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PATHOGENESIS OF CHRONIC DISEASE ASSOCIATED WITH
PERSISTENT LYMPHOCYTIC CHORIOMENINGITIS
VIRAL INFECTION

II. RELATIONSHIP OF THE ANTI-LYMPHOCYTIC CHORIOMENINGITIS IMMUNE
RESPONSE TO TISSUE INJURY IN CHRONIC LYMPHOCYTIC
CHORIOMENINGITIS DISEASE*

By MICHAEL B. A. OLDSTONE,† M.D., AND FRANK J. DIXON, M.D.

(From the Department of Experimental Pathology, Scripps Clinic and Research
Foundation, La Jolla, California 92037)

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The possibility of an immunologic basis for the tissue injury associated with persistent lymphocytic choriomeningitis (LCM)¹ viral infection in mice is suggested by several pieces of evidence. First, chronic LCM infection per se usually is not associated with cytopathogenicity. Mice persistently infected with LCM (LCM carriers) of most strains demonstrate high titers of virus in virtually all tissues and yet most of these tissues show little, if any, evidence of cellular injury (1-6). Also, LCM infection of a variety of cells in tissue culture is usually not accompanied by cell injury despite active viral replication (7-10). Second, adult mice inoculated with LCM virus develop an acute fatal disease only when they make an anti-LCM response. In such adult infections, immunosuppression, by several methods, prevents acute disease (11-15).

The LCM carrier state develops in mice inoculated with LCM virus shortly after birth (5, 16, 17) or infected transplacentally *in utero* (18). Such chronically infected mice carry high titers of virus in blood and organs and apparently make an anti-LCM antibody response throughout their life (6, 19). LCM carrier mice of several strains develop an associated chronic disease. Those strains carrying the largest amount of LCM virus and apparently making the greatest anti-LCM antibody response develop the earliest and most severe disease.

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¹ Abbreviations used in this paper: CNS, central nervous system; i.p., intraperitoneally; i.s., intraspinaly; i.v., intravenously; LCM, lymphocytic choriomeningitis; LCM carriers, mice persistently infected with LCM; PMN, polymorphonuclear leukocytes.

The present studies further define the role of the anti-LCM immune response in production of lesions in chronic LCM disease. Parabiosis of immune mice to isologous LCM carriers or transfer of immune lymphoid cells to isologous LCM carriers initiated and/or intensified chronic disease in the carriers of several murine strains. In addition, the passive transfer of isologous or heterologous anti-LCM antibody to persistently infected carrier mice caused the prompt appearance of acute necrotizing inflammatory lesions. This phenomenon seemed to result from the interaction of circulating anti-LCM antibody with antigen (virus or virus-induced) associated with cells. The acute inflammation was replaced several days later by a mononuclear infiltration reminiscent of that associated with classical delayed type hypersensitivities.

Materials and Methods

Host.—

Mice: Breeders for SWR/J, B10D2 old line and C₃H inbred strains were obtained from Jackson Memorial Laboratories, Bar Harbor, Maine. NZB breeders were obtained from Laboratory Animal Center, Medical Research Council, Carshalton, Surrey, England. Breeding was performed in our laboratories.

Virus carrier state: Newborn mice were inoculated within the first 15 hr of life with 1000 × LD₅₀ doses of virus prepared from infected isologous mouse brain. Details of virus passage, inoculation procedure, development of the LCM carrier state and associated chronic disease have been previously reported (20).

Immune state: Animals immune to LCM virus were obtained by both natural and acquired infection. Babies neonatally infected with LCM excrete large amounts of virus, thereby naturally infecting their mothers who do not develop overt disease. At the time of weaning, the mothers have good titers of circulating complement-fixing anti-LCM antibody. Similarly, adult mice artificially infected with intraperitoneal (i.p.) injections of sublethal doses of virus prepared from infected isologous tissue develop complement-fixing anti-LCM antibody. Adult male and female Hartley guinea pigs infected with i.p. and subcutaneous injections of virus demonstrated both anti-LCM-neutralizing and complement-fixing antibodies.

Virus.—

Source: Seed LCM virus used was mouse brain passage NIH strain CA 1371, lot 7022 provided by Dr. Wallace Rowe of National Institute of Allergy and Infectious Disease, Bethesda, Md. Methods of handling and diluting virus for inoculation have been previously described (20).

Titration: Virus titrations were carried out by intracerebral inoculation of serial 10-fold dilutions of virus into groups of 6–10 Swiss-Webster mice. Mice weighed 10–14 g and LD₅₀ titration end points were calculated by Reed and Muench method (21).

Anti-LCM Antibody.—

Determinations: Complement fixation and neutralizing tests for anti-LCM antibody have been previously reported (19). LCM complement-fixing antigen was kindly provided by Dr. Hercules and Dr. Parker of Microbiological Associates, Inc., Bethesda, Md.

Parabiosis, Cell and Serum Transfer.—

Parabiosis: Parabiotic union using coelomic attachment to obtain a common peritoneal cavity was performed by Dr. Sun Lee. Isologous mice of similar weights and ages were paired.

Combinations of parabionts used were: (a) immune to virus carrier, (b) immune to immune, (c) virus carrier to virus carrier, and (d) normal (LCM virgin) to virus carrier.

Cell transfer: Splens from immune, normal, or virus carrier mice were minced and passed through a 100-mesh nylon filter. The cells were washed twice in cold medium 199 and viability was checked with trypan blue. Specific procedures used to obtain, prepare, count, and test for viability of cells have been previously reported (22, 23). Nonviable cell preparations were obtained by rapidly freezing and thawing three times and cell viability was tested to ensure that all of the cells were dead. Isologous recipients were sacrificed at 4–6 hr, and 1, 3, 5, 7, and 14 days after intravenous (i.v.) injection of 7×10^6 viable or nonviable spleen cells. In several experiments, 7×10^8 viable spleen cells from immune donors were employed.

