DISEASE ACCOMPANYING *IN UTERO* VIRAL INFECTION

The Role of Maternal Antibody in Tissue Injury after Transplacental Infection with Lymphocytic Choriomeningitis Virus*

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During pregnancy, generalized infection of the mother with viremia may lead to infection of the fetus. When the virus is cytopathic, the resultant injury usually leads to fetal death and abortion. On the other hand, infection with a relatively noncytopathic virus may not kill the fetus but can cause damage to tissues *in utero* or shortly after birth. Although congenital viral infections are not uncommon (1) and are important in human disease (i.e. rubella and cytomegalovirus infections), the pathogenetic mechanism(s) of injury remain unclear. In order to define some of these mechanisms, we have chosen to study the pathogenesis of tissue injury which follows *in utero* infection of mice with lymphocytic choriomeningitis (LCM)[1] virus.

After *in utero* infection with LCM virus, mice carry high titers of virus in their blood and tissues until death, make an immune response to the virus, and develop manifestations of immune complex disease (2). Circulating IgG-virus-complement complexes can be detected on the day of birth and apparently persist. Study of the renal glomeruli of such mice showed progressive accumulations of complexes that led to death from nephritis in some mice by 2 wk of age. Most of the glomerular-bound IgG recovered from the kidney was anti-LCM viral antibody. This suggested that maternal factors might play a role in the pathogenesis of the early disease in these mice and the present experiments were undertaken to evaluate this possibility.

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[1] *Abbreviations used in this paper*: FITC, fluorescein isothiocyanate; LCM, lymphocytic choriomeningitis; V-ab, virus–antiviral antibody.

Materials and Methods

Mice.—SWR/J mice for breeding were obtained from Jackson Laboratories, Bar Harbor, Maine, and HA/ICR outbred mice from L. C. Strong Research Laboratories, Del Mar, Calif. In random tests all mice were free of LCM, polyoma, and lactic dehydrogenase viral infections.

Parental Virus Carrier State.—Newborn mice were inoculated within the first 15 hr of life with a 1000 × LD_{50} dose of virus prepared from a pool of infected isologous brains. Details of virus passage, inoculation procedure, and development of the LCM virus carrier state have been previously described (3). Brother and sister virus carriers were mated to establish colonies of naturally infected LCM carrier mice, all of which were assayed for circulating infectious virus.

Nursing Mother Mice.—Mouse mothers nursed some of their own offspring in addition to foster nursing other babies. Foster nursing began within 2–8 hr after birth. Virus-carrier mouse mothers were persistently infected with LCM virus and had circulating virus–antiviral antibody (V-Ab) complexes; they have been described in detail (2). Babies neonatally infected excreted large amounts of LCM virus; during the nursing period they thereby infected their mothers. Such naturally infected mothers (immune mouse mothers) had high titers of circulating complement-fixing anti-LCM viral antibody and no circulating virus (4). Immune mouse mothers were mated with noninfected male mice. In addition, nurses which had never before been exposed to LCM virus were used (noninfected mouse mothers). In this case nurses were replaced every 4–5 days.

Virus.—Seed virus used was mouse brain–passed National Institutes of Health strain CA1371 provided by Dr. Wallace Rowe, National Institute of Allergy and Infectious Diseases, Bethesda, Md. Procedures for handling, titrating, and passing viruses have been described (3, 5).

Evaluation of Disease.—Mice were sacrificed at 3, 7, 10, 14, 28, and 60 days after birth. Tissues were taken for both immunofluorescent and routine histopathologic study as previously noted (3). Procedures for making antisera and their conjugation to fluorescein isothiocyanate (FITC) have been reported (3). Rabbit antiserum to mouse IgA was kindly supplied by Dr. Hans Spiegelberg and was absorbed with purified mouse IgG and IgM. Rabbit antiserum to mouse IgM was absorbed with pooled newborn mouse sera, while antiserum to mouse IgG was absorbed with purified mouse IgA and IgM. After absorption, all rabbit antisera to the various mouse Ig's were monospecific by both Ouchterlony and immunoelectrophoresis analysis. Immune complex disease was defined as the presence of glomerular deposits of Ig, viral antigen, and C3 (third component of complement) in a granular pattern along the glomerular basement membranes and in the mesangia. Detailed descriptions of immune complex disease accompanying this viral infection have been published (2, 3).

