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Passive Immunity to Fatal Reovirus Serotype 3-Induced Meningoencephalitis Mediated by Both Secretory and Transplacental Factors in Neonatal Mice

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The role of passively acquired immunity to reovirus-induced meningoencephalitis in neonatal mice was examined. It was determined that female mice were capable of conferring protection against viral infection and meningoencephalitis in neonates depending on the route by which the dams were immunized and the serotype of the immunizing virus. Female mice immunized with homotypic virus via the oral route developed the most potent response. Infected neonates born and nursed by these females developed no signs of disease, and no virus was recoverable from their small intestines, livers, or brains following infection. Neonates born to females immunized with homotypic virus by the subcutaneous route manifested no evidence of meningoencephalitis or virus dissemination, yet virus was recovered from neonatal intestines. Mice immunized with heterotypic virus by either the subcutaneous or the oral route also conferred protection against disease; however, virus was recovered in small intestines and livers of infected neonates. Based on results from foster-nursing experiments, it appears that factors obtained both during suckling and by transplacental transfer contribute to protection. Passive transfer of reovirus-immune mouse serum also protected neonates from disease. These results demonstrate that passive immune mechanisms can mediate the protection of neonates against reovirus infection and provide further evidence of the importance of the mucosal immune response in protection against pathogens that invade the host via mucosal tissues.

Murine reovirus infection has been used as a model to study pathogenesis and mechanisms of immunity to enteric viruses, but the precise mechanisms of immunity to murine reovirus infection have not been fully defined. Previous studies have shown that both cellular (2, 7, 11–13) and humoral (1, 5, 10, 13) immune responses to reovirus develop in immunized mice. These specific immune responses may play a role in protection. Innate mechanisms, such as nonspecific immunity (11) and age-related changes in host cells (23), have also been shown to be important in resistance to infection.

It has been reported that several strains of serotype 3 reovirus including the Dearing strain (3/D) exhibit a tropism for central nervous system tissue in mice. Neonatal mice inoculated either intracerebrally (i.c.) or intramuscularly (i.m.) with reovirus 3/D develop a severe meningoencephalitis characterized by viral replication and pathologic changes in the brain, which may result in death (3, 15, 18, 22, 24, 25, 27). Passive immunity to reovirus-induced meningoencephalitis has been characterized by several investigators. A report by Gaulton et al. (3) described maternal transfer of immunity to serotype 3-induced meningoencephalitis. They found that female mice immunized with either type 3/D- or serotype 3-specific anti-idiotope antibody protected neonates against the development of neurologic abnormalities and against death following i.m. infection with type 3/D reovirus. Furthermore, studies have shown that rabbit anti-reovirus antiserum and murine anti-reovirus monoclonal antibodies are capable of protecting neonatal mice from serotype 3/D-induced meningoencephalitis following i.c. or i.m. inoculation (26).

In this report we have used a murine isolate of serotype 3 reovirus (designated type 3 clone 9 or T3c9) (6, 8) which replicates in the small intestine and disseminates to the central nervous system when inoculated orally (p.o.) into neonatal mice (8, 25). The infection results in the development of a highly lethal meningoencephalitis in nonimmune neonates. A previous report (25) has demonstrated that monoclonal antibodies directed against the serotype-specific hemagglutinin can protect neonatal mice after p.o. inoculation of T3c9 reovirus. We attempted to determine whether pups can be passively protected against reovirus infection by dams immunized prior to mating. Since it has been reported that antibodies to the serotype-specific hemagglutinin as well as specific antibodies against nonhemagglutinin determinants are capable of neutralizing infectivity in vitro and in vivo (1, 5, 10, 19, 25, 26), it was of interest to determine whether cross-serotype immunity to reovirus infection could be generated and whether this immune response would be protective. Finally, we wanted to ascertain whether the route of maternal immunization had an effect on protective immunity.

We have found that immunity can be passively transferred from immune mothers to pups following immunization of mothers with either the reovirus 3/D or type 1/Lang (1/L) and that protection can be mediated either transplacentally or by factors obtained during suckling. It also appears that different levels of immunity may develop depending on the route of immunization and the serotype of the immunizing virus. Oral inoculation of mothers with homotypic virus was found to be the most efficacious immunization protocol if

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measured by protection of neonates against meningoencephalitis following p.o. challenge and the absence of virus titers in tissues of infected neonates, including the intestines. Furthermore, serum from adult mice which were immunized with reovirus type 1/L or 3/D can protect against infection in neonates. Finally, the data suggest that prior experimental immunization may inhibit the development of maximum immune responses after incidental exposure of dams to virus.

MATERIALS AND METHODS

Viruses. Reoviruses 1/L, 3/D, and T3c9 (6, 8) were obtained from B. N. Fields, Harvard Medical School, Boston, Mass. Viral stocks were grown in L cells and purified as described elsewhere (12, 13, 20). A third-passage stock of reovirus was used for all immunizations and infections. Viruses purified on CsCl gradients banded in CsCl at a density of approximately 1.35 g/ml, and their titers on L-cell monolayers were determined prior to use.