Serum transfer: Sera collected from immune isologous and heterologous donors were titered for anti-LCM antibody activity by complement fixation (mouse, guinea pig) and neutralization (guinea pig) tests. High titered anti-LCM mouse sera (complement-fixing titers above 1:256) or guinea pig anti-LCM sera (complement-fixing titer greater than 1:256 and neutralization titer 1 log or more protection) were pooled separately, passed through a 0.2μ Millipore filter and stored at -70°C until use. Pooled serum was injected i.v. or intraspinally (i.s.) (24). The i.v. schedule was injection of 0.2 ml serum i.v. and 0.25 ml i.p. at time 0, 12, 24, and 30 hr. Animals were sacrificed 36 hr, 4, 7, and 14 days after the initial injection. Animals injected i.s. received 0.02 ml serum intrathecally via a high lumbar-low thoracic intravertebral space. A short bevel 26 gauge needle was used and animals were sacrificed 4–6 hr, 1, 3, and 7 days after inoculation.

Immunofluorescent and Histopathologic Techniques.—

Immunofluorescence: Tissue blocks were snap-frozen in liquid nitrogen and sections 4μ thick were cut, fixed, and examined by the direct immunofluorescent technique (7, 25). Fluorescein-labeled rabbit anti-mouse γ -globulin, anti-mouse albumin, anti-mouse C3 (third component of complement), anti-guinea pig C3 and anti-rat fibrinogen, plus guinea pig anti-LCM monospecific antisera were used. Details of the preparation of these reagents have been reported (25, 26).

Histology: Formalin-fixed paraffin sections of tissue were stained by hematoxylin and eosin and periodic acid-Schiff (PAS) methods. In addition to single sections of lymph node, thymus, heart, lung, skeletal muscle, kidney, adrenal, spleen, pancreas, and omental fat, multiple sections of liver and sections of lumbar, thoracic and cervical cord, medulla, pons, cerebellum including fourth ventricle, mesencephalon, diencephalon, and cerebral cortex were studied.

RESULTS

Parabiosis Between LCM Carriers and Isologous Partners Immunized Against LCM Virus.—

Mortality in LCM carrier strains: In certain murine strains a spectrum, extending from focal tissue damage to death, occurs after parabiotic union with more severe involvement occurring in the LCM carrier parabiont (Table I). Of the 4 strains tested, the SWR/J strain was most susceptible with over 60% of LCM carrier parabionts dying at the end of the 1st wk and 77% dying by the end of the 2nd wk. The NZB and B10D2 old line had a LCM carrier parabiont mortality of 40% at 2 wk, while the C_3H parabionts had no adverse changes during the 30 day observation period.

Histopathologic manifestations: Tissue injury occurred after the parabiosis of LCM carriers to immune isologous partners in SWR/J, NZB, and B10D2 old

line strains (Table I). The liver, kidney, and brain were the most severely injured tissues and, while both parabionts suffered, the LCM carrier was always more severely involved. The most affected organ was the liver where injury ranged from scattered foci to large confluent areas of necrosis with mixed inflammatory infiltrates. The large confluent necrotic liver lesions occurred commonly and early (by the 4th day) in SWR/J carriers but were infrequent at any time in NZB and B10D2 old line carriers (Fig. 1). These necrotic areas contained both plasma proteins and viral antigen and occurred in no particular anatomic distribution. In the kidney in SWR/J and NZB strains, there was an acceleration in the progression of tissue injury from basement membrane thickening, usually seen in carrier mice of this age, to intracapillary hyalinization and, finally, capillary occlusion, while in the B10D2 old carrier parabiont, mesangial proliferation and basement membrane thickening were the predominant lesions. In the brain, an increased incidence of lymphoid proliferation and mononuclear infiltrates in SWR/J, NZB, and B10D2 old carriers were noted in the perivascular spaces and meninges. In contrast, no apparent tissue injury in any organ was seen in either the carrier or immune parabionts of the C₃H strain (Table I).

Parabiosis Between LCM Carriers and Normal (LCM Virgin) Isologous Partners.—

The most striking change in these pairs was the apparent trapping of circulating antigen-antibody complexes in the glomeruli of the isologous normal parabiont. In three separate experiments using SWR/J mice the normal parabiont's right kidney was removed at time of parabiotic union, and the left kidney, 6 days later. Immunofluorescent study of the post parabiotic kidney revealed deposition of LCM antigen, γ -globulin, and C3 in the glomeruli along the basement membrane of capillaries and in the mesangia in a granular type pattern (Fig. 2), while the preparabiotic kidney as well as kidneys taken from normal to normal or immune to immune parabionts did not reveal any significant deposits.

Transfer of Spleen Cells from Immune Donors to Isologous LCM Carrier Recipients.—

Production of tissue injury: Spleen cells from SWR/J immune donors were transferred to 4-6-week old SWR/J carriers. Normal SWR/J donors and recipients served as controls and results from one of four such experiments are shown in Table II. 8 of 10 virus carrier recipients showed leptomeningeal and/or perivascular round cell infiltrations in the brain 14 days after transfer (Fig. 3), while in contrast, only 5 out of 40 noninoculated virus carrier litter mates demonstrated mild perivascular round cell infiltrations. The expected back-

ground of chronic disease lesions was not increased when normal or nonviable immune cells were given to SWR/J carriers.

The transfer of 7×10^8 immune C₃H spleen cells i.v. to C₃H carriers did not cause any detectable injury in C₃H carrier recipients.

TABLE I
Parabiosis of Immune to LCM Carrier Mice

Parabionts		Cumulative mortality (days)*			Necrotizing lesions (30 days survivors)
		7	14	30	
SWR/J	VC-I†	11/18	14/18	14/18	4/4
NZB	VC-I	0/8	3/8	3/8	5/5
B10D2 old	VC-I	0/8	3/8	3/8	5/5
C ₃ H	VC-I	0/12	0/12	0/12	0/12
SWR/J	VC-VC	0/4	0/4	0/4	4/4§
SWR/J	I-I	0/4	0/4	0/4	0/4

* Number of parabiotic pairs in which one partner died/total number of parabiotic pairs. Mice used were 2-3 months old.

† VC, virus carrier; I, immune.

§ Usual chronic disease lesions consisting of either mild renal glomerular injury or focal liver necrosis with predominant round cell infiltration (6).

TABLE II
Intravenous Transfer of Isologous Immune Spleen Cells to 4-6 Wk Old LCM Carrier Mice

Recipients	Cells		Histopathologic CNS lesions*	
	Number	Type	Lepto- meningitis	Perivascular round cell cuffing
SWR/J carriers	7×10^6	Immune	5/10‡	8/10
	7×10^6	Immune freeze-thaw	0/5	1/5
	7×10^6	Normal	1/10	1/10
	—	—	2/40	5/40
C ₃ H	7×10^8	Immune	0/8	0/8

* Animals sacrificed 14 days after i.v. cell transfer.