Suckling mouse sera and colostrum from nursing mothers were collected and analyzed for presence of various mouse Ig's by immunoelectrophoresis. Using modified Mancini radial diffusion antibody-agar plates (6, 7), the Ig's in individual samples or pools from seven or more animals were quantitated.

RESULTS

Immune Complex Glomerulonephritis after Natural In Utero Infection.—Kidneys from mice infected *in utero* and nursed by their own or other virus-carrier mouse mothers were studied by immunofluorescence for glomerular Ig deposits. By the 3rd day after birth and thereafter nearly all these mice had glomerular-bound IgG (Table I). IgG, viral antigen, and C3 were deposited in a granular distribution in the glomeruli along the basement membranes of capillaries and in the mesangia (Fig. 1 *a*). In contrast to the strict glomerular

localization of IgG and C3, viral antigen appeared in tubular, endothelial, and interstitial cells as well as in the glomeruli. When transplacentally infected littermates were nursed by noninfected mouse mothers, neither IgG nor C3 glomerular deposits were detected on the 3rd or 7th day after birth (Table I, Fig. 1 *b*), although LCM viral antigen was found throughout the kidney tissue. Assay for the amount of infectious virus in renal tissue from mice nursed by virus-carrier or noninfected mouse mothers showed similar concentrations of virus. By the 28th day after birth, nearly all *in utero*–infected mice nursed

TABLE I

*Maternal Influence in the Development of Immune Complex Disease after In Utero LCM Virus Infection**

Suckling baby infected *in utero*‡	Nursing mother§	IgG glomerular deposits on day			
		3	7	14	28
Yes	Virus carrier	9/10‖	10/10	6/7	21/22
No	Virus carrier	0/10	2/19	12/20	9/10
Yes	Noninfected	0/10	0/10	1/7	9/10
No	Noninfected	0/10	0/10	0/10	1/20
Yes	Immune	10/10	5/5	ND¶	ND
No	Immune	0/10	0/10	ND	0/10

* Immune complex disease was determined by IgG deposits in the glomeruli using a fluorescein-conjugated rabbit antiserum to mouse IgG.

‡ Mice naturally infected via *in utero* route.

§ Mouse mothers nursed some of their babies in addition to foster nursing others. "Virus carrier" pertains to those mice persistently infected with LCM virus and demonstrating high titers of virus in tissues and circulating V-Ab complexes. Noninfected mouse mothers had never before been exposed to LCM virus and were changed every 4–5 days. Immune mouse mothers had high titers of circulating anti-LCM antibody and no detectable virus (see Materials and Methods).

‖ Number of mice showing specific immunofluorescence per total number of mice assayed.

¶ Not done.

by noninfected mouse mothers had significant V-Ab glomerular deposits) (Table I).

Enhancement of Immune Complex Glomerulonephritis in In Utero–Infected Mice Nursed by Immune Mouse Mothers.—When *in utero*–infected mice were nursed by immune mouse mothers, the deposition of V-Ab complexes was enhanced and the development of early tissue injury accelerated. Hence, while less than 5% of *in utero*–infected mice nursed by virus-carrier mouse mothers had histologic evidence of immune complex disease by 14 days after birth, approximately 20% of littermates nursed by immune mouse mothers did. Fig. 1 *c* shows marked Ig glomerular deposits present 7 days after birth in a mouse