Mice. Female BALB/cByJ mice were obtained from Jackson Laboratory, Bar Harbor, Maine, and housed in the animal facility in the Department of Biology, University of Pennsylvania. The mice were immunized at 10 to 12 weeks of age with two inoculations, 1 week apart, of 10^7 PFU of third-passage stocks of either reovirus 1/L or 3/D. Adult mice were inoculated p.o. by suspending the virus on 50 μl of gelatin containing borate-buffered saline (pH 7.4) (gel saline) and introducing it by means of a sterile stainless steel feeding tube directly into the stomachs of the female mice. Subcutaneous (s.c.) immunizations were performed by suspending virus in 50 μl of phosphate-buffered saline (PBS) and injecting it under the skin on the dorsal sides of the animals. Four or five mice were used for each immunization protocal. Reovirus-immune mice were housed in separate rooms away from stock mice. Neonatal mice were infected p.o. with 3×10^6 PFU of T3c9 suspended in 35 μl of gel saline by using a piece of PE10 tubing attached to a 30-gauge needle. Neonates were infected within 48 h of birth.

Preparation of immune serum. Adult mice were inoculated twice s.c. with 10^7 PFU of 1/L or 3/D 1 week apart as described above. Two weeks after the second inoculation the mice were anesthetized and exsanguinated by cardiac puncture. Serum was also similarly obtained from nonimmune mice.

Tissue handling and preparation. At various times after infection, neonatal mice were sacrificed by decapitation, and livers, small intestines, and brains were removed for virus titration. To assess the protection of each litter in a given immunized group, one pup from each of three separate litters was randomly chosen for virus titration. Virus titers were determined by using a plaque assay as previously described (20), and protein determinations were performed by using a micro-Lowry method as descrobed below. Briefly, viral titers were determined by making 10-fold dilutions of tissue samples in gel saline which were disrupted by three cycles of freezing and thawing followed by sonication. Samples (100 μl) were incubated on L-cell monolayers in 12-well plates (Costar, Cambridge, Mass.) at 37°C for 45 min, and then an agar overlay containing medium 199 Earle salts, 5% fetal bovine serum (Hyclone Laboratories, Inc., Logan, Utah), 4 mM L-glutamine (GIBCO Laboratories, Grand Island, N.Y.), 5 μg of gentamicin (GIBCO) per ml (complete medium), and 1% agar was added. Virus cultures were incubated for 7 days at 37°C. Cultures were overlaid on day 3 with medium containing agar. On day 6 cultures were fed with medium containing agar supplemented with 0.02% neutral red to visualize viral plaques. Protein determinations on tissues were made in 96-well microdilution plates by preparing appropriate dilutions of homogenized tissues in water to a final volume of 50 μl. Lowry solution (100 μl) (14) was added to each well and incubated at room temperature for 10 min, and then 10 μl of 1 N Folin reagent was added. Color development was read at 620 nm on an enzyme-linked immunosorbent assay plate reader and compared with standards of bovine serum albumin. Virus titers are expressed as $\log_{10}$ PFU per milligram of protein. We prepared brains from 22-day-old (20 days postinfection) mice for pathologic examination by fixing whole brains in Bouin solution for 3 h and cutting coronal sections, which were processed and stained with hematoxylin and eosin by the Pathology Department, Hospital of the University of Pennsylvania. Pathologic changes in brains were assessed by evaluating sections in a blinded manner.

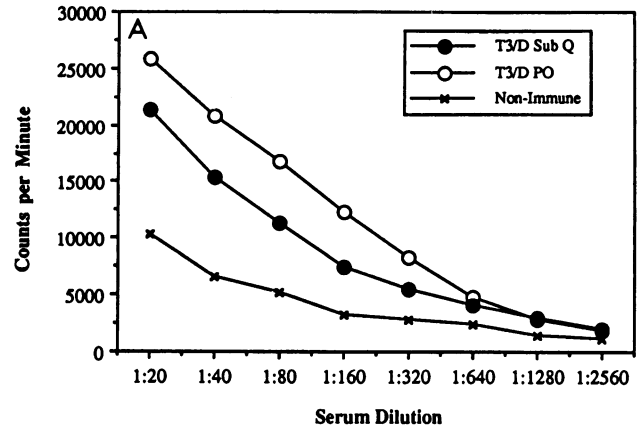
TABLE 1. Virus neutralization titers of various sera following immunization

Immunization	No. of mice	Neutralization titers against reovirus serotype[a]:	
		1/L	3/D
Nonimmune	3	<1:20	<1:20
Serotype 1/L (p.o.)	4	1:160	<1:20
Serotype 1/L (s.c.)	4	1:1280	<1:20
Serotype 3/D (p.o.)	3	<1:20	1:160
Serotype 3/D (s.c.)	2	<1:20	1:320

[a] Neutralization titers are expressed as the highest dilution of serum which inhibits the virus-mediated lysis of an L-cell monolayer formed in flat-bottom 96-well microdilution plates as described in Materials and Methods. Results are reported as the mean titers of serum samples obtained from mice immunized with two inoculations of 10^7 PFU of reovirus. Sera were obtained 2 weeks following the second immunization. All titers were within one dilution of the mean.