‡ Number with lesions/total number of mice.

Temporal sequence of lesions: 24 SWR/J 4-6-wk old carriers were given 7×10^6 isologous viable immune spleen cells i.v. and sacrificed 4-6 hr and 1, 3, 5, 7, and 14 days later. The central nervous systems (CNS) of these mice were examined for appearance of lesions because of the low background of chronic disease lesions in this tissue (10-15%). No lesions were detected until 7 or more days after transfer when perivascular round cell infiltrations occurred regularly in about 90% of recipients. When lesions occurred they were scattered with a predominance in the hind brain and cerebellum. Even when as many as 7×10^8

isologous viable immune spleen cells were injected into SWR/J carriers lesions did not occur until after the 7th day.

Transfer of Immune Sera to LCM Carrier Mice.—

Effect of i.v. transfer of immune isologous and homologous serum to LCM carrier recipients: Virus carriers were injected with a total of 1.8 ml of pooled immune serum over a 30 hr period and sacrificed 6 hr after the last injection. 75% of SWR/J carriers receiving serum from SWR/J or C₃H immune mice showed acute inflammatory lesions in the liver and/or brain, while no C₃H carriers receiving either immune serum developed lesions (Table III). Histo-

TABLE III
*Intravenous Transfer of Immune Serum to 4-6-Wk Old LCM Carrier Mice**

Recipients	Serum		Histopathologic lesions† (6 hr)	
	Source	Type	Liver	Brain
SWR/J carriers	SWR/J	Immune§	9/12	5/12
	C ₃ H	Immune	4/6	2/6
	Guinea pig	Immune	4/6	3/6
	SWR/J	Normal	2/11	0/11
	—	—	4/40	0/40
C ₃ H carriers	C ₃ H	Immune	0/10	0/10
	SWR/J	Immune	0/10	0/10
	Guinea pig	Immune	0/10	0/10
Normal SWR/J	SWR/J	Immune	0/10	0/10
	Guinea pig	Immune	0/10	0/10

* Transfer of 0.2 ml serum i.v. at time 0, 12, 24 and 30 hr. Injections of 0.25 ml given i.p. at similar times. Animals sacrificed 6 hr after final injection.

† Acute inflammatory lesions with predominant polymorphonuclear leukocyte infiltration.

§ Pooled anti-LCM antibody showing high complement fixing (mouse, guinea pig) and/or neutralizing (guinea pig) activity.

|| Number with lesions/total number of mice.

pathologically, scattered areas of severe focal necrosis with a predominant polymorphonuclear leukocyte (PMN) infiltration occurred in the livers of virus carriers of the SWR/J strain. These areas were easily distinguished from the usual round cell infiltrations of chronic disease. In the brain a similar acute perivascular infiltration and leptomeningitis was seen (Fig. 4). Normal SWR/J recipients injected with immune serum and SWR/J carriers injected with normal serum served as controls and did not show acute inflammatory lesions. When SWR/J carriers receiving immune serum were sacrificed 4 or more days after transfer, lesions contained mostly mononuclear cell infiltrates consisting almost entirely of histiocytes and lymphocytes. Plasma cells were occasionally

seen and comprised up to 2-4% of the cellular infiltrate. Some of the plasma cells contained Russell bodies. Eosinophils and polymorphonuclear leukocytes were rarely seen at this time.

Effect of i.v. transfer of heterologous serum: SWR/J carriers receiving 1.8 ml of guinea pig anti-LCM serum over a 30 hr period developed acute inflammatory brain and liver lesions of varying severity 4-6 hr after final injection (Table III). Cellular infiltrate appeared identical to that occurring 4-6 hr after i.v. isologous or homologous immune serum transfer to SWR/J carriers. Immunofluorescent study of glomeruli showed deposits of guinea pig γ -globulin, presumably anti-LCM antibody. Sections were stained by direct immunofluorescent technique with fluoresceinated rabbit anti-guinea pig γ -globulin that had been absorbed with mouse γ -globulin (Fig. 5). No immunoglobulin

TABLE IV
Intraspinal Transfer of Immune Serum to 4-6 Wk Old SWR/J Carrier Mice

Recipients	Number	Serum		Histopathologic lesions*	
		Source	Type	(Acute poly) 4-6 hr	(Round cell) 7 day
SWR/J carriers	16	SWR/J	Immune	7/8‡	8/8
	10	Guinea pig	Immune	5/5	5/5
Normal SWR/J	20	SWR/J	Normal	0/10	2/10
	15	SWR/J	Immune	0/10	0/5
	15	Guinea pig	Immune	0/10	0/5

* Histologic lesion 4-6 hr after intraspinal transfer of 0.02 ml anti-LCM antibody consists of a polymorphonuclear infiltration in leptomeninges and choroid plexus, especially choroid plexus of the fourth ventricle. The lesion seen at 7 days after transfer is a predominantly mononuclear infiltration.

‡ Number with lesions/total number of mice.

deposits occurred after the injection of this heterologous antiserum into non-infected SWR/J mice nor with normal guinea pig serum inoculated into SWR/J carriers.

Intraspinal transfer of isologous anti-LCM serum to SWR/J carriers: SWR/J carriers developed an acute necrotizing polymorphonuclear response in the leptomeninges and choroid plexus 4-6 hr after i.s. injection of only 0.02 ml isologous anti-LCM serum (Table IV, Fig. 6b). When sacrificed at 7 days these same areas had a predominantly lymphocytic infiltration with occasional plasma cells (Figure 6c). These plasma cells, occasionally containing Russell bodies, comprised up to 3-4% of the total cellular infiltration. Animals sacrificed at 1 or 3 days showed varying gradations of a mixed inflammatory response which consisted mainly of round cells at the 3rd day. The i.s. inoculation of heterologous guinea pig anti-LCM serum caused similar histologic lesions. Early

polymorphonuclear response was not observed in SWR/J carrier controls receiving normal mouse serum and normal SWR/J recipients receiving LCM immune serum.

