer, Purkinje cells, and granule cells, with cellular infiltrate in molecular layer. See Fig. 3 b for experimental conditions. $\times 250$. (b) Cerebellum, to show spongy vacuolization of white matter, perivascular cuffing, and foci of granule cell necrosis. See Fig. 3 b for experimental conditions. $\times 225$.

in the brain several other lesions were seen, but were relatively mild and infrequent. In the olfactory bulbs of about half the animals focal granule cell necrosis (Fig. 3 *a*) and focal spongy changes in the white matter were seen. All animals had scattered perivascular cuffs.

Carriers given immune spleen cells 24 days after infection, also developed choroiditis and leptomeningitis. However, this was always relatively mild and similar to that seen in mice dying after receiving only an intracerebral injection of virus (Table II). Perivascular inflammation was similar to that described above. Also, a few mice had definite focal softenings of white matter, usually in the pons, and sometimes associated with a perivascular cuff.

A striking constellation of lesions was present in the cerebellums of these mice. The most widespread was focal necrosis of granule cells, which involved

TABLE III
*Virus and Antibody Levels in Drug-Induced LCM Carriers after Adoptive Immunization with Immune or Normal Spleen Cells**

Spleen cell donor	Clinical status when sacrificed	Virus titers		Immunofluorescence in choroid plexus	CF titer
		Brain	Plasma		
Immune	Morbund	5.7	1.5	4	<12
Normal	Normal	5.8	1.6	4	<12
None	Normal	6.0	1.8	3	<6

* Mice adoptively immunized 5 days after infection and sacrificed 10 days after infection. Virus titers expressed as $\log_{10}/0.03$ g or 0.03 ml, and immunofluorescence graded on scale where 3 represents 50-75%, and 4 represents 75-100% of cells infected. Data are medians for three or more mice.

an estimated 25% of the total cell population (Figs. 3 *b* and 4 *b*). Individual nuclei became pyknotic, but did not disappear by the time of death. A few inflammatory cells were seen among necrotic granule cells. Common but less widespread was a spongy softening of the inner molecular layer, often accompanied by outfall of Purkinje cells. An infiltrate, primarily of monocytes, was usually seen in such areas (Fig. 4 *a*). Molecular layer involvement was often contiguous with necrosis of the underlying granule cell stratum. Finally, most animals showed focal softenings of the white matter of cerebellum, associated in some cases with perivascular inflammation (Fig. 4 *b*). After transfer of spleen cells from normal isogenic donors, drug-induced carriers showed no histological lesions in the CNS (Table II), consistent with their healthy appearance.

Neonatal carriers: Neonatal carriers as described above, remained asymptomatic after transfer of isogenic spleen cells from immune donors. When sacrificed 8 and 17 days after cell transfer, neither choroiditis (Fig. 1 *b*) nor leptomeningitis (Fig. 2 *b*) nor vasculitis was seen in their brains.

Virological and Serological Observations after Cell Transfer.—

Drug-induced carriers: Drug-induced carriers, after receiving spleen cells from immune donors, were compared with similar mice given cells from normal donors. There was no evidence of a terminal decrease in virus titer in brain or plasma or in the immunofluorescent staining of the brain (Table III). Plasmas from mice dying 5 days after immune cell transfer (10 days after infection) had little or no CF antibody (median <1:12).

Neonatal carriers: Neonatal carriers were tested 17 days after transfer of immune spleen cells (Table IV). Viremias, which were moderate to high in untreated carriers (median titer $10^{4.1}$ LD₅₀/0.02 ml) had dropped to trace levels in adoptively immunized carriers (median titer $10^{0.5}$). Conversely, CF antibody, which was not detected in untreated carriers, rose to very high levels

TABLE IV
*Viremia and CF Antibody Titers in Neonatally Inoculated LCM Carriers: Effect of Adoptive Transfer of Immune Spleen Cells**

		Carriers not given cells	17 days after cell transfer
Viremia	Median	4.1	.5
	Range	3.8-4.3	<0.5-0.7
CF antibody	Median	<6	3000
	Range	<6	200-12,000

* Adoptively immunized mice received cells 132 days after infection and all animals were killed 149 days after infection. Medians based on 3-14 animals. Viremia expressed as log₁₀ LD₅₀/0.03 ml plasma.

after cell transfer (median titer 1:3000). These findings, consistent with Volkert's prior studies (7), documented the efficacy of immune cell transfer in the same group of neonatal carriers which failed to show any CNS lesions.

Passive Immunization with Serum

A pool of immune serum was obtained from mice used as donors of spleen cells in adoptive immunization experiments. This was administered to drug-induced carriers, 5 days after infection, in a single dose calculated to give a low antibody titer, similar to that seen in carriers dying after receipt of immune spleen cells (Table III). Table V shows that a low mortality (5/22) was seen after serum transfer, but this was only slightly greater than that in the observed controls (7/51).

Titers of the donor serum pool and of plasmas of recipients are also shown in Table V. Antibody titers in recipients were similar to those predicted by the relationship between the volume of administered serum and the volume of the plasma compartment. Titers were very similar in viremic and normal recipients,

as reported by Volkert (13). The rate of decrease in antibody titer was roughly consistent with the 3–5-day half-life of homologous IgG in rodents (14).