Serum neutralization titers. Serum samples were assayed by using a microneutralization assay. L cells (5×10^4) were dispensed into each well of 96-well flat-bottom microdilution plates and incubated overnight at 37°C in 5% CO_2 in air to form an adherent monolayer. The next day, duplicate samples of 20 μl of twofold serum dilutions in gel saline were mixed in separate microdilution plates with 20 μl of virus diluted to 10^7 PFU/ml. The samples were incubated for 30 min at room temperature. They were then pipetted onto L-cell monolayers and incubated for an additional 45 min at 37°C. After the second incubation the cells were overlaid with 80 μl of 1.5× complete medium and incubated for 3 days at 37°C in 5% CO_2 in air. On day 3, medium was removed and the plates were stained with 25 μl of crystal violet per well for 3 min and then washed with distilled water. The serum neutralization titer was determined to be the highest dilution of serum in which a confluent, stained monolayer was observed. This method has been compared with the plaque reduction method and provides similar titers of virus-specific antisera. The results have been published previously (28).

Radioimmunoassay. Antireovirus activity in serum was determined by using a radioimmunoassay. Wells of 96-well microdilution plates were coated with 2.5×10^{10} particles of either reovirus type 1/L or type 3/D and incubated at 4°C overnight. The plates were blocked with 200 μl of 3% bovine serum albumin in PBS for 2 h and washed extensively with PBS. Samples (20 μl) of twofold serial dilutions of serum in triplicate were added to the plates, which were incubated at



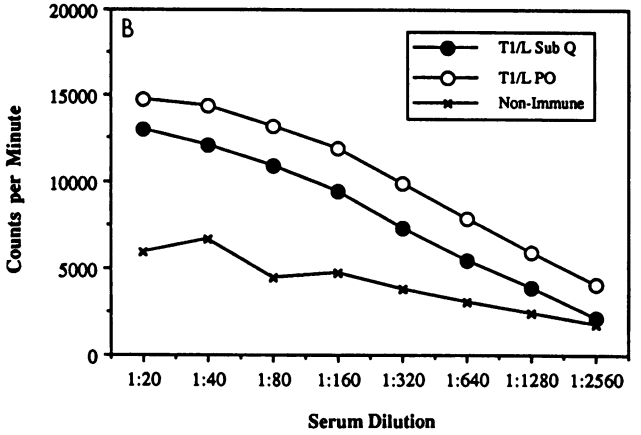


FIG. 1. Antireovirus activity in serum as measured by radioimmunoassay. Dilutions of serum obtained from mice immunized with type 3/D (A) or type 1/L (B) in PBS were incubated on plates coated with reovirus type 1/L. Each point represents the mean value of three replicates from four separate samples. The standard error of the mean did not exceed 15% at any point shown. Similar results were obtained with plates coated with reovirus type 3/D (data not shown). Abbreviations: T3/D Sub Q, mice immunized s.c. with type 3/D virus; T3/D PO, mice immunized p.o. with type 3/D virus.

room temperature for 4 h and then washed extensively. ^{125}I-labeled goat anti-mouse Fab antibody (100 μl) was pipetted into each well, and the plates were incubated at room temperature overnight. Following incubation, the plates were washed four times with PBS and were cut into individual wells. Radioactivity was measured with a gamma counter.

RESULTS

Development of serum antibody titers after immunization. Serum samples were obtained from immunized female mice 2 weeks after the second inoculation with reovirus by retroorbital sinus puncture. Mice immunized with reovirus types 1/L or 3/D either s.c. or p.o. developed titers of both serotype-specific neutralizing antibody (Table 1) and non-serotype-specific antibodies (Fig. 1; data not shown) as measured by radioimmunoassay. Immunization by the p.o. route appeared to induce slightly higher non-serotype-specific antireovirus antibody as measured by RIA, but lower serum neutralization titers. A pool of serum immunized with 1/L subcutaneously had a neutralization titer of <1:20 against T3c9 (data not shown).

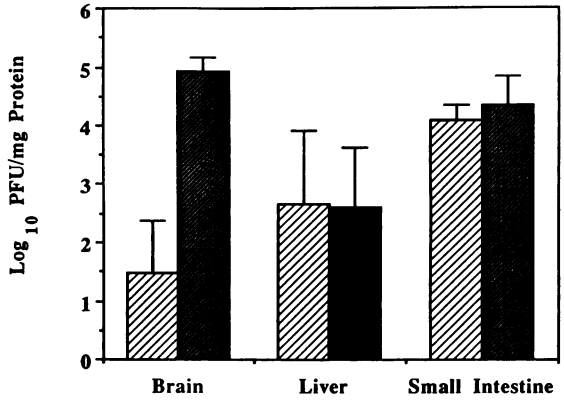


FIG. 2. Virus titers of small intestines, livers, and brains in infected nonimmune neonates. Two-day-old neonatal mice were orally inoculated with 3×10^6 PFU of reovirus T3c9. Seven pups were sacrificed by decapitation 4 (▨) or 7 (▨) days after inoculation, and tissues were removed and processed as described in Materials and Methods. Virus titers are expressed as geometric mean ± standard deviation of $\log_{10}$ PFU per milligram protein.