DISCUSSION

The most obvious lesions in several murine strains persistently infected with LCM virus are chronic glomerulonephritis, focal hepatic necrosis, and interstitial lymphoid infiltrations in many of the body's tissues. Less frequently, there is a mild perivascular round cell infiltration in the CNS. We have previously demonstrated that the glomerulonephritis has an immune complex pathogenesis (6). First, the virus calls forth a host anti-viral antibody response with the resultant formation of circulating infectious virus-antibody complexes. This can be shown by the immunologic precipitation of either host IgG or C3 from the serum, which removes approximately 2 logs of titratable infectivity, while precipitation of albumin, as a control, has no such effect. The trapping and associated accumulation of virus, anti-viral antibody and C in the glomeruli leads to the induction of glomerulonephritis. Hence, it is not surprising to demonstrate either deposition of γ -globulin from a guinea pig immunized against LCM virus in the glomeruli after heterologous serum transfer, or the deposition of γ -globulin (presumably anti-LCM antibody), viral antigen, and C in the glomeruli of a normal mouse several days after parabiosis to an isologous LCM carrier parabiont.

The necrotizing lesions in the liver and elsewhere in carrier mice of certain strains are probably in part the result of an immunologic reaction with LCM antigen(s) in infected cells at the lesion site. Intravenous transfer of anti-LCM immune serum to LCM carrier mice results initially in acute necrotizing lesions predominantly in the liver and brain. Viral antigen can be located in the cytoplasm and on or near the surface of many infected cells, i.e., hepatic parenchymal cells, choroid cells in the brain, and in endothelial cells of blood vessels. Presumably, antibody meets the cell-associated antigen and combines with it, polymorphonuclear leukocytes accumulate probably as a result of C activation, and acute inflammation ensues. The involvement of the C system is suggested by preliminary experiments where the appearance of PMN's and ensuing necrosis was inhibited by deplementing SWR/J carriers at the time of serum transfer.² In addition, *in vitro* experiments have shown that anti-LCM antibody and complement have a rapid cytotoxic effect on LCM-infected culture cells. This cytotoxic effect is apparently closely dependent on the proportion of culture cells infected (27).

Polymorphonuclear leukocytes present in the acute necrotizing lesion after an anti-LCM antibody transfer are replaced within 3 days by mononuclear

² Oldstone, M. B. A., and F. J. Dixon. Unpublished observation.

cells. This round cell infiltration appears similar to those infiltrations associated with classic delayed hypersensitivities, consisting predominantly of lymphocytes and histiocytes with occasional plasma cells (28). In the CNS of SWR/J carriers, this round cell infiltration is hardly distinguishable from the classic perivascular round cell infiltrations seen in allergic encephalomyelitis (25, 29) and differs from a fading Arthus reaction by its paucity of plasma cells (30). The recency of these mononuclear infiltrates after antibody transfer is suggested by the presence of cells of lymphoblastic series as well as lymphoidal cells undergoing mitosis. If, as appears to be the case, serum antibody can induce mononuclear inflammatory lesions similar or identical to those in presumed classical cellular hypersensitivity, it is extremely difficult to ascribe with certainty any of the changes seen in this disease exclusively to cellular mechanisms (3).

Other pathogenetic pathways besides the antibody-C-mediated system may also cause tissue injury in chronic LCM infection. Spleen cells from isologous immune donors injure LCM infected tissue culture cells in the absence of added C, although the time needed to produce this injury is longer than is needed to produce comparable injury with serum antibody in the presence of C (27). Similarly, the lesions developing in vivo after transfer of cells take a number of days, while those after transfer of antibody in the doses employed here, appear in a few hours. Whether the immune splenocytes elaborate humoral factors such as antibody and C, a cytotoxic substance, or cause injury by direct cell-cell interaction is not as yet clear. However, the susceptibility of several C5-deficient strains of mice both to lethal effects of inoculation with LCM virus as adults and to chronic disease associated with persisting LCM infection suggests either an alternative non-C-mediated pathway or a system in which C components beyond activation of C3 are not prerequisites for tissue injury (31, 32).

Although all mice infected at birth with LCM virus carry the virus in their blood and organs and make an anti-LCM antibody response throughout life, only certain of these strains develop associated chronic disease (6). Failure to appreciate this variation in strain susceptibility led to disagreement as to the presence or absence of chronic disease. Volkert (33), who did not observe disease in LCM carriers, worked with both C₃H and AKR strains. In careful experiments utilizing isologous anti-LCM immune cell or serum transfer in C₃H carriers, he was not able to detect clinical illness and did not look for histological injury (4, 34, 35). We too have used parabiosis, cell and serum transfer and also have been unable to detect either clinical or histopathological injury in C₃H carriers. Although the infected C₃H mouse carries LCM virus in its tissues and makes an anti-LCM antibody response, it quantitatively has significantly less (i.e., 2 logs) virus and apparently makes less anti-viral antibody than other strains carrying LCM which do develop tissue injury (6, 20).

The importance of the quantitative aspects of viral infection in relationship to cellular damage or the appearance of clinical symptoms is not without precedent. The safety of attenuated live vaccines are to a great extent due to low levels of virus production. Furthermore, in studies of naturally or experimentally occurring infections such as inapparent neurotropic arbor virus infections, lack of clinical or pathological disease is not associated with failure of viral invasion or replication, but rather with the number of cells infected. Although infectious arbor virus can be found in both apparent and nonapparent infections, neither clinical symptoms nor histologic evidence of encephalitis occur until virus titer reaches approximately 10^5 LD₅₀ per 0.03 g of brain (36).

The tissue injury associated with persistent LCM infection is best seen in mice of strains such as SWR/J which carry large amounts of virus. Hotchin (3, 37), who first described in part chronic LCM disease, worked with Swiss Albino herd-bred strain (NYLAR-A) which is probably closely related to the inbred Swiss Albino SWR/J strain we have employed. However, Hotchin was not able to induce tissue injury in NYLAR-A carriers after the injection of immune anti-LCM serum from mice, rabbits, or monkeys, or after transfer of viable immune spleen cells, although he did report induction of a disease in carriers after parabiotic union to immune mice (38). His inability to induce disease in carrier mice with either anti-LCM antibody or immune cells, plus his failure to detect anti-LCM antibody led him to postulate an "autoimmune" basis for the chronic disease (3, 16, 38). From the present studies it seems much more likely that the chronic disease is the result of an anti-LCM immune response interacting with the virus. Hotchin's failure to induce tissue injury in carrier mice after the injection of anti-LCM serum may be related to the virus or mouse strain employed or to the time and method of assessing injury. Failure to detect an effect of transferred cells between incompletely inbred animals could also reflect rejection of the transferred cells on the basis of histoincompatibility. 4-8 wk after parabiosis between carrier and immune mice, Hotchin noted the occurrence of a clinical runting syndrome (ruffled hair, wasting, and anemia) (38). Histologic studies of liver and spleen sections from parabionts did not reveal any significant findings. The description presented by Hotchin might possibly have been the result of histoincompatibility but the differences in patterns of injury noted in this report and in Hotchin's could also be due to different mouse or virus strains used.