DISCUSSION

Choriomeningitis after Adoptive Immunization.—The salient observations in the present study were: (a) Adoptive immunization of CY-induced adult LCM carriers with spleen cells from immune donors produced acutely lethal CNS disease with great regularity, particularly when cells were transferred within 1 wk after infection. (b) Cells from nonimmune donors or immune serum had little or no effect. (c) Neonatally infected carriers did not develop acute disease after adoptive immunization.

TABLE V
*Mortality and CF Antibody Titers after Passive Immunization of Drug-Induced Carrier Mice with Immune Serum**

Type of recipient	Type and titer of donor serum	Titer in recipients at indicated times after serum			Mortality for 30 days after serum
		6 hr	2 days	6 days	
Drug-induced carrier	Immune	6	12	6	5/22
	50	6	12	6	
		12	25	6	
Drug only	Immune	6	6	6	0/9
	50	6	6	6	
		25	12	6	
Drug-induced carrier	None				7/51

* Sera were administered as a single intravenous dose of 0.25 ml given 5 days after infection, to 20-g mice with an estimated 0.8 ml plasma volume. CF titers are given for individual animals.

This is the first report of an experimental system in which acutely fatal LCM can be produced with regularity by transfer of lymphoid cells. Prior studies of adoptive immunization (3, 7, 8, 10, 11) have utilized neonatally infected carriers as recipients. The reports of Volkert and associates (9, 15) indicated that before transfer such recipients had high virus titers in the brain and other tissues, and that grafting of cells from immune donors resulted in a marked reduction of virus titers in blood and tissues over a period of 2–4 wk with the concomitant appearance of high titers of CF and N antibodies. No clinical illness accompanied these changes. Oldstone and Dixon (11) grafted neonatally infected LCM carriers with isogenic immune spleen cells. Depending upon mouse strain, minimal or no round cell infiltrates were produced in leptomeninges or around vessels, but none of the recipients died of acute disease nor was choroiditis reported. Hotchin (3, 10) also transferred isogenic

immune lymphocytes, thymocytes, or bone marrow cells, and parabiosed immune mice to neonatally infected carrier mice. Acute CNS disease was not seen, although virus suppression was induced in some instances. Our results are in essential agreement, in that adoptive immunization of neonatally infected carriers with immune spleen cells, sufficient to suppress viremia and induce high levels of CF antibody, failed to produce clinical or histological evidence of CNS disease.

Since the same immune spleen cells which failed to produce disease in neonatal recipients, did produce acutely fatal choriomeningitis in drug-induced carriers, it appears that the type of carrier used as a recipient in transfer experiments plays a critical role in determining the subsequent occurrence of disease. One important difference between neonatally induced and drug-induced carriers is the distribution of viral antigen in the brain. In neonatal carriers, we found (12) relatively few immunofluorescent antigen-bearing cells in the choroid plexus or ependyma, consistent with the reports of Mims (16) and of Oldstone and Dixon (17). Drug-induced carriers, in contrast, have heavy infection of choroid plexus, with 50–100% of cells antigen-positive, as do adult mice dying of acute LCM after intracerebral injection of virus only (12, 18). This suggests that the choroid plexus of the drug-induced carrier presents a more extensive target for the anti-LCM immune response than does that of the neonatally induced carrier.

Another difference between the two types of LCM carriers is the degree of extraneural infection. Neonatal carriers have much more widespread tissue infection with higher levels of viremia than do CY-induced carriers. This may divert immune lymphoid cells so that fewer reach the choroid plexus as suggested by studies of delayed hypersensitivity or experimental allergic encephalitis (EAE).

Desensitization studies of delayed hypersensitivity (19) demonstrated a quantitative relationship between the dose of desensitizing antigen and the subsequent decreased response to graded intradermal doses of antigen. It was calculated that a delayed response could be elicited when the intradermal concentration of antigen exceeded the serum concentration by two- to fourfold, illustrating the powerful influence of intravascular antigen concentration on the local expression of cell-mediated immunity.

Studies of EAE indicated that immunopathological CNS disease, presumably dependent upon cell-mediated immunity, was prevented or suppressed (20) by encephalitogenic basic proteins (BP). Furthermore, it was recently reported (21) that BP could interfere with cellular transfer of EAE. When syngenic lymphoid cells from donor rats with actively induced EAE were transferred intravenously to normal rats, the recipients consistently developed EAE, but were protected if BP was injected intravenously shortly after adoptive immunization.

These considerations underline the potential importance of the approxi-

mately 100-fold difference in viremia titers between neonatal carriers (10^3 - 10^4 LD₅₀/0.02 ml) and drug-induced carriers (10 - 10^2 LD₅₀) at the time of immune spleen cell transfer. Consistent with this view is the observation that if CY-induced carriers received immune cells 5 days after infection, when they had little if any viremia, the severity of choroiditis was much greater, survival time shorter, and mortality higher than in drug-induced carriers given cells a month or more after infection when viremia was always present (12).