Infection of neonatal mice with reovirus T3c9. Neonatal mice which were infected p.o. with 3×10^6 PFU of reovirus T3c9 developed a highly lethal meningoencephalitis. Previous experiments indicated that the p.o. 50% lethal dose for this virus was approximately 10^4 PFU (data not shown). Thus, the challenge dose was approximately 300 times the 50% lethal dose. In these experiments, 3×10^6 PFU routinely killed 75 to 100% of infected control mice.

Figure 2 illustrates the development of virus titers in tissues 4 and 7 days postinfection. Nonimmune neonates developed high virus titers in the small intestines and livers 4 days after infection. Most nonimmune neonates developed detectable virus titers in brain tissue by day 4, and high virus titers were found in all brains from nonimmune pups by day 7. The mean time to death was approximately 10 days (data not shown). Pathologic changes in the brains of survivors at day 20 included foci of necrosis and neuronal cell dropout, which were found throughout the cortex, hippocampus, and medulla. Surrounding neurons exhibited oval or spherical hyaline inclusions. There was moderate to severe perivascular cuffing of capillaries by both mononuclear cells and occasional polymorphonuclear leukocytes. The leptomeninges were severely infiltrated with similar inflammatory cells (Fig. 3). This is consistent with previous reports of serotype 3 reovirus-induced meningoencephalitis following i.c., i.m., or p.o. inoculation (3, 15, 18, 25, 26).

Passive protection against fatal reovirus infection. Groups of four or five female mice were immunized with two inoculations of 10^7 PFU of 1/L or 3/D reovirus by the p.o. or s.c. routes. At 2 weeks after the second immunization, reovirus-immune female mice were mated with nonimmunized male mice. The resulting pups were infected p.o. with T3c9 2 days after birth. Although 10 of 13 control animals died, all neonates from immune dams (T1 (s.c.), $n = 13$; T1 (p.o.), $n = 18$; T3 (s.c.), $n = 17$; T3 (p.o.), $n = 17$) survived the infection (Fig. 4).

Virus titers in selected tissues were determined from neonates in each group by randomly selecting single neonates from each of three separate litters. Four days after infection, mice were sacrificed and brains, livers, and small