Some of the major characteristics of chronic LCM disease may be found in other chronic viral infections. Thus in Aleutian disease of mink (39, 40) and lactic dehydrogenase virus infection of mice (6, 41, 42) chronic viral infection, viremia, circulating virus-gamma globulin complexes, and deposition of these complexes in the glomeruli occur. In visna (43) there also is persistence both of circulating virus and anti-viral antibody. It is of interest that in Aleutian disease little morphologic damage occurs in the early stage of infection, despite high

viral titers. It is only after 2-3 wk of infection, with the appearance of host immune response to viral antigen(s), that tissue injury occurs (44).

The presence of anti-viral antibody is associated with recovery from or immunity to many virus infections. But such antibody may not be universally beneficial, since tissue damage in many acute viral diseases occurs at the end of the viremia stage when antibodies are first appearing. It seems possible that the interaction of antibody and virus may precipitate or contribute to tissue damage caused by virus replication. In addition, genetically determined predispositions controlling viral multiplication and/or degree and type of host immune response may influence the severity and perhaps nature of the disease. Although all evidence to date suggests that viral antigen and anti-viral antibody is involved in chronic LCM disease, the possibility must be considered that new (neo) antigen coded for by the virus also may be involved.

SUMMARY

Tissue injury (chronic disease) associated with persistent LCM infection is apparently caused by the host immune response to the virus. Employing parabiosis or cell transfer from hyperimmune donors to isologous virus carriers, the tissue injury of chronic disease could be initiated and/or intensified. Furthermore, the transfer of anti-LCM antibody to SWR/J carrier mice results in acute necrotizing inflammatory lesions in regions of viral persistence, followed by chronic mononuclear infiltrates quite similar to those seen after the transfer of immune cells.

The pathogenesis of the nonglomerular tissue injury of chronic LCM disease is apparently at least in part related to the interaction of circulating anti-LCM antibody with viral antigen at the tissue site. Trapping of circulating virus-antibody complexes in the glomerular filter is apparently the major cause of the glomerulonephritis.

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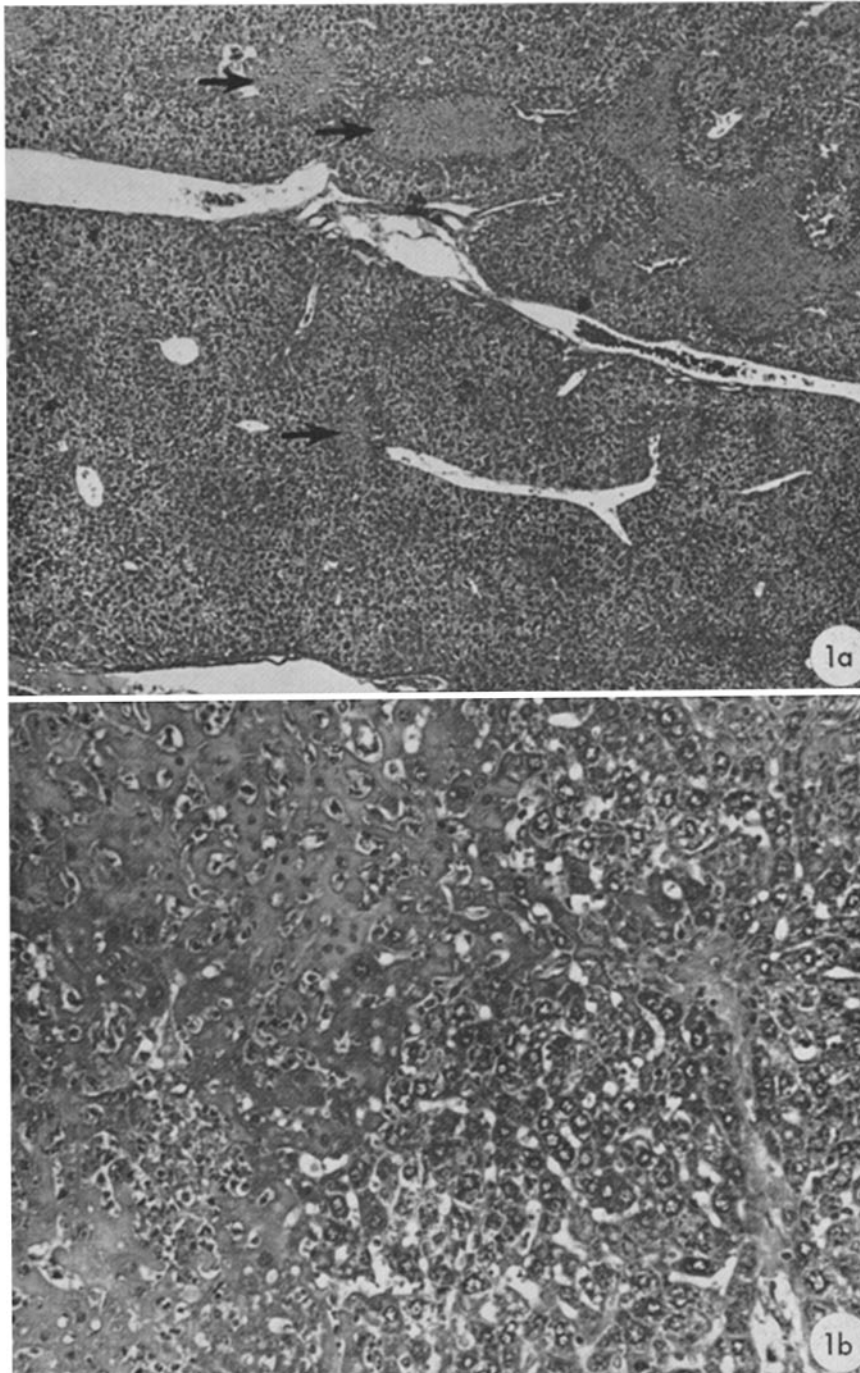


FIG. 1. Light photomicrographs of liver tissue from 2-3-month old LCM carrier parabiosed to an immune isologous partner. Sections stained with hematoxylin and eosin. (a) Scattered foci of necrosis (arrows) in a B10D2 old LCM carrier sacrificed 7 days after parabiotic union. Location of areas of necrosis have no apparent relationship to hepatic architecture. (b) Higher power photomicrograph of a large confluent area of necrosis at left in an SWR/J LCM carrier sacrificed 4 days after parabiosis.