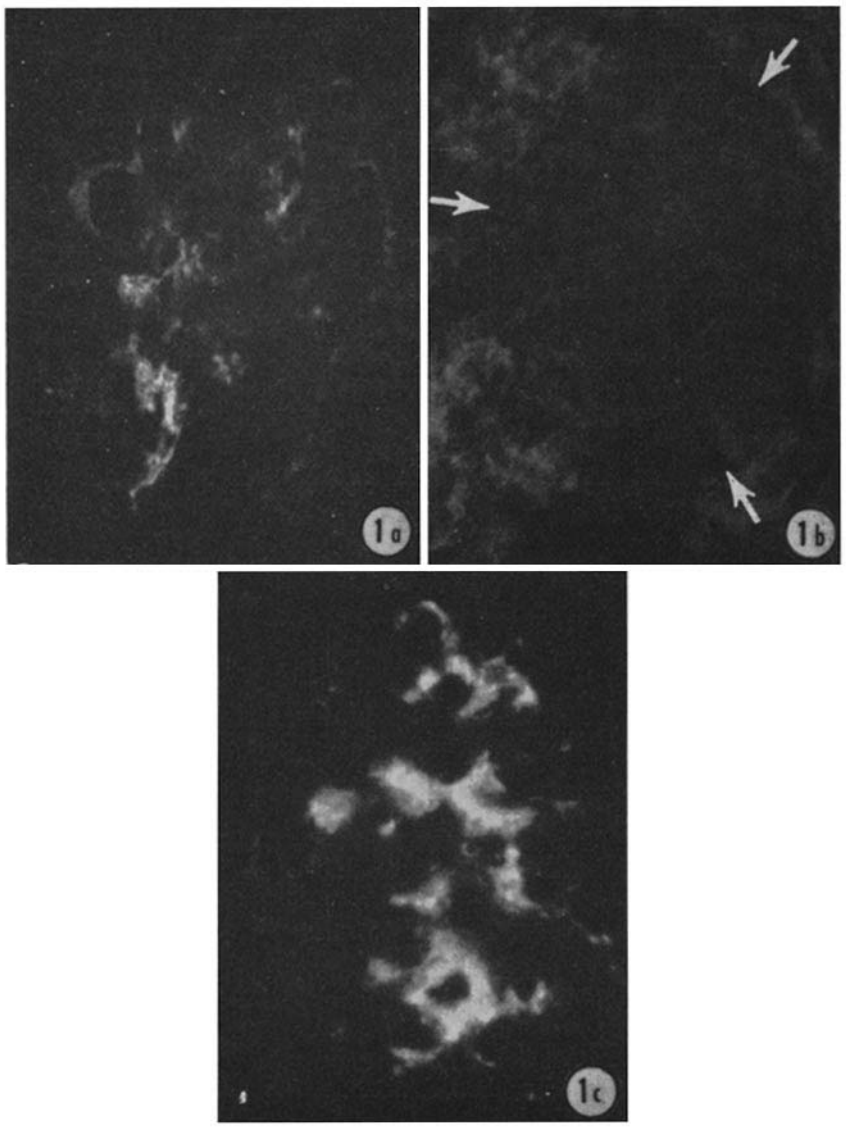

FIG. 1. Fluorescent photomicrographs of renal glomeruli from 7-day-old SWR/J mice infected *in utero* with LCM virus. The preparations were stained with fluorescein-conjugated rabbit antiserum to mouse IgG. (*a*) Infected newborn nursed by its own virus-carrier mouse mother. IgG deposits are seen along peripheral walls of glomerular capillaries and in the mesangial area. (*b*) Infected offspring foster nursed by a noninfected mother. IgG deposits are absent. Arrows outline glomerulus and nonspecific autofluorescence of adjacent tubules is seen. (*c*) Infected littermate foster nursed by an immune mouse mother. Heavy IgG deposits are present along peripheral walls of glomerular capillaries and in mesangial area. Magnification, Fig. 1 *a*, *b*, and *c*: roughly × 750.

suckled by an immune mouse mother, while Fig. 2 depicts severe glomerular disease in a similarly nursed mouse 14 days after birth.