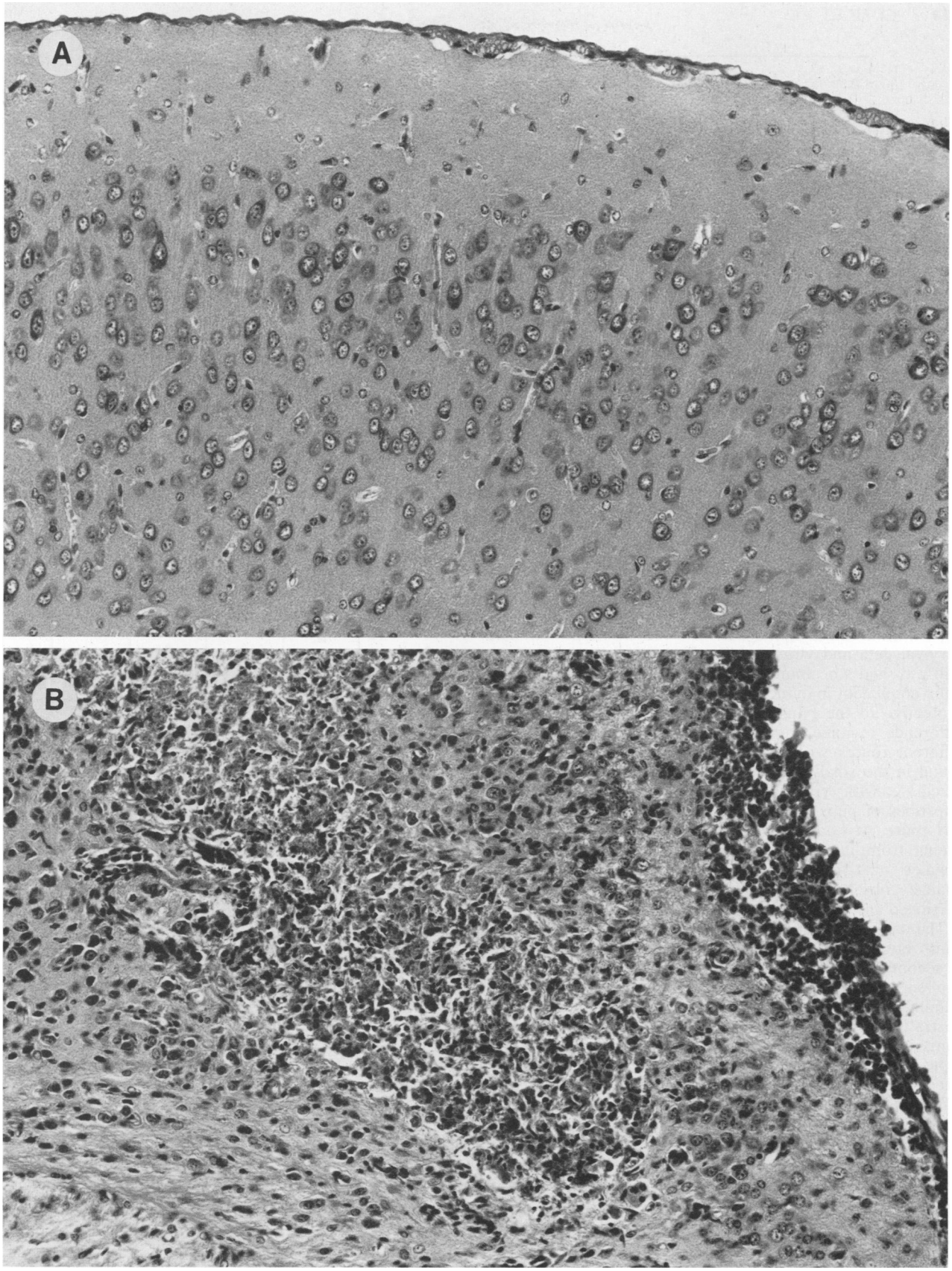


FIG. 3. Pathologic changes in survivors following reovirus T3c9 infection. Hematoxylin and eosin stains of brain sections of young mice are shown. (A) Brain section from a 22-day-old mouse nursed on a dam previously orally immunized with type 3/D reovirus. This section exhibits no evidence of meningoencephalitis. (B) Brain section from a nonimmune survivor taken 20 days after infection. This section demonstrates typical reovirus type 3-induced meningoencephalitis. Magnification, ×200.

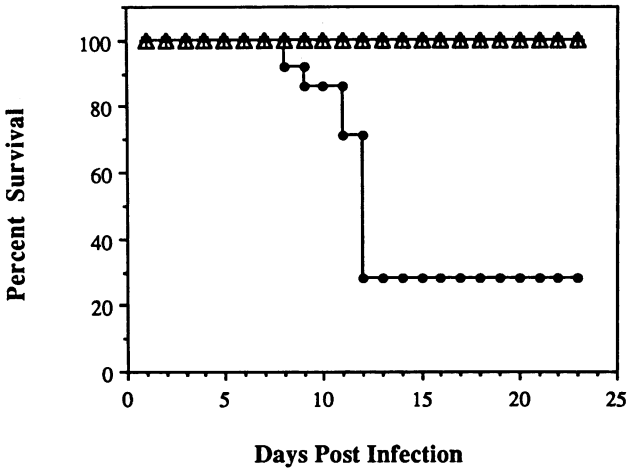


FIG. 4. Survival of infected neonates from immune mothers. Neonates from nonimmune mice or from mice previously immunized s.c. or p.o. with reovirus type 1/L or reovirus type 3/D were orally infected with 3×10^6 PFU of reovirus T3c9. The following numbers of neonates were infected: T1 (p.o.), $n = 18$ (◆); T1 (s.c.), $n = 13$ (□); T3 (p.o.), $n = 17$ (△); T3 (s.c.), $n = 17$ (|); normal, $n = 13$ (●).

intestines were taken for virus titration (Fig. 5). Although all of the pups from immune mothers survived, detectable virus was found in the small intestines and livers in the groups of pups from mothers immunized with reovirus type 1/L. The survival data and virus isolation from tissues demonstrate that p.o. and s.c. immunization with type 1/L were essentially equivalent in inducing an immune response which was protective to the pups. However, there was a qualitative difference in mice from mothers immunized by the two different routes with serotype 3/D. Detectable virus was found in the small intestines of mice from mothers immunized s.c. with type 3/D, but no virus was detected in the intestines of pups whose mothers were immunized by the p.o. route. At 4 days postinfection, no virus was detected in brains from infected neonates born to reovirus-immune females (data not shown), nor were consistent pathologic changes observed when all of the remaining mice were sacrificed and their brains examined 20 days after infection.

The dams were remated, and the second litters from each dam were infected 2 days after birth. As expected, the pups in second litters from the immune mothers all survived the challenge. In addition, the litters from the previously nonimmune mothers also survived. The results in Fig. 6 demonstrate the differential effects of nursing infected pups on dams previously immunized by homotypic versus heterotypic virus or not deliberately immunized. These differential effects are revealed by the degree of protection conferred to offspring in second litters. Although virus was still detected in the small intestines of pups from dams immunized with type 1/L, no virus was found in any tissues from pups whose mothers were immunized with type 3/D, nor in pups whose mothers were not previously immunized by experimental p.o. or s.c. immunization at day 4 or day 7. The observation that nonimmune dams conferred immunity to second litters of pups was made with three separate groups of experiments. A pool of serum taken from three such female mice was found to have a type 3-specific neutralizing titer of 1:16, and contained anti-reovirus activity by radioimmunoassay when diluted to 1:160, indicating that the dams were probably immunized by contact with the infected first litters.