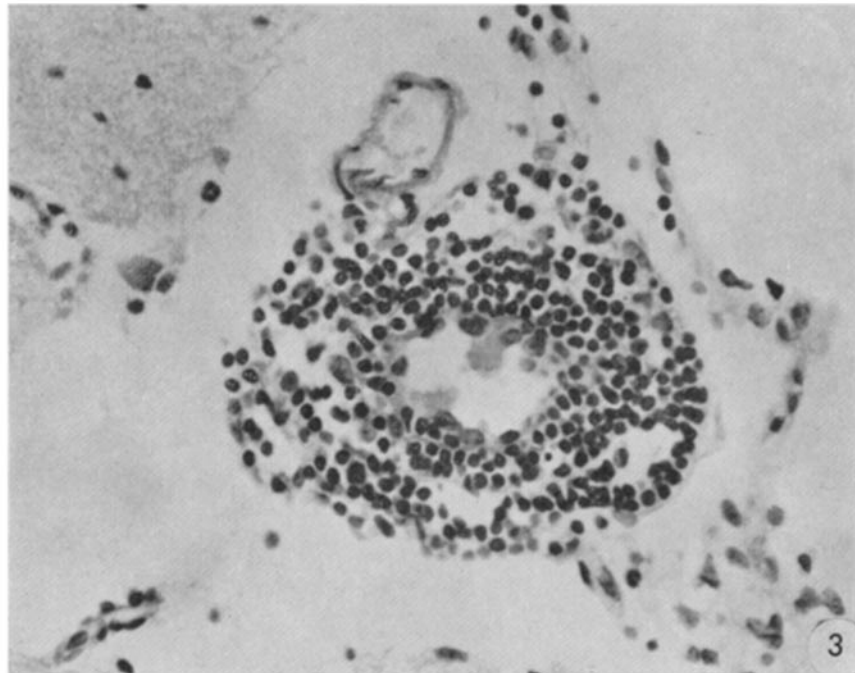
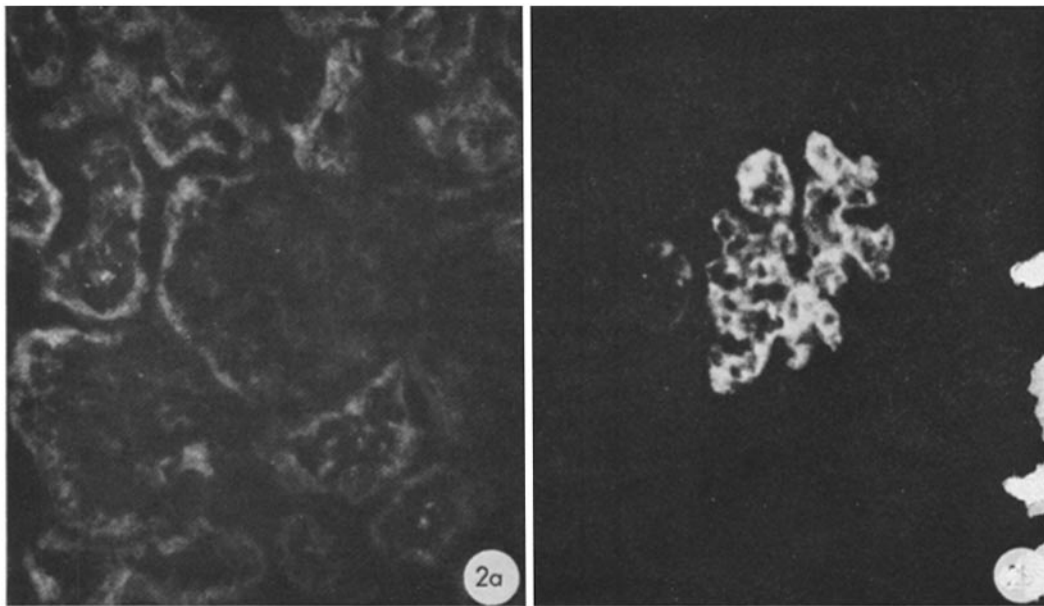


FIG. 2. Renal glomeruli from an 8-wk old SWR/J normal (LCM virgin) mouse stained with fluorescein-conjugated rabbit antiserum to mouse $7S\gamma$ -globulin. (a) Kidney removed at time of parabiotic union to an SWR/J LCM carrier. There is no detectable γ -globulin observed. Surrounding tubules are indicated by natural auto-fluorescence brought out by overexposure. (b) Remaining kidney removed after 6 days parabiosis. Mouse γ -globulin can be seen outlining the glomerular basement membranes of capillaries in peripheral areas as well as being deposited in mesangia.

FIG. 3. Light photomicrograph of a hematoxylin and eosin stained brain section from a 6-wk old SWR/J LCM carrier mouse 14 days after transfer of 7×10^6 isologous immune spleen cells intravenously. Predominant round cell perivascular infiltration is seen in the pons.