Development of Immune Complex Disease in Noninfected Mice Nursed by Virus-Carrier Mouse Mothers.—Normal noninfected mice nursed by virus-carrier

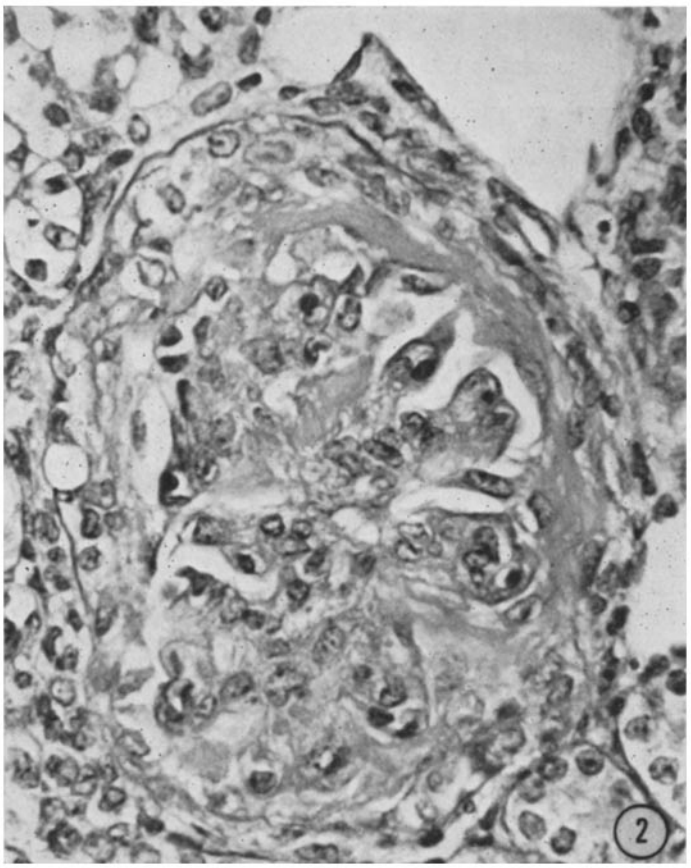

FIG. 2. Light photomicrograph of a renal glomerulus from a 14-day-old SWR/J mouse infected *in utero* with LCM virus and foster nursed by an immune mouse mother. The preparation was stained with periodic acid–Schiff reagent. Extensive glomerular injury with proliferation of mesangial cells, obliteration of capillary lumens, and proliferation of cells lining Bowman's capsule with crescent formation are present. Magnification: roughly × 750.

mouse mothers also developed evidence of immune complex disease. While approximately 10% showed mild V-Ab glomerular deposits at 7 days, over 50% showed deposits by 14 days after birth (Table I). At 28 days after birth nearly all weanlings were infected with LCM virus and showed significant V-Ab glomerular deposits (Fig. 3).

Time of Appearance and Class of Glomerular-Bound Ig.—Glomeruli from SWR/J and HA/ICR mice, 3–60 days old, were studied for the presence of the various mouse Ig's. *In utero*–infected mice nursed by LCM virus carriers or immune mouse mothers had heavy IgG and IgA deposits by the 3rd day after birth, while IgM deposits were absent (Table II). IgG persisted throughout the 60 day observation period, but IgA diminished in intensity. In contrast, IgM deposits were not common 10 days after birth and became frequent at 60 days.