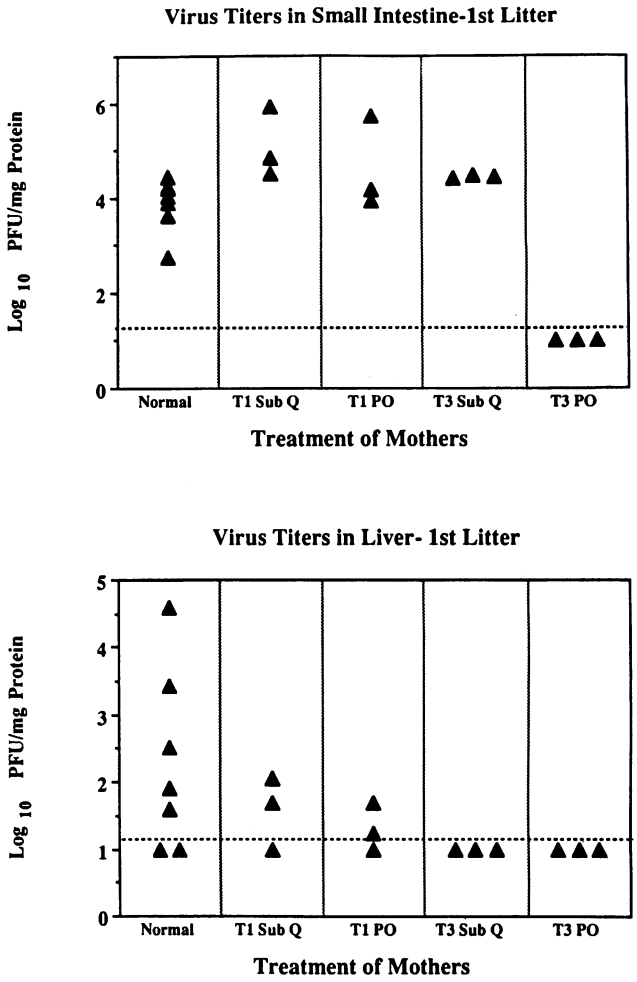


FIG. 5. Virus titers in small intestines and livers from first litters of infected neonates. Neonates were randomly selected from each group and sacrificed. Small intestines and livers were removed and processed as described in Materials and Methods. Each point represents the titer obtained from one animal. The dashed line represents the detectable limit of the assay for the virus.

Foster nursing. From the previous experiments it was not clear whether the protection was mediated by passive transfer of antibody in utero or by factors in milk which neonates obtained by nursing on immune mothers. To determine whether one or both mechanisms were responsible for protection, female mice were immunized with two p.o. inoculations of type 3/D and then mated with nonimmune mice. The offspring from this group were delivered by cesarean section to ensure that newborns received no milk from immune mothers. These pups were placed in cages with lactating nonimmune female mice. Two days later the pups were infected with virus. All of the infected mice ($n = 4$) survived and displayed no evidence of meningoencephalitis on pathologic examination of brains at day 20. The converse experiment was also performed. After natural delivery, 10 pups from nonimmune females were caged with lactating serotype 3-immune females and infected 2 days later. These pups also survived, and no evidence of pathology was found at day 20 (Table 2).

Passive protection mediated by passive transfer of immune serum. Mice (1 to 2 days old) were given 30 μl of reovirus type 1/L-immune, type 3/D-immune, or nonimmune serum

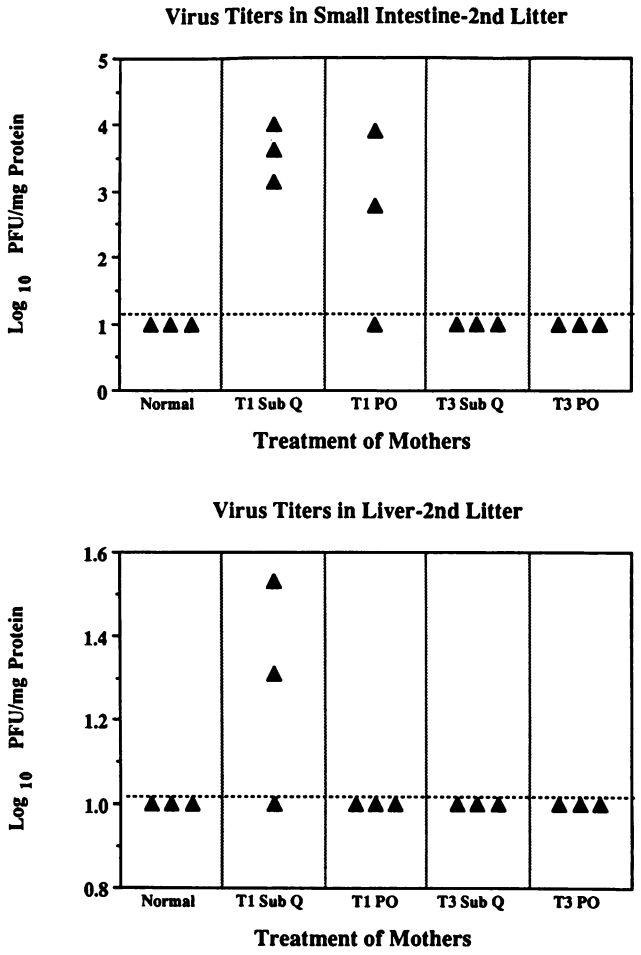


FIG. 6. Virus titers in small intestines and livers from second litters of infected neonates. Following completion of the first experiments, infected neonates were sacrificed and dams were remated. Second litters of pups were infected 2 days after birth. Neonates were randomly selected from each group and sacrificed. Small intestines were removed and processed as described in Materials and Methods. Each point represents the titer obtained from one animal. The dashed line represents the detectable limit of the assay for virus.

s.c. and inoculated p.o. with 3×10^6 PFU of T3c9 1 day later. Both type 1/L-immune and type 3/D-immune serum protected the neonates against lethal infection. Nonimmune serum had no detectable protective activity (Fig. 7). No

TABLE 2. Protection in foster-nursed neonates

Status of foster mother	Status of neonate	No. of neonates	No. surviving/ total no.	No. showing pathology[a]/no. of survivors
Normal	Normal	6	2/6	2/2
3/D immune[b]	Normal	10	10/10	0/10
Normal	3/D immune[c]	4	4/4	0/4

[a] At day 20 postinfection.
[b] Mothers were immunized with two p.o. inoculations of 10^7 PFU of reovirus type 3/D 1 week apart. The mice were mated 2 weeks after the second immunization.
[c] Neonates were born by cesarean section to preclude the possibility that milk was obtained by nursing following birth.