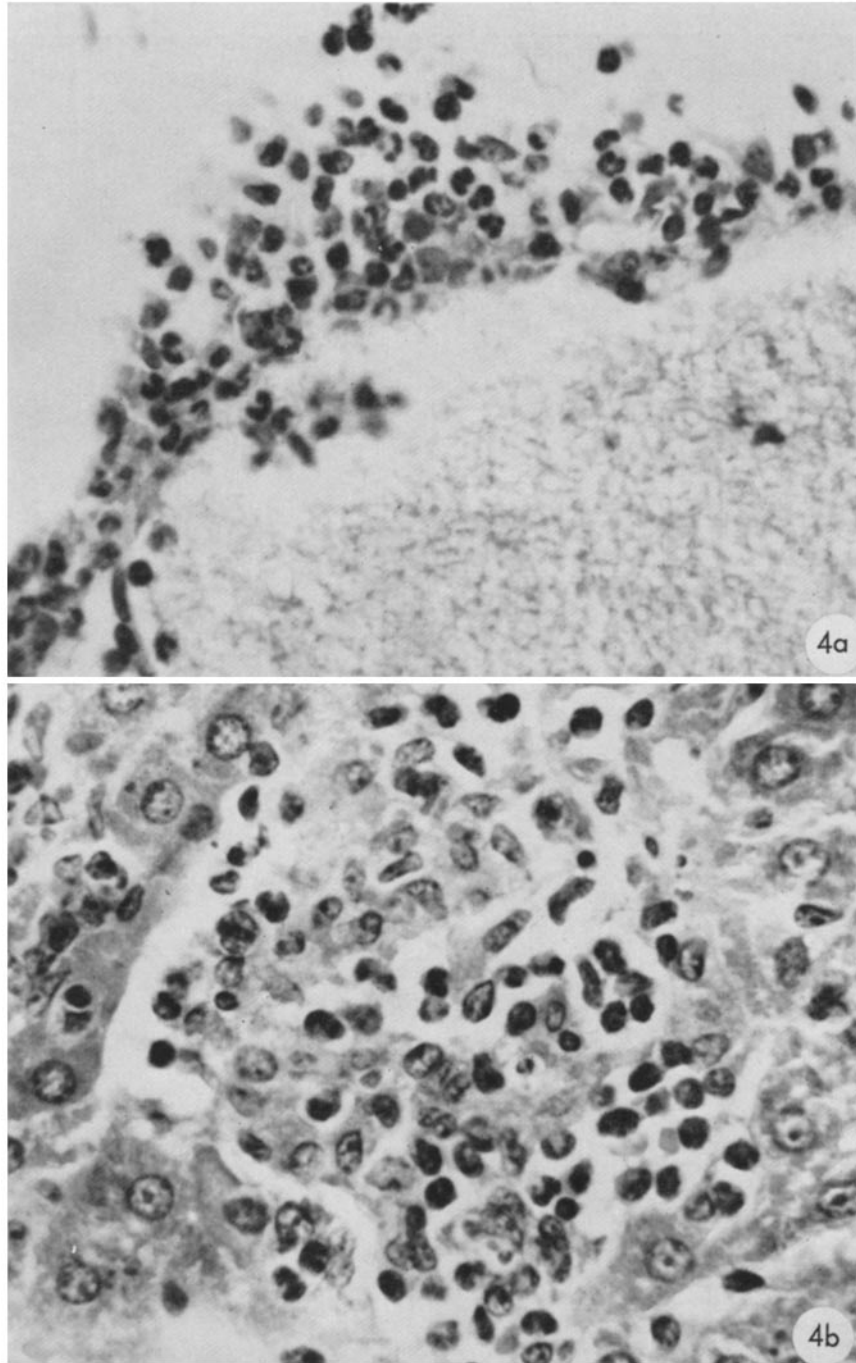


FIG. 4. Light photomicrographs of hematoxylin and eosin stained tissues from a 6-wk old SWR/J LCM carrier mouse 6 hr after last intravenous and intraperitoneal injection of high titer isologous antisera to LCM virus. Mixed cell infiltration with large numbers of polymorphonuclear cells are seen in: (a) leptomeninges of cerebral cortex, (b) liver. Normal SWR/J recipients injected with immune sera and carriers with normal sera served as controls and did not show similar lesions.