The immunological mechanism of acute choroiditis is illuminated by several observations: (a) Immune spleen cells transferred disease with great consistency, and the evolving pathology was strikingly similar in its tempo and character to classical LCM. (b) Antibody was not detectable in mice dying of acute LCM and, likewise, little or no antibody was present in plasmas of carrier mice moribund with LCM after immune spleen cell transfer. Furthermore, staining with fluorescein-conjugated anti-mouse immunoglobulin reveals few if any plasma cells and little bound immunoglobulin in the brains of mice with acute choroiditis (G. A. Cole and N. Nathanson, unpublished observations). (c) Immune serum failed to produce acutely fatal LCM when given to drug-induced carriers. Oldstone and Dixon (11) injected neonatal LCM carriers with immune serum by intravenous or intraspinal routes. Although they did not kill mice or produce convulsions, they did observe histological lesions in the CNS. The salient features of these lesions were an initial appearance of an acute, sometimes necrotizing polymorphonuclear response gradually resolving into a round cell infiltrate over the following week, and localization in perivascular and leptomeningeal areas after intravenous injection. In our view these features suggest that Oldstone and Dixon were producing lesions which differed significantly from the immunopathology pathognomonic of classical acute LCM. Since the weight of current evidence indicates that immune lymphoid cells are required for the successful transfer of acute choriomeningitis, it appears likely that the cell-mediated immune response plays a central role in the pathogenesis of classical LCM disease.

Cerebellar Necrosis.—The ability of LCM virus to cause cerebellar necrosis has now been observed in this laboratory under several circumstances: (a) in young rats, which are not killed by the E-350 strain of LCM, and are left with a marked ataxia due to almost total destruction of the cerebellum (22); (b) in 4-day-old mice which develop focal necrosis before death 8-12 days after intracerebral injection (23); (c) in adult drug-induced LCM carrier mice adoptively immunized 1 month or more after infection. Furthermore, other members of the arenavirus group appear to produce a similar lesion, as exemplified by Tacaribe virus (24) and Tamiami virus (D. H. Gildea, unpublished observations) infections of newborn mice.

The immunopathological nature of this lesion is suggested by its occurrence after adoptive immunization in the present study. Furthermore, anti-lymphoid serum (ALS) protects rats against LCM (A. A. Monjan, unpublished data) and

mice against Tacaribe-induced cerebellar disease (24). The relative paucity of inflammatory cells in areas of granule cell necrosis, in comparison with the marked inflammation of the choroid plexus, raises questions regarding the precise mechanism of the cerebellar lesion. This discrepancy is particularly notable in the 4-day-old rat where the cerebellum undergoes devastating liquefaction in the almost total absence of inflammation apparent in the light microscope (22).

An inconsistency in the relationship between sites of LCM infection in the brain and the subsequent localization of necrosis has been repeatedly observed. Pathological lesions occur only in areas with moderate to heavy concentrations of immunofluorescent antigen, but certain regularly infected nuclei are consistently uninvolved by subsequent necrosis. In the drug-induced adult carrier, immunofluorescent antigen was present in the cerebellum, both in the molecular layer and in granule cells, where pathological changes subsequently developed. However, necrosis was not seen in other parts of the brain, where there were infected cells, with the exception of a few minimal foci in the olfactory bulb. This discrepancy is much more dramatic in the 4-day-old mouse and rat, where there is widespread infection of the neural parenchyma and necrosis is confined to the granule cells of cerebellum (24). The mechanism whereby an immunopathological process selectively affects a few among many infected CNS nuclei is presently unknown.

SUMMARY

Lymphocytic choriomeningitis (LCM) virus carriers were established by intracerebral inoculation of adult BALB/c mice followed by a single dose of cyclophosphamide (CY) (150 mg/kg) 3 days after infection, and by intracerebral injection within 24 hr of birth. These carriers were then adoptively immunized with spleen cells or serum from immune or normal BALB/c donors.

Transfer of immune spleen cells into drug-induced carriers consistently resulted in acutely fatal choriomeningitis, histologically strikingly similar to classical LCM. Normal spleen cells or immune serum failed to produce either central nervous system (CNS) pathology or illness with any regularity. In addition, focal necrosis of the cerebellum was seen after adoptive immunization of drug-induced carriers but only when mice received cells at least 3 wk after inoculation, which is probably explained by the gradual spread of infection from membranes to the neural parenchyma during the first month after establishment of the carrier state in adult mice.

Immune spleen cells, when transferred to neonatal carriers, led to a decrease in virus titers in blood and brains and to development of antibody without acute CNS disease.

It appears that the production of fatal choriomeningitis after LCM infection is determined in part by the distribution of viral antigen, and this is markedly different in neonatal and drug-induced carriers at the time of cell transfer.

Another factor of potential importance is the much higher level of circulating viral antigen in the plasma of neonatal than in that of drug-induced LCM carriers.

Classical LCM disease can only be transferred by immune lymphoid cells and not by antiserum. Furthermore, little or no complement-fixing (CF) antibody was found in the plasma of mice dying of acute choroiditis. These observations strongly suggest that acute choroiditis is dependent upon the cell-mediated immune response.

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