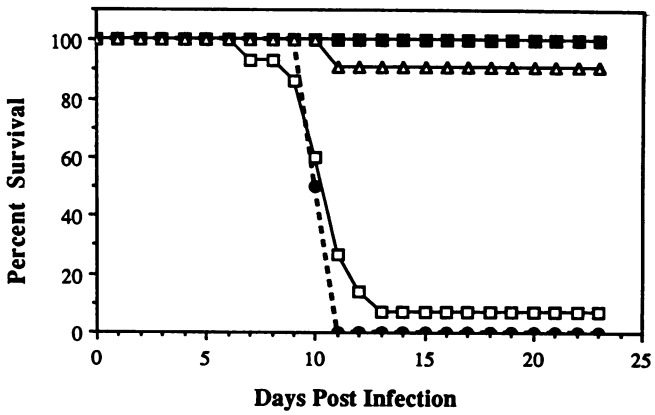


FIG. 7. Serum-mediated passive protection of neonates. Groups of mice were given 30 μl of 1/L-immune, 3/D-immune, or nonimmune serum at 1 to 2 days of age. The neonates were infected p.o. with 3×10^6 PFU of T3c9 1 day later and observed for survival. The following numbers of neonates were infected: no treatment (●), n = 12; nonimmune serum (□), n = 15; 1/L-immune serum (△), n = 14; 3/D-immune serum (■), n = 15.

pathologic changes were observed in brain sections from randomly selected survivors 20 days after infection, indicating that immune serum protected against reovirus-induced meningoencephalitis (data not shown).

DISCUSSION

This report further characterizes the lethal serotype 3 reovirus infection of neonatal mice and demonstrates that various levels of immunity develop in dams depending on the route of immunization and serotype of the immunizing virus. Previous reports of protection against type 3-induced disease in neonates described infections following either i.c. or i.m. injection of virus (3, 26) as well as p.o. inoculations with T3c9 (25). Because the normal portal of entry for reovirus is the gastrointestinal tract, infection via the p.o. route allows a more complete characterization of mucosal immune mechanisms. Mucosal immunity may play a pivitol role in mediating protection against pathogens which normally gain entry to the host through the gastrointestinal tract.

It was determined that the immune response induced in female mice following immunization with live reovirus can confer protection to neonates. This immunity is at least partially cross-serotype specific. Reovirus type 1-immune females could confer immunity to pups challenged with reovirus type 3 virus. Passively acquired immunity protected the pups from developing severe meningoencephalitis, although significant virus titers were detectable in the small intestines and livers of two of three of the neonates tested, suggesting that virus did replicate and disseminate in these pups. The cross-serotype protection was not due to reovirus type 3/D contamination of the type 1/L virus preparations which were used to immunize the dams, because (i) serum from reovirus type 1-immune mice neutralized type 1/L reovirus but not type 3/D reovirus in the in vitro neutralization assay (Table 1) and (ii) neonates inoculated i.c. with reovirus type 1/L manifested only characteristic ependymal cell pathology (9), with no pathology characteristic of reovirus type 3 infection (data not shown). This report extends the findings of Virgin et al. (26), in which serotype-specific monoclonal antibodies and immune rabbit serum could protect against reovirus type 3-induced meningoencephalitis in

neonates following i.m. inoculation, as well as those of Tyler et al. (25), who demonstrated antibody-mediated protection against meningoencephalitis following i.m. or p.o. inoculation of reovirus.

The observation that virus titers were found in the tissues of neonates as a result of immunization with homotypic or heterotypic virus is a significant finding because it implies that there are both serotype-specific and non-serotype-specific passively acquired factors which contribute to complete immunity. The route of immunization was also important in the development of transferable immunity in the dams. The findings reported here are complementary to those reported by Offit and Clark (16, 17) with a rotavirus model of infection. These investigators found that p.o. immunization with heterotypic rotavirus failed to induce in dams an immune response which was protective to neonates after p.o. challenge. Offit and Clark used the development of diarrhea in infected neonates as an index of protection. In their model, diarrhea is an indication of viral replication in the gastrointestinal tract, resulting in destruction of absorptive epithelial cells. Although we characterize infected neonates that failed to develop meningoencephalitis as protected, neonates whose mothers were immunized with heterotypic virus did have significant virus titers in their small intestines.