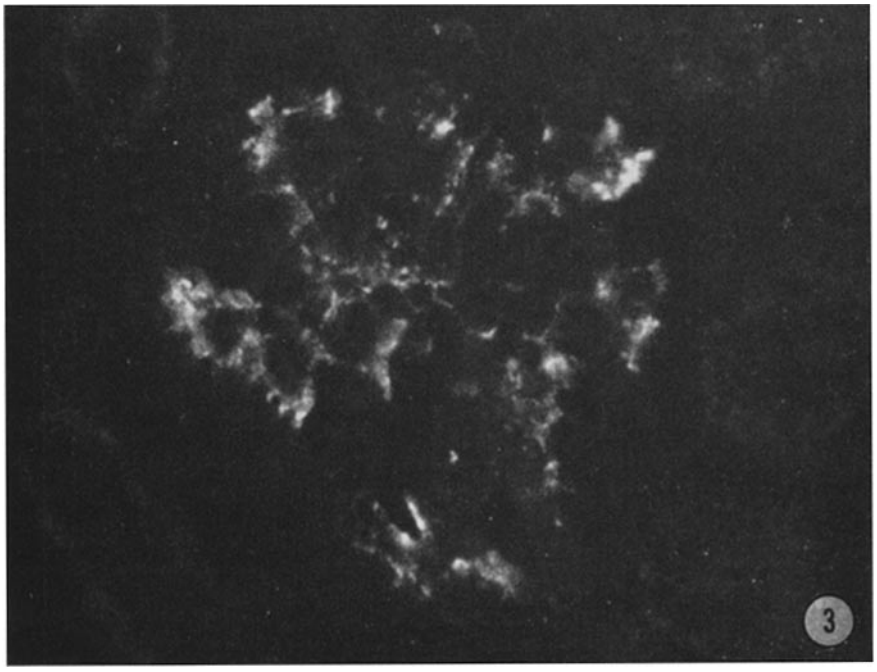

FIG. 3. Fluorescent photomicrograph of a renal glomerulus from a 28-day-old HA/ICR mouse noninfected *in utero* but nursed by a virus-carrier foster mouse mother. The section was stained with fluorescein-conjugated rabbit antiserum to mouse IgG. IgG is deposited along the peripheral walls of glomerular capillaries and in the mesangial areas. Magnification: roughly × 750.

In addition, Table II shows that transplacentally infected mice nursed by noninfected mouse mothers did not have Ig deposits by the 10th day after birth. However, by the 60th day after birth 100% had IgG, 60%, IgM, and 20% IgA glomerular deposits.

Serum and Colostrum Ig Changes after Birth.—No differences in levels of serum Ig's occurred in either infected or noninfected offspring nursed by various mouse mothers. At birth, 200–400 μg IgG/ml of serum were detected; the level rose to 600–800 μg IgG/ml by 2 wk then dropped to approximately 200 μg IgG/ml by 28 days. In contrast, both IgA and IgM were detected only occa-

sionally by 2 wk of age and routinely by 6 wk in the range of 200–500 μg/ml. Using immunoelectrophoresis, both IgA and IgG were detected in the colostrum from all mouse mothers while IgM was not.

DISCUSSION

Judging from several lines of evidence, injury accompanying LCM viral infection is dependent on the interaction of the host antiviral immune response with

TABLE II

*Class of Ig Deposited in the Glomeruli after In Utero Infection with LCM Virus**

Class of Ig deposited at		*In utero*-infected mice nursed by mothers who were‡		
		Virus carriers	Noninfected	Immune
Day 3	IgG	10/10§	0/10	10/10
	IgA	9/10	0/10	10/10
	IgM	0/10	0/10	1/10
Day 10	IgG	10/10	0/10	10/10
	IgA	10/10	0/10	10/10
	IgM	1/10	0/10	2/10
Day 60	IgG	10/10	10/10	10/10
	IgA	4/10	2/10	3/10
	IgM	7/10	6/10	6/10

* Direct immunofluorescent technique was employed to detect mouse IgG, while indirect immunofluorescence was used to demonstrate IgA and IgM.

‡ Mouse mothers nursed some of their own babies in addition to foster nursing others. "Virus carrier" pertains to those mice persistently infected with LCM virus and demonstrating high titers of virus in tissues and circulating V-Ab complexes. Noninfected mouse mothers had never before been exposed to LCM virus and were changed every 4–5 days. Immune mouse mothers had high titers of circulating anti-LCM antibody and no detectable virus (see Materials and Methods).

§ Number of mice showing specific immunofluorescence per total number of mice assayed.

the infecting agent. Many laboratories have reported that immunosuppression of the host protects against the ordinarily fatal adult infection despite viral persistence (8–14). Others have shown that viruses grown in cultured cells were relatively noncytopathic (15–19), and many tissues from persistently infected mice contained high titers of infectious virus and the presence of viral antigen(s) but suffered little injury (2, 4, 15, 20, 21). Furthermore, passive transfer of antiviral antibody or lymphoid cells sensitized against virus initiated or enhanced cellular injury in areas of viral persistence both in vivo (4, 14) and in vitro (22–25).