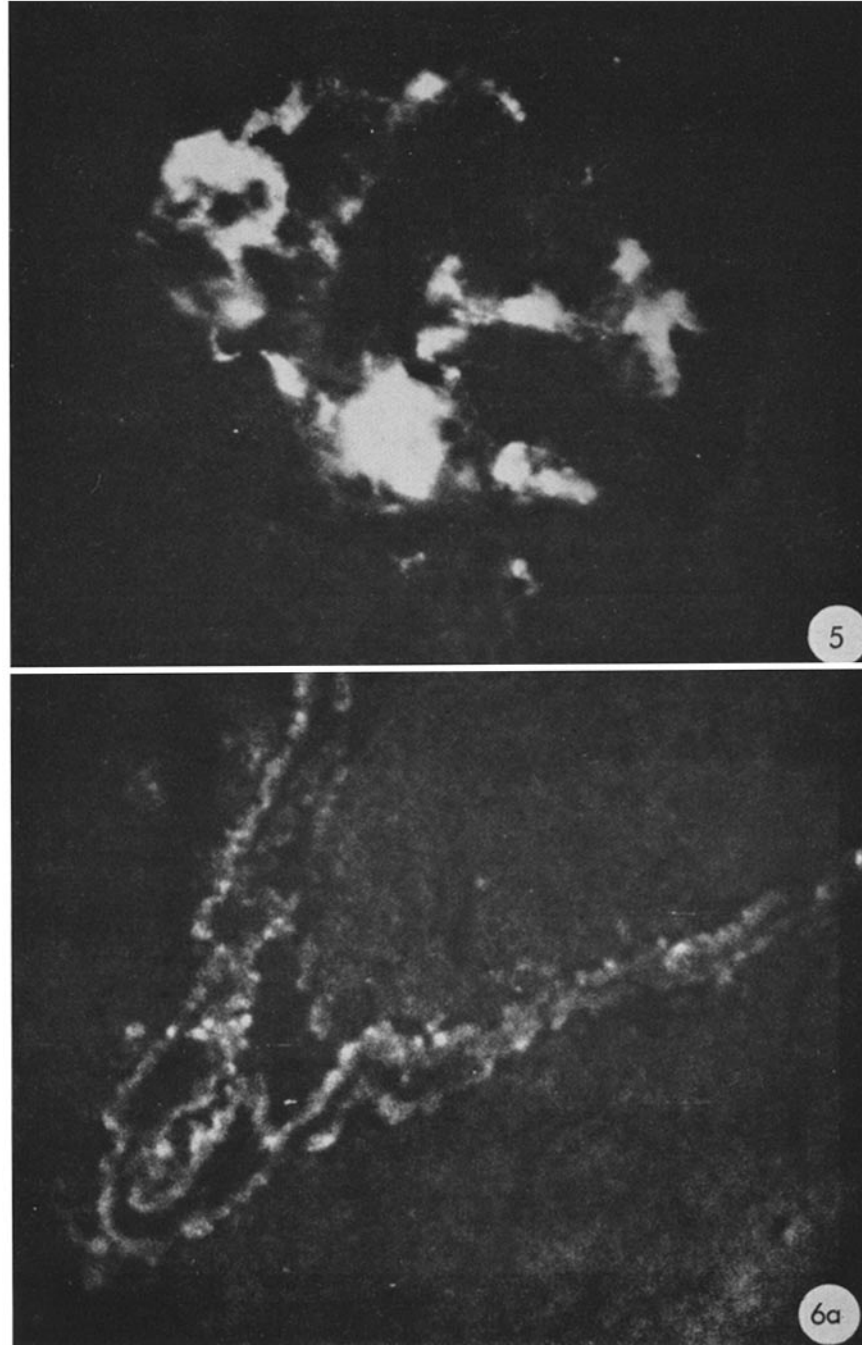


FIG. 5. Renal glomerulus from a 6-wk old SWR/J LCM carrier receiving guinea pig antiserum to LCM virus. The preparation was stained with fluorescein-conjugated rabbit antiserum to guinea pig 7S γ -globulin that had been absorbed with mouse γ -globulin. Guinea pig γ -globulin, presumably antibody to LCM virus, is heavily deposited in mesangial areas and less on peripheral walls of glomerular capillaries.

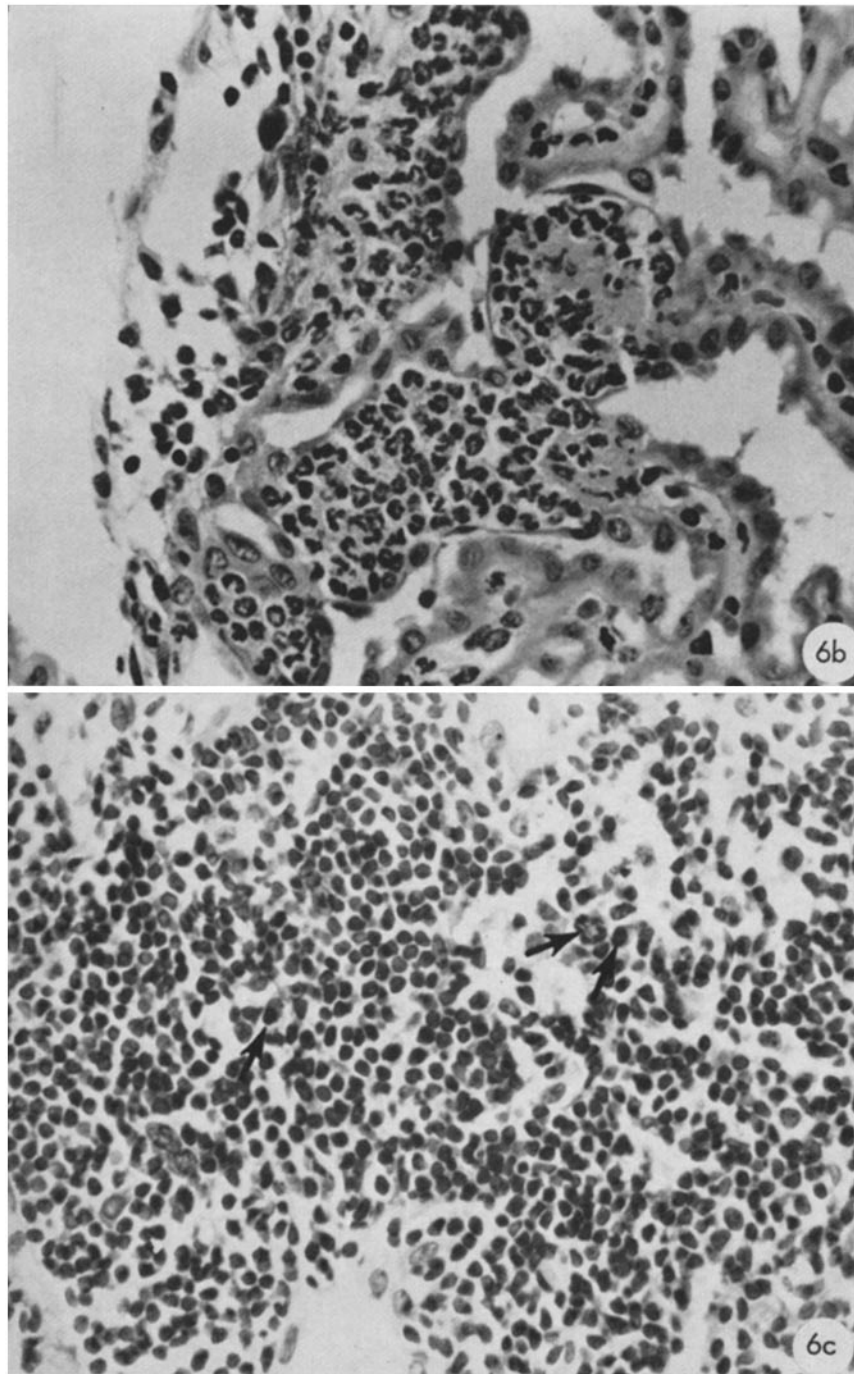


FIG. 6. Fourth ventricle choroid plexus from 6-wk old SWR/J carrier mice receiving isologous anti-LCM serum intrathecally via a high lumbar-low thoracic inoculation. (a) Preparation stained with fluorescein-conjugated guinea pig antiserum to LCM virus in an LCM carrier litter mate control not receiving serum. LCM viral antigen is seen in choroid cells and in endothelial cells of capillaries. (b) Light photomicrograph 6 hr after intrathecal serum transfer. Acute necrotizing polymorphonuclear response is evident. (c) Light photomicrograph 7 days after intrathecal injection showing predominant lymphocytic infiltrate. Occasional plasma cells and a large lymphoid cell undergoing mitosis (arrows) are seen in the infiltrate