Because mice which were born to dams previously immunized p.o. with reovirus type 3 manifested no detectable virus in any tissue tested, it is possible that a specific secretory immunoglobulin A (IgA) response to the homotypic virus resulting from p.o. immunization of dams provides the most potent protection. Since the virus hemagglutinin attachment polypeptide (sigma 1) determines serotype specificity, it is possible that a serotype-specific, secretory immune response which is transferred from immune dams to neonates is necessary to inhibit the replication of virus in the gastrointestinal tracts of infected neonates. These results are consistent with the findings of Offitt and Clark (16), who reported that newborn mice could be protected against rotavirus infection by IgA- or IgG-containing fractions of mouse milk from rotavirus-immune mice. It should be possible to test our hypothesis by feeding the pups monoclonal sigma 1-specific IgA antibodies. The foster-nursing experiment in which milk from immune dams could protect pups born to nonimmune dams further suggests that secretory factors, such as IgA antibodies, may be sufficient to protect against death and/or the development of severe pathology. This interpretation is consistent with the findings of London et al. (13), who reported that intraduodenal application of reovirus markedly increased the frequency of IgA memory cells in Peyer's patches of infected adult mice. It is also possible that non-IgA isotypes were transferred to neonates during suckling and absorbed before gut closure. High titers of neutralizing antibodies were found in the sera of s.c. immunized mice, and these antibodies should be present in milk. However, neonates from dams immunized s.c. with homotypic virus were not completely free of virus, since virus was recovered from the small intestines of pups reared by these dams. Therefore, it seems that serotype-specific, non-IgA antibody is not sufficient to protect neonates against virus replication in small intestines following infection. The recent report by Tyler et al. (25) suggests that systemic application of hemagglutinin-specific monoclonal antibodies blocks dissemination of the virus but does not inhibit viral replication in the intestines of neonates following p.o. inoculation of reovirus T3c9. The important factor in inhibiting viral replication in the intestines appears to be serotype-specific immunity (presumably mediated by IgA) which can be obtained during suckling.

The foster-nursing experiment also suggests that transplacental transfer of immunity is sufficient for protection against meningoencephalitis, because pups delivered by cesarean section from a reovirus type 3-immune mouse did not develop meningoencephalitis following infection when nursed by a nonimmune lactating dam. Virus titers were not determined in these animals. However, further studies will determine whether reovirus replicates in intestines or livers of infected pups.

Second litters of nonimmune females were protected against infection and manifested no detectable virus in any tissue tested, suggesting that the females were apparently immunized by contact with the first litters infected with T3c9. This particular aspect of the experiment was repeated two separate times, and although the data were shown for only three mice (taken from three separate dams), tissue titers were performed on a total of nine mice, with the same result. These females conferred better protection to a second litter of mice than did females previously immunized s.c. with reovirus type 1/L. It was not surprising to find that maternal contact with infected pups induced a strong immune response in the previously nonimmune mice. Keroac and Fields (8) demonstrated that reovirus, particularly the T3c9 isolate used for p.o. infection of the neonates, is capable of being spread from infected pups to noninfected pups. The reovirus type 1-immune females that nursed T3c9-infected pups apparently did not develop the immune responses which were necessary to inhibit virus replication in the small intestines of infected neonates in second litters. It is possible that a regulatory mechanism was activated in those mice which inhibited the further development of immunity. Reovirus-specific suppressor cell activity has been demonstrated following infection in mice (4, 21). These observations further reinforce the commonly observed phenomenon of the failure of one serotype of virus to prime effectively for a secondary response against other serotypes of the same virus.

Our finding that passive transfer of immune serum could protect against disease provides further evidence that virus-specific antibody can protect against meningoencephalitis. Virgin et al. (26) and Tyler et al. (25) previously showed that hyperimmune rabbit immunoglobulin and mouse monoclonal antibodies specific for the neutralizing epitope on the sigma 1 polypeptide could protect against serotype 3/D-induced meningoencephalitis. The experiments described in this report demonstrate that in addition to the findings of Virgin et al. (26) and Tyler et al. (25), murine as well as rabbit antibodies directed against non-serotype-specific epitopes could protect against disease. Our source of non-serotype-specific protective antibodies differed significantly from those of Virgin et al. (26) and Tyler et al (25). In our experiments, protective antibodies were induced in homologous hosts after immunization with virus in the absence of adjuvant. In contrast, the rabbit antiserum used by Virgin et al (26) and Tyler et al (25) was prepared by repeated injection of rabbits with virus and adjuvant, resulting in a high-titer preparation. It is not known whether low-titer mouse serum arrests the spread of reovirus in a manner similar to that observed with hyperimmune rabbit sera. Further studies are needed to define the precise site of action for protection by the homologous antibodies used in this model.

In conclusion, protection against reovirus-induced meningoencephalitis can be passively transferred either transplacentally or by suckling, and protection is serotype cross-

reactive, because dams immunized with reovirus type 1/L protected neonates infected with T3c9. The most effective immune response develops following p.o. immunization with homotypic virus, presumably because p.o. immunization induces a strong secretory immune response which is transferred to neonates via suckling. A combination of transplacental immunity and antibodies or other factors obtained by suckling appears to inhibit virus replication in the intestines of infected neonates. It may be important to consider these findings in developing strategies of immunization against enteric pathogens such as cholera and rotavirus or against pathogens such as human immunodeficiency virus, which enter the host through mucosal surfaces.

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