Shortly after birth, mice infected *in utero* showed glomerular immune complex deposits of virus and Ig which increased during the suckling period. Experiments using foster mothers suggested that most if not all the Ig in these

deposits was maternal in origin, since mice nursed by noninfected mouse mothers did not have such deposits during the first 10 days after birth. Furthermore, nursing of LCM virus-infected babies by foster mouse mothers having high titers of circulating antiviral antibody enhanced immune complex deposition and resultant disease. Others have reported that the fetus, when stimulated by appropriate antigens, was capable of making antibodies (26–28). Because of the excess of antigen and the presence of maternal antibody, a measure of fetal antibody is not possible. The fact that Ig glomerular deposits and associated glomerular injury did not occur in fetuses (2) may be related to both limited anti-natal glomerular function and to relatively low levels of maternally transmitted Ig and therefore anti-LCM antibody.

IgA glomerular deposits were present on the 3rd day after birth and lessened after the suckling period. This paralleled the time of expected absorption of antibodies from the gut in the nursing mouse (29, 30) and indicated that these deposits were most likely maternal in origin. Although IgA was present in the colostrum from all nursing mothers, IgA renal deposits occurred only in those infected offspring nursed by mouse mothers possessing antiviral antibody. Similarly, IgG glomerular deposits occurred during the first 3 days of life only in mice nursed by mouse mothers having antiviral antibody. Usually, infected littermates nursed by mouse mothers not having antiviral antibody failed to develop Ig deposits until the 28th day after birth. IgM glomerular deposits were uncommon early after birth, increased as the mouse aged, and were most likely made by the host.

Maternal antibody might participate in offspring tissue injury by various means. Antiviral antibody might complex with circulating virus or viral antigen(s) in the offspring to form V-Ab complexes with resultant immune complex disease. Also, antibody could react at the cell surface with budding virus and result in cell membrane damage through activation of the complement system. We have described such a mechanism for LCM virus–infected cells in vitro (24, 25). Others have reported this type of antibody-mediated injury in rabies (31), herpes simplex (32), vaccinia (32), Newcastle disease,[2] influenza,[2] Sendai (33, 34), SV5 (35), and oncorna viruses (36–42), and we have in mumps (25) and Moloney sarcoma (43) viral infections.

The relationship between human congenital rubella infection and fetal or newborn tissue injury was recognized over 30 yr ago (44) and is attributed to viral effects on developing organs (1, 45, 46). However, one might wonder whether maternal and/or fetal antiviral antibody might not also contribute to this tissue injury. In this regard, it is worth making the following comparisons between rubella and LCM viruses. Both are relatively noncytopathic (15– 19, 47, 48), bud from plasma membranes, and are thereby accessible to interact with antiviral antibody and sensitized cells. Both virus and antiviral antibody

[2] Notkins, Abner. Personal communication.

occur in young children who have contracted rubella *in utero* (49–53). The current practice of passively transferring hyperimmune Ig to pregnant mothers exposed to rubella virus would be expected to be beneficial when such antibody prevented the viremic stage and hence infection of the fetus. Theoretically, transfer of antibody once the fetus was infected might, however, result in immunologically mediated injury. The fact that infectious virus can persist in the presence of antiviral antibody and that this immune response to viral antigens may be injurious to the host poses both difficult and challenging problems.

SUMMARY

Early, after *in utero* infection with LCM virus, SWR/J and HA/ICR mice developed manifestations of immune complex disease. Observations based on nursing such mice with virus-infected, immune, or noninfected mouse mothers indicated that maternal antiviral antibody was responsible for the early immune complex glomerulonephritis. Despite comparable viral persistance, *in utero*–infected offspring failed to develop glomerulonephritis when nursed by noninfected mouse mothers, but did when suckled by virus-infected mouse mothers. Nursing by mouse mothers carrying high titers of anti-LCM viral antibody markedly enhanced the Ig glomerular deposits and the resultant nephritis.

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